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Title: Behavioral Characterization of Pentylenetetrazole-Induced Seizures: Moving Beyond the Racine Scale

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **08/08/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 19
Number of Shots: 36 (21 SC)

Introduction

1.1. **Antonio Jhones Rocha:** Our research focuses on characterizing and quantifying two types of ictal behaviors, the spasms and myoclonus, after administering pentylenetetrazole (PTZ). We're trying to unveil how these behaviors contribute to the onset of limbic seizures and how their patterns evolve over time.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1* APF Timecode: 68112_Interview_1 (1.1.1_1.2.1_1.3.1).mp4 00:02-00:22

What are the most recent developments in your field of research?

1.2. **Antonio Jhones Rocha:** Lately, there's been a lot of progress in understanding how focal seizures spread through neuronal networks and eventually generalize. With the help of computer-assisted video analysis, we're now able to capture much finer details about how seizures unfold.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1* APF Timecode: 68112_Interview_1 (1.1.1_1.2.1_1.3.1).mp4 00:23-00:41

What research gap are you addressing with your protocol?

1.3. **Antonio Jhones Rocha:** Most seizure studies rely on the Racine scale, but that approach often misses important behaviors triggered by PTZ. Our protocol helps fill that gap by identifying and quantifying events that are usually overlooked, both before and after the limbic seizure actually appears.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1* APF Timecode: 68112_Interview_1 (1.1.1_1.2.1_1.3.1).mp4 00:44-01:02

What advantage does your protocol offer compared to other techniques?

1.4. **Ana Augusta C. Rangel:** Our protocol allows for a much more detailed analysis of behavior. Instead of relying on a single score like the Racine scale, we can measure multiple parameters for each ictal event, giving us a deeper understanding of what's actually happening during seizures.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1* APF Timecode:

68112_Interview_2 (1.4.1).mp4 00:02-00:22 (0:02 – 0:20 s, TAKE 1), (0:22 – 0:38 s, TAKE 2), choose one

What research questions will your laboratory focus on in the future?

1.5. **Claudio Marcos Queiroz:** Right now, our lab is focused on exploring whether alternative antiseizure medications can prevent, delay, or lessen spasms and myoclonus, and how that might affect the development of limbic seizures.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1* APF Timecode: 68112_Interview_3 (1.5.1).mp4 (0:03 – 0:20 s, TAKE 1), (0:29 - 0:42 s, TAKE 2), choose one

Ethics Title Card

This research has been approved by the local ethical committee at the Federal University of Rio Grande do Norte

Protocol

NOTE: All the files are named as per the shot number. The timecodes indicated by the authors are added in red font below. The writer has not reviewed footage for regular talent shots

2. Administration of PTZ (Pentylenetetrazol) to the Animal

Demonstrator: Antonio Jhones Rocha

2.1. To begin, use a scale to measure the body weight of the animal **[1]**.

2.1.1. WIDE: Talent weighing the animal using a digital scale on a benchtop. (0:02 – 0:09 s, TAKE 1), (0:10 – 0:13 s, TAKE 2), (0:15 – 0:26, TAKE 3), please choose

2.2. Calculate the volume of pentylenetetrazol solution needed for each animal based on their body weight **[1]**.

2.2.1. Talent entering the animal's weight into a calculator or spreadsheet on computer. (0:02 – 0:16 s).

2.3. Record the animal's behavior inside the arena for at least 1 hour prior to the pentylenetetrazol injection **[1]**. This habituation period reduces novelty-induced ambulation and anxiety and allows extraction of baseline activity **[2]**.

2.3.1. Talent placing the animal in the arena. (0:02 – 0:18 s)

2.3.2. Shot of the animal activity in the recording arena. (0:02 – 0:26 s, TAKE 1), (0:28 – 0:49 s, TAKE 2), (0:51 – 0:56 s, TAKE 3), please choose

2.4. Prepare the pentylenetetrazol injection **[1]**. For subcutaneous administration, use one hand to secure the animal **[2]** and gently pinch the skin between its shoulder blades **[3]**.

2.4.1. Talent preparing the syringe for pentylenetetrazol injection. (0:02 – 0:24 s)

2.4.2. Talent holding the animal with one hand. (0:02 – 0:06 s)

2.4.3. Talent gently pinching the skin at the scruff to expose the subcutaneous space. (0:06 – 0:09 s).

2.5. Using a 1 milliliter syringe attached to a 26 and a half-gauge needle, insert the needle into the subcutaneous space in parallel with the body orientation **[1]** and inject the solution slowly **[2]**. Once the injection is complete, gently remove the needle while

holding the skin at the exit point to prevent leakage due to internal cavity pressure **[3]**.

2.5.1. Close-up of needle being inserted subcutaneously along the spine. (0:02 – 0:06 s)

2.5.2. Talent pushing the syringe plunger. (0:07 – 0:13 s, TAKE 1 – far away), (0:18 – 0:24 s, TAKE 2 – close up)

2.5.3. Talent removing the needle while pressing the injection site gently with fingers. (0:25 – 0:29 s)

2.6. Observe and make a note of any solution leakage from the injection site **[1-TXT]**. If leakage is present, consider administering an additional 50 microliters of the same pentylenetetrazol solution **[2]**.

2.6.1. Talent inspecting the injection site for any leakage. **TXT: Administer 50 µL of the same solution if leakage is observed** (0:02 – 0:12 s)

2.7. Now, place the animal back into the arena **[1]** and record continuously for 1 hour **[2]**.

2.7.1. Talent gently returning the animal to the arena. (0:02 – 0:08 s)

2.7.2. Talent pressing the record button on the camera. (0:02 – 0:11 s)

2.8. One hour or more after pentylenetetrazol injection, stop the recording and copy the video file for post-processing **[1]**. Use this for video tracking and behavioral coding **[2]**.

2.8.1. Talent stopping the recording. 0:02 – 0:15 s

2.8.2. SCREEN: 68112_Shot_12 (2.8.2).mp4 00:02-00:10.

3. Software Configuration for Post-Processing

Demonstrator: Ana Augusta C. Rangel

3.1. Open the **Solomon Coder** software **[1]**.

3.1.1. SCREEN: 68112_screenshot_2 (3.1.1) 00:11-00:18.

3.2. If a configuration file is available, select **File** and click **Load Configuration** to load predefined settings **[1]**.

3.2.1. SCREEN: 68112_screenshot_3 (3.2.1). 00:09-00:20.

3.3. To open a video, select **File** and click **Open Video [1]**.

3.3.1. SCREEN: 68112_screenshot_4 (3.3.1).00:09-00:21.

3.4. Press **Play** to begin video playback **[1]**.

3.4.1. SCREEN: 68112_screenshot_5 (3.4.1). 000:04-00:10.

3.5. Click the left mouse button or press the assigned keyboard key to mark observed behaviors during playback **[1]**.

3.5.1. SCREEN: 68112_screenshot_6 (3.5.1) 00:12-00:18.

3.6. To save the data, go to **Analyze** and select **Save Output As** to create a comma-separated values file **[1]**. For the coding sheet, go to **File** and choose **Save Coding Sheet [2]**.

3.6.1. SCREEN: 68112_screenshot_7 (3.6.1_3.6.2) 00:15-00:35.

3.6.2. SCREEN: 68112_screenshot_7 (3.6.1_3.6.2) 00:34-00:47

4. Coding Behaviors Post-PTZ Injection

4.1. Tag the pentylenetetrazol administration time at the moment the mouse reappears in the video frame after being returned to the observational arena **[1]**. Use this moment as time zero to serve as the temporal reference point for all subsequent behaviors and latency calculations **[2]**.

4.1.1. SCREEN: 68112_screenshot_8 (4.1.1_4.1.2). 00:04-00:15.

4.1.2. SCREEN: 68112_screenshot_8 (4.1.1_4.1.2). 00:16-00:28.

4.2. Observe for spasms and myoclonus a few minutes after pentylenetetrazol injection **[1]**. Identify spasms as fast, transient contractions of axial muscles, including bilateral ear flexion, back muscle contraction, tail raising, and rarely, a startle jump **[2]**. Code these spasms as discrete, instantaneous events, associating them with a single video frame **[3]**.

4.2.1. SCREEN: 68112_screenshot_9 (4.2.1). 00:16-00:25.

4.2.2. SCREEN: 68112_screenshot_10 (4.2.2) 00:15-00:25.

4.2.3. SCREEN: 68112_screenshot_11 (4.2.3) 00:20-00:30

4.3. To code myoclonus, identify events longer than 200 milliseconds, marked by a strong,

abrupt myoclonic jerk of the head, neck, and paws **[1]**. Classify subtypes based on associated tremors or directional posture loss including forward, leftward, or rightward **[2]**. Code the event across multiple frames, as the duration typically ranges from 1 to 2 seconds **[3]**.

4.3.1. SCREEN: 68112_screenshot_12 (4.3.1). 00:15-00:28.

4.3.2. SCREEN: 68112_screenshot_13 (4.3.2). 00:12-00:25.

4.3.3. SCREEN: 68112_screenshot_14 (4.3.3) 00:10-00:25.

4.4. For limbic seizures, identify behaviors aligned with the Racine scale, starting with behavioral arrest or staring **[1]**. Follow with automatisms, orofacial movements, forelimb or head myoclonus, and escalation to rearing, jumping, or tonic seizures **[2]**. Use the modified Racine scale to assign subcategories to events lasting longer than 10 to 15 seconds **[3]**.

4.4.1. SCREEN: 68112_screenshot_15 (4.4.1) 00:10-00:20.

4.4.2. SCREEN: 68112_screenshot_16 (4.4.2) 00:08-00:18.

4.4.3. SCREEN: 68112_screenshot_17 (4.4.3) 00:38-00:47.

4.5. Finally, mark the end of the recording session on a single video frame exactly 1 hour after pentylenetetrazol injection **[1]**. ~~If the animal dies following a tonic seizure, identify the time of death with this final frame mark **[2]**~~.

4.5.1. SCREEN: 68112_screenshot_18 (4.5.1) 00:20-00:32.

~~4.5.2. SCREEN: If applicable, label the final frame with "Death Timepoint" based on tonic seizure outcome.~~

Results

5. Results

5.1. The figure details parameters that can be extracted from this coding system, including latency to each ictal behavior, number of events, event duration, inter-event interval, and severity of limbic seizures **[1].**

5.1.1. LAB MEDIA: Figure 4

5.2. Spasms typically involved trunk stiffening in majority of events **[1]**, while only 6% of spasms exclusively activated the tail **[2]**.

5.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the large dark red section in each bar representing "tr 55%"*

5.2.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the small light section in each bar representing "tl 6%"*

5.3. Myoclonus presented predominantly as tremors in 64% of cases **[1].** Forward **[2]**, rightward **[3]**, and leftward motions were also noted **[4]**.

5.3.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the dark purple segments in all the bars labelled "tremor"*

5.3.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the light blue segments labelled "forward"*

5.3.3. LAB MEDIA: Figure 5C. *Video editor: Highlight the light yellowish segments labelled "rightward"*

5.3.4. LAB MEDIA: Figure 5C. *Video editor: Highlight the light purple segments labelled "leftward"*

5.4. The latency to onset was significantly different among spasms, myoclonus, and limbic seizures **[1]**, with spasms occurring earliest **[2]**.

5.4.1. LAB MEDIA: Figure 5D

5.4.2. LAB MEDIA: Figure 5D. *Video editor: Highlight the first box (spasms)*

5.5. The total number of spasms was significantly higher **[1]** than that of myoclonus or limbic seizures **[2]**.

5.5.1. LAB MEDIA: Figure 5E. *Video editor: Highlight the first bar "spasms"*

5.5.2. LAB MEDIA: Figure 5E. *Video editor: Highlight the bars for "myoclonus" and "limbic seizures"*

5.6. The median inter-event interval did not differ significantly between spasms and myoclonus **[1]**, but the limbic seizures lasted significantly longer than myoclonus **[2]**.

5.6.1. LAB MEDIA: Figure 5F.

5.6.2. LAB MEDIA: Figure 5G. *Video editor: Highlight the second boxplot "limbic seizures"*

5.7. In the daily PTZ injection protocol, spasm latency and inter-event interval significantly decreased by day 8 **[1]**, whereas the overall spasm events increased **[2]**.

5.7.1. LAB MEDIA: Figure 6C and E. *Video editor: Emphasize the boxplot for day "8" (extreme right) in both C and E*

5.7.2. LAB MEDIA: Figure 6D. *Video editor: Emphasize the boxplot for day "8" (extreme right)*

1. Pentylenetetrazol

- **Pronunciation Link:** No entry found in Merriam-Webster or OED. Reliable audio available at AudioEnglish:

  `https://www.audioenglish.org/z/pentylenetetrazol.htm`

- **IPA (American):** /ˌpɛn.tɪ.liˌnɛt.ɪˈtræz.ɔl/
- **Phonetic Spelling:** pen-ti-li-net-i-TRAZ-awl

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2. Myoclonus

- **Pronunciation Link:** Merriam-Webster provides clear audio and phonetic transcription:

  `https://www.merriam-webster.com/dictionary/myoclonus`
  wordpanda.net+3YouTube+3youglish.com+3WikipediaWikipedia+12Merriam-Webster+12Cambridge Dictionary+12

- **IPA (American):** /ˌmaɪ-ˈɑː-klə-nəs/ Cambridge Dictionary+2Merriam-Webster+2
- **Phonetic Spelling:** my-O-cluh-nuhs

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3. Spasms

- **Pronunciation Link:** Standard entry available in Merriam-Webster:

  `https://www.merriam-webster.com/dictionary/spasm`

- **IPA (American):** /spæzms/
- **Phonetic Spelling:** spaz-mz

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4. Limbic

- **Pronunciation Link:** Merriam-Webster entry with audio:

  `https://www.merriam-webster.com/dictionary/limbic`

- **IPA (American):** /ˈlɪm.bɪk/
- **Phonetic Spelling:** LIM-bik

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5. Subcutaneous

- **Pronunciation Link:** Merriam-Webster entry with audio:

  `https://www.merriam-webster.com/dictionary/subcutaneous`

- **IPA (American):** /ˌsʌb.kjuˈteɪ.ni.əs/
- **Phonetic Spelling:** sub-kyoo-TAY-nee-us

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6. Pentylenetetrazol *(same as #1)*

We've already covered this above as it was the main scientific term in your text.

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7. Racine (as in Racine scale)

- **Pronunciation Link:** HowToPronounce provides several audio recordings:

  https://www.howtopronounce.com/racine Collins Dictionary+15How To Pronounce+15Cambridge Dictionary+15howjsay.com+6Merriam-Webster+6Wikipedia+6Collins Dictionary+1How To Say Guide+10Wikipedia+10Wikipedia+10howjsay.com

- **IPA (American):** /rəˈsiːn/ or /rəˈsin/ (most common) wordpanda.net
- **Phonetic Spelling:** ruh-SEEN

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8. Myoclonic (adjective form of myoclonus)

- **Pronunciation Link:** Merriam-Webster entry includes the adjective form:

  https://www.merriam-webster.com/dictionary/myoclonic

- **IPA (American):** /ˌmaɪ.əˈklä.nɪk/
- **Phonetic Spelling:** my-uh-KLOH-nik

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9. Tonic

- **Pronunciation Link:** Merriam-Webster entry available:

  https://www.merriam-webster.com/dictionary/tonic

- **IPA (American):** /ˈtɑː.nɪk/
- **Phonetic Spelling:** TAH-nik

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10. Latency

- **Pronunciation Link:** Merriam-Webster entry available:

  https://www.merriam-webster.com/dictionary/latency

- **IPA (American):** /ˈleɪ.tən.si/
- **Phonetic Spelling:** LAY-tun-see