Title: Application of Dixon's Up-and-Down Design to Estimate the Minimum Alveolar Concentration of Sevoflurane in Rats with Refined Movement Classification

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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? **No**

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: **07/09/25**

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

Current Protocol Length

Number of Steps: 05
Number of Shots: 09

Introduction

REQUIRED:

1.1. **Roxana Polojintef-Corbu:** Our research focuses on the need for a personalized management of drug combinations in anesthesiology to achieve the appropriate anesthetic depth, considering the complex interactions among different drugs.

    1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.1.1*

What significant findings have you established in your field?

1.2. **Roxana Polojintef-Corbu:** Our previously published studies showed significant reductions in sevoflurane and propofol requirements following THC, ethanol, or pregabalin administration, highlighting notable and clinically relevant drug-anesthetic interactions.

    1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.1.2*

What research gap are you addressing with your protocol?

1.3. **Roxana Polojintef-Corbu:** Basic animal studies on hypnotic–vasopressor interactions date back to the 1970s, often using few subjects and focusing on outdated agents like halothane, leaving limited data on modern anaesthetics such as sevoflurane.

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

Ethics Title Card

This research has been approved by the Intramural Committee for Animal Experimentation of the Medical University of Vienna and the competent regulatory authority (Austrian Federal Ministry of Women, Science and Research)

Protocol

2. **Assessing Nociceptive Responses in Rats Under Sevoflurane Anesthesia Using Tail Clamp Stimulation**

Demonstrator: Johannes Müller

NOTE: Protocol was scripted from author provided footage

2.1. To begin, use an airtight, transparent chamber for placing the rat **[1]**. Connect the inflow and outflows line to sevoflurane and monitor sevoflurane concentrations **[2]**.

2.1.1. LAB MEDIA: video1.positive 00:03-00:04 **TXT: Chamber dimensions: 15 cm × 20 cm × 9 cm; opening: 15 mm**
Video Editor: Please freeze frame here

2.1.2. LAB MEDIA: video2.negative 00:03-00:05

2.2. Ensure a constant oxygen flow of 3 litres per minute **[1-TXT]**. Now, identify the distal third of the tail and lock the clamp in place **[2]**.

2.2.1. LAB MEDIA: video2.negative 00:00-00:01   *Video Editor: Please freeze frame here*
TXT: Determine sevoflurane concentration individually per animal; Start with 2.6 %

2.2.2. LAB MEDIA: video1.positive 00:06-00:10

2.3. Apply the pain stimulus until the rat shows a positive movement response **[1-TXT]**.

2.3.1. LAB MEDIA: video1.positive 00:11-00:19

TXT: Maximum: 1 min; Record presence or absence of a motor reaction with brief description

2.4. Classify as rolling when the behavioral response involves an axial rotation of the body, typically along the longitudinal axis **[1]**, or as stretching when the behavioral response is characterized by a bilateral, symmetric outward extension of the limbs and body **[2]**.

2.4.1. LAB MEDIA: video3.rolling 00:10-00:20

2.4.2. LAB MEDIA: video4.stretching 00:13-00:27

2.5. Classify as saltatory movement when the behavioral response consists of sudden, hindlimb-focused kicks or jerks resembling short, hopping-like bursts of the whole body **[1]** and as curling when the behavioral response involves uncoordinated, inward-directed movement of varying intensity, causing the body to bend toward its center

[2].

2.5.1.   LAB MEDIA: video5.saltatory 00:11-00:24

2.5.2.   LAB MEDIA: video6.curling 00:17-00:27

Results

3. **Results**

3.1. The MAC *(M-A-C)* was significantly reduced in the 10 milligram per kilogram THC group compared to the THC control group **[1]**, and in both 1 gram per kilogram and 2 gram per kilogram ethanol groups compared to the ethanol control group **[2]**.

3.1.1. LAB MEDIA: Figure 1. *Video editor: Please highlight the curves for "THC control" and "THC 10 mg/kg"* **TXT: MAC: Minimum Alveolar Concentration ; THC: Tetrahydrocannabinol**

3.1.2. LAB MEDIA: Figure 1. *Video editor: Please highlight the curves for "Ethanol 1 g/kg" , "Ethanol 2g/kg"and "Ethanol control"*

3.2. Curling was the most frequently observed movement in the THC study **[1]**, whereas rolling predominated in the ethanol study **[2]**.

3.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the black bar under "Curling"*

3.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the light gray bar under "Rolling"*

3.3. In the THC study, movement types such as curling, rolling, stretching, and saltatory appeared at similar sevoflurane concentrations, with no evident pattern linking them to anesthetic depth **[1]**.

3.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight all dot clusters for curling, rolling, stretching, and saltatory*

3.4. In the ethanol study, no clear separation between movement types and sevoflurane concentration was observed, indicating no obvious relationship with anesthetic depth **[1]**.

3.4.1. LAB MEDIA: Figure 4. *Video editor: Sequentially highlight the scatter of dots from Curling to Saltatory*

Pronunciation Guides:

 Sevoflurane
Pronunciation link: https://www.merriam-webster.com/dictionary/sevoflurane
IPA: /ˌsɛv.oʊˈflʊəˌreɪn/
Phonetic: sev-oh-FLUH-rayn
 Tetrahydrocannabinol
Pronunciation link: https://www.merriam-webster.com/dictionary/tetrahydrocannabinol
IPA: /ˌtɛtrəˌhaɪdroʊˌkænəˈbɪnɒl/
Phonetic: tet-ruh-hy-droh-kan-uh-BIN-awl

☐ **Saltatory**
Pronunciation link: https://www.merriam-webster.com/dictionary/saltatory
IPA: /ˈsæltəˌtɔri/
Phonetic: SAL-tuh-TOR-ee

☐ **Alveolar**
Pronunciation link: https://www.merriam-webster.com/dictionary/alveolar
IPA: /ˌælviˈoʊlər/
Phonetic: al-vee-OH-ler

☐ **Anesthesia**
Pronunciation link: https://www.merriam-webster.com/dictionary/anesthesia
IPA: /ˌænəsˈθiziə/
Phonetic: an-uhs-THEE-zhuh

☐ **Distal**
Pronunciation link: https://www.merriam-webster.com/dictionary/distal
IPA: /ˈdɪstəl/
Phonetic: DIS-tuhl

☐ **Transparent**
Pronunciation link: https://www.merriam-webster.com/dictionary/transparent
IPA: /trænsˈpɛrənt/
Phonetic: trans-PEH-rent

☐ **Peristalsis**
Pronunciation link: https://www.merriam-webster.com/dictionary/peristalsis
IPA: /ˌpɛrɪˈstɔlsɪs/
Phonetic: per-ih-STOL-sis