

Oct 07, 2025

An operant rodent orofacial nociceptive assay

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l6z1jdgqe/v1>

Airam Vivanco-Estela¹, Bruna De Paula¹, Basak Donertas-Ayaz¹, Robert Caudle¹

¹University of Florida

RE-JOIN



Airam Vivanco-Estela

University of Florida

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l6z1jdgqe/v1>

Protocol Citation: Airam Vivanco-Estela, Bruna De Paula, Basak Donertas-Ayaz, Robert Caudle 2025. An operant rodent orofacial nociceptive assay. [protocols.io https://dx.doi.org/10.17504/protocols.io.bp2l6z1jdgqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l6z1jdgqe/v1)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 04, 2025

Last Modified: October 07, 2025

Protocol Integer ID: 224024

Keywords: Orofacial Pain, Temporomandibular Joint, Orofacial Assay, Nociception, Pain behavior, Neuropathic pain, Pain assessment, Rodent model, Operant assay, orofacial pain sensitivity in rodent, operant rodent orofacial nociceptive assay this assay, orofacial pain assessment device, orofacial pain sensitivity, reliable for orofacial pain model, orofacial pain model, nociceptive behavior, nociceptive assay, quantitative measures of pain, operant rodent orofacial, step procedures for animal habituation, animal habituation, voluntary facial contact by positive reinforcement, voluntary facial contact, thermal stimuli, responses with minimal stress, consistent licking, pain, testing session

Funders Acknowledgements:

NIH/NIAMS

Grant ID: UC2AR082196

Abstract

This assay is designed as an efficient method to evaluate nociceptive behaviors and orofacial pain sensitivity in rodents through voluntary facial contact by positive reinforcement using mechanical or thermal stimuli. The protocol includes step-by-step procedures for animal habituation, training, and testing, including device setup, sensor calibration, and optimal task performance of licking/contact detection in controlled mechanical or noxious thermal conditions. The aim is to conduct daily training sessions to ensure consistent licking and contact behavior to achieve accurate data during testing sessions, providing quantitative measures of pain-related responses with minimal stress and allowing reproducibility. This assessment is reliable for orofacial pain models in preclinical studies.

Stoelting: OroFacial Pain Assessment Device (OPAD) Supplier

Safety warnings

 This protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee

Ethics statement

All animal work conducted followed the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida (Protocol n° 202300000001)



A. Preparation:

1 Animal Transfer & Acclimatization

- Transfer the animals from the housing room to the behavioral room.
- Allow enough time for habituation to minimize stress caused by relocation.

2 Reward Solution Preparation

- Prepare the reward solution (1 part condensed milk: 2 parts water) and allow it to reach room temperature.
- Fill the bottles for each OPAD (Orofacial Pain Assessment Device) box.

STEP CASE

Reward Solution Preparation 10 steps

Materials:

- 1 can of sweetened condensed milk (approximately 300mL)
- Large beaker ($\geq$ 1L capacity)
- Magnetic stir bar
- Water
- Hot plate stirrer
- Parafilm
- Labeling tape and marker

Instructions:

1. Open one can of sweetened condensed milk.
2. Place a magnetic stir bar into a large beaker.
3. Pour the sweetened condensed milk into the beaker (approx. 300mL).
4. Add 600mL of water to the beaker to reach a final volume of approximately 900mL.
5. Warm the solution using a hot plate stirrer. Set the desired temperature and stir speed, and mix for the required duration.
6. **IMPORTANT:** Do not leave warming milk unattended!
7. Cover the beaker with parafilm once mixing is complete.
8. Label **"NOT FOR HUMAN CONSUMPTION"**
9. Expiration date after 1 week after preparation.
10. Store the solution in the refrigerator after use.
11. Make sure the reward solution is at room temperature before using it.

3 OPAD Device Setup

- Put all the parts together and connect all the plugs to the device.
- Turn on the computer and open the Any-Maze file associated with the assay.
- Power on the OPAD device and verify that all devices are recognized by the software.

4 Bottle Installation & Cleaning Check

- Carefully place the milk bottles into position, avoiding any milkdrops or spillage of milk on the sensors or the device's components.
- Adjust the spout forward to encourage the animals to lick.



- Ensure devices remain clean and dry to prevent data interference or equipment damage, as it can deteriorate the devices over time.

5 **System Check**

- Test device functionality before starting.
- Verify licking detection by touching each bottle spout.
- Confirm the contact sensors are functional by touching the spikes (mechanical assay) or thermodes (thermal assay).

B. Training Phase

6 **Session Setup**

- Place the animals individually into the acrylic OPAD boxes.
- Run each session for 10min per animal.
- After the session, return the animals to their cages.

7 **Training Frequency**

- Train the animals daily for 2 weeks (10 sessions).
- Alternate assay types every other day (mechanical one day, thermal the next).
- Use a non-noxious thermal stimulus of 37°C during thermal training sessions.

8 **Behavioral Goal**

- Allow the animals to learn how to reach the milk (positive reinforcement) while voluntarily exposing their faces to the contact stimulus, touching the sensor with their face.
- Adjust the bottle and contact distance to optimize licking/contact detection.
- Use a measuring tool to register the final positions.
- Licking data must appear as a solid red block in the software.
- Contact data should appear as a continuous empty block, confirming sensor contact during licking.

**Note: If licking data shows gaps, move the bottle slightly backward until licking is continuous.*

C. Testing Phase

9 **Pre-test Preparation**

- Shave the animals one day before testing to ensure proper skin contact with the sensor, avoiding hair interference.

10 **Testing Sessions**

- Perform the testing daily for 1 week (5 sessions).
- Run each session for 10 minutes per animal.
- Alternate mechanical and thermal assay every other day.



- For thermal testing session, set thermodes to 45°C (noxious temperature) or any desired temperature between 4 and 60°C.

11 **OPAD Device Setup**

- Put all the parts together and connect all the plugs to the device.

D. Post-session Cleanup

- 12
- Remove all animals from OPAD boxes and return them to their cages.
 - Wash the acrylic boxes with water and soap.
 - Clean any spilled milk off of the OPAD hardware.
 - Allow boxes to air dry completely before the next session.