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# 🌐 Protocol for Social Partner and Solitary Platform-Mediated Avoidance (PMA) Task in Rats

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✓ Peer-reviewed method

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PLOS ONE Lab Protocols

Spotlight series



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**Protocol status:** Working

**We use this protocol and it's working**

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## Disclaimer

### Spotlight Video

The video below is a supplement with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

<https://www.youtube.com/embed/gFz4tfh5t98?si=NBi7-JE2vB5Aax49>

## Abstract

This protocol details methodology to study platform-mediated avoidance (PMA) in solitary and social partner contexts.

## Guidelines

Prior to performing any study methods described, investigators must obtain approval from their respective Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s).

## Materials

### Biological Materials and Reagents:

- Adult (3-5 months) male and female Sprague-Dawley rats
- Sucrose pellets [1], used for reward
- DI water, for cleaning operant chambers between sessions
- 70% ethanol, for cleaning operant chambers at the end of the day

### Operant Box and Equipment – Solitary PMA :

- Coulbourn modular test cage [2] containing a house light [3], pellet feeder [4], lever [5], speaker [6], and food dish [7].
- Drop pan [8] and shock floor grid [9] to place in bottom of operant box
- Square acrylic platforms (custom-built, 14.0 cm each side, 0.33 cm tall)
- Wide-angle webcam [10]
- Custom sound-attenuating cubicle (opaque sides, noise-reducing) for containing operant shuttle box

#### Note

Coulbourn Instruments is no longer producing equipment. Operant boxes and other materials may be purchased from other vendors such as Med Associates, San Diego Instruments, or can be custom built to fit the dimensions listed above.

### Additional Apparatus and Wiring:

- ANY-maze digital interface [11]
- ANY-maze relay interface [12]
- Coulbourn Programmable Animal Shocker [13] and shock cable [14]
- Power Distribution Board [15\*]
- DC Power Supply (0-10A/0-30V) [16\*]
- Electrical Junction Enclosure Box [17\*]
- Panel Mount Power Distribution Fuse Module Board [18\*]
- 18 AWG Gauge Electrical Wire [19\*]
- Hook Up Wire Kit [20\*]
- RJ11/RJ12 Keystone jacks [21\*]
- Digital Sound Level Meter for assessing speaker levels [22\*]

### Software:

- ANY-maze video-tracking software [23]

## Biological Materials and Reagents

|  | A    | B       | C                                      | D           | E                           |
|--|------|---------|----------------------------------------|-------------|-----------------------------|
|  | Item | Vendor  | Item Names                             | Item Number | Specifications              |
|  | 1    | Bioserv | Dustless Precision Pellets, 50,000/Box | F0021       | 45 mg, Rodent Purified Diet |

## Operant Box and Equipment

|  | A    | B              | C                 | D             | E                              |
|--|------|----------------|-------------------|---------------|--------------------------------|
|  | Item | Vendor         | Item Name         | Item Number   | Specifications                 |
|  | 2    | Coulbourn      | Modular Test Cage | H10-11R-TC    | This model is specific to rats |
|  | 3    | Coulbourn      | House Light       | H11-01R-LED   | N/A                            |
|  | 4    | Coulbourn      | Pellet Feeder     | H14-23R       | N/A                            |
|  | 5    | Coulbourn      | Lever             | H21-03R       | N/A                            |
|  | 6    | Coulbourn      | Speaker           | H12-01R       | N/A                            |
|  | 7    | Med Associates | Food Dish         | ENV-200R1AM   | N/A                            |
|  | 8    | Coulbourn      | Drop Pan          | H10-11R-TC-DP | N/A                            |
|  | 9    | Coulbourn      | Shock Floor       | 10-11R-TC-SF  | N/A                            |
|  | 10   | Spedal*        | Wide-Angle Webcam | MF920Pro      | 1080P, 120° viewing angle      |

## Additional Apparatus and Wiring

|  | A    | B         | C                          | D           | E              |
|--|------|-----------|----------------------------|-------------|----------------|
|  | Item | Vendor    | Item Name                  | Item Number | Specifications |
|  | 11   | Stoelting | ANY-maze Digital Interface | 60064       | N/A            |
|  | 12   | Stoelting | ANY-maze Relay Interface   | 60063       | N/A            |

|  | A  | B                    | C                                    | D            | E                                                                                                                                                                                                                  |
|--|----|----------------------|--------------------------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | 13 | Coulbourn            | Programmable Animal Shocker          | H21-03R      | N/A                                                                                                                                                                                                                |
|  | 14 | Coulbourn            | Shock Cable                          | H93-01-25    | Length: 25ft                                                                                                                                                                                                       |
|  | 15 | Evemodl*             | Power Distribution Board             | PCB005       | <ul style="list-style-type: none"> <li>• 3 Inputs</li> <li>• 2×10 Outputs for DC AC voltage</li> <li>• Max voltage: 30V</li> </ul>                                                                                 |
|  | 16 | TUFFIOM*             | DC Power Supply                      | B01NACT99O   | <ul style="list-style-type: none"> <li>• Input Voltage: AC 220V/ 110V±10%, 50Hz/ 60Hz</li> <li>• Output Voltage: DC 0-30V</li> <li>• Output Direct Current: 0-10A</li> </ul>                                       |
|  | 17 | Zulkit*              | Electrical junction enclosure box    | B07RT6NWT R  | <ul style="list-style-type: none"> <li>• Outer Size of electrical enclosure: 7.9 × 4.7 × 2.95 (L*W*H)</li> <li>• Inner Size of electrical enclosure 7.6×4.4×2.7 (L*W*H)</li> <li>• Thickness: 0.12"/3mm</li> </ul> |
|  | 18 | Electronics-Salon*   | Power Distribution Fuse Module Board | MD-D1125D-1  | <ul style="list-style-type: none"> <li>• Work Voltage: AC or DC 5~32V</li> <li>• Total current rating 40Amp</li> <li>• Dimensions: 150mm x 72.5mm x 29mm (W x L x H)</li> </ul>                                    |
|  | 19 | MILAPEAK*            | 18 AWG Gauge Electrical Wire         | 2468         | <ul style="list-style-type: none"> <li>• Color: Red &amp; Black</li> <li>• Voltage: 12V DC</li> </ul>                                                                                                              |
|  | 20 | Electronics Express* | Hook Up Wire Kit                     | 27WK22STR 25 | <ul style="list-style-type: none"> <li>• Authentic Stranded</li> <li>• 300 volts</li> <li>• 22 gauge copper</li> <li>• 6 colors</li> <li>• 150ft</li> </ul>                                                        |
|  | 21 | C&E*                 | RJ11/RJ12 Keystone jacks             | CNE584393    | <ul style="list-style-type: none"> <li>• 90 degree connection</li> </ul>                                                                                                                                           |
|  | 22 | Meterk               | Digital Sound Level Meter            | E245122YH    | N/A                                                                                                                                                                                                                |

## Software

|  | A    | B         | C                               | D           | E              |
|--|------|-----------|---------------------------------|-------------|----------------|
|  | Item | Vendor    | Item Name                       | Item Number | Specifications |
|  | 23   | Stoelting | ANY-maze videotracking software | 60000       | Full-License   |

\*Items indicated by an asterisk were purchased from Amazon, but could be sourced from elsewhere if items are made using the same parameters

## Operant Box and Equipment – Social Partner PMA

- Coulbourn operant shuttle box [24] containing two house lights [3], two pellet feeders [4], two levers [5], two speakers [6], and two food dishes [7], one on each side of the shuttle box.
- Plexi-glass, perforated divider for separating sides of operant box (custom-built, 28.24 cm x 25.17 cm x 0.32 cm, 0.48 cm holes)
- Drop pan [25] and shock floor grid [26] to place in bottom of operant box
- Two rectangular acrylic platforms (custom-built, 10×15 cm), one per side of the operant box
- Two wide-angle webcams [10\*], one per side of the operant box
- Sound-attenuating cubicle [27] (noise-reducing) for containing operant box

## Additional Apparatus and Wiring:

- ANY-maze digital interface [11]
- ANY-maze relay interface[12]
- Coulbourn Programmable Animal Shocker [13] and shock cable [14]
- Power Distribution Board [15\*]
- DC Power Supply (0-10A/0-30V) [16\*]
- Electrical Junction Enclosure Box [17\*]
- Panel Mount Power Distribution Fuse Module Board [18\*]
- 18 AWG Gauge Electrical Wire [19\*]
- Hook Up Wire Kit [20\*]
- RJ11/RJ12 Keystone jacks [21\*]
- Wire Crimping Tool
- Digital Sound Level Meter for assessing speaker levels [22\*]

## Software

ANY-maze video-tracking software [23]

## Operant Box and Equipment Unique to Social Partner PMA

|  | A    | B         | C                   | D             | E                              |
|--|------|-----------|---------------------|---------------|--------------------------------|
|  | Item | Vendor    | Item Name           | Item Number   | Specifications                 |
|  | 24   | Coulbourn | Operant Shuttle Box | H10-11R-SC    | This model is specific to rats |
|  | 25   | Coulbourn | Drop Pan            | H10-11R-XX-DP | N/A                            |
|  | 26   | Coulbourn | Shock Floor         | 10-11R-XX-SF  | N/A                            |

|  | A  | B                   | C                         | D         | E   |
|--|----|---------------------|---------------------------|-----------|-----|
|  | 27 | Med Associates Inc. | Sound-Attenuating Cubicle | ENV-018MD | N/A |

**Note**

Biological materials and reagents, additional apparatus and wiring, and software are identical to those specified in Table 1

## Husbandry

- 1 Sprague-Dawley rats are bred in-house from rats purchased from a commercial vendor (Charles River Laboratories, Wilmington, MA) and housed in same-sex dyads or triads. Rats are handled and weighed twice a week, or daily if actively participating in an experiment. When all rats within a cage reach the weight indicated by the standard growth chart for Sprague-Dawley rats at approximately 8-10 weeks of age, they are placed on a restricted diet (16-18 g/rat/day) of standard laboratory rat chow to facilitate lever pressing for sucrose pellets. Weights are closely monitored to ensure that rats remain within the healthy range of body weights outlined by the standard growth chart for Sprague-Dawley rats. A general experimental overview for both Solitary and Social Partner PMA is shown in Figure 1.

## Experimental Setup (Solitary)

- 2 A Rat Modular Test Cage is used as the operant box for the Solitary platform-mediated active avoidance (PMA) task (see Figure 2). The operant box includes a house-light, speaker, lever, and food dish. In the bottom of the operant box, a drop pan, and shock floor grid are connected and placed to serve as the floor of the chamber and to administer foot shocks. A square acrylic platform is placed in the corner opposite the lever and food dish. The legs of the platform extend through the shock floor grid down to the drop pan beneath the shock floor grid. An overhead camera is placed at the top of the operant box for monitoring the rat during PMA sessions.
- 3 Place the operant box inside of a sound-attenuating cubicle to minimize outside noise and visible distractions during the session.
- 4 Wire the operant chamber and apparatuses into ANY-maze interfaces to allow for communication with the ANY-maze software.

## Lever-Press Conditioning (LPC)

- 5 Rats in the experiment are food-restricted (see troubleshooting), and weighed daily to ensure that animals remain within the healthy range of body weights outlined by the standard growth chart for Sprague-Dawley rats. Each day of conditioning, food is allotted after each animal completes daily LPC sessions to avoid satiation prior to the task.
- 6 To condition rats to press a lever to receive a sucrose reward, rats undergo three shaping schedules (lever-press conditioning phases 1-3). Training takes place over 5-7 days, beginning at the same time each day. A rat is placed into an operant box, which must be the same box in which Solitary PMA is eventually run. Further, rats are run in the same

order and within the same operant box to maintain consistent conditions. Each LPC session is 30 minutes. The house light is on with the shock floor grid (with shockers left unplugged) and platform present to keep consistent conditions between LPC and eventual platform-mediated avoidance. Each day, prior to the start of the session, boxes are tested to ensure levers and food dispensers are working properly. The operant box and surrounding cubicle are closed to minimize outside distractions.

- 7 In LPC phase 1, place the rats under a fixed-ratio one (FR1) reinforcement schedule, receiving one sucrose pellet every time the rat presses the lever.

#### Note

Passing criteria for this phase required rats to reach a pressing rate of 2 presses per minute (ppm) by the end of the session.

- 8 After completion of phase 1, move the rats to LPC phase 2 and place them on a variable-interval reinforcement schedule where rats receive a response-contingent pellet every 15 seconds on average (VI-15).

#### Note

Passing criteria for this phase required rats to reach a pressing rate of 5 ppm by the end of the session.

- 9 Finally, move the rats to LPC phase 3, where they are increased to a VI-30 reinforcement schedule.

#### Note

Passing criteria for this phase required rats to reach a pressing rate of 12 ppm.

- 10 After completing phase 3 of LPC, rats advance to the platform-mediated avoidance (PMA) task.

## Solitary Platform-Mediated Avoidance Conditioning

- 11 Rats within the experiment are food-restricted following the same guidelines as LPC. Each day of training, daily food is allotted after completion of a PMA session to avoid satiation prior to the task.

- 12 Rats are conditioned with a pure tone (30 sec, 4 kHz, 75 dB) co-terminating with a scrambled footshock delivered through the floor grids (2 sec, 0.4 mA). The shortest inter-trial interval (ITI) is approximately 120 seconds while the longest ITI is approximately 200 seconds. All rats receive 9 tone-shock pairings per day. Access to the lever is unrestricted throughout all training and test sessions. Sucrose reward is given on a VI-30 schedule.
- 13 Rats are conditioned across 10 days. The availability of reward on the side opposite to the platform motivates rats to leave the platform during the ITI, facilitating trial-by-trial assessment of avoidance.
- 14 Upon the conclusion of each session, the platform, tray, and grid are removed and replaced with clean parts after wiping down the inside of the box with DI water. After all sessions are completed for the day, the box is disinfected with 70% ethanol.

## Procedure - Experimental Setup (Social Partner)

- 15 The operant box for the Social Partner platform-mediated active avoidance (PMA) task consists of a shuttle box modified with the addition of a transparent, perforated acrylic barrier separating the chamber into two size-equivalent, mirrored sides (see Figure 2). Each side of the operant box includes a house-light, speaker, lever, and food dish. In the bottom of the operant box, a drop pan and shock floor grid are connected and placed to serve as the floor of the chamber, and to administer foot shocks. A rectangular acrylic platform is placed in the corner opposite of the lever and food dish, one for each side of the operant box. Two overhead cameras are placed in the top of the operant box, one for monitoring each rat during PMA sessions.
- 16 Experimental setup is otherwise identical to Solitary PMA.

## Procedure - Lever-Press Conditioning (LPC)

- 17 Lever-Press Conditioning (LPC) follows the same procedures described for LPC during Solitary PMA above. Rats are placed with their cagemate into the operant box, one rat per side separated by a perforated divider. Rats are run in the same order and within the same operant box and side until reaching the response criteria for each LPC phase.
- 18 Upon the conclusion of each session, the platform, drop pan, and shock grid are removed and replaced with clean parts after wiping down the inside of the box with DI water. After all sessions are completed for the day, the box is disinfected with 70% ethanol.

## Procedure - Social Partner Platform-Mediated Avoidance

- 19 Rats are assigned a same-sex and age-matched partner that is not their cagemate. Partner rats are placed on a designated side of the perforated acrylic barrier, where they are still able to see, smell, and hear one another. Rats complete 10 days of training within the same operant box and side of barrier.
- 20 Rats within the experiment are food-restricted following the same guidelines as Solitary PMA.
- 21 Partner rats are conditioned with the same experimental parameters as described for Solitary PMA. Partner rats are conditioned across 10 days. The availability of food on the side opposite to the platform motivates rats to leave the platform during the ITI, facilitating trial-by-trial assessment of avoidance.
- 22 Cleaning procedures upon the conclusion of training sessions and days are identical to procedures described for Solitary PMA.

## Troubleshooting

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| A                                 | B                                                                 | C                                                                                                                                                           | D                                                                                                                                                                                                                                                                                                        |
|-----------------------------------|-------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Stage                             | Problem                                                           | Possible Reason                                                                                                                                             | Solution                                                                                                                                                                                                                                                                                                 |
| General                           | A rat is seemingly uninterested in the sucrose reward.            | <ol style="list-style-type: none"> <li>1. The rat is not hungry enough to seek the reward.</li> <li>2. The rat does not like the sucrose reward.</li> </ol> | <ol style="list-style-type: none"> <li>1. Ensure a rat is not fed prior to behavioral session (LPC or PMA). Adjust homecage food as needed to maintain appropriate levels of motivation for sucrose reward.</li> <li>2. Consider alternate variety of reward (ex. chocolate-flavored pellet).</li> </ol> |
| General                           | The rat is moving the platform away from its designated position. | 1. The rat is attempting to bring the platform closer to the sucrose reward.                                                                                | 1. The platform can be fixed more rigidly (e.g., duct-taped underneath to the drop pan or grid floor) to further secure it in place.                                                                                                                                                                     |
| Leve-Press Conditionin<br>g (LPC) | The rat is not reaching the criterion for passing LPC.            | 1. The rat does not understand the association between the lever and receiving a sucrose                                                                    | 1a. Introduce additional shaping procedures to strengthen the relationship between the lever and the reward                                                                                                                                                                                              |

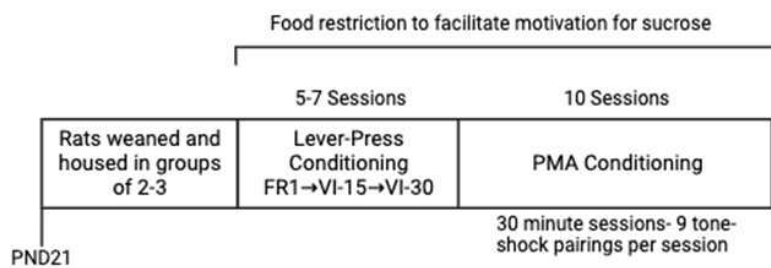


| A                                    | B                                                                                                       | C                                                                                                                                                                                                    | D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------------------|---------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                      |                                                                                                         | <p>reward.</p> <p>2. The rat is not hungry enough to seek the sucrose reward.</p> <p>3. The rat decreases responding when increased to a leaner schedule of reinforcement.</p>                       | <p>(ex: crush a sucrose pellet and sprinkle on the lever).</p> <p>1b. Consider hand-shaping where the experimenter will manually control the pellet delivery via the computer software when the rat gets increasingly close to pressing the lever. Alternatively, the experimenter can physically guide the rat's paw to the lever to demonstrate lever pressing for sucrose delivery.</p> <p>2. Ensure a rat is not fed prior to LPC. Adjust homecage food as needed to maintain appropriate levels of motivation for sucrose reward while ensuring that IACUC protocols are met.</p> <p>3. Rather than continue running the rat on leaner schedule (e.g. VI-30), revert to VI-15 schedule and run until reliable responding is achieved.</p> |
| Platform-Mediated Avoidance Training | The rat is not reacting (e.g., not retracting paws, not jumping, not running to platform) to the shock. | <p>1. The shock level may be too low to elicit a proper behavioral response.</p> <p>2. The shocker may not be plugged in.</p> <p>3. Rat may not be making two-points of contact with grid floor.</p> | <p>1. Raise the shock in increments of 0.02 mA and reassess behavior at each trial.</p> <p>2. Ensure shocker and grid are working before beginning session.</p> <p>3. Ensure there is sufficient distance between platform and the lever.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Platform-Mediated Avoidance Training | The rat is not leaving the platform for the majority of the session.                                    | <p>1. The shock level may be too high to elicit a proper behavioral response.</p> <p>2. The rat may not be motivated</p>                                                                             | <p>1. Raise the shock in increments of 0.02 mA and reassess behavior at each trial.</p> <p>2. Consider food restricting the rat further, while ensuring</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

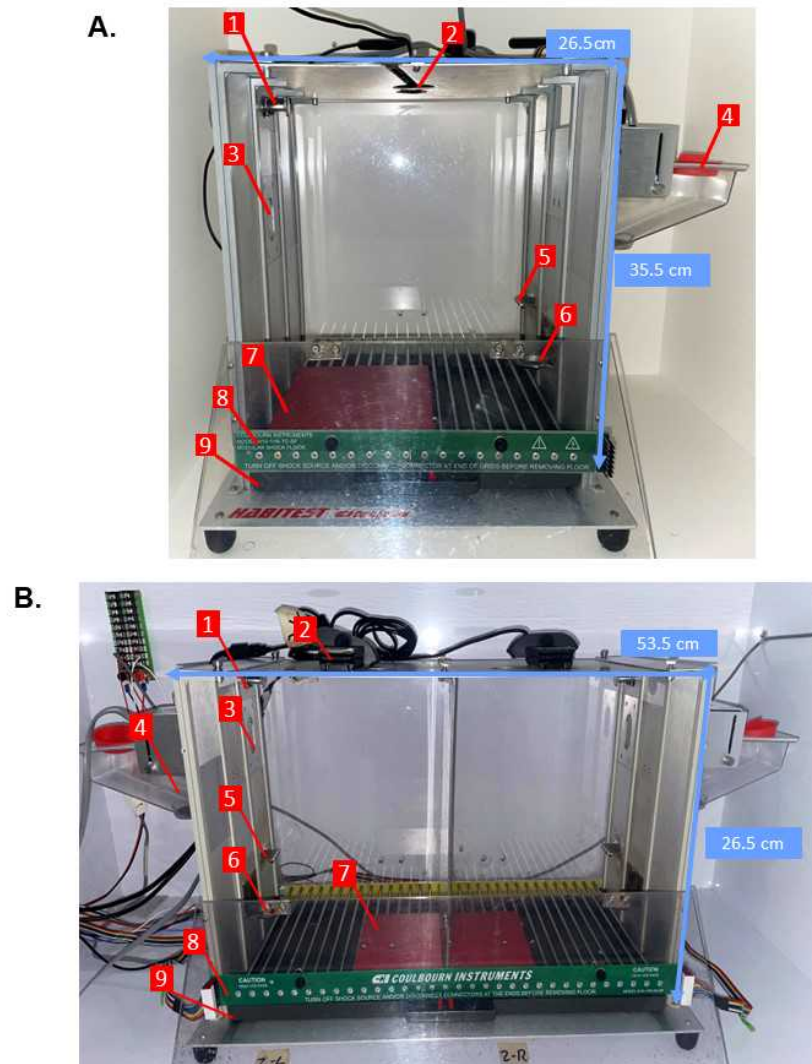
|  | A | B | C                             | D                        |
|--|---|---|-------------------------------|--------------------------|
|  |   |   | enough to leave the platform. | IACUC protocols are met. |

## Figures

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**Figure 1.** Timeline of Solitary or Social Partner PMA conditioning.



**Figure 2.** Solitary and Social Partner PMA apparatus.