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# Title: Using a Murine Model of Psychosocial Stress in Pregnancy as a Translationally Relevant Paradigm for Psychiatric Disorders in Mothers and Infants

**Authors and Affiliations:**
**Sandra P Zoubovsky[1,2,3], Akil Wilder[4], Louis Muglia[1,2,3*]**

[1]Molecular Developmental Biology Program, Cincinnati Children's Hospital Medical Center
2Division of Human Genetics, Cincinnati Children's Hospital Medical Center
3Department of Pediatrics, University of Cincinnati College of Medicine
[4]Division of Veterinary Services, Cincinnati Children's Hospital Medical Center

*Present address: Burroughs Wellcome Fund, Research Triangle Park, NC, US

**Corresponding Authors:**
Louis Muglia (louis.muglia@cchmc.org)

**Email Addresses for All Authors:**
zoubovsp@mail.uc.edu
akil.wilder@cchmc.org
louis.muglia@cchmc.org

# 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? **No**

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?

   ☒ 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: 11
Number of Shots: 31

# Introduction

**1. Introductory Interview Statements**

**REQUIRED:**

1.1. **Sandra Zoubovsky:** Our protocol is significant because it represents a new paradigm for evaluating the effects of chronic stress, specifically psychosocial stress, on pregnancy outcomes. We expose pregnant mice to various psychosocial insults of different intensities, in an unpredictable fashion.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.2.2, 2.4.2*

1.2. **Akil Wilder:** Previous animal model paradigms employed physical stressors or a limited number of stressors administered repetitively, which does not reflect the experience of women during pregnancy. Our chronic psychosocial stress model overcomes these limitations.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.7.2, 2.8.2*

**OPTIONAL:**

1.3. **Sandra Zoubovsky:** This method has wide ramifications for various research areas, including maternal mental health and adverse infant development-related research, and more broadly, developmental origins of adult disease through adverse intrauterine events.

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

**Ethics Title Card**

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Cincinnati Children's Medical Center.

# Protocol

**2. Performing the Chronic Psychosocial Stress (CGS) Paradigm**

2.1. Before starting each stressor in the CGS *(C-G-S)* paradigm, bring the CGS group mice from the housing room into the room designated for CGS **[1-TXT]**. Expose the mice to different stressors following an 11-day stressor regimen **[2]** running from gestational day 6.5 to 17.5 **[3]**.

2.1.1. WIDE: Establishing shot of talent bringing and placing down the CGS mouse cages in the CGS room. **TEXT: Leave control mice undisturbed in the housing room**

2.1.2. LAB MEDIA: Figure 1. *Video Editor: Show only the left half of the figure (timeline from TM up to G17.5 with the table depicting the schedule of the morning, afternoon, and overnight stressors)*

2.1.3. LAB MEDIA: Figure 1. *Video Editor: Show only left half as above; Emphasize the 'Day' column.*

2.2. To expose the mice to foreign objects, randomly distribute six marbles or six legos of different shapes into a clean static cage with mouse bedding without the mouse nestlets **[1]**. Place all mice from one home cage into the static cage with foreign objects for 2 hours **[2]**, then return the mice to their home cage **[3]**.

2.2.1. Talent placing marbles and legos in the static cage. *Videographer: This step is important!*

2.2.2. Talent transferring mice from their home cage to the static cage with foreign objects.

2.2.3. Talent returning the mice to their home cage.

2.3. To expose the mice to predator odor, place 1-centimeter-thick dirty bedding from female rats into a clean static cage with no mouse bedding or nestlets **[1]**. Place all mice from one home cage into the static cage with dirty rat bedding for 2 hours **[2]**, then return the mice to their home cage **[3]**.

2.3.1. Talent placing dirty rat bedding in the static cage. *Videographer: This step is important!*

2.3.2. Talent transferring mice from their home cage to the static cage with dirty rat bedding.

2.3.3. Talent returning the mice to their home cage.

2.4. To expose the mice to a cage tilt, place all mice from one home cage into a clean static cage with mouse bedding without the mouse nestlets **[1]**. Keep the cage tilted at a 30-degree angle against the wall for 2 hours **[2]**, then return the mice to their home cage **[3]**.

2.4.1. Talent transferring mice from their home cage to a static cage with mouse bedding. *Videographer: Obtain multiple usable takes because this will be reused in 2.5.1, 2.7.1, 2.8.1*

2.4.2. Talent tilting the cage. *Videographer: This step is important!*

2.4.3. Talent returning the mice to their home cage. *Videographer: Obtain multiple usable takes because this will be reused in 2.5.3, 2.7.3, 2.8.3*

2.5. To expose the mice to frequent bedding changes, place all mice from one home cage into a clean static cage with mouse bedding without the mouse nestlets **[1]**. Every 10 minutes, gently move the mice to a different clean cage **[2]** and replace the mouse bedding **[3]**. After 2 hours, return the mice to their home cage **[4]**. *Videographer: This step is important!*

2.5.1. *Use 2.4.1. Talent transferring mice from their home cage to a static cage with mouse bedding.*

2.5.2. Talent moving mice to a different cage.

2.5.3. Talent replacing the mouse bedding.

2.5.4. *Use 2.4.3. Talent returning the mice to their home cage.*

2.6. To expose the mice to bedding removal, place all mice from one home cage into an empty, clean static cage with no mouse bedding or nestlets for 2 hours **[1]**, then return the mice to their home cage **[2]**.

2.6.1. Talent transferring mice from their home cage to static cage with no mouse bedding. *Videographer: This step is important!*

2.6.2. Talent returning the mice to their home cage.

2.7. To expose the mice to movement on a shaker, place all mice from one home cage into a clean static cage with mouse bedding without the mouse nestlets **[1]**. Place the static cage atop a reciprocal lab shaker set to 140 strokes per minute for 2 hours **[2]**, then return the mice to their home cage **[3]**.

2.7.1. *Use 2.4.1. Talent transferring mice from their home cage to a static cage with mouse bedding.*

2.7.2. Talent placing the cage on a shaker. *Videographer: This step is important!*

2.7.3. *Use 2.4.3. Talent returning the mice to their home cage.*

2.8. To expose the mice to lights overnight, place all mice from one home cage into a clean static cage with mouse bedding without the mouse nestlets **[1]**. Keep the lights on overnight to interfere with the dark cycle **[2-TXT]**. On the following day, return the mice to their home cage **[3].**

2.8.1. *Use 2.4.1. Talent transferring mice from their home cage to a static cage with mouse bedding.*

2.8.2. Shot of mice in the static cage in the room with lights on. TEXT: **Lights on: 8:00 pm - 6:00 am** *Videographer: This step is important!*

2.8.3. *Use 2.4.3. Talent returning the mice to their home cage.*

2.9. To expose a mouse to new cage mates, transfer the mouse into a clean static cage with mouse bedding containing two unfamiliar female mice **[1]**. Leave the mouse in the static cage with the unfamiliar cage mates overnight **[2]**. On the following day, return the mouse to its home cage with familiar cage mates **[3]**.

2.9.1. Talent transferring a mouse to a static cage with two female mice.

2.9.2. Shot of mouse in the cage with unfamiliar cage mates. *Videographer: This step is important!*

2.9.3. Talent returning the mouse to its home cage.

2.10. To expose the mice to wet bedding, saturate the mouse bedding in a static cage with clean water kept at 24 degrees Celsius **[1]**. Place all mice from one home cage into the static cage with wet bedding overnight **[2]**. On the following day, return the mice to their home cage **[3]**.

2.10.1. Talent adding clean water to the mouse bedding. *Videographer: This step is important!*

2.10.2. Talent transferring mice from their home cage to a static cage with wet bedding.

2.10.3. Talent returning the mice to their home cage.

2.11. After the stressor regimen is complete, on gestational day 17.5, single-house all the experimental mice to prepare for parturition and downstream functional assessments **[1]**.

2.11.1. Talent placing each experimental mouse in a separate cage.

# Results

**3. Results: Effects of Chronic Psychosocial Stress on Dams and Pups**

3.1. Exposing pregnant female mice to CGS causes changes in chronic stress-relevant parameters **[1]**, such as a reduction in body weight gain during pregnancy **[2]** and increased adrenal gland weights in the early postpartum period **[3]**.

3.1.1. LAB MEDIA: Figure 2.

3.1.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize the red bar in Figure 2A*

3.1.3. LAB MEDIA: Figure 2. *Video Editor: Emphasize the red squares in Figure 2B*

3.2. Importantly, CGS dams exhibit postpartum abnormalities in maternal neuroendocrine function **[1]**, as evidenced by increased serum corticosterone levels following a novel acute insult **[2].**

3.2.1. LAB MEDIA: Figure 3.

3.2.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize the red bar*

3.3. Furthermore, CGS dams display alterations in maternal care as reflected by an increase in the degree of fragmentation of maternal signals received by the pups **[1]**. The average duration of licking-grooming bouts is reduced **[2]** while the mean number of bouts is increased **[3]**, indicating numerous short episodes of nurturing behavior **[4]**.

3.3.1. LAB MEDIA: Figure 4A and 4B.

3.3.2. LAB MEDIA: Figure 4A and 4B. *Video Editor: Emphasize red bar in figure 4A*

3.3.3. LAB MEDIA: Figure 4A and 4B. *Video Editor: Emphasize red bar in figure 4B*

3.3.4. LAB MEDIA: Figure 4A and 4B.

3.4. Sucrose preference is also depressed in CGS dams **[1]** when compared to control dams **[2]**, suggesting the presence of anhedonia **[3]**. Lastly, the CGS dams also display increased anxiety-related behaviors **[4]** as measured by a reduction in the time spent in the open quadrants of the EZM **[5]** when compared to control dams **[6].**

3.4.1. LAB MEDIA: Figure 4C. *Video Editor: Emphasize the red bar*

3.4.2. LAB MEDIA: Figure 4C. *Video Editor: Emphasize the white bar*

3.4.3. LAB MEDIA: Figure 4C.

3.4.4. LAB MEDIA: Figure 4D.

3.4.5. LAB MEDIA: Figure 4D. *Video Editor: Emphasize the red bar*

3.4.6. LAB MEDIA: Figure 4D. *Video Editor: Emphasize the white bar*

3.5. In the offspring, exposure to CGS in-utero **[1]** results in decreased weight gain during the postnatal period, from postnatal day 7 to 21 **[2]**, although there are no differences at birth **[3]**. This reduction in body weight gain is present in offspring of both sexes **[4]**.

3.5.1. LAB MEDIA: Figure 5.

3.5.2. LAB MEDIA: Figure 5. *Video Editor: Successively emphasize the red squares and red inverted triangles at PN7, PN15, and PN21.*

3.5.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize the no difference in weights at PN0 and PN2.*

3.5.4. LAB MEDIA: Figure 5.

# Conclusion

**4. Conclusion Interview Statements**

4.1. **Sandra Zoubovsky:** When attempting this procedure, establishing a schedule for obtaining timed pregnancies and consistently implementing the entire series of variable, unpredictable psychosocial stressors is critical.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: LAB MEDIA: Figure 1*

4.2. **Akil Wilder**: This technique can help answer interesting questions such as: Can maternal psychosocial stress cause sex-specific effects on placental function or offspring brain development and behavior, and can these effects be reversed or modified by pharmacological intervention or postnatal maternal care?

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