OVERVIEW
The lab uses the Cold Pressor Task (CPT) although others—such as the Trier Social Stress Test—have been used as well (e.g. see Kischbarum et al., 1993).

Participants should be randomly assigned to a stress or control group. Participants in the stress group undergo the cold pressor task, during which they submerge their arms in a 0-4°C ice-water bath for three minutes. The cold-pressor task is used widely in the laboratory to induce stress levels comparable to that which participants may experience in everyday life and has been shown to elicit subjective, physiological, and neuroendocrine increases in stress (references below). The control participants submerge their arms in room-temperature water (30-40°C) for three minutes. To assess subjective levels of stress after the task, all participants should somehow indicate how subjectively stressful they find the stress or control task to be (e.g. scale from 1 (not stressful) to 10 (most stressful)).

METHODS

<table>
<thead>
<tr>
<th>Materials</th>
<th>Participants</th>
<th>Task Procedure</th>
<th>Salivary Samples</th>
<th>References</th>
</tr>
</thead>
</table>

- Salivary sample kit
- Gloves
- Bag of Ice
- Bucket
- Paper Towels
- Timer
- Thermometer

Click [here](#) for how to order supplies from Salimetrics.

**Stress Group:** Ice water temperature: 0°C - 4°C

**Control Group:** Room temperature water: 28°C - 30°C

**Age:** 18-40 years old

**Scheduling:** 12PM - 6PM

**For Females:** Make sure to take note of menstrual cycle
General Script:

“Before we begin, we’re going to have you complete a task that includes submerging your hand to elbow in a bucket of water. You have been randomly assigned to be in the (WARM v.s. COLD) group. This simply entails you pull up your sleeve and place your arm in the water for approximately 3min, after which you may remove your arm.”

Stress group:

The water should be ice-cold—specifically, 0-4°C. A good heuristic if you don’t have a thermometer is for the water to be so cold that the ice no longer melts. Directly beforehand (but not before the baseline saliva sample) participants should be told they’ve been selected for the cold-water group, and that this means the water will be ice-cold. They should know that they might experience discomfort during this task, but that this task poses no risk to them and widely used in research laboratories. It is important that they hold their arm in the water for the full 3-minute duration. If the discomfort level becomes too much they can remove their arm for a few seconds, but if this happens more than once, or for more than a few seconds, it is recommended to terminate the experiment.

Control group:

Take temperature of water (should be room temp; 30-40 degrees Celsius). Ask participants to submerge hand to elbow in water for 3 minutes. Afterwards, controls should be given a 10 minutes break (or whatever amount of time the stress group received as well).

Neuroendocrine markers of stress

Pilot work here in the lab has shown that SCR and shock responses are markedly diminished directly after cold pressor, so stressed participants will need 5-10min for their arm/hand to warm up before SCR or shock may commence.
To confirm that participants show viable stress responses as a result of the manipulation, we collect two types of neuroendocrine data: **alpha-amylase** and **cortisol**. Alpha-amylase (AA) is an enzyme secreted from the salivary glands is thought to reflect norepinephrine release (i.e., salivary AA remains unchanged under stress when NE is blocked). Cortisol is a glucocorticoid released via the Hypothalamic-Pituitary-Adrenal (HPA) axis. AA appears to reflect the earlier, rapid catecholamine response of stress, while cortisol reflects the delayed recovery response (lag times range from 10-20min).

**Storing & Shipping Samples**

Samples should be labeled with the Salimetrics bar codes on the outer test tube and stored in the cryostorage boxes in a freezer set to -20°C for preservation. Cortisol is quite robust, so samples can be handled and organized outside of the freezer for up to 30-60 minutes; AA is slightly less robust, but still can be handled outside the freezer for some time. Samples should be shipped using overnight delivery through Fedex or UPS. Only ship samples Monday – Wednesday; if you need to ship on a Thursday, confirm this ahead of time with a Salimetrics contact representative. To ship samples, the cryostorage boxes should be secured with a rubberband, and each box should be placed in a gallon sized Ziploc freezer bag. Fill a styrofoam shipping container (we use the one that Salimetrics sends our cortisol supplies in) about ¼ of the way with dry ice (we typically use ice from the histology lab on the 10th floor). Place a crumbled layer of newspaper on top of the ice as a barrier, and the sample boxes on top of the newspaper. A copy of the sample roster (i.e., a list of sample IDs) as well as a Test Order Form, should be placed inside a Ziploc bag and inside the shipping container before it is closed. Close the shipping container and place it in a corrugated cardboard shipping carton before taping up the box. For UPS, we place a UN 3373 label as well as a label stating that 2.5kgs of dry ice are in the carton (2.5kgs DRY ICE), but different carriers require different labeling.

**Detailed directions for shipping samples for testing** can be found on the Salimetrics website: [http://www.salimetrics.com/lab/shipping-instructions/](http://www.salimetrics.com/lab/shipping-instructions/)


**Trier-Social Stress Test (TSST)**