Chronic Unpredictable Stress Directions

1 Vinegar Water

Measure out approximately 400 mL of vinegar water using the graduated cylinder for each of the rats and pour it into the vinegar water bottles. Record the amount of vinegar water for each rat in the CUS binder. At the end of the stressor, pour each one of the vinegar water bottles into the graduated cylinder and record the amount. Calculate the amount of vinegar water consumed, then pour the leftover vinegar water back in the original container and put the regular water bottles back in the cages.

2 Damp Bedding

Using the graduated cylinder, fill it with approximately 400 mL of water. Pour this into the cage through the cage top (you don't need to take it off). Do not get water on the food pellets. At the end of the stressor, you will need to change the cage.

3 Tail Clip

Take a small hair clip from the box on the top shelf (brown and black) and clip it onto the base of a rat's tail. They figure out how to take them off fairly quickly so it is best to do 4 at a time so that you can effectively put them back on when they take them off. The tail clip should last 5 minutes.

4 Predator Sounds

Turn on and press play on the black CD/VCR(?) player that is on the bottom shelf and make the speakers are plugged in. If it is not already, change the volume so it is at a level where it is loud enough for the animals but would not be heard by the animals in the other rooms. The sounds will turn off on their own.

5 Novel Stimulus

Place one of the giant lego pieces into each of the cages and keep it in the cage for 5 minutes. Clean the toys with ethanol and return them to the storage box.

6 Strobe Light

Turn on the strobe light by pressing the power button until it reaches the mode where the light is flashing. Turn off the room light. At the end of the stressor, turn off the strobe light and press the room's light switch to continue the light cycle.
7 **White Noise**

Turn on the blue and silver CD player and it should automatically play white noise. If not, set the radio station to 87.5 and turn the volume to a level where it is loud but not audible by the rats in the other rooms. At the end of the stressor, turn the player off.

8 **Altered Light Cycle**

Contact Animal Care Services to alter the pre-programmed light cycle.

9 **Open Field**

Spray the open field apparatus with clidox and wipe it down with paper towel. Place a rat in the apparatus for 5 minutes. Repeat the open field test and cleaning for all other rats. Record number of vocalizations, defecations and any other variables of interest. Return to cage after 5 minutes and clean the maze thoroughly before the next animal.

10 **Tilted Cage**

Put one of the red enrichment huts underneath one side of the cage so that the cage is at an angle. Remove and put back the enrichment at the end of the stressor.

11 **Predator Odour**

Take a plastic pipette and put a couple drops of fox urine onto the cotton and distribute these around the outside of the cages around the room. At the end of the stressor, place the pieces of cotton back in the plastic bag and transfer the rats back into the animal unit. The odor is very potent so only do this in a room where the door can be closed and where other animals cannot be disturbed.