

## Creating a Rat Model of Chronic Variate Stress

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**[Abstract]** Stress is a condition of human experience and an important factor in the onset of various diseases. There are numerous studies showing how stress can accelerate cell aging, immune senescence and some age-related diseases such as neurodegenerative disorders and osteoporosis. However, the effects of stress have different consequences depending on the type, duration or severity and predictability of the stressor applied. Although stress can be beneficial in its acute phase, repeated and severe stressful stimuli produce adverse effects. There are different models of stress depending on the exposure time; acute (when the stressor is applied for a short time, e.g. hours or days, and intensely) or chronic (when the stressor is applied for a long time, e.g. weeks or months, and less intensely). In these cases, the stressor can be repeated each time or different stressors can be used). The latter model is most frequently used to achieve similar conditions to those found in human diseases related to stress. Also, there are several different paradigms depending on the purpose of the study [development of drug therapies or modeling depressive behaviors; for the different paradigms see Dagnino-Subiabre, (2012)]. Here, we describe a 9-day variable-stressor paradigm with repeated and prolonged stimulation and a random daily stressor over days or weeks to minimize its predictability. This protocol has been adapted from other models of variable stress with significant modifications. The absence of predictability of the stressor applied is an important characteristic of this model compared to other models in which repeated stress is used. We avoid the use of a strong stressor, such as foot shock or tail pinch, and describe an easily reproducible new chronic mild stress model. Some models of chronic mild stress have been reported to lead to a wide range of behavioral disturbances and have been proposed as models of depression in animal studies (Cryan *et al.*, 2005).

### **Materials and Reagents**

1. Male albino Wistar rats (250-270 g)

### **Equipment**

1. Glass tank (44 x 33 x 30 cm)

2. Plastic tube (21 x 6 cm, 6 cm diameter)
3. Individual cages (47 x 32 x 20 cm)
4. Cold room or refrigerator (4 °C)

## **Procedure**

The stressors schedule used in this protocol is listed in Table 1. Application of stress starts at a different time every day to minimize its predictability (from 8:00 a.m. to 8:00 p.m.).

**Table 1. Schedule of stressors used during chronic variant stress treatment**

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Day	Stressor	Time
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1	Forced swimming	10 min
2	Restraint	3 h
3	Water deprivation	24 h
4	Restrain at 4 °C	90 min
5	Isolation	24 h
6	Food deprivation	24 h
7	Water deprivation	24 h
8	Restrain at 4 °C	2 h
9	Food deprivation	24 h
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### Day 1: Forced swimming

1. Fill a glass tank 22 cm deep with water at  $23 \pm 2$  °C (Figure 1).
2. Place the animal in the glass tank for 10 min. While the animal is swimming it may try to get out of the tank, so to prevent escape it may be necessary to carefully close it with a heavy lid.
3. Return the animal to a clean and dry cage with fresh bedding in order to avoid chills and colds.



**Figure 1. Picture of a typical glass tank filled with cold water**

#### Day 2: Restraint

1. Place the animal in a 21 x 6 cm plastic tube; adjust it with plastic tape on the outside so the animal is unable to move (Figure 2). The tube must have a 6 cm hole at the far end to allow regular breathing. To place the animal in the plastic tube, it is necessary to place the head of the animal close to the entrance, after which it should enter by itself (Figure 3).



**Figure 2. Picture of the tube with tape and animal inside**



**Figure 3. Pictures showing the restraint procedure using a plastic tube**

2. Wait for 3 h. Although the plastic tube should be sufficient to prevent the animal coming out is desirable to leave the immobilizer inside the cage.
3. Return the animal to its cage. The best way to extract the animal is to make a sudden movement downwards, dropping it into the cage. Try to avoid pulling from the tail.

#### Day 3: Water deprivation

1. Remove the bottle of water from the cage during 24 h. If the animal house is supervised by staff, indicate that the water/food must not be replaced in that cage.
2. Place the bottle of water back after the time point is reached.

#### Day 4: Restraint at 4 °C

1. Place the animal in the above-mentioned plastic tube.
2. Place the tube with the animal inside a cold room or a refrigerator at 4 °C for 90 min. Cold is a well-documented stressor (Krishnamurthy *et al.*, 2011). Therefore, it is necessary to find a place with these features to put the animals in.
3. Return the animal to its cage at room temperature.

#### Day 5: Isolation

1. Place the animal alone in a new cage.
2. Return the animal to the cage with their mates.

#### Day 6: Food deprivation

1. Remove the food from the cage for 24 h.
2. Place the food back.

#### Day 7: Water deprivation

1. Remove the water container from the cage for 24 h.
2. Place the bottle of water back.

#### Day 8: Restraint at 4 °C

1. Put the animal in the plastic tube described above.
2. Place the tube with the animal inside a cold room or a refrigerator at 4 °C for 2 h. It has been observed that after the first cold-environment exposure, 90 min at 4 °C is not enough time any more to reach adequate stress levels, that is why 2 h at 4 °C is more convenient.
3. Return the animal to its cage at room temperature.

#### Day 9: Food deprivation

1. Remove the food from the cage for 24 h.
2. Place the food back.

#### Representative data

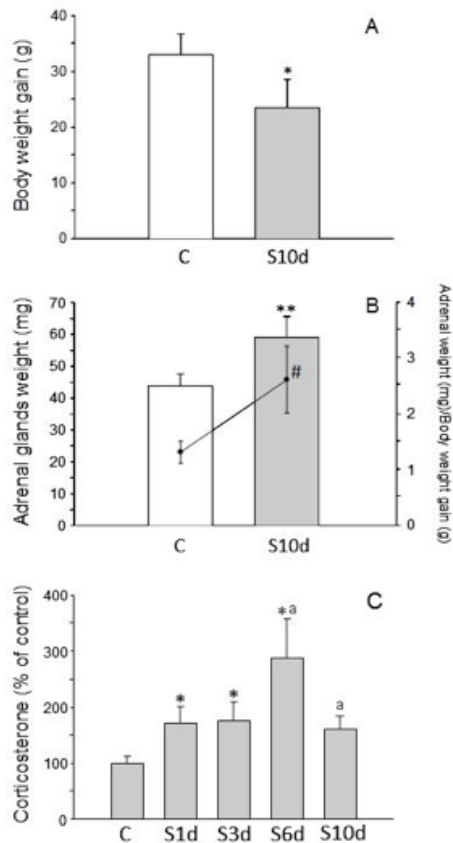
Validation of the stress model: Changes in body and adrenal glands weight, in the blood levels of corticosterone, and in dopamine (DA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) levels in the prefrontal cortex are typical effect of stress and are used as methods to assess stress models. With this chronic variant stress protocol, the body weight of the animals decreases, whereas adrenal weight and the blood levels of corticosterone increase (Figure 4). It has also been found that increased levels of dopamine and DOPAC in the prefrontal cortex are observed (Table 2).

*Notes about reproducibility and variability: Stress perception is very subjective and each animal, just as each person, is stressed to a different degree. Therefore, it is normal that the results present some variability. To avoid this, glucose preference test is recommended (Hu et al., 2010); then, only animals that have been actually stressed must be included in the study.*

**Table 2. DA and DOPAC amount in brain cortex in control and stressed rats**

Days	DA		DOPAC	
	Control	Stress	Control	Stress
0	45.1 ± 2.5	45.8 ± 2.2	57.0 ± 8.8	56.9 ± 6.7
2	45.2 ± 2.2	168.9 ± 22.3**	56.2 ± 7.9	122.8 ± 22.0*
4	45.3 ± 2.6	47.3 ± 11.4	55.8 ± 7.0	183.9 ± 31.8*
8	45.5 ± 3.2	85.8 ± 15.2*	59.8 ± 3.9	26.2 ± 4.5**
10	45.7 ± 1.7	55.4 ± 14.4	54.8 ± 5.3	62.6 ± 12.8

Animals were killed at different time points after treatment (0, 2, 4, 8, and 10 days respectively), and the prefrontal cortex was harvested and processed for DA and DOPAC quantification by HPLC as described in de Pablos *et al.* (2006). Numbers are expressed as nanograms per gram of wet tissue and are Mean ± SD of five independent experiments. \*p<0.05, \*\*p<0.01, statistical significance (Student's *t* test) compared with control animals.



**Figure 4.** A. Body weight gain (g); B. Adrenal glands weight (mg, bars) and ratio between adrenal glands weight and body weight gain (scatter plot and line). C. Serum corticosterone (percentage of control animals). Statistical signification: Student's *t* test comparing before C and after 10 days of variate stress (S10d); \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; #,  $p < 0.01$  (for the ratio adrenal glands weight/body weight gain). One-way ANOVA followed by the LSD post hoc test for multiple range comparisons,  $p < 0.01$ ; \*, compared with the control; a, compared with the previous time point (S1d to S10d indicate the days subjected to variate stress).

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Chronic-variable stress was adapted from other models of variable stress (Gamero *et al.*, 2003; Konarska *et al.*, 1990; Murua and Molina, 1992; Muscat *et al.*, 1992; Papp *et al.*, 1991; Willner *et al.*, 1987) with significant modifications. This work was supported by grant SAF-2012-39029 from the Spanish Ministry of Economy and Competitiveness and P10-CTS-6494 (Proyecto de Excelencia of Junta de Andalucía).

## References

1. Cryan, J. F. and Holmes, A. (2005). [The ascent of mouse: advances in modelling human depression and anxiety](#). *Nat Rev Drug Discov* 4(9): 775-790.
2. Dagnini-Subiabre, A. (2012). [Modelos animales para el estudio del estrés y las conductas depresivas](#). *Rev Farmacol Chile* 5(1):19.
3. de Pablos, R. M., Herrera, A. J., Espinosa-Oliva, A. M., Sarmiento, M., Munoz, M. F., Machado, A. and Venero, J. L. (2014). [Chronic stress enhances microglia activation and exacerbates death of nigral dopaminergic neurons under conditions of inflammation](#). *J Neuroinflammation* 11: 34.
4. de Pablos, R. M., Villaran, R. F., Arguelles, S., Herrera, A. J., Venero, J. L., Ayala, A., Cano, J. and Machado, A. (2006). [Stress increases vulnerability to inflammation in the rat prefrontal cortex](#). *J Neurosci* 26(21): 5709-5719.
5. Gamaro, G. D., Manoli, L. P., Torres, I. L., Silveira, R. and Dalmaz, C. (2003). [Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures](#). *Neurochem Int* 42(2): 107-114.
6. Hu, H., Su, L., Xu, Y. Q., Zhang, H. and Wang, L. W. (2010). [Behavioral and \[F-18\] fluorodeoxyglucose micro positron emission tomography imaging study in a rat chronic mild stress model of depression](#). *Neuroscience* 169(1): 171-181.
7. Konarska, M., Stewart, R. E. and McCarty, R. (1990). [Predictability of chronic intermittent stress: effects on sympathetic-adrenal medullary responses of laboratory rats](#). *Behav Neural Biol* 53(2): 231-243.
8. Krishnamurthy, S., Garabadu, D., Reddy, N. R. and Joy, K. P. (2011). [Risperidone in ultra low dose protects against stress in the rodent cold restraint model by modulating stress pathways](#). *Neurochem Res* 36(10): 1750-1758.
9. Murua, V. S. and Molina, V. A. (1992). [Effects of chronic variable stress and antidepressant drugs on behavioral inactivity during an uncontrollable stress: interaction between both treatments](#). *Behav Neural Biol* 57(1): 87-89.
10. Muscat, R., Papp, M. and Willner, P. (1992). [Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline](#). *Psychopharmacology (Berl)* 109(4): 433-438.
11. Papp, M., Willner, P. and Muscat, R. (1991). [An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress](#). *Psychopharmacology (Berl)* 104(2): 255-259.
12. Willner, P., Towell, A., Sampson, D., Sophokleous, S. and Muscat, R. (1987). [Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant](#). *Psychopharmacology (Berl)* 93(3): 358-364.