

Supplementary Material:

Manuscript: Monte et al. **Sex influences in the preventive effects of N-Acetylcysteine in a two-hit animal model of schizophrenia.**

Peripubertal unpredictable stress (PUS) exposure (2nd-hit)

This procedure was adapted from elsewhere (Sandra Giovanoli et al., 2013). PUS was induced using a variable sub-chronic stress protocol, which included five distinct stressors applied on alternate days starting from postnatal day (PND) 40 (GIOVANOLI et al., 2013 adapted). The five distinct stressors are described in detail below and were applied in the following order: 1. Electric foot shock (day 40); 2. Restraint stress (day 42); 3. Water deprivation (day 44); 4. Forced swimming stress (day 46); 5. Restraint stress (day 48).

Exposure to electric foot shock (PND40): We used a passive avoidance apparatus (Insight, São Paulo, Brazil). Each animal was first placed into the chamber for an initial period of 3 min, after which it received 3 mild electric foot shocks (0.3 mA) separated by intervals of 3 min. The electric foot shocks lasting 1 s. The session ended with an additional 3 min period, in which no shocks were delivered.

Exposure to restraint stress (PND42): Each animal was kept for 45 min in transparent plastic tube (diameter: 3 cm; length: 11.5 cm). The restrainer tubes contained drilled holes (2 mm in diameters) so as to facilitate oxygen supply. The tubes were tapped on a table which was placed in testing room. The animals were immediately returned to their home cages at the end of the restraint stress procedure.

Exposure to water deprivation (PND44): Animals were kept in their home cages, and the water bottles were removed at 16h00 on PND44 and added again the next day at 08h00. Animals had free access to food during the entire water deprivation period.

Exposure to forced swim stress (PND46): The apparatus used for the forced swim stress was made of a circular white fiberglass tank (diameter: 22 cm, height: 50 cm) filled with water (temperature: 18 °C, depth: 30 cm). The water tank was placed in a brightly lit testing room. Each animal underwent two sessions of forced swimming, each lasting 5 min. The two-swimming sessions were separated by a 5-min interval, during which the animals were kept in a waiting box containing sawdust bedding. The animals were dried with a towel and immediately brought back to their home cages after the second swimming session.

Exposure to restraint stress (PN4D8): Restraint stress was repeated.