

Total Sleep Deprivation Apparatus



To reduce stress and physical fatigue, our study employed a modified automatic total sleep-deprivation water box apparatus (BorjSanatazma Co, Tehran, Iran) [Fig. 1A]. The apparatus comprised a Plexiglas water-tank (120 cm × 30 cm × 50 cm), divided into four equal boxes (30 cm × 30 cm × 50 cm), containing temperature-regulated water at 30° C. To maintain social stability, four rats were simultaneously submitted to the tank (one rat in each box). Two small square platforms (15 cm in diameter, with 3mm high edges), were closely adjusted side by side. There were some holes in the surface of each platform (3 mm in diameters) to facilitate water evacuation during upward movements, and to help the rats avoid slipping or getting their paws wet. The platforms moved independently with an automatically-set plan. Initially, both platforms slightly emerged from the water level. Then, each platform alternatively moved below and above the surface of the water, forcing the animal to continuously move from one platform to another to avoid water contact. The speed of movement was 1.5 cm/s. Each platform motion cycle required 20 seconds. During this period, each platform remained stable over the surface of the water (at 12.5 cm) for 20 sec at the highest position (holding time). All rats had free access to clean water bottles and food pellet baskets which were placed on the top of the box [Fig. 1B]. The platform went down and immediately reverted until reaching the initial position. One day before the induction of different protocols for sleep deprivation, rats were allowed 30 min to

become familiar with the water box. As such, they learned to stay at the junction of both platforms, allowing them to escape from the sinking platform and avoid contact with water [Fig. 1C]. The behavioral observation during a 10-hr sleep deprivation period in earlier reports has documented that animals tend to remain awake 100% of the time in the water-box (Pierard, Liscia, Chauveau, Coutan, Corio, Krazem, and Beracochea, 2011; Pierard, Liscia, Philippin, Mons, Lafon, Chauveau, Van Beers, Drouet, Serra, Jouanin, and Beracochea, 2007).

In the present research, two models for sleep deprivation including total sleep deprivation (TSD; whereby animals were continuously deprived from sleep for 12, 24 or 36 hrs) and chronic-partial sleep restriction (CPSR; during which rats were submitted to daytime sleep deprivation for 12 hrs over 3 days with the opportunity to catch up sleep over night) were used. All animals were kept under standard conditions [12:12-h light-dark cycle at a controlled temperature ($23 \pm 1^{\circ}\text{C}$)] during the sleep deprivation period. To eliminate the possible stress effects of environmental novelty, some rats were assigned to control (sham) groups undergoing similar situation when the apparatus was turned off.

[Figure 1, somewhere here]