Contextual Spaced Fear Conditioning and Extinction Protocol

Contextual fear conditioning is the most basic of the conditioning procedures. It involves taking an animal and placing it in a novel environment, providing an aversive stimulus, and then removing it. When the animal is returned to the same environment, it generally will demonstrate a freezing response if it remembers and associates that environment with the aversive stimulus. Freezing is a species-specific response to fear, which has been defined as "absence of movement except for respiration." This may last for seconds to minutes depending on the strength of the aversive stimulus, the number of presentations, and the degree of learning achieved by the subject (Curzon et al., 2009).

Fear Conditioning and Extinction paradigm

One fear conditioning chamber with two distinct contexts was used in the experiments: a Plexiglas chamber 20X20X30 cm (Ugo Basil, Italy) with chessboard-like wallpapers and pre-cleaned with 75% ethanol served as a conditioned context (CC+). The same size Plexiglas chamber but with plain grey walls and pre-cleaned with Meliseptol® was used as a neutral context (CC-). All experiments were performed in a sound-attenuated constantly ventilated cubicle 60X60X60 cm illuminated with warm lamp (500 Lux) and protected from external electro-magnetic waves by a metal Faraday cage suitable for EEG recordings (Ugo Basil, Italy, fear conditioning system). Delivery of footshocks, their intensity and duration was controlled by a touch-screen handheld computer (Ugo Basil, Italy).

Handling: days -2 and -1

All animals were handled transferring the mouse from one cage to another one at least 10 times per day and wait for the mouse to run 1-2 minutes in every cage. This process can be repeated some hours later if the mouse is still nervous when you try to catch it from the home cage. Mice were also habituated for 5 min into the home cage to the experimental conditions two days before the training session.

Training session: day 0

Each mouse was placed into the neutral chamber (CC-) and allowed to freely explore this chamber for 5 min. Two hours later, the mouse was placed into the conditioned context (CC+) for 2.5 min. In this context and after the first minute of free exploration, the mouse was subjected to 3 footshocks (0.5mA, 1s) separated by 30 s. After this, the mouse was returned to the home cage. One hour later, the mouse was placed again into the conditioned context (CC+) and the footshocks were applied as before. After this, mice were returned to the home cage.

Recalls: days 2 and 9

Each mouse was placed into the conditioned context (CC+) and allowed to freely explore this chamber for 5 min without applying any footshock. Two hours later, the mouse was placed into the neutral chamber and allowed to freely explore this chamber for 5 min. After this, mice were returned to the home cage.

Extinction sessions: days 5,6 and 7

In order to extinct fear memory, 9 extinction sessions were performed, placing each mouse into the conditioned context (CC+) and allowing them to freely explore this chamber for 5 min. These sessions were performed during 3 consecutive days with 3 sessions per day and separated by 1 hour.

Behavioural analysis

All the sessions were recorded on Intel Xeon workstation (CPU 3 GHz, 6 GB RAM) using an USB video camera (Microsoft, USA) and a special behavioural video acquisition and analysis software (AnyMaze, USA). All recorded movies were then analyzed using AnyMaze automatic analysis. Total

time freezing was taken as a measure of fear-related memory and quantified as described previously (Senkov et al., 2006).

References:

Curzon P, Rustay NR, Browman KE. Cued and Contextual Fear Conditioning for Rodents. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press; 2009. Chapter 2.

Senkov O, Sun M, Weinhold B, Gerardy-Schahn R, Schachner M, Dityatev A. Polysialylated neural cell adhesion molecule is involved in induction of long-term potentiation and memory acquisition and consolidation in a fear-conditioning paradigm. J. Neurosci. 2006;26:10888–109898.