Rodent Fear Conditioning: Development and Pharmacological Validation of a Video-Based Freezing Detection System

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Abstract

Rodent fear conditioning is a relatively simple, cognitive-based paradigm extensively used to study the neurobiological mechanisms underpinning learning and memory. Prior to the introduction of video-based acquisition systems, manual observer scoring was the only available method to capture the freezing responses of animals. There are however considerable downsides to this approach including the risk of inter-animal and/or study variability as well as the risk of unintentional operator bias. These issues can lead to inaccurate results and difficulties in replicating studies across laboratories. The relatively recent introduction of video-based behavioral acquisition systems now allows investigators to record and analyse fear conditioning studies using locomotor detection algorithms. Our preference for a video based system over other fear conditioning recording systems is that this allows the investigator to compare the system generated data with the actual animal behaviour. We entered into a joint risk-sharing collaboration with Biobserve, Germany, with the objective to develop a bespoke fear conditioning video acquisition and analysis system.

This collaboration started in spring 2010 and after a four month design and data exchange period, followed by trial running a beta-version, the final system was delivered to us in December 2010. The software package does not require object identification, but analyses differences between subsequent video images to quantify the activity (or lack of) of the rat. It also includes unique features in its functionality including individual software based camera sensitivity filters that can be altered dependent on the types of the cameras used. This functionality also allows the operator to individually adjust each camera's sensitivity so that small differences in the test cage environment (e.g. subtle differences in illumination) can be balanced across the test chambers. The software can also drive additional hardware (e.g. shock grids, speakers) using basic programming skills. In addition, the package also contains statistical analysis capabilities (details are available at http://www.biobserve.com/products/fear-test/fear-test.html).

The fear conditioning test involves a conditioning phase, during which animals are placed in a test chamber for 4 min and receive an electric foot shock after 2 min (conditioning stimulus, CS). The second phase 24h later consists of a recall test, where the animals are placed in the same test chamber (context, or unconditioning stimulus, US) for 8 min, but do not receive a foot shock. After the foot shock in phase 1, and during phase 2, the animals show freezing behaviour. This freezing is represents a fear response to the foot shock, and the duration of freezing during phase 2 is taken as a measure of how well the animals can still associate the context (test chamber, US) to the foot shock (CS). Test compounds were administered just before phase 1 to assess their effect on memory consolidation. The Institutional Ethical Committee on Animal Experimentation approved the experimental protocols, in compliance with Belgian law (Royal Decree on the protection of laboratory animals dd. April 6, 2010) and the facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Identification of freezing identified by the algorithm showed a high correlation with that by an observer blinded to treatment. The pharmacological validation studies using compounds that target the cholinergic system, (scopolamine, a muscarinic agonist and donepezil), revealed some unexpected effects during the conditioning phase (CS/US pairing). For example, whilst there was no effect of scopolamine during the initial habituation phase before the foot shock, significant dose-dependent reductions in shock-induced freezing were observed in scopolamine treated animals, suggestive of hyperactivity. During the recall phase after 24h, freezing was reduced compared to previously unshocked rats, indicating recall impairment. Interestingly, while donepezil attenuated this hyperactive response and reversed the scopolamine-induced recall impairment, the selective M1

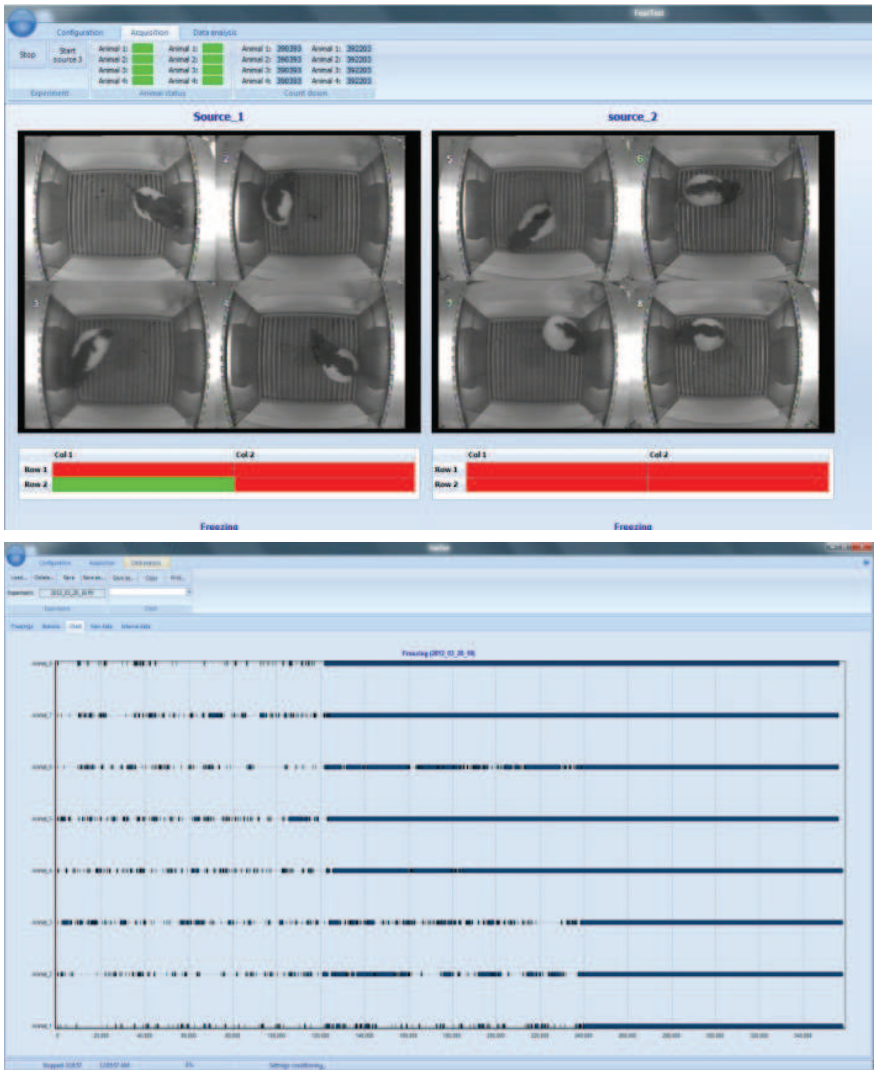

Figure 1. Screen captures of the fear conditioning test software. Top panel shows the image acquisition window; bottom panel shows the ethogram comparison: each horizontal line represents automated scoring of freezing behaviour (bold segments) of a rat.

muscarinic agonist compound-A (3-{1-[4-(2-Methoxyethoxy)-1-methylcyclohexyl]piperidin-4-yl}-2-oxo-2,3-dihydro-1,3-benzoxazole-5-carbonitrile) [1] only reversed the effects of scopolamine on conditioning, but not on recall.

In summary, we have developed a bespoke fear conditioning acquisition software package that allowed us to detect previously unobserved differences in cholinergic compounds. These studies underscore the importance of collecting and interrogating response data during the conditioning phase as interesting differences in pharmacology can be detected.

Reference

1. Cooper et al. (2009). *Compounds which have activity at M1 Receptor and their uses in medicine*. WIPO Patent Application WO/2009/037296.