Machine analysis of conditional and unconditional freezing behavior in rats

Pawel M. Boguszewski[1], Sun Jung Bang[1], Thomas H. Brown[1,2]

Department of Psychology, Yale University[1] and Department of Cellular and Molecular Physiology, Yale School of Medicine[2]

e-mail: pawel.boguszewski@yale.edu or pmbogusz@gmail.com

Abstract

Freezing is often measured as a conditional response (CR) in studies of fear conditioning. The analysis can be done by a human observer with a stop watch and video recordings of behavior. Over the last two years we have developed software for video-based monitoring of rat motor activity and freezing detection. High image resolution and time precision were available using inexpensive hardware. An MPEG2 encoder was used to digitize the analog signal from an infra-red sensitive analog camera. Movement is detected by comparing successive video images. The first stage of image processing and filtering adjusts for different light and video image conditions. Spatial pixel filtering plus a tuning parameter resulted in successful elimination of noise caused by the video source. Minor movements, such as those associated with breathing or the elicitation of 22 kHz ultrasonic vocalizations, could be excluded without loosing sensitivity. Real-time visualization of pseudocolored images provides immediate feedback that is useful for learning how to control exactly what is scored as freezing (or immobility). Movement records are plotted as a function of time. In the second stage, these settings are used for rapid analysis of the rat activity. Freezing can be defined to include a minimum freezing duration. Video images of freezing behavior are easily related to a range of experiment-controlling systems. For example, machine scoring of freezing can be combined with neurophysiological recordings of sleep spindles to distinguish freezing from sleeping. The correlation between the computer-based analysis and the results of human-scored data was nearly perfect ($r = 0.97$, $p<0.005$). The exercise of capturing freezing (or immobility) using video-analysis software is instructive in developing an operational definition of the behavior. We invite discussion of other experiences using machine scoring of freezing.

Introduction

In studies of classical fear conditioning, freezing, a natural rodent defensive behavior, is generally measured as a conditional response (CR). Manual scoring of freezing behavior can be done with a stop-watch and video recordings. However, manual scoring requires well-trained observers who are blind to the experimental condition. To improve the reliability and increase the speed of scoring freezing, we developed an automatic freezing-recognition software. The approach is described here along with its application. We invite discussion of other experiences using machine scoring of freezing.

Methods

Subjects

Experiments were performed using a total of 12 male Sprague-Dawley rats (Charles River) weighing 230-350 g. Subjects were individually housed, and had *ad libitum* access to food and water.

Experimental procedures

Two conditioning chambers (H10-11R-TC, Coulbourn Instruments, Allentown, PA) were used in both conditioning and testing sessions. The grid shock US was delivered by a shock generator (ENV-410, MED Associates) and grid scrambler (ENV-412, MED Associates). During conditioning each animal received 5 CS-US pairings. After a 2 min baseline, a USV CS (60 dB, 7.8 s, 19 kHz) was presented along with a co-terminating footshock US (0.5 s, 0.5 mA). Intertrial intervals (ITIs) ranged from 90 s to 150 s (mean = 117 s). Rats were returned to their cages 1 min after the last CS-US presentation. Freezing to the cue and the conditioning context was tested individually on a separate days in a counterbalanced order. In the test for cue-elicited freezing, each rat was placed in a novel chamber in which the CS was continuously presented for 6 min after a 2 min baseline. In the test for context-elicited freezing, rats were placed in the conditioning context, where they remained for 6 min.

Field potential recordings

Field potentials were recorded from perirhinal cortex using a chronically-implanted bundle electrode (8 wires, tungsten, 25 µm diameter, formvar insulated). Field potentials were acquired on each channel in parallel with single-unit activity. A National Instruments 64-channel A/D subsystem further processed the field potentials. The field potentials were visualized with Sort Client software.

Video recording

Behavioral sessions were video recorded. Live images from an IR-CCD camera (Circuit Specialists, Mesa, AZ) were digitized, encoded and stored in MPEG-2 format by hardware encoder (WinTV-PVR-USB2, Hauppauge Computer Works, Inc., Hauppauge, NY). The image files were stored directly on a PC computer hard drive. Later, files were trimmed by VideoReDo software and stored on DVD discs.

Software development platform

The software was written in Borland Delphi Personal version 7.0, build 4.453 (Borland Software Corporation, Austin, TX). Delphi 7 was used under personal, "non commercial" license (registration key: 11414387). The digital video capturing component DSPack ver. 2.3.4 (http://www.progdigy.com/modules.php?name=DSPack) was used to provide images from video files and live source. DScaler 5 - Alpha MPEG Filters ver 5.0.0.8 (http://sourceforge.net/projects/deinterlace) was used to provide proper MPEG2 codec.

1. Stage I - Activity detection

1.1 Video capture and image pre-processing

Fig 1. Stage 1 - main window

Fig 2. Time lapse series of consecutive images for processing

Video source
- MPEG2 file / other files
- Live image (webcam)

Time lapse capturing
- Intervals are flexible
- For rats, it is usually 5 frames per second (FPS).

Image pre-processing
- Blur filter (for noise elimination)
- Luminosity adjustment
- Highlights cutoff

Limiting analysis to region of interest (ROI)
- Eliminates unwanted signal sources.
- Cuts off permanent noise (ex. video tape artifacts).
- Decreases computational demands

1.2 Detection of between-frames differences

Fig 3. Differential bitmap

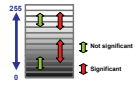

Fig 4. Luminosity threshold

Difference detection:
Consecutive frames are subtracted pixel-by-pixel (Fig 5.) and significant changes in luminosity are written in differential black and white bitmap (Fig 3.) Threshold for significant difference is adjusted to eliminate intrinsic noise of digital video (Fig 4).

Detection of "Hot spots":
The software detects changes of image in special zone, ex. infrared LED activity to synchronize physiological data like field potential (sleep spindles detection, see Frame 3), EMG, or single-unit activity. Various visible events can be used to synchronize video images with other data source.

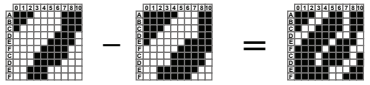

Fig 5. Subtracting consecutive frames produces differential black and white bitmap.

1.3 Spatial noise filtering, visual feedback and data export

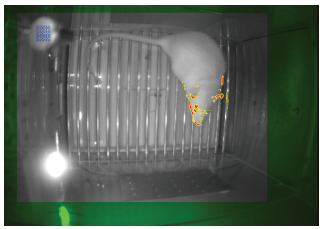

Fig 6. Real-time visual feedback with pseudo-colored image

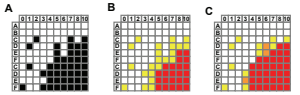

Fig 7. Spatial noise filtering. Differential bitmap (A) is processed with "neighbors" filter. Conservative filtering with factor 8 (B) and loose filtering with factor 4 (C).

Spatial noise filtering:
"Neighbors filter": each pixel is considered as a significant change (signal – red color, see Fig 6 and 7) when proper numbers of the immediate neighbors are changed as well. All other pixels are considered as not significant (noise – yellow). This filter eliminates "salt-and-pepper" type noise and slight respiratory movements. Filtering level is adjusted to image resolution and video quality.

Visual feedback
Difference detection as well as signal filtering results are plotted against pseudo-colors over the live images for real-time visual feedback (Fig. 6)

Data export
Data is exported in common plain data formats (CSV, TXT) for freezing recognition processing. Also, data can be exported in subtitle formats for easy freezing verification.

2. Stage II - Freezing recognition

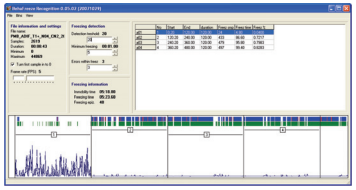

Fig 8. Stage 2 - main window

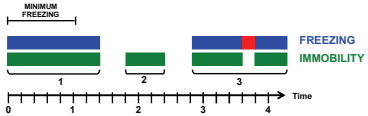

Fig 9. Freezing recognition from activity plot.

Freezing recognition
Raw data from Stage I are processed in a series of steps. Movement records are plotted as a function of time (activity plot, see Fig 8, lower panel). When the number of the pixels is under a given threshold, this time point is classified as "immobilization" (see Fig. 9, green bar). Freezing is defined here as immobilization that lasts longer than one second (but the duration can be selected to be any value). In Fig. 9, the first immobilization episode is long enough to be classified as freezing—marked with a blue bar (Fig. 9, period 2). If an episode of immobility is too short, it is classified as non-freezing (Fig 9, period 2). The red bar indicates that this frame was considered an error.

Data export
Results, percentage of freezing, are calculated within defined time bins and stored as text file. Analysis can be performed automatically on a large set of files, producing a single array of results.

3. Freezing vs. sleep

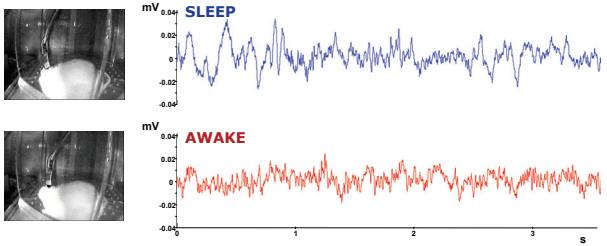

Fig 10. "Hot-spots" detection provides easy and a convenient time synchronization mechanism between freezing and elecrophysiology (see 1.2). Combining behavioral activity recordings of sleep spindles makes it possible to distinguish between sleep and freezing.

4. Human vs. machine scoring

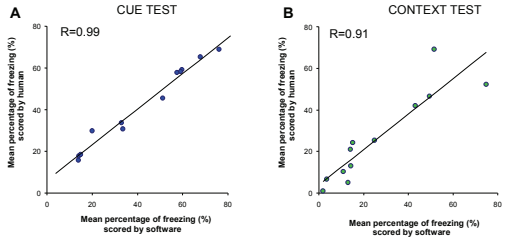

Fig 11. Correlation between the computer-based analysis and the human-scored data for the cue test (Fig 11A, $r = 0.99$, $p < 0.005$, $n=12$) and the context test (Fig 11B, $r = 0.91$, $p < 0.005$, $n=12$). Human scoring was performed by a highly trained person blind to experimental condition. Behavior was videotaped and scored off-line using a stopwatch. For machine scoring, tapes were digitalized with the hardware described above.

Results and Discussion

- **Video-based computer software is a versatile and convenient solution for scoring freezing behavior.**
- **High correlation with results obtained by human observer, both in cue and in context tests, ensure the validity of the software.**
- **Precise and flexible synchronization of animal activity with electrophysiological measures provides a convenient way to distinguish various behavioral states.**

Research supported by MH58405 grant to Thomas H. Brown and by Yale University