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An improved approach to separating startle data from noise

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HIGHLIGHTS

- We explored the fundamental movement of the ASR in mouse, utilizing high-speed video to record startle movements.
- We created an automated program that classifies raw force traces into startles and non-startles.
- The accuracy of this new approach was then compared with other common methods for startle data analysis.
- We suggest a method for normalizing for animal mass by combining raw force data with each individual animal's mass into a simple mathematical equation.

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ABSTRACT

Background: The acoustic startle reflex (ASR) is a rapid, involuntary movement to sound, found in many species. The ASR can be modulated by external stimuli and internal state, making it a useful tool in many disciplines. ASR data collection and interpretation varies greatly across laboratories making comparisons a challenge.
New method: Here we investigate the animal movement associated with a startle in mouse (CBA/CaJ). Movements were simultaneously captured with high-speed video and a piezoelectric startle plate. We also use simple mathematical extrapolations to convert startle data (force) into center of mass displacement ("height"), which incorporates the animal's mass.
Results: Startle plate force data revealed a stereotype waveform associated with a startle that contained three distinct peaks. This waveform allowed researchers to separate trials into 'startles' and 'no-startles' (termed 'manual classification'). Fleiss' kappa and Krippendorff"s alpha (0.865 for both) indicate very good levels of agreement between researchers. Further work uses this waveform to develop an automated startle classifier. The automated classifier compares favorably with manual classification. A two-way ANOVA reveals no significant difference in the magnitude of the 3 peaks as classified by the manual and automated methods (P1: $p = 0.526$, N1: $p = 0.488$, P2: $p = 0.529$).
Comparison with existing method(s): The ability of the automated classifier was compared with three other commonly used classification methods; the automated classifier far outperformed these methods.
Conclusions: The improvements made allow researchers to automatically separate startle data from noise, and normalize for an individual animal's mass. These steps ease inter-animal and inter-laboratory comparisons of startle data.

1. Introduction

The acoustic startle reflex (ASR) describes a reflexive movement in response to an auditory stimulus, typically one that is sudden and of high intensity (Landis and Hunt, 1939). The ASR is found in many species and is believed to have evolved as a rapid defense mechanism (Koch, 1999). Though the ASR is fundamentally a reflex movement, both the amplitude and probability of a resulting startle movement can be modulated by a number of external

Abbreviations: ASR, acoustic startle reflex; P1(*t*)(win), first positive peak of the startle waveform (timing)(window); P2(*t*)(win), second positive peak of the startle waveform (timing)(window); N1(*t*)(win), first negative peak of the startle waveform (timing)(window); LED, light emitting diode; pT, postitive threshold; nT, negative threshold; COMd, center of mass displacement; dB SPL, decibels sound pressure level.

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stimuli, and changes in internal state (Hoffman and Wible, 1970; Davis et al., 1982; Acocella and Blumenthal, 1990). Modulators of an ASR startle include: preceding visual pulses (Buckland et al., 1969; Ison and Hammond, 1971), preceding auditory pulses (Graham, 1975; Carlson and Willott, 1996; Franklin et al., 2007), concurrent background noise (Gerrard and Ison, 1990; Ison and Russo, 1990; Longenecker and Galazyuk, 2012), and gaps of silence in background noise preceding the startle sound (Ison, 1982; Walton et al., 1997; Allen et al., 2008). Modulation of the ASR is used in behavioral paradigms to investigate a wide range of disorders in a range of disciplines including schizophrenia (Swerdlow and Geyer, 1993; Grillon et al., 1992; Parwani et al., 2000), alcoholism (Krystal et al., 1997; Stanley-Cary et al., 2002) and psychopharmacology (Phillips et al., 2000; Davis and Menkes, 1982). The ASR is also used to develop behavioral tests for neurological disorders such as post-traumatic stress disorder (Weston, 2014) and tinnitus (Turner et al., 2006).

Current practice in the recording and analysis of the ASR in lab animals is greatly varied. One of the biggest challenges when interpreting data, or in comparing research from different laboratories, is the variation in how ASR data are collected and analyzed. To date, there has been little effort to standardize the method of analyzing data collected from ASR experiments.

In this paper, we use a variety of tools to methodically explore the ASR in mouse, and describe an easy to implement method for the detection and analysis of ASR that is substantially more accurate than the commonly adopted approaches.

First, we explored the fundamental movement of the ASR in mouse, utilizing high-speed video to record startle movements (Horlington, 1968). Recorded animals were placed upon the same startle plates used during behavioral experiments. A light pulse, synchronized with the acoustic stimulus, allowed us to correlate the animal's movements during a startle with the raw data produced by the piezoelectric startle plate. This initial step allowed us to identify a stereotyped waveform output produced by the plate during a startle movement. The discovery of this waveform allowed experienced researchers to visually separate trials with startle data from those without. The visual separation of trials in this manner was termed 'manual classification' and was used as a baseline to compare automated methods.

We then developed a mathematical method to separate trials with this stereotyped waveform, indicative of true startle movements, from those without, indicative of noise. Further, we used the criteria found to separate trials mathematically to create an automated program that separates trials where a startle has occurred from those where no startle occurred.

The accuracy of this new approach was then compared with other methods for startle data analysis, on a novel set of mouse data. Accuracy was determined by comparing the ability of each method to separate startles from noise relative to the manual classification of trials by three experienced researchers. For manual classification, trials were visually reviewed to identify startle trials and no-startle trials. Each classification method was then compared to the manual classification; values of percentage correct were calculated to quantitate the success of each method. In respect to a sample dataset, our automated method far outperformed all other methods.

Finally, we put forward an approach to normalize startle data for an individual animal's mass. By adopting some simple mathematical conversions used in the field of animal locomotor mechanics, the mass of each animal is used to convert force into center of mass displacement (COMd) or "height". This mathematical conversion has two benefits: first, the procedure normalizes for mass, allowing legitimate comparisons between animals of different mass. Second, it converts the forces sensed by the piezoelectric startle plate into a more readily understandable unit of "height": the center of mass displacement (COMd).

2. Material and methods

2.1. Animals

A total of 24 adult male CBA/CaJ mice (4–9 months of age) made up the four datasets used in this study. All mice were obtained from Jackson Laboratories. Mice were housed in pairs within a colony room with a 12-h light–dark cycle at 25 °C. Experiments were performed during the light phase of the light-dark cycle. All procedures used in this study were approved by the Institutional Animal Care and Use Committee at Northeast Ohio Medical University.

2.1.1. High-speed video recordings

Adult CBA/CaJ mice were filmed using a digital high-speed camera (HiSpec Lite, Fastec Imaging, San Diego, CA). Videos were captured at 1000 frames per second onto a notebook computer (Latitude Ultrabook, Dell Computers, Austin, TX) using Fastec HiSpec video capture software. Mice were placed upon a Kinder Scientific piezoelectric startle platform that was connected to a TDT RZ6 multi-processor running custom OpenEx software. The OpenEx software controlled the production of acoustic and visual stimuli and synchronized the acquisition of all data. The stimulus used to elicit a startle, solely for video recording purposes, was a wide band noise burst (107 dB, 20 ms duration 1ms rise/fall, 5–100 kHz) delivered through a loudspeaker (FT17H, FOSTEX). The loudspeaker was calibrated with a 0.25-in. microphone (Brüel and Kjaer 4135) attached to a measuring amplifier (Brüel and Kjaer 2525). Speaker calibration was performed to increase output voltages for frequencies where speaker roll-off occurred. The resulting speaker output had a flat (±3 dB) response across all frequencies of the mouse audiogram (5–100 kHz). A short (25 ms) voltage pulse that was time-locked to the startle stimulus onset was delivered to an LED mounted in front of the piezoelectric startle platform (Fig. 1). This light pulse served as a timing reference for the high-speed video recording. The synchronizing LED voltage pulse, acoustic startle stimulus signal and piezoelectric startle plate signal (Fig. 1) were continuously recorded (25 kHz sampling rate) in OpenEx to enable precise pre- and post-stimulus analysis of the startle waveform.

Two separate video recording sessions were performed. In the first session, the mouse was restrained in a small cage placed on top of the startle plate. In the second session, the mouse was placed on the startle plate without a restrainer. The restrained paradigm allowed for evaluation of the startle waveform produced in conditions close to those of typical experimental conditions. During typical experimental conditions mice are restrained in an acoustically transparent restrainer (Longenecker and Galazyuk, 2012). The unrestrained paradigm permitted the mouse to use its full range of motion during an ASR.

2.2. Developing a mathematical, automated, startle waveform classifier

Video analysis revealed that an ASR in mouse produced a stereotyped waveform from the piezoelectric startle plate (Fig. 2). Further analysis revealed that the first three peaks of this waveform, the first positive (P1), the first negative (N1), and the second positive (P2) peak, are necessary to identify it from non-startle waveforms. An automated classifier was developed to identify this stereotyped waveform and separate startle data from noise. Three criteria are used by the classification. First, the waveform must contain the three peaks of interest, second, these peaks have the appropriate timing, and third, each peak's magnitude is greater than the trial-specific threshold.

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