Journal of Neuroscience Methods 275 (2017) 45–49

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Short communication

Air-puff induced vocalizations: A novel approach to detecting negative affective state following concussion in rats

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HIGHLIGHTS

- A method to studying negative affect following concussion in rats is proposed.
- Concussion resulted in greater aversive vocalization following mTBI in rats.
- Ultrasonic vocalization may be a useful tool to assess mood following mTBI in rats.

ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form 27 October 2016
Accepted 28 October 2016
Available online 29 October 2016

Keywords:
Ultrasonic vocalizations
Concussion
22kHz vocalizations
Negative affect
Projectile concussive impact model

ABSTRACT

Background: Negative emotional states resulting from concussion are of increasing concern. In the current study, we developed a model to investigate negative affect following concussion in the projectile concussive impact (PCI) model. High frequency ultrasonic vocalizations (22 kHz USVs) are associated with negative affective stimuli in rats. Changes in negative affective state were examined following PCI using a mild air-puff stimulus to elicit 22 kHz USVs.

New method: Forty-eight hours post-injury, animals were placed into a clean acrylic box lined with bedding. A 5 min baseline recording was followed by 15 air puffs (55 psi) spaced 15 s apart aimed at the upper back and neck.

Results: Injured animals produced on average 153.5 ± 55.13 more vocalizations than shams, vocalizing on average 4 min longer than shams. Additionally, concussed animals vocalized to fewer air-puffs, exhibiting a 1.5 fold lower threshold for the expression of negative affect.

Comparison with existing methods: Studies currently used to test negative affective states following concussion in animals, such as the elevated plus maze and forced swim task have, as of yet, been unsuccessful in demonstrating injury effects in the PCI model. While the air-puff test has been applied in other fields, to our knowledge it has not been utilized to study traumatic brain injury.

Conclusion: The current study demonstrates that the air-puff vocalization test may be a valuable tool in assessing negative mood states following concussion in rat models and may be used to evaluate novel therapies following brain injury for the treatment of mood dysfunction.

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1. Introduction

Traumatic brain injury (TBI) is a major concern to the US military. Over 300,000 service members were diagnosed with TBI between 2000 and 2014, and the vast majority (82.4%) of these diagnoses resulted from concussions (Helmick et al., 2015). Evidence suggests that service members who have suffered concussions while deployed may be at a greater risk for developing psychiatric disorders such as anxiety and post-traumatic stress disorder (PTSD) (Leo and McCrea, 2016). Currently very little is known about the mechanisms behind emotional dysregulation following concussion and there are limited treatment options.

There have been varied results when assessing negative affect resulting from concussion in rodents. The elevated plus maze is frequently used to assess anxiety following brain trauma and has been applied to several concussion models, yielding mixed results. Investigators show more (Turner et al., 2015; Pandey et al., 2009; Shultz et al., 2011; Kovesdi et al., 2012), less (Mychasiuk et al., 2015; Shultz et al., 2012a), or equal (Fidan et al., 2016; Mychasiuk et al., 2014; Shultz et al., 2012b) time spent in the open arms following

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http://dx.doi.org/10.1016/j.jneumeth.2016.10.017
0165-0270/Published by Elsevier B.V.

mild TBI. Similarly, results vary using the forced swim task to assess depressive state (Fidan et al., 2016; Mychasiuk et al., 2014, 2015; Shultz et al., 2012a, 2012b). The disparity in these results demonstrate the need for a reliable measure of negative affect following concussion that can be used to study the etiology of negative affect and examine novel therapeutics.

The goal of the present study was to develop a testing paradigm to facilitate detection of negative affective state in a model of closed-head concussion. Rats emit ultrasonic vocalizations (USVs) in the range of 22–25 kHz in response to negative affective stimuli (Knutson et al., 2002; Wohr and Schwarting, 2013). These vocalizations are known as 22 kHz USVs, aversive vocalizations, or aversive calls.The air-puff test was developed by Knapp and Pohorecky (1995) to induce a negative affective state in rats, leading to the production of aversive calls, and has been well-established as a methodof studying study drug and alcohol withdrawal (Berger et al., 2013; Mutschler and Miczek, 1998b, 1998a; Williams et al., 2012; Barros and Miczek 1996; Mutschler et al., 2001; Vivian and Miczek 1991; Vivian et al., 1994; Kissler et al., 2014). The air-puff test is ideal for experiments in which multiple behavioral paradigms are used because it is non-invasive and does not require training. In addition, unlike other affective models used to study brain injury, such as the elevated plus maze and open field, the air-puff induced vocalization test does not rely on locomotor activity which is potentially altered following brain injury.

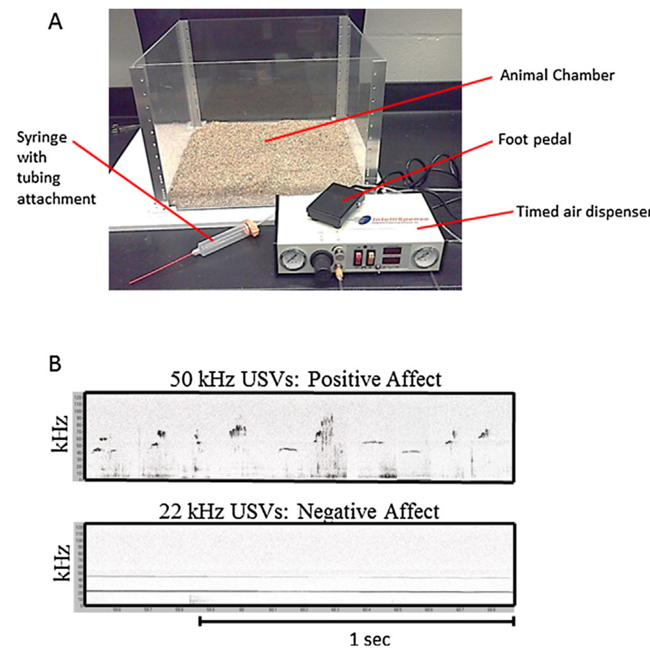

Fig. 1. Air-puff test setup (A) and sample USVs (B).

2. Methods

2.1. Animals

Two month old male Sprague-Dawley rats (300 g) were single housed in a reverse 12 h light/dark cycle (lights on at 9:00am) in a temperature (21 ± 1 °C) and humidity (50–70%) controlled room. All procedures and conditions were approved by the Walter Reed Army Institute of Research, Institutional Animal Care and Use Committee and adhere to the principles stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council; 2011 Edition), and other federal statutes and regulations relating to animals and experiments involving animals. Injury and testing were performed from 1:00pm to 6:00pm. Rats were handled once per day for 3 days prior to injury. Animals were fed ad lib.

2.2. Projectile Concussive Impact (PCI) injury

The PCI injury apparatus consisted of an elevated platform and a computer-controlled electro-pneumatic pressure release system used to launch a small projectile (3.52 g sphere) targeted at the rat's head (Leung et al., 2014). Following anesthetization with 5% isoflurane, a custom-designed helmet (Army Research Lab, Aberdeen Proving Ground, MD) was securely fastened onto the rat's head. Pressure sensor films (Fujifilm pre-scale pressure sensitive film) adhered to the inner and outer surfaces of the helmet were used to record the distribution and magnitude of the pressure from the impact of the projectile. The rat was anesthetized using 5% isoflurane and placed in a supine position on the platform with its head positioned above an oval opening in the elevated platform such that the right hemisphere of the helmet-protected head was exposed to the projectile angled 45° from the sagittal plane. A computer program was used to trigger the targeted release of the projectile at the rat's head. Immediately following PCI injury, the helmet was removed and the rat was returned to its home cage.

In the current study, a total of 37 animals were randomly divided into 2 groups and exposed to sham treatment (n = 19) or to repeated concussive injury (4 impacts, spaced 1 h apart) to the right frontal-parietal cortex (n=18). Animals assigned to sham treatment were anesthetized, fitted with the helmet and placed on the PCI device the same number of times, but did not receive a concussive impact injury. Immediately following each PCI or sham procedure, the animals were returned to their home cage and the righting reflex was measured and recorded.

2.3. Air-puff testing procedure

One day following PCI injury, animals were transported to the testing room for a 1 h habituation period, during which time they were exposed to the transportation and the environmental stimuli in the testing room while remaining in the home cage.

USV testing took place at 48 h post-injury using an air-puff procedure adapted from Knapp and Pohorecky (1995). Vocalizations were recorded with UltraSoundGate 116Hnbm recording system at a sampling rate of 250 kHz with 16-bit resolution. Rats were placed in a clean acrylic box (43 × 43 × 31 cm) with fresh bedding (Fig. 1A). The P48 Electret Ultrasound microphone (Avisoft Bioacoustics, Knowles FG) was mounted to the top of the testing arena approximately 27 cm above the floor. The testing room was illuminated with a dim red light, located 25 cm from the top of the testing arena. Light level on the floor of the testing arena was 2–10 lx. A 5 min baseline recording was followed by 15 air-puffs (55 psi), spaced 15 s apart, which were delivered to the dorsal upper back/neck of the rats. The pressure and the duration of the air-puff stimulus were controlled with a timed-air digital dispensing system attached to an air tank (Integrated Dispensing Solutions Inc., Agoura Hills, CA). This system is designed to deliver a precise volume of liquid at a set pressure. Using the syringe that comes with the machine without liquid or the plunger enables delivery of a precisely timed puff of air at a set pressure. To increase the accuracy of delivery to the animal, a hard plastic tube (inner diameter 1 mm, length 12.5 cm) was affixed to the end of the syringe with rubber cement. The tip of the tube attached to the syringe was held approximately 5 cm away from the back of the rats' necks when the air-puff was delivered. The air-puff was delivered via depression of a foot pedal. Following air-puffs, rats were monitored and recording was terminated following a 1 min discontinuation of aversive vocalization.