Respiratory Physiology & Neurobiology 204 (2014) 112–119

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Respiratory responses following blast-induced traumatic brain injury in rats☆

Sherry Adams[a], Jillian A. Condrey[a], Hsiu-Wen Tsai[a], Stanislav I. Svetlov[b,c], Paul W. Davenport[a,*]

[a] Department of Physiological Sciences, University of Florida, Gainesville, FL, USA
[b] Banyan Biomarkers, 13400 Progress Blvd, Alachua, FL, USA
[c] Departments of Medicine and Psychiatry, University of Florida, 1600 SW Archer Road, Box 100274, Gainesville, FL, USA

ARTICLE INFO

Article history:
Accepted 21 August 2014
Available online 19 September 2014

Keywords:
Overpressurization blast
Traumatic brain injury
Respiration
Electromyographic recording

ABSTRACT

Blast overpressure (OB) injury in rodents has been employed for modeling the traumatic brain injury (TBI) induced by an improvised explosive device (IED) in military service personnel. IED's can cause respiratory arrest if directed at the thorax due to the fluid–tissue interface of the lungs but it is unclear what respiratory changes occur in a head-directed OB injury. The diaphragm is the primary muscle of inspiration and electromyographic (EMG) recordings from this muscle are used for recording breathing in anesthetized and conscious rats. The breathing pattern of the rodents will be recorded during the OB injury. Our results indicate that a dorsal directed closed-head OB injury results in a neurally mediated apnea followed by respiratory timing changes.

1. Introduction

Traumatic brain injury (TBI) affects 1.7 million people annually in the United States (Faul et al., 2010). The CDC reports that TBI rates are higher for males than females in every age group. Soldiers in combat are most susceptible to sustain a TBI as a result of an overpressure blast (OB) wave from an improvised explosive device (IED). Overpressure wave causes damage to air-filled organs and air-fluid interfaces due to the interaction between the stress wave and shear wave (Guy et al., 1998a). Blast directed and localized to the dorsal surface of the head between bregma and lambda induces closed-head TBI if the pressure force is of sufficient magnitude. Closed-head OB injuries send shearing and stressing forces throughout the brain including the brainstem resulting in observational disruptions in breathing. TBI with body protection is known to result in observational apneic periods in rodent models (Cheng et al., 2010; Dixon et al., 1987; Guy et al., 1998b; Kuehn et al., 2011). These apneas may result from neuronal disruption in the brainstem respiratory control center, transmission of the pressure force vector throughout the body via the cerebrospinal fluid and circulatory system or other unknown reasons. The OB TBI disruption of breathing is, however, not well understood, especially during OBI exposure, hence our primary goal is to determine the respiratory rhythm pattern during an OB TBI isolated to the head.

1.1. OB TBI

The OB wave is experimentally produced by a shock tube driven by compressed air. An OB wave directed at the skull of a rodent results in an OB TBI if the pressure is of sufficient magnitude. OB waves directed at the dorsal skull of a rat between bregma and lambda may cause apnea based on anecdotal evidence. The OB shock tube can generate a controlled pressure wave which can be replicated under experimental conditions. A shock tube was designed, constructed and tested by the Florida Institute of Technology at Banyan Biomarkers (see their Fig. 1a) (Svetlov et al., 2010). There are two sections of the shock tube separated by a metal diaphragm. The two sections include the gas at high pressure (driver) and the gas at low pressure (driven) separated by a diaphragm. At a predetermined threshold level, the diaphragm ruptures which generates a shock wave propagating through the low-pressure section (driven) to the end of the shock tube. The peak and duration of the overpressure blast is determined by the driver/driven ratio, thickness and type of diaphragm material. Stainless steel diaphragms 0.05-mm thick with driver/driven ratio of 15–1 was used to produce the shock wave. An internal cutter was used to initialize the rupture of the diaphragm so the low-pressure

☆ This paper is part of a special issue entitled "Non-homeostatic control of respiration", guest-edited Dr. Eugene Nalivaiko and Dr. Paul Davenport.

* Corresponding author. Tel.: +1 352 294 4025; fax: +1 352 392 5145.
E-mail address: pdavenpo@ufl.edu (P.W. Davenport).

http://dx.doi.org/10.1016/j.resp.2014.08.015
1569-9048/

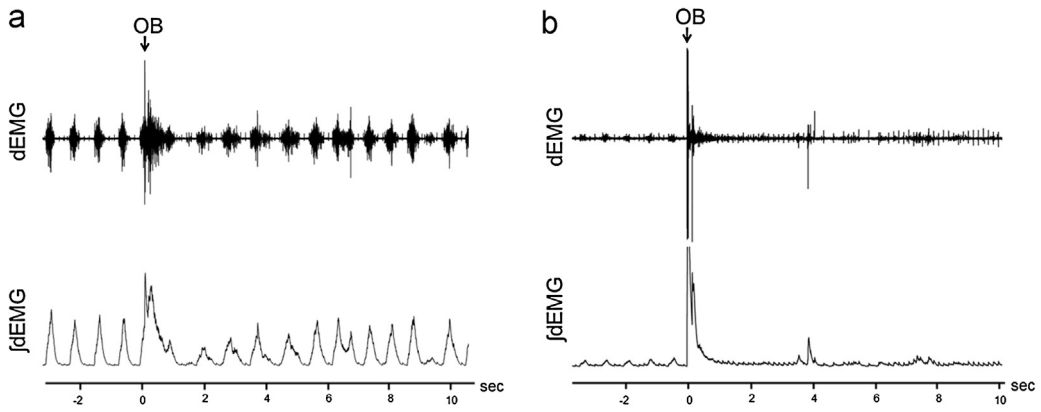

Fig. 1. Recordings of raw (top trace) and $\int$dEMG (bottom) for OB-1 animals. (a) dEMG in an OB-1, Group 1 animal, Psi = 69.9. (b) dEMG in an OB-1, Group 2 animal, Psi = 90.3.

air mixes with the high-pressure gas resulting in a shock wave. The blast pressure waveform (Svetlov et al., 2010) has a peak overpressure and gas venting phase, believed to cause the most damage due to the prolonged time spent in this phase. The blast wave is 10 ms at variable Psi levels measured at the shock tube. The blast pressure data was acquired from piezoelectric blast pressure transducers.

When the animal is positioned directly under the shock tube nozzle, it is known as a composite blast. Composite blast exposure (directed at the skull) can result in diffuse brain injury and neurodegeneration in the rostral and caudal diencaphalon and mesencephalon (Svetlov et al., 2010). OB injury can also result in intracranial hematomas as well as brain swelling (Svetlov et al., 2010). Upon autopsy of the animals (5/27) that succumbed to blast injury, hematomas were found on the dorsal aspect of the brain between bregma and lambda along with evidence of a disruption of vascular supply within the circle of Willis. The kinectic force of the OB on the superficial and interior portion of the brain is evident when mortality occurred (Svetlov et al., 2010).

The OB blast injury is reproducible and causes significant damage throughout the brain as evidenced by gross inspection and histology (Svetlov et al., 2010). Observational disruptions in breathing have been seen in multiple rodent models during both closed and open head injuries (Cheng et al., 2010; Dixon et al., 1987; Guy et al., 1998b; Kuehn et al., 2011). However, breathing pattern immediately before, during and immediately after OB TBI remains unknown. Specifically, it is unknown if inspiratory motor drive ceases (apnea) during and after OB exposure. It is also unknown if there is a relationship between the magnitude of the OB and apnea. Further, the effect of repeated OB on apnea is unknown.

1.2. TBI and respiration

Respiration is controlled both consciously and autonomically via the brainstem respiratory network. The respiratory control network receives afferent and efferent input and this information is processed to maintain homeostasis. Autonomic brainstem respiratory function is controlled by the pons and medulla. The pons and medulla have a network of respiratory neurons that generate the respiratory motor pattern. Inspiratory and expiratory neurons send axons to the inspiratory and expiratory motor units to provide the neuromuscular drive and breathing pattern. A disruption in this region can result in an absence of breathing (apnea) or an altered breathing pattern evidenced by gasping, sighs or irregularities in the breathing pattern. Apnea is defined as an absence in diaphragmatic muscle activity for a minimum of two respiratory cycles. Observational apneic periods have been seen in humans and animals during TBI. Changes in breathing patterns including apnea, partial recovery of respiration, followed by slow and deep respiration were observed in rodents during a skull-directed detonator blast (Cheng et al., 2010).

Apnea followed by a period of irregular breathing is believed to result from OB injury of sufficient magnitude but specific respiratory motor activity has not been recorded during OB exposure. It is likely that the respiratory control network is disrupted at the medullary level by the intracranial high pressure wave. Disruption within the respiratory network is hypothesized to result in an apneic period and the apneic duration may be a function of the magnitude of the pressure wave. It is also likely that the initial OB injury would cause this respiratory neural network to become hypersensitive to a second smaller magnitude OB, resulting in a longer apneic period. The present study will directly record diaphragm EMG (dEMG) during an OB to determine duration of the apneic period and subsequent irregular breathing pattern following the OB.

We hypothesized that blast-induced TBI: (1) will induce an apneic period during and immediately following blast exposure induced acute TBI directly related to the magnitude of the OB pressure wave; (2) will induce post-apnea irregular breathing pattern that is directly related to the magnitude of the OB pressure wave; (3) will induce apnea at lower pressures and longer durations with repeated OB injury (2 weeks after the first OB); and (4) will induce irregular breathing pattern directly related to magnitude of the second OB pressure wave with repeated OB injury (2 weeks after the first OB). To test these hypotheses, dEMG recordings were obtained in chronically instrumented rats immediately before, during and immediately after the dorsal head OB exposure.

2. Materials and methods

2.1. Animals

These experiments were performed on male Sprague-Dawley rats weighing 250–300 g. The animals were housed in the University of Florida animal care facility. They were exposed to a 12-h light/12-h dark cycle with food and water ad libitum. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Florida.

2.2. Surgical procedure

Animals were anesthetized using isoflurane. The abdominal wall was incised and costal borders retracted to expose the diaphragm muscle. dEMG electrodes (stainless steel, Teflon-coated wire AS631, Cooner Wire, Chatsworth, CA, USA) were introduced into the diaphragm at the intercostal border on the right side and advanced through the diaphragm muscle and glued securely into