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Autonomic responses to blast overpressure can be elicited by exclusively exposing the ear in rats

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ABSTRACT

Blast overpressure has become an increasing cause of brain injuries in both military and civilian populations. Though blast's direct effects on the cochlea and vestibular organs are active areas of study, little attention has been given to the ear's contribution to the overall spectrum of blast injury. Acute autonomic responses to blast exposure, including bradycardia and hypotension, can cause hypoxia and contribute to blast-induced neurotrauma. Existing literature suggests that these autonomic responses are elicited through blast impacting the thorax and lungs. We hypothesize that the unprotected ear also provides a vulnerable locus for blast to cause autonomic responses. We designed a blast generator that delivers controlled overpressure waves into the ear canal without impacting surrounding tissues in order to study the ear's specific contribution to blast injury. Anesthetized adult rats' left ears were exposed to a single blast wave ranging from 0 to 110 PSI (0–758 kPa). Blast exposed rats exhibited decreased heart rates and blood pressures with increased blast intensity, similar to results gathered using shock tubes and whole-body exposure in the literature. While rats exposed to blasts below 50 PSI (345 kPa) exhibited increased respiratory rate with increased blast intensity, some rats exposed to blasts higher than 50 PSI (345 kPa) stopped breathing immediately and ultimately died. These autonomic responses were significantly reduced in vagally denervated rats, again similar to whole-body exposure literature. These results support the hypothesis that the unprotected ear contributes to the autonomic responses to blast.

1. Introduction

Blast, such as that produced by explosive devices, has become a frequent cause of injury in both military and civilian populations (Cernak and Noble-Haeusslein, 2010; Taber et al., 2006). Injuries from blast wave overpressure are a source of uncertainty in that they often lack clinically apparent signs and can be insidious, only presenting themselves after other injuries have been managed (Bass et al., 2012; Burgess et al., 2010; Lemonick, 2011; Phillips, 1986). Detection, management, and prevention of blast injury has become an active area of study in blast literature, and more clinical diagnoses can be attributed to the effect of blast overpressure than in the past (Burgess et al., 2010; Svetlov et al., 2009).

Traumatic brain injury, including blast-induced neurotrauma (BINT), is the “signature injury” of U.S. soldiers involved in recent conflicts in Iraq and Afghanistan (Huber et al., 2013; Mac Donald et al., 2014; Moss et al., 2009; Pham et al., 2015). Blast exposure

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may cause BINT through multiple pathways such as direct passage of the blast wave through the skull, compression of the torso resulting in transfer of the blast wave's kinetic energy to the brain via hydraulic oscillations within the vasculature, and hypoxia through over-activation of the parasympathetic nervous system (Cernak, 2010; Cernak et al., 2001; Courtney and Courtney, 2009; Long et al., 2009; Moss et al., 2009; Simard et al., 2014). The parasympathetic response, specifically, is hypothesized to be a result of hyperinflation of the lungs stimulating alveolar juxtacapillary J-receptors innervated by vagal fibers, leading to apnea then tachypnea, bradycardia, and hypotension, which in turn lead to a Bezold-Jarish reflex further deepening bradycardia and hypotension (Cernak, 2010; Krohn et al., 1942; Zucker, 1986; Zuckerman, 1940). Another proposed mechanism suggests that the foramina of the skull (e.g., the acoustic meatus, optic canal, nasal cavity, or foramen magnum) can provide a conduit for the blast wave to enter the cranial vault in addition to the established mechanisms mentioned above (Hicks et al., 2010; Ropper, 2011; Sundaramurthy et al., 2012). The advanced combat helmet, the current helmet of the U.S. Army, uses layers of Kevlar and a foam suspension to protect the skull from penetrating and blunt-force injuries, but leaves the ears, eyes, nose, and mouth all exposed to the surrounding air, permitting pressure from a blast to interact (Meaney et al., 2014; Moore et al., 2009). Nonetheless, soldiers frequently wear little to no ear protection, citing a necessity for situational awareness through unhindered sound localization (Abel, 2008; Brown et al., 2015; Clasing and Casali, 2014; Jones and Pearson, 2016). A perforated eardrum is the most frequently reported blast injury (Cernak and Noble-Haeusslein, 2010; Choi, 2012; Darley and Kellman, 2010; DePalma et al., 2005; Gan et al., 2016; Garth, 1994; Helling, 2004; Katz et al., 1989; Kronenberg et al., 1993; Mayorga, 1997; Patterson and Hamernik, 1997; Phillips, 1986). We hypothesize that energy from blast overpressure could enter the unprotected ear canal, traverse these soft tissues into the cranial vault, and directly impact the brain.

In this study, we tested the hypothesis that the ear provides a vulnerable locus for blast energy to impact the brain while causing acute autonomic responses typically observed in whole-body paradigms of blast exposure (Guy et al., 1998; Krohn et al., 1942; Sawdon et al., 2002). To do so, we developed a blast generator that delivers precisely controlled blast overpressure waves targeted to a small area of tissue, with minimal impact on the surrounding tissues (Fig. 1A). Though placing an animal in a “shock tube” is widely accepted to be the most accurate laboratory model of primary blast, i.e. the shockwave effect of blast (Alay et al., 2017; Needham et al., 2015), there is not a feasible way to prevent the blast wave from causing flexion of the skull and compression of the torso that would introduce confounding factors in our study of the ear's role. Instead, our blast generator produces an overpressure wave similar to that of shock tube literature, but isolates the wave's impact to the ear and minimizes exposure of the rest of the body, including the lungs. This approach allows us to specifically study the ear's role in blast injury, minimizing confounding factors encountered when blast is delivered over the whole animal (Cernak, 2005; Mediavilla Varas et al., 2011; Xiong et al., 2013; Yarnell et al., 2013).

Blast-induced injuries to the organs of the inner ear are active areas of study using whole-body exposure paradigms (Akin and Murnane, 2011; Chandler and Edmondt, 1997; Chen et al., 2013; Cho et al., 2013; Choi, 2012; Cohen et al., 2002; Darley and Kellman, 2010; Dougherty et al., 2013; Fausti et al., 2009; Gan et al., 2016; Garth, 1994; Helling, 2004; Hoffer et al., 2010; Jagade et al., 2008; Kerr and Byrne, 1975; Niwa et al., 2016; Scherer et al., 2011; Singh and Ahluwalia†, 1968; Teter et al., 1970; Tungsinmunkong et al., 2007). However, to the best of our knowledge, this is the first study of the ear's specific contribution to the systemic autonomic responses induced by blast injury.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley (SD) rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 250–500 g were used for this experiment. In total, 31 rats were used in this study; 22 Rats were used for the autonomic response measurements, and 9 rats were used for the subsequent vagal denervation study. Rats were assigned to exposure intensities randomly. All procedures were performed in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

2.2. Blast wave generator and calibration

A modified high-power airgun was used as the basis for the blast generator (Fig. 1A). All seals and connectors were replaced and/or reinforced with high-quality o-rings and poly-tetra-fluoro-ethylene (PTFE) tape to minimize unwanted pressure escape and increase repeatability. An M1-3KPSI digital pressure gauge (Crystal Engineering Corp. San Luis Obispo, CA) was connected to the air canister to measure input air pressure at a resolution of 0.1 PSI (0.7 kPa). The muzzle of the blast generator was threaded and an aircraft-grade aluminum t-fitting (Eaton, Dublin, Ireland) was affixed for use as a sensor bung. A high-frequency integrated-circuit-piezoelectric (ICP) pressure sensor connected to a signal conditioner (102B04, 480C02, PCB Piezotronics, Depew, NY) was installed in the sensor bung. The blast generator was installed on a stereotaxic frame (David Kopf Instruments, Tujunga, CA) using an adjustable multi-arm instrument holder. A 3.0 mm stainless steel speculum was attached to the instrument holder and held even with the level of the muzzle, leaving open space for excess blast wind to escape. For calibration, a second arm was attached holding another high-frequency ICP pressure sensor and adjusted such that the sensor was in the position of the rat's tympanic membrane (2.5 mm from the tip of the speculum). The blast generator was charged to 500 PSI (3447 kPa) input pressure, allowed to come to equilibrium, and then activated with both the bung sensor and the output sensor recording at 500 kHz. After the blast, the generator was given time to return to equilibrium and then activated again. This was repeated until the air canister was depleted (about 45 blasts). This process was repeated twice to verify repeatability. Each of the waveforms produced by the blast generator were then analyzed for rise time, total positive pressure time, maximum pressure, and return to baseline pressure. The blast pressures were linearly related to the input pressure as well as the bung pressure. Thus, desired blast pressure was achieved by adjusting the input pressure and was verified by the bung pressure.

2.3. Blast exposure

Rats were maintained under 2% inhaled isoflurane anesthesia. Before blast exposure, a photograph of the left and right tympanic membranes was captured using a digital macroview otoscope (Welch-Allyn, Skaneateles Falls, NY) and evaluated for intactness and lack of erythema or effusion. If either factor was present, the rat was not used for this study. A PE-50 polyethylene catheter (Intramedic by BD, Franklin Lakes, NJ) was implanted in the left femoral artery and connected to a disposable transducer (Argon Medical, Athens, TX) for blood pressure monitoring. If a pulsatile waveform was recorded, the catheterization was deemed successful. If such a

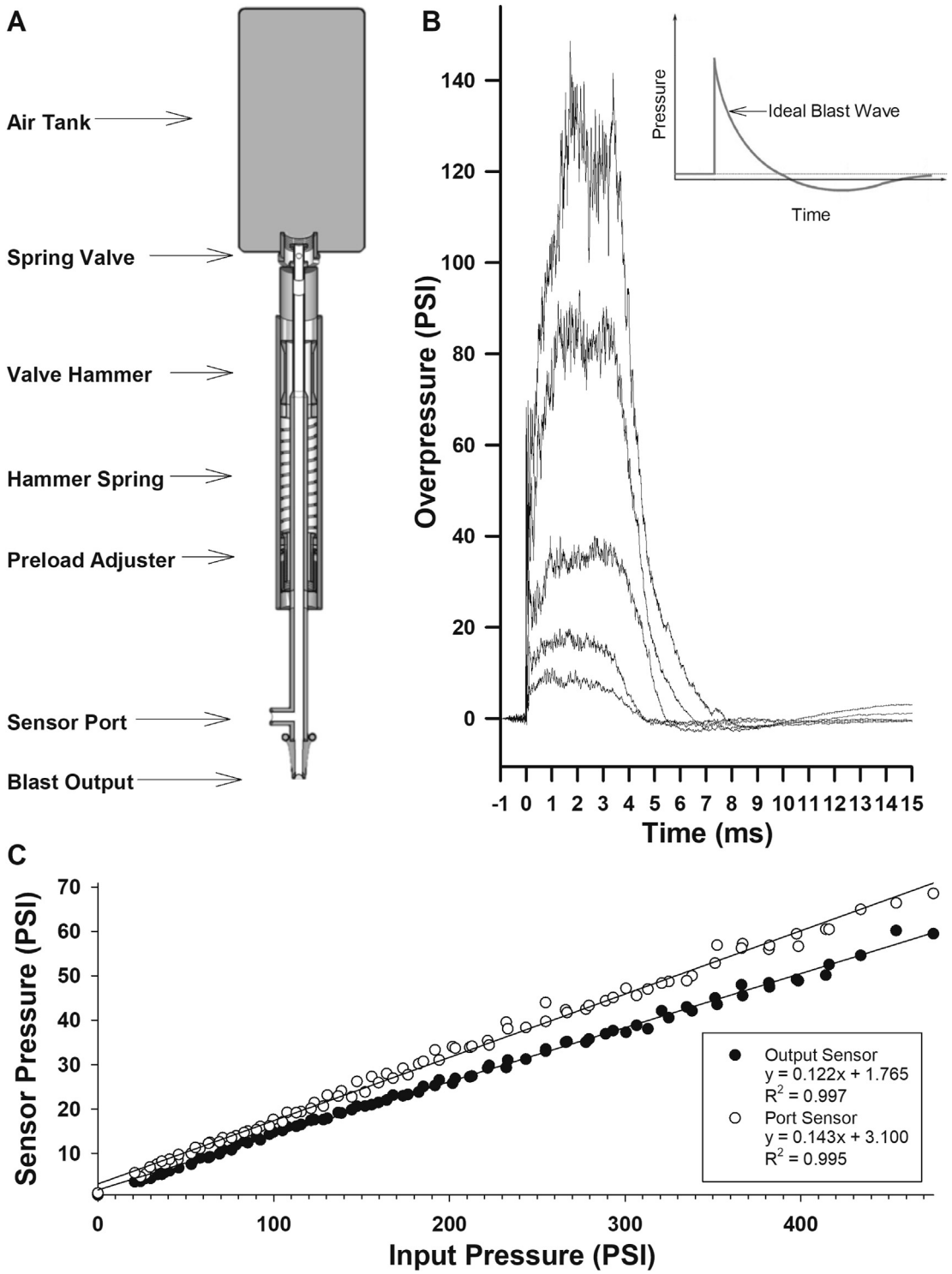

Fig. 1. The isolated blast generator. A. Schematic of the blast generator. B. Waveforms of blast waves at different intensities. Note the primary change due to varying pressures of calibration is the peak overpressure while rise time remains stable. Total positive phase increases with increasing blast pressure, but negative phase time remains stable. Insert: "Ideal" blast wave in an open space (Friedlander waveform), adapted from Goel et al. (2012) for comparison. C. Input-Output regressions for sensor port peak pressure (empty circles) and actual blast pressure measured at the speculum (filled circles) at varying input pressures.

waveform was not present or if the catheterization was incomplete, the catheter was left in place and the experiment was carried out normally, but the blood pressure data was not recorded. Stainless steel 27-gauge subdermal needle electrodes (CareFusion, Middleton, WI) were placed at the ventral right shoulder, left shoulder, and left hip of the rat for electrocardiography using a D360 isolated patient amplifier (Digitimer, Ft. Lauderdale, FL). The rat was then laid in the right lateral decubitus position and a piezo transducer (Radio Shack, Ft. Worth, TX) was placed under the ribcage to detect inspiratory effort. The blast generator was lowered into the left ear

canal, and the blast apparatus was rotated away to allow direct visualization of the speculum position within the ear canal using the digital macroview otoscope. The speculum was lowered further and the rat's head position adjusted until the head was held in place on the platform by the speculum, and the tympanic membrane was in the center of the speculum's line of sight. The blast generator was then rotated back into position and locked. Electrocardiography (ECG), pulse oximetry, piezo respiratory effort sensing, and blood pressure were continuously monitored prior to, during, and after blast exposure to evaluate the physiologic effects of the blast. The rats were monitored for stabilization of the heart rate and respiratory rate. One blast of varying pressure was delivered. At first, a wide range of blast pressures were experimented with (0–110 PSI). However, when it became evident that blasts of 50 PSI and above could be immediately lethal, the remaining blasts were performed below that threshold. Control rats underwent the same anesthesia, preparation, and monitoring, except the blast generator was not charged (0 PSI, or 0 kPa). After blast exposure and autonomic response recording, rats were sacrificed via intracardiac perfusion with 4% Paraformaldehyde in phosphate-buffered saline for tissue fixation for separate studies.

2.4. Vagus nerve cervical segment ligation and blast exposure

Rats were maintained under isoflurane anesthesia and placed supine on a feedback-controlled heating pad (FHC Inc. Bowdoin, ME). A midline incision was made on the neck and the fat and submandibular glands were retracted. The left sternocleidomastoid muscle was rotated laterally to expose the carotid sheath. The sheath was opened at the level of the thyroid cartilage and the vagus nerve bundle separated from the carotid artery and jugular vein using blunt dissection with fine forceps. A 4-0 silk suture was loosely tied around the nerve, which was then allowed to rest back under the sternocleidomastoid muscle. The contralateral nerve was then isolated in the same fashion. Once the cervical portion of the vagus nerve had been isolated bilaterally, the nerves were lifted using the suture, the sutures were tied tightly, and the nerves were cut using micro-scissors. An increase in heart rate when tying and cutting the nerves was used to verify adequate separation. Rats receiving sham surgery underwent the midline incision, retraction of fat and glands, and reflection of both sternocleidomastoid muscles, but the carotid sheath was not opened. Rats then underwent blast exposure as outlined above.

2.5. Data collection and analysis

The blast sensor signal conditioners, blood pressure monitor, ECG amplifier, and respiratory rate monitor were connected to a CED Power1401 data acquisition system connected to a PC running CED Spike2 data acquisition and analysis software (Cambridge Electronic Design Limited, Cambridge, England). Respiratory rate and heart rate were computed as the inverse of the period between breaths and heartbeats, respectively. Baseline respiratory rate and heart rate were calculated as the mean rates for 1 min prior to blast exposure. Peak/trough respiratory rate and heart rate were defined as the highest or lowest value after blast exposure that was not due to a double/absent breath or heartbeat. Blast pressure was calculated using the bung pressure. Relationships were analyzed using linear regression and t-tests using SigmaPlot software (Systat Software Inc. San Jose, CA). P-value of 0.05 was used to determine significance of the results, expressed as mean ± standard error.

3. Results

3.1. Blast waveforms and calibration

The waveforms produced by the blast generator exhibited a rise time of 2.2 ± 0.3 ms and a total positive phase duration of 7.3 ± 0.5 ms (Fig. 1B), resembling the waveforms reported in the literature using shock tubes based on the Friedlander waveform, i.e., the ideal waveform produced by a blast in an open space (Fig. 1B insert) (Cernak et al., 2011; Friedlander, 1946; Goel et al., 2012; Mediavilla Varas et al., 2011; Sundaramurthy et al., 2012). The output pressure was linearly related to the input tank pressure ($P_{Output} = 0.12 \times PInput + 1.76$, $R^2 = 0.997$, $p < 0.0001$) and the bung pressure ($P_{Output} = 0.85 \times PBung$, $R^2 = 0.995$, $p < 0.0001$) (Fig. 1C). These relationships allowed us to deliver blast waves with the desired intensities.

3.2. Autonomic responses to blast waves delivered to the external ear canal

Blast waves delivered to the ear canal evoked well-defined autonomic responses in anesthetized rats. Exposure to a single blast wave of 50 PSI (345 kPa) or above through the ear canal caused an immediate and permanent apnea in some rats, resulting ultimately in death. Fig. 2A shows such an example: this rat had a stable respiratory rate of 35 breaths/minute before blast exposure, which dropped to 0 breaths/minute immediately after blast exposure and remained for 2 min, at which point the rat was sacrificed. Exposure to a single blast wave lower than 50 PSI (345 kPa), however, resulted in an increase in respiratory rate. Fig. 2B shows that exposure to a single blast shockwave increased the rat's respiratory rate from 28 breaths/minute to 40.2 breaths/minute (~42.8% increase). The blast-induced increases in respiratory rate were linearly related to blast intensity (0.59% per PSI, $R^2 = 0.69$, $p < 0.0001$) (Fig. 2C). When it was found that blasts above 50 PSI had the potential to kill the rats outright, we refocused our efforts on survivable blast pressures (those below 50 PSI).

Rats exposed to blast waves ranging from 2 to 110 PSI (14–758 kPa) exhibited an immediate decrease in heart rate that was more pronounced with increasing blast intensity. Fig. 3A shows a typical record of blast-induced changes in heart rate. The rat exhibited a stable heart rate of 230 beats/minute before blast, which decreased to 192.6 beats/minute (~16% drop) and slowly recovered over the next several minutes. After the blast, the rat occasionally exhibited both extra and skipped beats, resulting in spikes in the instantaneous rate demonstrated on the graph. The decrease in heart rate was more pronounced for blasts with higher intensity (heart rate: −0.51% per PSI, $R^2 = 0.70$, $p < 0.0001$, Fig. 3B).

Rats exposed to blasts of 30 PSI and above exhibited an immediate decrease in blood pressure. Rats exposed to blasts below 30 PSI exhibited some variation in blood pressure, but those variations were not as pronounced as those above 30psi. Fig. 4A shows a record of blast-induced changes in blood pressure. Prior to blast, the rat's blood pressure was stable at approximately 120/80 mmHg. After blast, the rat's blood pressure dropped to approximately 45/20 mmHg and slowly rose over the next few minutes. Decreases in blood pressure were more pronounced for blasts with higher intensity (−1.07% per PSI, $R^2 = 0.88$, $p < 0.0001$, Fig. 4B).

Rats that were exposed to blasts of 10–50 PSI (69–345 kPa) did not display a statistically significant increase in P-R interval on ECG tracings. However, at higher pressures, some rats exhibited P-R interval prolongation with progressive heart beats until one QRS

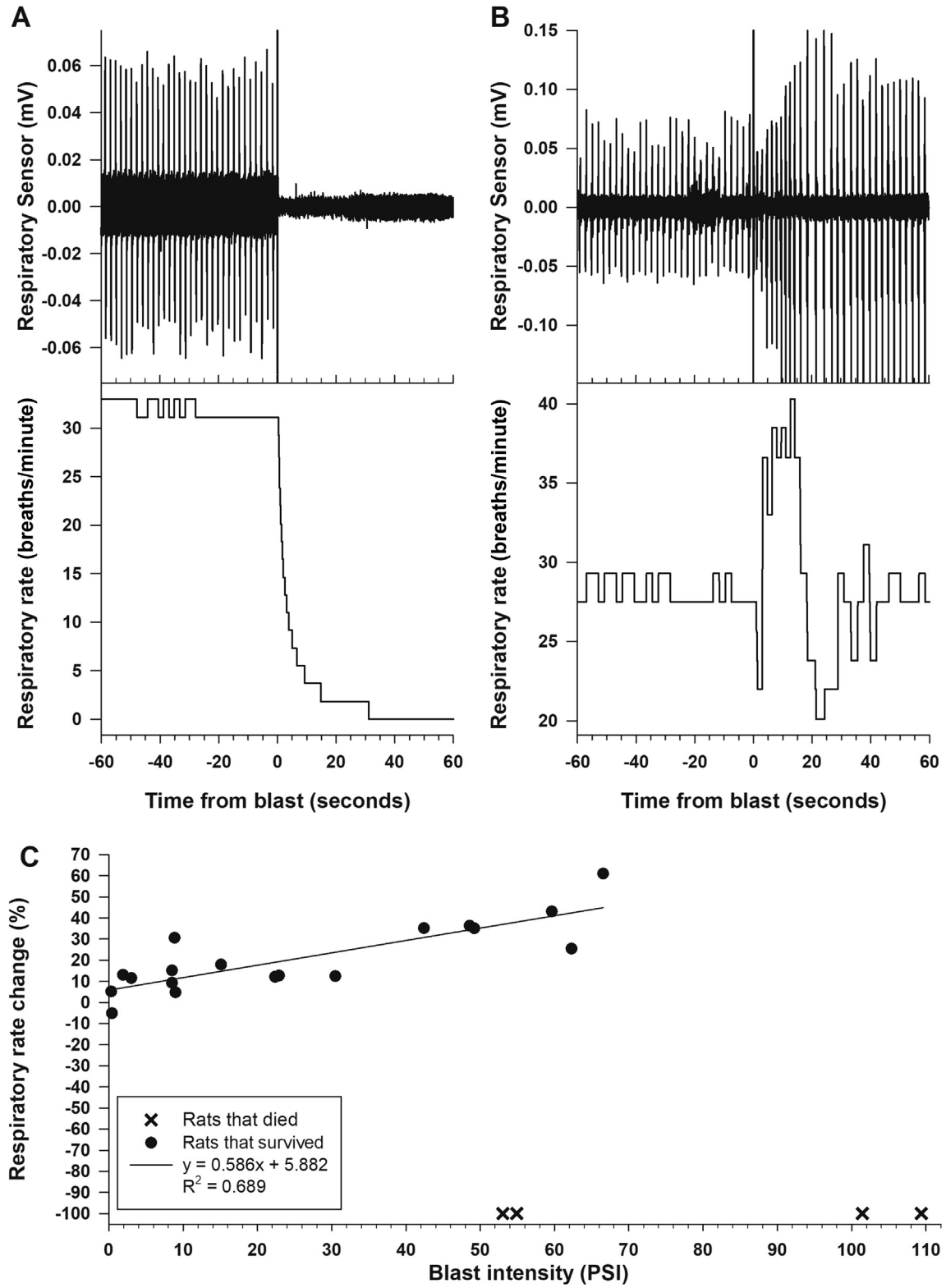

Fig. 2. Respiratory rate responses to blast via the ear. A. Apnea after a 53.0 PSI (365 kPa) blast in one rat. B. Respiratory rate increase after a 59.6 PSI (411 kPa) blast in one rat. C. Linear regression of blast intensity and respiratory rate change ($R^2 = 0.69$, $p < 0.0001$). Crosses are four rats that immediately stopped breathing after the blast. Circles are rats that survived the blast exposure.

complex was dropped completely, indicative of a type 2 heart block with Wenckebach phenomenon (Fig. 5). Ultimately, some rats in 50 + PSI (345 + kPa) groups expressed complete desynchronization of P-waves from QRS complexes, indicative of a type 3 heart block.

3.3. Vagal nerve denervation

To test the role of the vagus nerve in the blast-induced cardio-respiratory responses, the cervical segment of the vagus nerve was

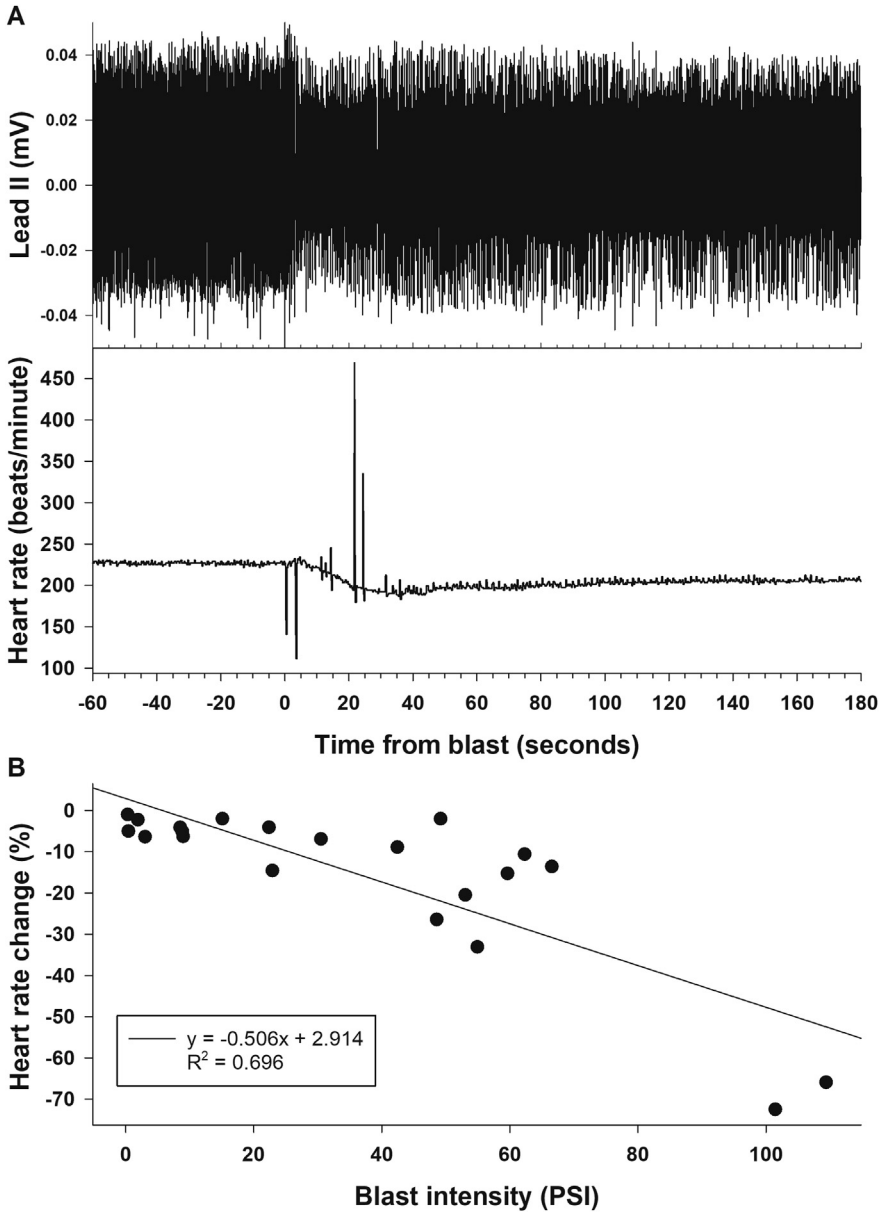

Fig. 3. Heart rate responses to blast via the ear. A. Heart rate decreased after exposure to a blast of 59.6 PSI (411 kPa). Spikes are due to occasional extra- and skipped-beats. B. Linear regression of blast intensity and heart rate change ($R^2 = 0.70$, $p < 0.0001$).

ligated and severed bilaterally prior to blast exposure. Rats that received a vagal ligation and severance exhibited significantly less respiratory rate elevation and heart rate depression than the rats with intact vagus nerves ($p = 0.015$ and $p = 0.001$, respectively) (Fig. 6). The changes in respiratory rate and heart rate of the rats with ligated and severed vagus nerves exposed to 50 PSI (345 kPa) blasts were not significantly different from that of the intact rats exposed to sham blast ($p = 0.431$ and $p = 0.281$, respectively).

4. Discussion

4.1. A novel targeted and repeatable blast model for elucidating tissue-specific responses to blast overpressure

We have developed a new blast generator that delivers blast overpressure energy specifically targeted a small area of tissue while minimizing blast overpressure on the surrounding tissues. It is highly adjustable and repeatable, and can be used to simulate blasts of varying overpressure intensities. The waveform of the blast closely approximates that of an ideal blast. We found that 15 PSI (103 kPa) was the 95–100% threshold of rupturing the rat tympanic membrane (TM), which was similar to the human values of 5–15 PSI (34–103 kPa) (Ritenour et al., 2008). This innovative model allows us to study the ear's specific contributions to the overall spectrum of blast injury, in contrast to models that expose the entire body or head to blast. Though the “shock tube” is widely accepted as the most accurate laboratory model of primary blast, there is not a feasible way to protect the rest of the animal from the blast and exclusively expose the ears; thus we would not have been able to definitively say the response was due to the ear. The results gathered with this model show that blast exposure to the ear resulted in substantial cardiorespiratory changes and even death, demonstrating that the ear is a vulnerable site for transferring blast energy to the central nervous system, and that the autonomic

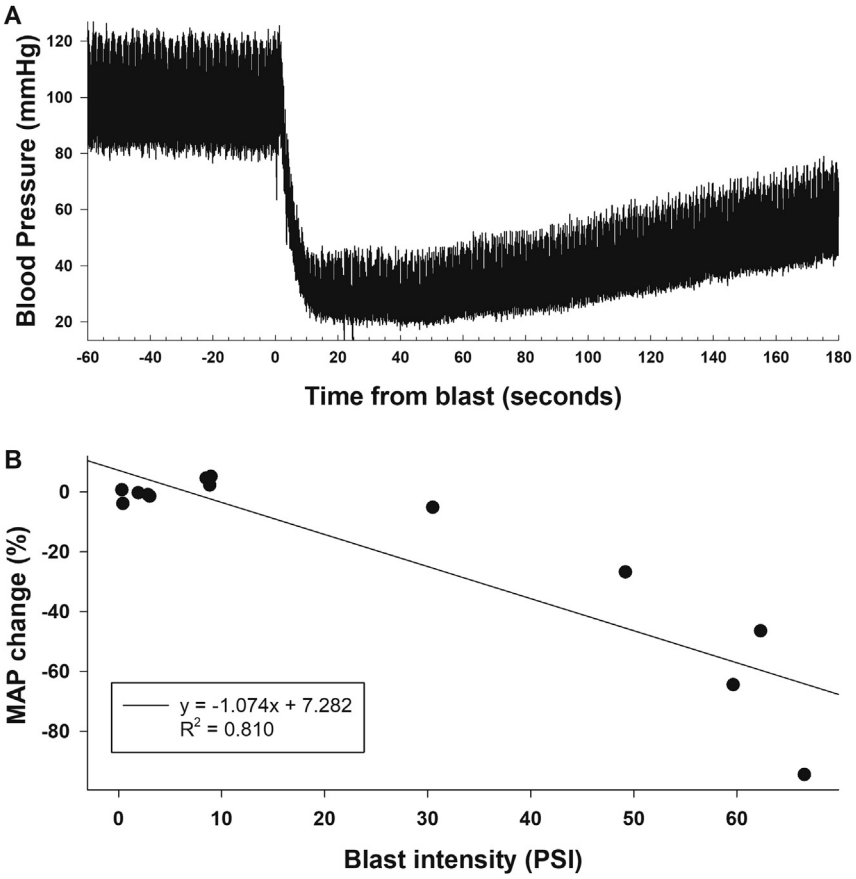

Fig. 4. Blood pressure responses to blast via the ear. A. Blood pressure changes after exposure to a blast of 60 PSI (414 kPa). B. Linear regression of blast intensity and mean arterial pressure (MAP) ($R^2 = 0.81$, $p < 0.0001$).

response to blast is not dependent on the lungs' exposure. This targeted-blast injury model could also be used to test other tissues' specific contributions to blast injury, including the eye, nose, neck, and more.

4.2. Blast delivered solely to the ear has significant physiologic effects

Rats exposed to medium-to high-level blasts (those above 30 PSI) exhibited increased respiratory rate, decreased heart rate, atrioventricular block, and decreased blood pressure in response to blasts delivered exclusively to the rats' ear canals. These results are similar to those seen in whole-body and whole-head exposure paradigms, where the prevailing hypothesis is that these autonomic responses are due to lung injuries (Cernak, 2010; Zucker, 1986; Zuckerman, 1940). One primary difference is that in our experiments the initial response was tachypnea, as opposed to apnea followed by tachypnea in whole-body exposure. This one difference is possibly due to our model not directly impacting the lungs, thus suggesting the apnea in other models is, indeed, due to initial blast injury to the lungs.

Lower-level blasts, e.g., those from 2 to 30 PSI, still resulted in tachypnea peaks and bradycardia troughs with slow recovery that would not be expected in rats under constant general anesthesia. However, only blasts at 30 PSI and above elicited a demonstrable hypotension response. This may represent a threshold where the blast impacts a new area of the brainstem, where the blast activates more vagal afferents in the ear canal, at which the autonomic response to blast overcomes the vestibulo-cardiac reflex, or other mechanisms may be in action. This is discussed further in section 4.3.

If humans exhibit similar ear-mediated autonomic responses to a blast, it is very likely they would experience syncope, which could cause further injury and would limit their ability to seek safety. Additionally, these responses have been shown to induce hypoxemic injury to the brain (Cernak, 2010). Finally, blasts of this magnitude frequently also induce profound secondary and tertiary injuries, e.g., shrapnel penetration and blunt-force trauma, all of which could cause profuse bleeding and ultimately hemorrhagic shock (Cooper et al., 1983; Elsayed and Atkins, 2010). In our tests, we show that blast experienced by the ear canal may inhibit responses typically mediated by the sympathetic and parasympathetic nervous system, e.g., heart rate and blood pressure. The sympathetic response is vital to surviving hemorrhagic shock, so victims of blast may be at greater risk of morbidity and mortality due to the combined effect of shock, sympathetic inhibition, and increased parasympathetic tone. Further investigation using this model in conjunction with a model of hemorrhagic shock may provide insight into the interaction between these processes.

4.3. Autonomic effects of ear-only blast exposure are mediated via the vagus nerve

The vagus nerve has been shown to mediate autonomic responses to blast using thoracic and whole-body exposure models, so we also tested whether the ear's exposure to blast activates the vagal response (Cernak et al., 1996b; Irwin et al., 1999; Sawdon et al., 2002; Wang et al., 2013). We noted on ECG that some rats

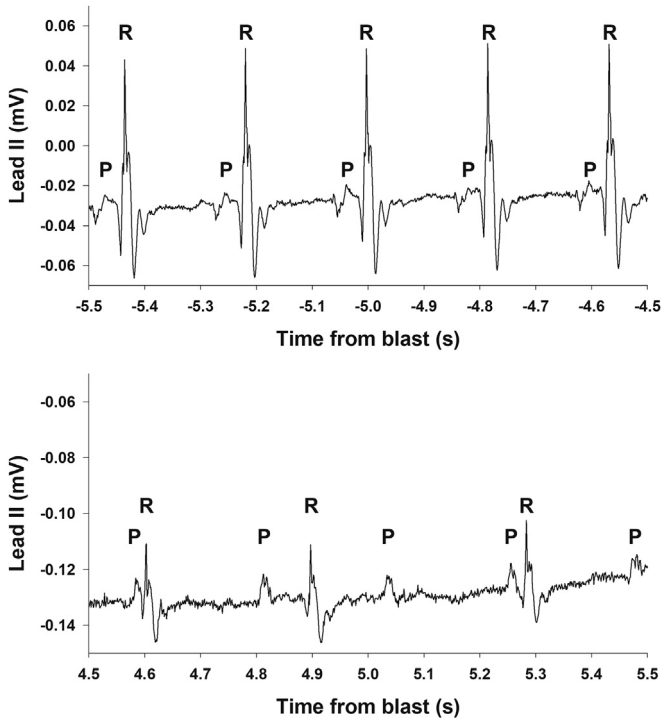

Fig. 5. ECG response to blast via the ear. ECG signals before and after exposure to a blast of 109 PSI (752 kPa). P-R interval were prolonged with progressive heart beats until one QRS complex was dropped completely, indicative of a type 2 heart block with Wenckebach phenomenon.

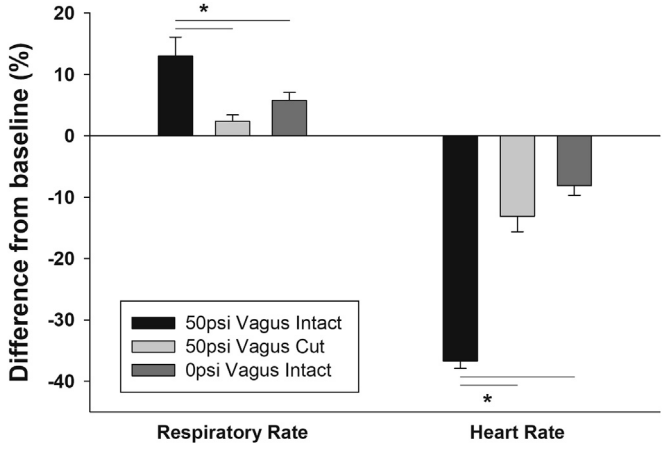

Fig. 6. Vagal mediation of autonomic response blast via the ear. Rats with bilateral vagus nerve denervation exhibited significantly less changes in respiratory rate and heart rate than the rats with intact vagus nerves ($p = 0.015$ and $p = 0.001$, respectively). Cardiorespiratory changes in rats with bilateral vagus nerve denervation were not significantly different from those in intact rats receiving 0 PSI (0 kPa) blasts ($p = 0.461$ and $p = 0.281$, respectively).

blasted at 50 PSI (345 kPa) and above experienced atrioventricular block immediately after blast exposure via the ear canal. That the blocks occurred acutely after blast exposure suggests that strong vagal stimulation may be inducing the blocks, and could also have a part in mediating the other physiologic responses. To determine the degree to which the vagus nerve mediates the physiologic response, we severed the cervical segment of the vagus nerve prior to blasts at the highest survivable pressures (~50 PSI, or 345 kPa). Rats with intact vagus nerves displayed tachypnea and bradycardia, but those whose vagus nerves were severed exhibited responses that were not significantly different from intact rats receiving a sham blast. Thus, the vagus nerve plays a critical role in mediating the autonomic responses induced by blast to the ear.

The parasympathetic response to blast has been hypothesized to be a result of hyperinflation of the lungs stimulating alveolar juxtacapillary J-receptors innervated by vagal fibers, leading to apnea then tachypnea, bradycardia, and hypotension, which in turn lead to a Bezold-Jarish reflex further deepening bradycardia and hypotension (Cernak, 2010; Krohn et al., 1942; Zucker, 1986; Zuckerman, 1940). However, in the present study we show that blast exclusively delivered to the ear also causes these autonomic effects, highlighting the unprotected ear's role as a vulnerable locus during blast exposure. There are at least two potential mechanisms that underlie the ear's role in the vagus nerve-mediated autonomic responses to blast. One is direct impingement of the blast shockwave on the central nervous system. The soft tissues of the inner ear and the vestibulocochlear nerve may provide a conduit of low resistance through the temporal bone, allowing the energy of the blast to transfer into the cranial vault. The internal acoustic meatus opens toward the brainstem, which contains centers implicated in cardiac, respiratory, and vascular function including the nucleus ambiguus, nucleus tractus solitarius, and the dorsal and ventral respiratory groups (Machado and Brody, 1988; Paxinos and Watson, 2007). The complex neural damage which blast induces could cause activation, suppression, or disruption of transmission of these groups, leading to the respiratory elevation and cardiovascular suppression exhibited by our rats. This hypothesis is further supported by the occurrence of complete apnea in rats exposed to blasts higher than 50 PSI (345 kPa): a threshold above which the blast energy possibly completely disrupted motor output to the diaphragm.

A second potential mechanism underlying the blast-induced cardiorespiratory responses is activation of vagal afferents located in the ear canal. The auricular branch of the vagus nerve (ABVN) innervates portions of the ear canal, the cymba of the concha, and the tragus of the ear, and projects to the nucleus tractus solitarius (NTS) (Berthoud and Neuhuber, 2000; Frangos et al., 2015; Peuker and Filler, 2002). The NTS, in turn, may activate the baroreflex by activating the caudal ventrolateral medulla, which inhibits the rostral ventrolateral medulla, ultimately inhibiting the sympathetic nervous system (Boron and Boulpaep, 2003; Howe, 1985; Mifflin and Felder, 1990). Histologic and electrophysiological studies investigating neuronal activation, inhibition, and damage in these areas may help elucidate the precise mechanisms of injury.

Stimulation of the vestibular system can result in activation of the sympathetic nervous system (Ishikawa and Miyazawa, 1980; Radtke et al., 2003, 2000; Ray and Carter, 2003; Tanaka et al., 2014; Tang and Gernandt, 1969). Due to the overwhelming parasympathetic responses seen in our animal model, vestibular stimulation by blast may play a role in tempering the responses we see in this model. Specifically, since hypotension is only reliably elicited by blasts of 30 PSI or above, this may represent a threshold above which the other mechanisms of blast affecting the autonomic responses overcomes this vestibulo-cardiac reflex. Electrophysiological studies of the vestibular response to blast may help elucidate the mechanisms of the autonomic responses.

4.4. Support for necessity of ear protection in blast-prone environments

Soldiers frequently wear little or no ear protection, citing a necessity for situational awareness through unhindered sound localization (Abel, 2008; Brown et al., 2015; Clasing and Casali, 2014; Jones and Pearson, 2016). If the hypotension induced in our

rats by high-intensity blast via the ear canal also occurs in humans, it would likely induce syncope, preventing an individual's ability to react to threats, seek safety or help, or treat themselves until help arrives. Additionally, the autonomic response to blast, now shown to be contributed to by the ear's exposure, has been shown to induce hypoxic brain injury (Cernak, 2010; Cernak et al., 1996a). Finally, very high-pressure blasts via the ear canal induced a permanent, lethal apnea in our rats. It should be noted that this experiment is conducted in anesthetized rats, which may have different autonomic responses to blast compared to humans in real-world blast exposure. Nonetheless, the degree to which the respiratory rate, heart rate, and blood pressure could be affected by a blast suggest that the unprotected ear could present a vulnerability to blast not covered by existing head and torso protections.

This vulnerability highlights the necessity for wearing ear protection in blast-prone locations, such as battlefield or industrial environments, not only to protect hearing, but also to protect the wearer from ear-conducted blast-induced neurotrauma or other injury. These data further reinforce the importance of development of advanced ear protection that would allow users normal or enhanced hearing acuity and localization while still protecting the brain from the blast shockwave via the ear canal and acoustic meatuses.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.joto.2018.01.001.

References

Abel, S.M., 2008. Barriers to hearing conservation programs in combat arms occupations. Aviat Space Environ. Med. 79, 591–598. https://doi.org/10.3357/ASEM.2262.2008.

Akin, F.W., Murnane, O.D., 2011. Head injury and blast exposure: vestibular consequences. Otolaryngol. Clin. North Am. https://doi.org/10.1016/j.otc.2011.01.005.

Alay, E., Skotak, M., Misistia, A., Chandra, N., 2017. Dynamic loads on human and animal surrogates at different test locations in compressed-gas-driven shock tubes. Shock Waves 1–12. https://doi.org/10.1007/s00193-017-0762-4.

Bass, C.R., Panzer, M.B., Rafaels, K.A., Wood, G., Shridharani, J., Capehart, B., 2012. Brain injuries from blast. Ann. Biomed. Eng. 40, 185–202. https://doi.org/10.1007/s10439-011-0424-0.

Berthoud, H.-R., Neuhuber, W.L., 2000. Functional and chemical anatomy of the afferent vagal system. Auton. Neurosci. 85, 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0.

Boron, W.F., Boulpaep, E.L., 2003. Medical Physiology: a Cellular and Molecular Approach. W.B. Saunders.

Brown, A.D., Beemer, B.T., Greene, N.T., Argo, T., Meegan, G.D., Tollin, D.J., Tollin IV, D.J., Meegan, G.D., Tollin, D.J., 2015. Effects of active and passive hearing protection devices on sound source localization, speech recognition, and tone detection. PLoS One 10. https://doi.org/10.1371/journal.pone.0136568 e0136568.

Burgess, P.E., Sullivent, E.M., Sasser, S.M., Wald, M., Ossmann, E., Kapil, V., 2010. Managing traumatic brain injury secondary to explosions. J. Emergencies Trauma Shock 3, 164–172. https://doi.org/10.4103/0974-2700.62120.

Cernak, I., 2005. Animal models of head trauma. NeuroRx 2, 410–422. https://doi.org/10.1602/neurorx.2.3.410.

Cernak, I., 2010. The importance of systemic response in the pathobiology of blast-induced neurotrauma. Front. Neurol. 1, 151. https://doi.org/10.3389/fneur.2010.00151.

Cernak, I., Noble-Haeusslein, L.J., 2010. Traumatic brain injury: an overview of pathobiology with emphasis on military populations. J. Cerebr. Blood Flow Metabol. 30, 255–266. https://doi.org/10.1038/jcbfm.2009.203.

Cernak, I., Savic, J., Malicevic, Z., Zunic, G., Radosevic, P., Ivanovic, I., 1996a. Leukotrienes in the pathogenesis of pulmonary blast injury. J. Trauma Inj. Infect. Crit. Care 40, 148S–151S. https://doi.org/10.1097/00005373-199603001-00032.

Cernak, I., Savic, J., Malicevic, Z., Zunic, G., Radosevic, P., Ivanovic, I., Davidovic, L., 1996b. Involvement of the central nervous system in the general response to pulmonary blast injury. J. Trauma 40, S100–S104. https://doi.org/10.1097/00005373-199603001-00023.

Cernak, I., Wang, Z., Jiang, J., Bian, X., Savic, J., 2001. Ultrastructural and functional characteristics of blast injury-induced neurotrauma. J. Trauma 50, 695–706. https://doi.org/10.1097/00005373-200104000-00017.

Cernak, I., Merkle, A.C., Koliatsos, V.E., Bilik, J.M., Luong, Q.T., Mahota, T.M., Xu, L., Slack, N., Windle, D., Ahmed, F.A., 2011. The pathobiology of blast injuries and blast-induced neurotrauma as identified using a new experimental model of injury in mice. Neurobiol. Dis. 41, 538–551. https://doi.org/10.1016/j.nbd.2010.10.025.

Chandler, D.W., Edmondt, C.V., 1997. Effects of blast overpressure on the ear: case reports. J. Am. Acad. Audiol. 8, 81–88.

Chen, W., Wang, J., Chen, J., Chen, J., Chen, Z., 2013. Relationship between changes in the cochlear blood flow and disorder of hearing function induced by blast injury in Guinea pigs. Int. J. Clin. Exp. Pathol. 6, 375–384.

Cho, S.-I., Gao, S.S., Xia, A., Wang, R., Salles, F.T., Raphael, P.D., Abaya, H., Wachtel, J., Baek, J., Jacobs, D., Rasband, M.N., Oghalai, J.S., 2013. Mechanisms of hearing loss after blast injury to the ear. PLoS One 8, e67618. https://doi.org/10.1371/journal.pone.0067618.

Choi, C.-H., 2012. Mechanisms and treatment of blast induced hearing loss. Korean J. Audiol 16, 103–107. https://doi.org/10.7874/kja.2012.16.3.103.

Clasing, J.E., Casali, J.G., 2014. Warfighter auditory situation awareness: effects of augmented hearing protection/enhancement devices and TCAPS for military ground combat applications. Int. J. Audiol. 53 (Suppl 2), S43–S52. https://doi.org/10.3109/14992027.2013.860489.

Cohen, J.T., Ziv, G., Bloom, J., Zikk, D., Rapoport, Y., Himmelfarb, M.Z., 2002. Blast injury of the ear in a confined space explosion: auditory and vestibular evaluation. Isr. Med. Assoc. J. 4, 559–562.

Cooper, G.J., Maynard, R.L., Cross, N.L., Hill, J.F., 1983. Casualties from terrorist bombings. J. Trauma Inj. Infect. Crit. Care 23, 955–967. https://doi.org/10.1097/00005373-198311000-00001.

Courtney, A.C.C., Courtney, M.W.W., 2009. A thoracic mechanism of mild traumatic brain injury due to blast pressure waves. Med. Hypotheses 72, 76–83. https://doi.org/10.1016/j.mehy.2008.08.015.

Darley, D.S., Kellman, R.M., 2010. Otologic considerations of blast injury. Disaster Med. Public Health Prep. 4, 145–152. https://doi.org/10.1001/dmphp.D-08-00057R2.

DePalma, R.G., Burris, D.G., Champion, H.R., Hodgson, M.J., 2005. Blast injuries. N. Engl. J. Med. 352, 1335–1342. https://doi.org/10.1056/NEJMra042083.

Dougherty, A.L., MacGregor, A.J., Han, P.P., Viirre, E., Heltemes, K.J., Galarneau, M.R., 2013. Blast-related ear injuries among U.S. military personnel. J. Rehabil. Res. Dev. 50, 893–904. https://doi.org/10.1682/JRRD.2012.02.0024.

Elsayed, N.M., Atkins, J.L., 2010. Explosion and Blast-related Injuries: Effects of Explosion and Blast from Military Operations and Acts of Terrorism, second ed. Academic Press, Waltham.

Fausti, S.A., Wilmington, D.J., Gallun, F.J., Myers, P.J., Henry, J.A., 2009. Auditory and vestibular dysfunction associated with blast-related traumatic brain injury. J. Rehabil. Res. Dev. 46, 797–810. https://doi.org/10.1682/JRRD.2008.09.0118.

Frangos, E., Ellrich, J., Komisaruk, B.R., 2015. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. Brain Stimul 8, 624–636. https://doi.org/10.1016/j.brs.2014.11.018.

Friedlander, F.G., 1946. The diffraction of sound pulses. I. Diffraction by a semi-infinite plane. Proc. R. Soc. A Math. Phys. Eng. Sci. 186, 322–344. https://doi.org/10.1098/rspa.1946.0046.

Gan, R.Z., Nakmali, D., Ji, X.D., Leckness, K., Yokell, Z., 2016. Mechanical damage of tympanic membrane in relation to impulse pressure waveform - a study in chinchillas. Hear. Res. https://doi.org/10.1016/j.heares.2016.01.004.

Garth, R.J., 1994. Blast injury of the auditory system: a review of the mechanisms and pathology. J. Laryngol. Otol. 108, 925–929. https://doi.org/10.1017/S0022215100128555.

Goel, M., Vasant, M., Gupta, A., 2012. An abridged review of blast wave parameters. Defence Sci. J. 62, 300–306. https://doi.org/10.14429/dsj.62.1149.

Guy, R.J., Kirkman, E., Watkins, P.E., Cooper, G.J., 1998. Physiologic responses to primary blast. J. Trauma 45, 983–987. https://doi.org/10.1097/00005373-199812000-00001.

Helling, E.R., 2004. Otologic blast injuries due to the Kenya embassy bombing. Mil. Med. 169, 872–876. https://doi.org/10.7205/MILMED.169.11.872.

Hicks, R.R., Fertig, S.J., Desrocher, R.E., Koroshetz, W.J., Pancrazio, J.J., 2010. Neurological effects of blast injury. J. Trauma 68, 1257–1263. https://doi.org/10.1097/TA.0b013e3181d8956d.

Hoffer, M.E., Balaban, C., Gottshall, K., Balough, B.J., Maddox, M.R., Penta, J.R., 2010. Blast exposure: vestibular consequences and associated characteristics. Otol. Neurotol. 31, 232–236. https://doi.org/10.1097/MAO.0b013e3181c993c3.

Howe, P.R.C., 1985. Blood pressure control by neurotransmitters in the medulla oblongata and spinal cord. J. Auton. Nerv. Syst. 12, 95–115. https://doi.org/10.1016/0165-1838(85)90054-2.

Huber, B.R., Meabon, J.S., Martin, T.J., Mourad, P.D., Bennett, R., Kraemer, B.C.,

Cernak, I., Petrie, E.C., Emery, M.J., Swenson, E.R., Mayer, C., Mehic, E., Peskind, E.R., Cook, D.G., 2013. Blast exposure causes early and persistent aberrant phospho- and cleaved-tau expression in a murine model of mild blast-induced traumatic brain injury. J. Alzheim. Dis. 37, 309–323. https://doi.org/10.3233/JAD-130182.

Irwin, R.J., Lerner, M.R., Bealer, J.F., Mantor, P.C., Brackett, D.J., Tuggle, D.W., 1999. Shock after blast wave injury is caused by a vagally mediated reflex. J. Trauma Inj. Infect. Crit. Care 47, 105–110. https://doi.org/10.1097/00005373-199907000-00023.

Ishikawa, T., Miyazawa, T., 1980. Sympathetic responses evoked by vestibular stimulation and their interactions with somato-sympathetic reflexes. J. Auton. Nerv. Syst. 1, 243–254. https://doi.org/10.1016/0165-1838(80)90020-X.

Jagade, M.V., Patil, R.A., Suhail, I.S., Kelkar, P., Nemane, S., Mahendru, J., Kalbande, V., Kewle, P., 2008. Bomb blast injury: effect on middle and inner ear. Indian J. Otolaryngol. Head Neck Surg. 60 https://doi.org/10.1007/s12070-008-0110-1.

Jones, G.H., Pearson, C.R., 2016. The use of personal hearing protection in hostile territory and the effect of health promotion activity: advice falling upon deaf ears. J. Roy. Army Med. Corps 162, 280–283. https://doi.org/10.1136/jramc-2015-000420.

Katz, E., Ofek, B., Adler, J., Abramowitz, H.B., Krausz, M.M., 1989. Primary blast injury after a bomb explosion in a civilian bus. Ann. Surg. 209, 484–488. https://doi.org/10.1097/00000658-198904000-00016.

Kerr, A.G., Byrne, J.E.T., 1975. Concussive effects of bomb blast on the ear. J. Laryngol. Otol. 89, 131–144. https://doi.org/10.1017/S002221510008018X.

Krohn, P.L., Whitteridge, D., Zuckerman, S., 1942. Physiological effects of blast. Lancet 239, 252–259. https://doi.org/10.1016/S0140-6736(00)57842-8.

Kronenberg, J., Ben-Shoshan, J., Wolf, M., 1993. Perforated tympanic membrane after blast injury. Am. J. Otol. 14, 92–94.

Lemonick, D.M., 2011. Bombings and blast Injuries : a primer for physicians. Am. J. Clin. Med 8, 134–140.

Long, J.B., Bentley, T.L., Wessner, K.A., Cerone, C., Sweeney, S., Bauman, R.A., 2009. Blast overpressure in rats: recreating a battlefield injury in the laboratory. J. Neurotrauma 26, 827–840. https://doi.org/10.1089/neu.2008.0748.

Mac Donald, C.L., Johnson, A.M., Wierzechowski, L., Kassner, E., Stewart, T., Nelson, E.C., Werner, N.J., Zonies, D., Oh, J., Fang, R., Brody, D.L., 2014. Prospectively assessed clinical outcomes in concussive blast vs nonblast traumatic brain injury among evacuated US military personnel. JAMA Neurol 71, 994–1002. https://doi.org/10.1001/jamaneurol.2014.1114.

Machado, B.H., Brody, M.J., 1988. Role of the nucleus ambiguus in the regulation of heart rate and arterial pressure. Hypertension 11, 602–607. https://doi.org/10.1161/01.HYP.11.6.602.

Mayorga, M.A., 1997. The pathology of primary blast overpressure injury. Toxicology 17–28. https://doi.org/10.1016/S0300-483X(97)03652-4.

Meaney, D.F., Morrison, B., Dale Bass, C., 2014. The mechanics of traumatic brain injury: a review of what we know and what we need to know for reducing its societal burden. J. Biomech. Eng. 136, 21008. https://doi.org/10.1115/1.4026364.

Mediavilla Varas, J., Philippens, M., Meijer, S.R., van den Berg, A.C., Sibma, P.C., van Bree, J.L.M.J., de Vries, D.V.W.M., 2011. Physics of IED blast shock tube simulations for mTBI research. Front. Neurol. 2, 58. https://doi.org/10.3389/fneur.2011.00058.

Mifflin, S.W., Felder, R.B., 1990. Synaptic mechanisms regulating cardiovascular afferent inputs to solitary tract nucleus. Am. J. Physiol. 259, H653–H661.

Moore, D.F., Jérusalem, A., Nyein, M., Noels, L., Jaffee, M.S., Radovitzky, R.A., 2009. Computational biology - modeling of primary blast effects on the central nervous system. Neuroimage 47 (Suppl 2), T10–T20. https://doi.org/10.1016/j.neuroimage.2009.02.019.

Moss, W.C., King, M.J., Blackman, E.G., 2009. Skull flexure from blast waves: a mechanism for brain injury with implications for helmet Design. Phys. Rev. Lett. 103, 108702. https://doi.org/10.1103/PhysRevLett.103.108702.

Needham, C.E., Ritzel, D., Rule, G.T., Wiri, S., Young, L., 2015. Blast testing issues and TBI: experimental models that lead to wrong conclusions. Front. Neurol. 6, 72. https://doi.org/10.3389/fneur.2015.00072.

Niwa, K., Mizutari, K., Matsui, T., Kurioka, T., Matsunobu, T., Kawauchi, S., Satoh, Y., Sato, S., Shiotani, A., Kobayashi, Y., 2016. Pathophysiology of the inner ear after blast injury caused by laser-induced shock wave. Sci. Rep. 6, 31754. https://doi.org/10.1038/srep31754.

Patterson, J.H., Hamernik, R.P., 1997. Blast overpressure induced structural and functional changes in the auditory system. Toxicology 121, 29–40. https://doi.org/10.1016/S0300-483X(97)03653-6.

Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates Sixth Edition by, sixth ed. Academic Press.

Peuker, E.T., Filler, T.J., 2002. The nerve supply of the human auricle. Clin. Anat. 15, 35–37. https://doi.org/10.1002/ca.1089.

Pham, N., Sawyer, T.W., Wang, Y., Jazii, F.R., Vair, C., Taghibiglou, C., 2015. Primary blast-induced traumatic brain injury in rats leads to increased prion protein in plasma: a potential biomarker for blast-induced traumatic brain injury. J. Neurotrauma 32, 58–65. https://doi.org/10.1089/neu.2014.3471.

Phillips, Y.Y., 1986. Primary blast injuries. Ann. Emerg. Med. 15, 1446–1450. https://doi.org/10.1016/S0196-0644(86)80940-4.

Radtke, A., Popov, K., Bronstein, A.M., Gresty, M.A., 2000. Evidence for a vestibulo-cardiac reflex in man. Lancet 356, 736–737. https://doi.org/10.1016/S0140-6736(00)02635-0.

Radtke, A., Popov, K., Bronstein, A.M., Gresty, M.A., 2003. Vestibulo-autonomic control in man: short- and long-latency vestibular effects on cardiovascular function. J. Vestib. Res. 13, 25–37.

Ray, C.A., Carter, J.R., 2003. Vestibular activation of sympathetic nerve activity. Acta Physiol. Scand. 177, 313–319. https://doi.org/10.1046/j.1365-201X.2003.01084.x.

Ritenour, A.E., Wickley, A., Ritenour, J.S., Kriete, B.R., Blackbourne, L.H., Holcomb, J.B., Wade, C.E., 2008. Tympanic membrane perforation and hearing loss from blast overpressure in operation enduring freedom and operation iraqi freedom wounded. J. Trauma Inj. Infect. Crit. Care 64, S174–S178. https://doi.org/10.1097/TA.0b013e318160773e.

Ropper, A., 2011. Brain injuries from blasts. N. Engl. J. Med. 364, 2156–2157. https://doi.org/10.1056/NEJMe1102187.

Sawdon, M., Ohnishi, M., Watkins, P.E., Kirkman, E., 2002. The effects of primary thoracic blast injury and morphine on the response to haemorrhage in the anaesthetised rat. Exp. Physiol. 87, 683–689. https://doi.org/10.1113/eph8702432.

Scherer, M.R., Shelhamer, M.J., Schubert, M.C., 2011. Characterizing high-velocity angular vestibulo-ocular reflex function in service members post-blast exposure. Exp. Brain Res. 208, 399–410. https://doi.org/10.1007/s00221-010-2490-1.

Simard, J.M., Pampori, A., Keledjian, K., Tosun, C., Schwartzbauer, G., Ivanova, S., Gerzanich, V., 2014. Exposure of the thorax to a sublethal blast wave causes a hydrodynamic pulse that leads to perivenular inflammation in the brain. J. Neurotrauma 31, 1292–1304. https://doi.org/10.1089/neu.2013.3016.

Singh, D., Ahluwalia†, K.J.S., 1968. Blast injuries of the ear. J. Laryngol. Otol. 82, 1017–1028. https://doi.org/10.1017/S0022215100069802.

Sundaramurthy, A., Alai, A., Ganpule, S., Holmberg, A., Plougonven, E., Chandra, N., 2012. Blast-induced biomechanical loading of the rat: an experimental and anatomically accurate computational blast injury model. J. Neurotrauma 29, 2352–2364. https://doi.org/10.1089/neu.2012.2413.

Svetlov, S.I., Larner, S.F., Kirk, D.R., Atkinson, J., Hayes, R.L., Wang, K.K.W.W., 2009. Biomarkers of blast-induced neurotrauma: profiling molecular and cellular mechanisms of blast brain injury. J. Neurotrauma 26, 913–921. https://doi.org/10.1089/neu.2008.0609.

Taber, K.H., Warden, D.L., Hurley, R.A., 2006. Blast-related traumatic brain injury: what is known? J. Neuropsychiatry Clin. Neurosci. 18, 141–145. https://doi.org/10.1176/jnp.2006.18.2.141.

Tanaka, K., Ito, Y., Ikeda, M., Katafuchi, T., 2014. RR interval variability during galvanic vestibular stimulation correlates with arterial pressure upon head-up tilt. Auton. Neurosci. 185, 100–106. https://doi.org/10.1016/j.autneu.2014.04.001.

Tang, P.C., Gernandt, B.E., 1969. Autonomic responses to vestibular stimulation. Exp. Neurol. 24, 558–578. https://doi.org/10.1016/0014-4886(69)90158-7.

Teter, D.L., Newell, R.C., Aspinall, K.B., 1970. Audiometric configurations associated with blast trauma. Laryngoscope 80, 1122–1132. https://doi.org/10.1288/00005537-197007000-00010.

Tungsinmunkong, S., Chongkolwatana, C., Piyawongvisal, W., Atipas, S., Namchareonchaisuk, S., 2007. Blast injury of the ears: the experience from yala hospital, southern Thailand. J. Med. Assoc. Thai. 90, 2662–2668.

Wang, Y., Pan, L., Fan, W., Zhou, Z., Zhu, L., Wang, Y., Hu, R., 2013. Influence of vagal injury on acute traumatic reaction after blast injury. Eur. J. Trauma Emerg. Surg. 39, 385–392. https://doi.org/10.1007/s00068-013-0277-3.

Xiong, Y., Mahmood, A., Chopp, M., 2013. Animal models of traumatic brain injury. Nat. Rev. Neurosci. 14, 128–142. https://doi.org/10.1038/nrn3407.

Yarnell, A.M., Shaughness, M.C., Barry, E.S., Ahlers, S.T., McCarron, R.M., Grunberg, N.E., 2013. Blast traumatic brain injury in the rat using a blast overpressure model. Curr. Protoc. Neurosci. https://doi.org/10.1002/0471142301.ns0941s62.

Zucker, I.H., 1986. Left ventricular receptors: physiological controllers or pathological curiosities? Basic Res. Cardiol. 81, 539–557. https://doi.org/10.1007/BF02005179.

Zuckerman, S., 1940. Experimental study of blast injuries to the lungs. Lancet 236, 219–224. https://doi.org/10.1016/S0140-6736(01)08726-8.