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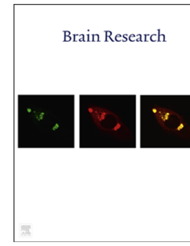

Research Report

Repeated blast exposure alters open field behavior recorded under low illumination

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ABSTRACT

Blast-induced traumatic brain injury (bTBI) can have devastating behavioral consequences. This study was designed to evaluate the behavioral consequences of single or repeated bTBI, as evaluated by an open field (OF) test conducted in near-darkness to avoid confounding effects of illumination and photophobia. Sprague-Dawley rats under isoflurane anesthesia were exposed to a series of 3 sub-lethal blasts into a compressed air-driven blast chamber separated by 2 week intervals ($n=11$). Sham controls received anesthesia but without blast exposure ($n=11$). OF tests were performed 1 or 7 days after each blast using a computerized video tracking system in near-darkness to monitor spontaneous activity. Spatial and temporal variables calculated for both blast and sham groups were: Distance moved (cm) and time (s) spent in the center or periphery zones of the field, total distance traveled, speed in center and periphery zones, rearing events and non-linear regressions of distance moved and rearing events on time. Results showed that the sham group expressed the expected decrease (habituation) in total distance walked, and distance walked as well as speed in center and periphery in successive exposures to the OF while the blast group did not, a sign of impaired learning. The blast group also walked more and faster and demonstrated more rearing behavior, both considered OF signs of anxiety. These results indicate that OF outcomes of bTBI in animals have resemblance to alterations observed in human subjects with this condition and might be useful in evaluating the response of behavioral outcomes of bTBI to experimental treatments.

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1. Introduction

Blast-related traumatic brain injury (bTBI) is a more frequently encountered injury in current conflicts. While there are no systematic evaluations of health consequences for survivors of blasts in the civilian population in areas of conflict, according to the "Defense and Veterans Brain Injury Center," 244,217 TBIs have been identified up to the

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first quarter of 2012 amongst all US armed forces. 76.8% of those TBI cases are considered mild and of those, approximately 50% are due to blast injuries (Defense and Veterans Brain Injury Center and Armed Forces Health Surveillance Center, 2011).

TBI and PTSD are considered signature injuries of the current war in Iraq and Afghanistan given the increased use of conventional and improvised explosive devices (Tanielan and Jaycox, 2008; Spelman et al., 2012). Even for mild TBI, defined as a confused or disoriented state which lasts less than 24 h; loss of consciousness for up to 30 min; memory loss lasting less than 24 h; and structural brain imaging (MRI or CT scan) yielding normal results, the functional deficits can be severe and long-lasting.

Animal models have been used to simulate blast TBI. However, the validation of the models requires comparison to human data in order to demonstrate that they reproduce one or more relevant features of human injury. Clinically, anxiety and depression are very prevalent mental health conditions in human subjects with mild TBI (Spelman et al., 2012; Moore et al., 2006; Mooney and Speed, 2001). Rodent models for these conditions have been proposed. Among them, unconditioned paradigms including avoidance of exposed, brightly lit spaces (elevated T or plus maze, light-dark box and open fields with bright illumination) have been widely used in rats and mice (Sartori et al., 2011). Preference of the covered arms of the first, the dark sector of the second and avoidance of the center of or freezing behavior in the third have been taken by many authors as “anxiety-like” behaviors (Cryan and Sweeney, 2011). However, in the case of TBI, photophobia is of common occurrence in humans (Tanielan and Jaycox, 2008; Spelman et al., 2012; Stovner et al., 2009; Kapoor and Ciuffreda, 2002; Bohnen et al., 1991) and possibly also in experimental animals. Moreover, light inhibits ambulation in rodents (Livesey and Egger, 1970; Valle, 1970; Blizard, 1971) and albinism, present in most of the rat strains used as TBI models, increases the light sensitivity even more (Dixon and DeFries, 1968). Thus, in order to examine the open field behavior of rats subjected to blast brain trauma, illumination should be minimized to realize the full ambulatory capacity of the animals and prevent the confounding effect of photophobia.

The present experiments were conducted in near-darkness with detection of movement by use of an infrared light source and an infrared-sensitive camera. In addition, and to model a common condition encountered in the field, multiple blasts, followed by OF tests in each case, were induced. This protocol also evaluated the magnitude of the inter-session habituation, i.e. a decrease in exploratory activity on successive exposures to the novel environment, that is considered a primitive form of learning, and its absence as an evidence of “forgetfulness” of the characteristics of the field environment, requiring renewed exploration at every exposure to it, (Russell, 1982a) that could model the memory problems reported in subjects with mild TBI (Terrio et al., 2009).

The more common blast injury models make use of blast tubes, which simulate field conditions in the open, showing a pressure profile similar to a Friedländer curve. In the present application, we have used a closed receiving chamber with a leak, providing a complex pressure profile to more realistically reproduce conditions encountered for blasts in urban environments (Cernak et al., 2011).

Soldiers are subjected to repeated blasts in training and in theater; however, there are limited studies on the neurological effects of repeated blast exposures. In second-impact syndrome as defined for sports, patients suffer an initial mild concussive head injury that is hypothesized to induce cerebral autoregulatory failure. Then, a second impact might produce a systemic stress-induced catecholamine surge and rapidly elevated blood pressure that results in devastating acute and massive brain swelling (Wetjen et al., 2010). A discrete return-to-play guideline has been established for all brain-injured athletes (Aubry et al., 2002). Limited studies looking at physiologic and anatomic changes from tightly coupled repeated bTBI in mice noted that neurodegeneration was found in various parts of the brain. Motor performance is also compromised in these mice (Wang et al., 2011).

Since 90% of rat genes possess strict orthologues in the human genome, they serve as appropriate subjects for the preclinical stage of research and for the sake of safety and time efficiency (Gibbs and Weinstock, 2004). This species has been selected to the present study carried out with a novel chamber device generating a blast of controlled intensity adjusted to induce a functional deficit without lethality. A repeated series of blast events was used to simulate field conditions with multiple exposures.

The main objective of this study was to determine if a simple procedure, the open field test, could demonstrate evidence of markers of anxiety or impaired spatial learning that are common to human cases of mild bTBI. A secondary objective was to determine which of the variables defined in this test showed the better power to differentiate between injured and control subjects.

2. Results

Peak pressures recorded inside the blast chamber were reproducible and no significant differences existed among the 1st, 2nd and 3rd blasts (Table 1). The pressure transient during the blast, measured with a piezoelectric sensor placed next to the animal head, oriented towards the mylar membrane indicated a fast (<2 ms) rise time, followed by a biphasic drop and a slightly negative pressure afterwards (Fig. 2).

Rats in the blast group walked significantly more in total distance (sum of distance traveled in the center and periphery of the field) than rats in the sham group ($P=0.035$) during the 20 min trial (Fig. 3 and Table 2). Animals in the SHAM group decreased their total distance traveled with repeated

Table 1 – Peak pressures (Mean and SD, kPa) recorded inside the blast chamber in all experiments. There were no significant differences among blast pressure means.

	Blast 1 (kPa)	Blast 2 (kPa)	Blast 3 (kPa)
Mean	175.0	174.2	163.9
SD	23.9	37.5	23.6