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14. ABSTRACT
The purpose of this study is to develop a survival model of blast-induced traumatic brain injury (BI-TBI) in swine. Two air guns were constructed, each having different lengths, air chamber volumes and barrel diameters. Air velocity was measured with a ballistics chronometer over a series of firings at different blast pressures. Calibration curves show linear and reproducible results through a range of firing pressures. Under anesthesia swine received BI-TBI with either one of the air guns at two different pressures and recovered for 7 days. Swine injured with the larger gun at the higher pressure took longer to return to normal behavior compared to the control or lesser blast animals. These animals demonstrated circling behavior, un-coordination, increased startle reflex, and hyperactivity during the recovery period. In a novel object test these injured animals took longer to start to investigate and the object was novel for a longer time. They showed electrocardiogram changes on Day 7 post blast indicating coronary ischemia and injury. The results show that this swine model of blast induced traumatic brain injury will provide a needed tool for the development of treatment for traumatic head injuries, rehabilitation and improvements in military and civilian protective body.

15. SUBJECT TERMS
Traumatic Brain Injury, Swine, Blast, Model Development

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Final Report for project W81XWH-08-2-00A blast model of traumatic brain injury in swine.

INTRODUCTION:

Blast-induced traumatic brain injury (BI-TBI) is a significant cause of morbidity and behavioral dysfunction in service men and women returning from Iraq and Afghanistan. Laboratory models are needed to study the mechanisms underlying this critical injury and develop new therapies to treat survivors. Many TBI models are performed in rodents, and data from these models have been used as a basis for several disappointing Phase III clinical trials in humans with TBI. The failure of these trials may, in part, be due to differences between the rodent and human (or pig) brain. The development of a large-animal model of BI-TBI will revolutionize the study of this pressing clinical problem and rapidly facilitate the development of novel therapies to treat injured military personnel. Therefore, the purpose of these experiments is to develop a survival model of BI-TBI in swine.

BODY:

Protocol Approval:

The protocol was submitted to the local IACUC and approval received on 20 March 2008. The protocol was then submitted to DOD on March 2008. The protocol PT 08-18-01 (ACORP) was approved by the ACURO on 7 July 2009.

Methods and Materials:

Calibration of air guns:

Two air guns were constructed from PVC piping, each having different lengths, air chamber volumes, barrel diameters and both designed to hold up to 100 pounds per square inch (psi) of pressurized air. The smaller gun has a 40-inch barrel with an internal diameter of one inch. The compression chamber has an outer diameter of 4 inches and is 26 inches in length. The larger gun has a barrel 54.25 inches in length with an inside diameter of 2.5 inches. The compression chamber is 4 inches in outside diameter with a length of 36 inches.

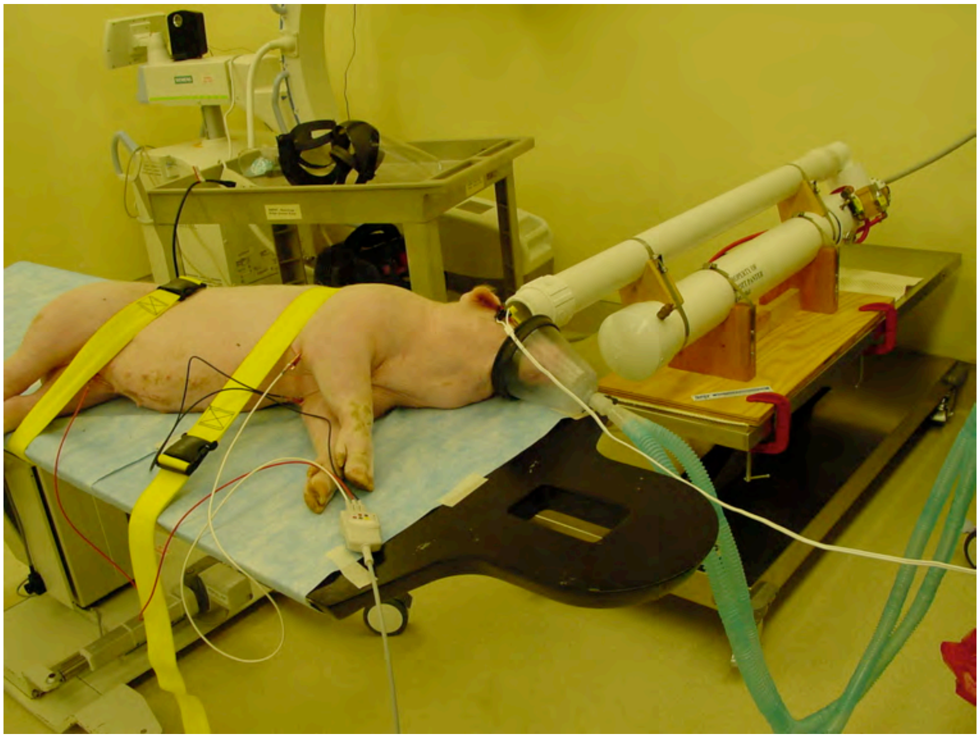
The air velocity was measured with a digital ballistics chronometer over a series of firings with controlled pressures in the air chambers. A digital ballistics chronometer measures the speed of an object such as a bullet, pellet, or an arrow through its sensing field. To characterize the guns and determine the velocity range, each gun, using room air and an air compressor, underwent two sets of 10 firings at 60, 70, 80, 90, and 100 psi in the air chamber. Air velocity in meters/second was measured at a distance of one foot from the end of the gun barrel.

Animals:

Ten female Yorkshire swine were obtained in 2 sets, 4 in the first set and 6 in the second set. The animals were acclimated in the animal care facility for at least one week prior to blast injury. The average body weight on the day of blast injury was 24 Kg, range 21 to 27 Kg. Animals were housed on wood shaving bedding together in groups of 3 or 4, water provided *ad libidum* and fed twice a day. Animals were fasted for 12 hours prior to blast injury.

Blast Induced Traumatic Brain Injury:

On the day of the blast injury animals were pre-medicated with ketamine i.m. (20 mg/kg) and glycopyrrolate i.m. (0.1 mg/kg) then masked with 4% isoflurane to achieve a deep plane of anesthesia. The animals in the first set were intubated with an endotracheal (ET) tube and the animals in the second set remained masked with a nose cone. Anesthesia was maintained with isoflurane (1-4%) using standard anesthesiology apparatus to deliver inhalation anesthetics. Once deeply anesthetized, the animals were placed in lateral recumbency and immobilized firmly on a table with 2-inch-wide nylon straps. The heads of the animals were capable of movement. The animals were monitored for blood oxygen saturation and electrocardiogram (ecg). Protective padding was placed over the swine's eyes. The air gun was secured to a nearby table and positioned with the barrel perpendicular to and centered between the ears and eyes of the swine with the barrel opening 5 cm from the top of the skull. Immediately prior to firing of the gun, the ET tube was disconnected from the anesthesia apparatus or the nose cone removed from the animal. The gun was fired, then the anesthesia tubing was reconnected or nose cone was replaced. The blast caused an acceleration/deceleration injury to the swine brain. The animals in the second set were given ketorolac (30 mg) s.c. for analgesia. The anesthesia was gradually reduced and the animals were moved to a mobile pen to recover from anesthesia. Animals were observed for 7 days post injury and weighed on day 7. The following photograph shows the equipment and animal prepared for the BI-TBI.



Four female Yorkshire swine were used in the first set of blast injury experiments, two were injured with the smaller gun at 100 psi, one was injured with the larger gun at 80 psi and one sham (no injury). In the second set of six animals, two pigs were injured with the larger gun at 80 psi, three pigs were injured with the larger gun at 100 psi, and one sham (no injury – anesthetized and strapped on table for same length of time as animals receiving blast).

Behavioral/learning/memory evaluation:
A learning/memory paradigm was used for the second set whereby the animals were given five minutes in a pen with three identical bins of different colors: stainless steel, pink, and blue, all containing a food treat, a piece of apple. Only the pink bin could be opened to retrieve food. The animals were expected to learn and remember to go directly to the pink bin and open it to obtain food, without attempting to open other bins. The time taken to complete the task (maximum 300 seconds) during each session was recorded, and a performance score was calculated as follows:

+2 = opens pink bin
+1 = touches pink bin but does not open it
+1 = does not approach the other two bins
+0 = smell either of the other two bins
-1 = tries to open either of the other two bins

The maximum score possible was +3 (opening of pink bin without approaching the other two bins). Each false attempt earned a negative score (no maximum). The trial ended as soon as the pink bin was opened or at 300 seconds. The order of the bins was changed with every trial so the animals had to associate color and not the position of the 3 bins. Prior to the blast injury the animals were trained and then scored for three trials. They were then tested on day 1 after injury with two trials. On day 2 or 3 after injury, the animals were tested for relearning by changing the color of the bin that could be opened. The pink bin was secured closed and the blue bin was enabled to open. The animals were given 3 trials. Scores per trial for each animal were averaged for each session, pre-blast, post-blast, and relearning.

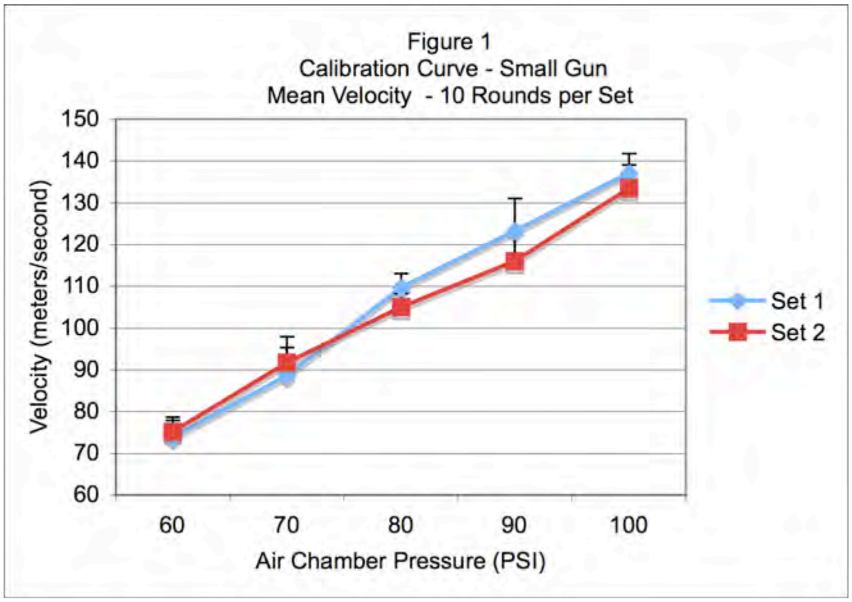
A novel object test was used on day 1 following injury for the second set of animals to measure inquisitive intrinsic exploration directed at stimuli of little biological consequence. Exploration decreases during exposure as the novelty decreases. Normal, uninjured pigs possess a high level of curiosity promoting inquisitive exploration. After injury the animals were individually placed in a pen with a novel object, an empty plastic water bottle, and the length of time taken to start investigating the object and the duration of the investigation was recorded.

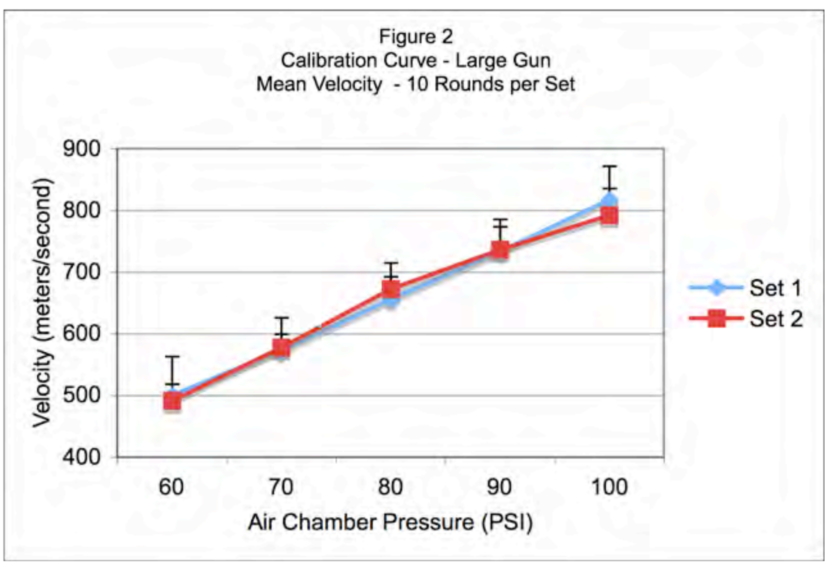
The animals for the second set were assessed for neurological status daily utilizing a neuroscore based on a 75-point scale (75 = normal; 21 = brain dead). The parameters assessed included level of consciousness, behavior, feeding, drinking, cranial nerve reflexes, motor and sensory function, co-ordination, and locomotion. Animals in the first set were observed daily but not given a score.

Euthanasia:
On day 7-post injury the animals were euthanized. The first set of 4 animals were transcardially perfused and the brain fixed. After sedation with ketamine, 20 mg/kg, i.m., the animals were deeply anesthetized with 1-4% isoflurane. A sternal incision was made, the sternum cut with a bone cutter, and a rib spreader used to open the sternum and expose the heart. The right ventricle was catheterized and approximately one liter of blood was removed. The animals were then perfused with heparinized ice-cold saline, and Zamboni's fixative. The second set of 6 animals were deeply anesthetized with isoflurane, then were euthanized with an i.v. injection of saturated KCl. When all cardiac activity ceased, the brains were removed and prepared for histology.

Results:

Calibration of air guns:
The test results of the first set of 10 rounds show the small and large guns produced a mean velocity in meters/sec of 73.9 (± 4.67) and 499.8 (± 63.02), respectively, using 60 psi; 88.6 (± 6.69) and 573.2 (± 26.14) at 70 psi; 109.7 (± 3.37) and 656.3 (± 36.24) at 80 psi; 123.2 (± 7.76) and 733.7 (± 39.45) at 90 psi and 137.1 (± 4.63) and 817.2 (± 54.37) at 100 psi. The test results for the second set of 10 rounds show the small and large gun produced a mean velocity of 75.1 (± 2.78) and 491.1 (± 26.67) at 60 psi, respectively; 91.7 (± 6.23) and 577.5 (± 48.10) at 70 psi; 105.0 (± 3.29) and 672.1 (± 42.47) at 80 psi; 116.0 (± 6.99) and 736.1 (± 49.32) at 90 psi and 133.4 (± 5.63) and 792 (± 43.46) at 100 psi. Figure 1 shows the mean velocity in meters/second for both sets of rounds for the small gun and Figure 2 shows the results for the large gun. These calibration curves show linear and reproducible results at known air velocities.





Body Weight:
There was no difference in body weight or weight gain between the groups. The control pig in the first set was not weighed seven days prior to euthanasia. The weights are shown in Table 1.

Table 1. Individual animal and group mean body weights (kg) on day of blast, day 7 post-blast and weight gain from blast to day 7.

	Control-no blast			**100 PSI small gun**			**80 PSI large gun**			**100 PSI large gun**		
	At Blast	**Day 7**	**Gain**	**At Blast**	**Day 7**	**Gain**	**At Blast**	**Day 7**	**Gain**	**At Blast**	**Day 7**	**Gain**
	21.0	23.0	2.0	27.0	32.0	5.0	25.0	26.0	1.0	24.0	27.5	3.5
				25.0	32.0	7.0	24.5	27.5	3.0	26.5	28.5	2.0
							22.0	24.0	2.0	22.5	24.5	2.0
Mean	**21.0**	**23.0**	**2.0**	**26.0**	**32.0**	**6.0**	**23.8**	**25.8**	**2.0**	**24.3**	**26.8**	**2.5**

Clinical Observations:
First set of animals: One swine injured with the smaller gun recovered quickly, was extubated 7 minutes post injury, did not need analgesia and was returned to the pig run 42 minutes after injury. The second swine injured with the smaller gun had a slower recovery, was extubated 14 minutes post injury, was given 100 mg carprofen IM one hour post injury because of the slow recovery although did not display signs of pain and was returned to the pig run 3 hours post injury. The swine that was injured with the larger gun at 80 psi was extubated 12 minutes post injury, showed signs of pain by excessively grinding teeth and was given 100 mg carprofen one hour post injury. The swine were observed daily for 7 days for behavioral and coordination abnormalities. All observations were normal.

Second set of animals: The control swine was pre-medicated, anesthetized and strapped to the table following the same protocol and for the same length of time (approximately 30 minutes) as the BI-TBI animals, but did not receive a blast. The control swine had a normal recovery, started chewing and opened eyes 18 minutes after anesthesia was discontinued, sat up at 55 minutes and stood at 60 minutes post anesthesia.

Two animals were injured with 80 psi using the large gun. Both animals recovered normally, one started chewing 15 minutes after anesthesia was discontinued and the other in 21 minutes. They displayed normal curiosity, sniffing and investigating their surroundings.

Three animals were injured with 100 psi using the large gun. One animal started chewing 12 minutes after anesthesia was discontinued and had a normal recovery. The second animal started chewing 7 minutes post-anesthesia, had difficulty standing, was unsteady walking, and circled, flailing and stumbling, in the recovery pen. She vocalized with distressed grunts even though she could see and touch noses with the swine in the adjoining pen. Two hours after the blast her balance was improving but still a little unsteady. Four and one half hours post injury she appeared normal and was returned to the pig run with the others. All of the swine in the study were offered pig chow as soon as they were sufficiently awake to safely eat (approximately one hour after anesthesia) and all the pigs promptly ate except this second 100 psi injured animal who did not eat until she was returned to pig run. The third animal also started chewing 7 minutes after anesthesia was discontinued. When food was offered she was startled and unable to get her footing, flailing, kicking the sides of the pen. She circled in her pen, unsteady on her feet for five minutes before starting to eat. She was steadier on her feet one and one half hours post blast and was very active in the recovery pen and overly startled by stimuli. She appeared normal and was returned to the pig run three and a half hours post injury. In summary, two of the three animals in the 100 psi blast group had a slower recovery to normal, displaying circling behavior, increased startle reflex, hyperactivity, flailing, kicking, unsteady on feet, and vocalizing, compared with the other animals in the study.

The injured animals in the second set were given ketorolac (30 mg) s.c. for analgesia prior to discontinuing the anesthesia. None of these animals required or were given additional analgesia during recovery.

The amount of time for each animal to awaken from anesthesia was measured from the time anesthesia was discontinued until the animal started chewing and swallowing. Results are shown in Figure 3. For the second set of animals, injured with the large gun, the amount of time taken for the animals to stand up after anesthesia was discontinued is shown in Figure 4. Animals awoke sooner with increasing blast injury but took longer to stand up.

Figure 3
Time to Awaken from Anesthesia

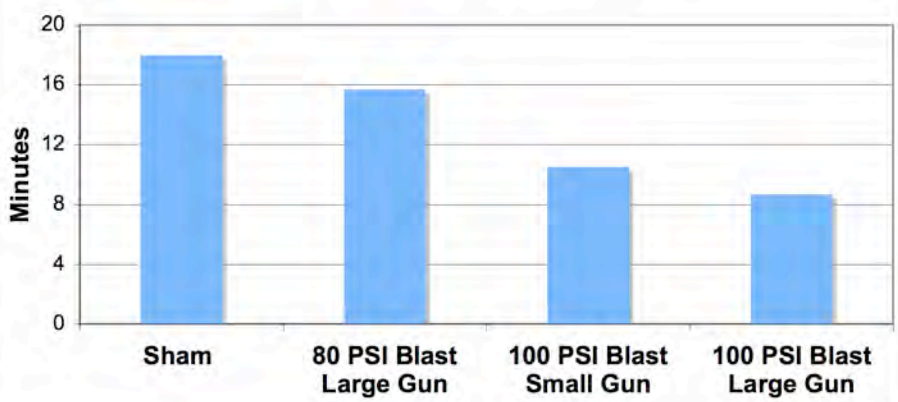
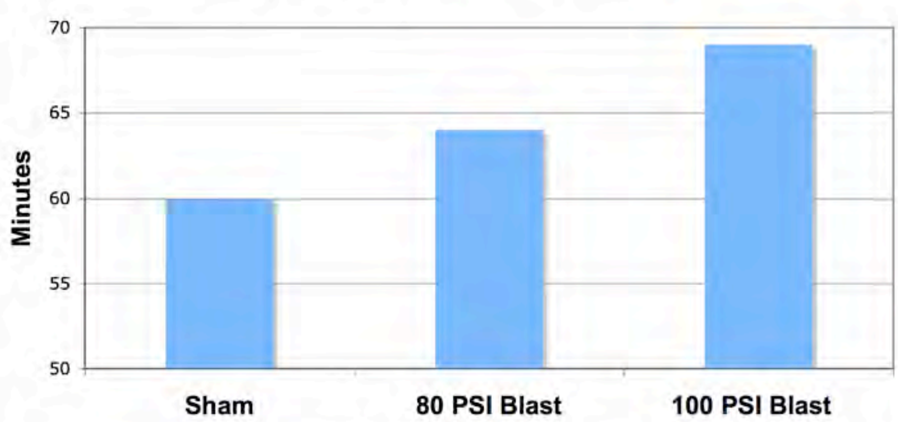


Figure 4
Time to Stand After Anesthesia Discontinued

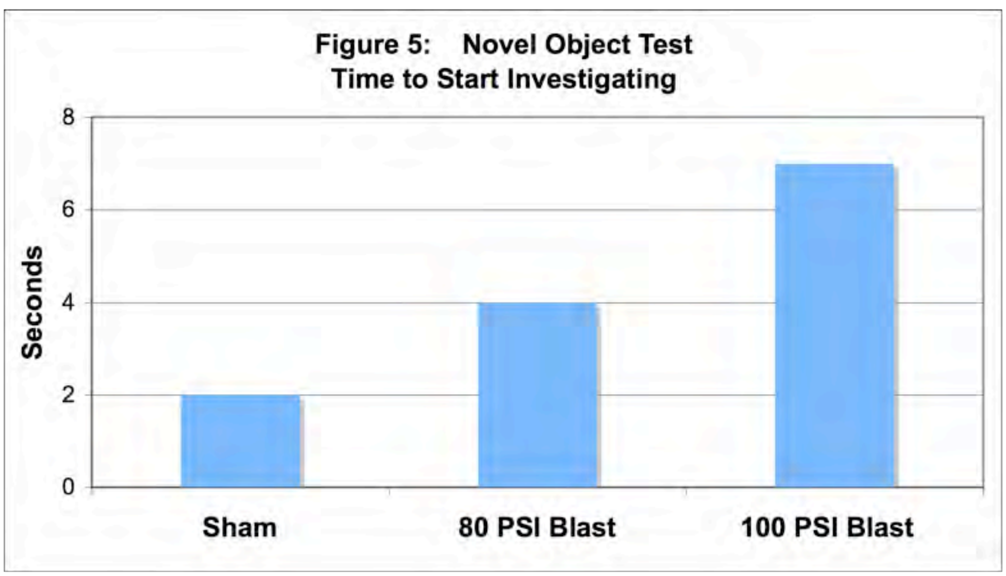


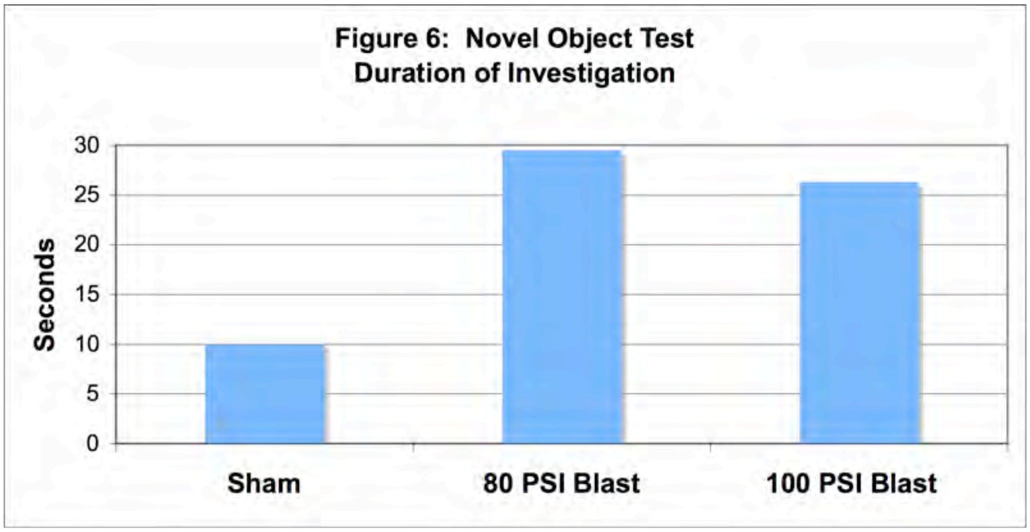
Learning and Memory Test: There was no difference in scores between the 80 psi and 100 psi blast groups or between the pre-blast, post-blast or relearning. The sham injury animal and one in the 100-psil-injury group performed poorly during the pre-injury training, being more concerned about being separated from the other animals than obtaining the food treat. By the third session, the re-learning, both were motivated and did not exhibit separation anxiety. The individual animal scores (average of trials per session per animal) and the group mean scores are shown in Table 2.

Table 2. Individual animal and group mean scores for Learning and Memory Test

	Sham			**80 PSI Blast**			**100 PSI Blast**		
	Pre Blast	**Post Blast**	**Re-learn**	**Pre Blast**	**Post Blast**	**Re-learn**	**Pre Blast**	**Post Blast**	**Re-learn**
	0	0.5	2.3	2.3	2.5	2.0	2.7	2.0	2.0
				2.7	2.5	2.3	1.0	1.5	2.7
							2.7	2.5	2.7
Group Mean	**0**	**0.5**	**2.3**	**2.5**	**2.5**	**2.15**	**2.13**	**2.0**	**2.47**

Novel Object Test: Each animal was placed individually in an empty pen with no bedding except for a novel object, an empty plastic water bottle. The animal was timed from when she was placed in the pen until she touched the object. This was defined as the time to start investigating. She was then timed until she lost interest in the object because it was no longer novel and left it. The time required to investigate the object increased with increasing blast injury. The sham injured animal lost interest and left the object sooner than the blast injured animals. The results are shown in Figures 5 and 6.





Neurological Assessment: Neurological assessments were done after the animals were returned to the home pig run. Animals in the first set were observed daily but not given a score. All animals were normal. All the animals for the second set scored 75 points (75 = normal; 21 = brain dead) at each daily observation.

Electrocardiography: Lead II of a 3-lead electrocardiogram was recorded immediately prior to the blast, during the blast, at 5 and 30 minutes post-blast and prior to euthanasia on Day 7 post-blast for the animals in the 100 psi large gun and control groups. The control animal showed a normal ecg. The animal that had an uneventful recovery (Swine #2) from the blast showed an abnormal ecg with an inverted T wave at 30 minutes post-blast and ST segment elevation and T wave elevation at Day 7. The tracings are shown on Figure 7A. Of the two animals that had a slower recovery (displaying circling behavior, increased startle reflex, hyperactivity, flailing, kicking, unsteady on feet) the first animal had a normal ecg and the second animal (Swine #4) had ST segment elevation at Day 7, shown on Figure 7B. Cardiac dysfunction can occur following acute neurologic injury. The most common repolarization abnormalities seen on ecg following brain injury include ST segment changes and T wave inversion. These changes can indicate coronary ischemia and injury.

Histology: Brains have been fixed. Histology has not been performed to date.

Problems: The larger gun had a design deficiency that caused a failure during the first set of animals. This deficiency was corrected and a second set of animals was then tested using the larger gun.

Figure 7A. Electrocardiograms, Lead II, Swine #2, 100 psi large gun, taken pre-blast, at 30 minutes and 7 days post-blast.

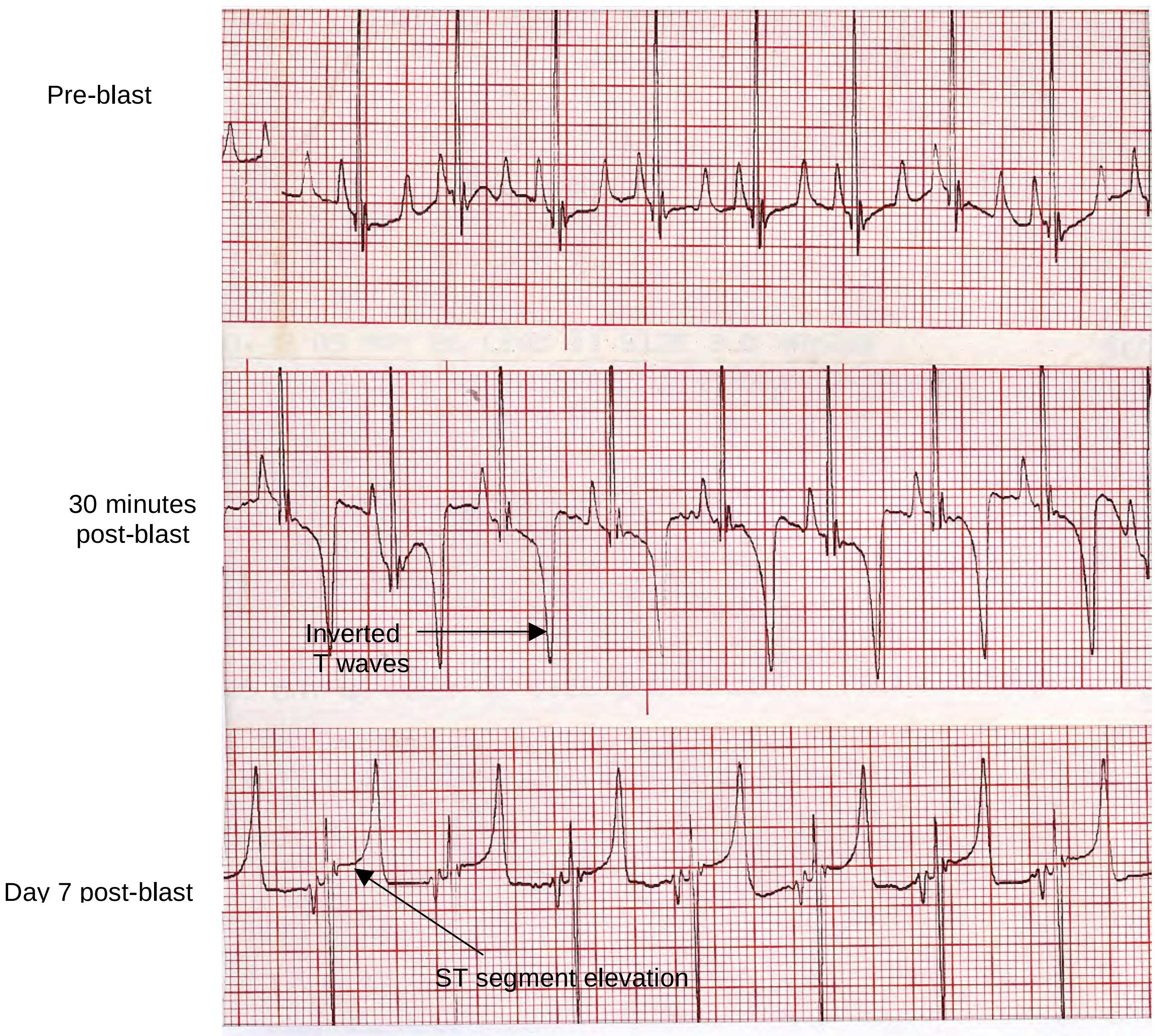
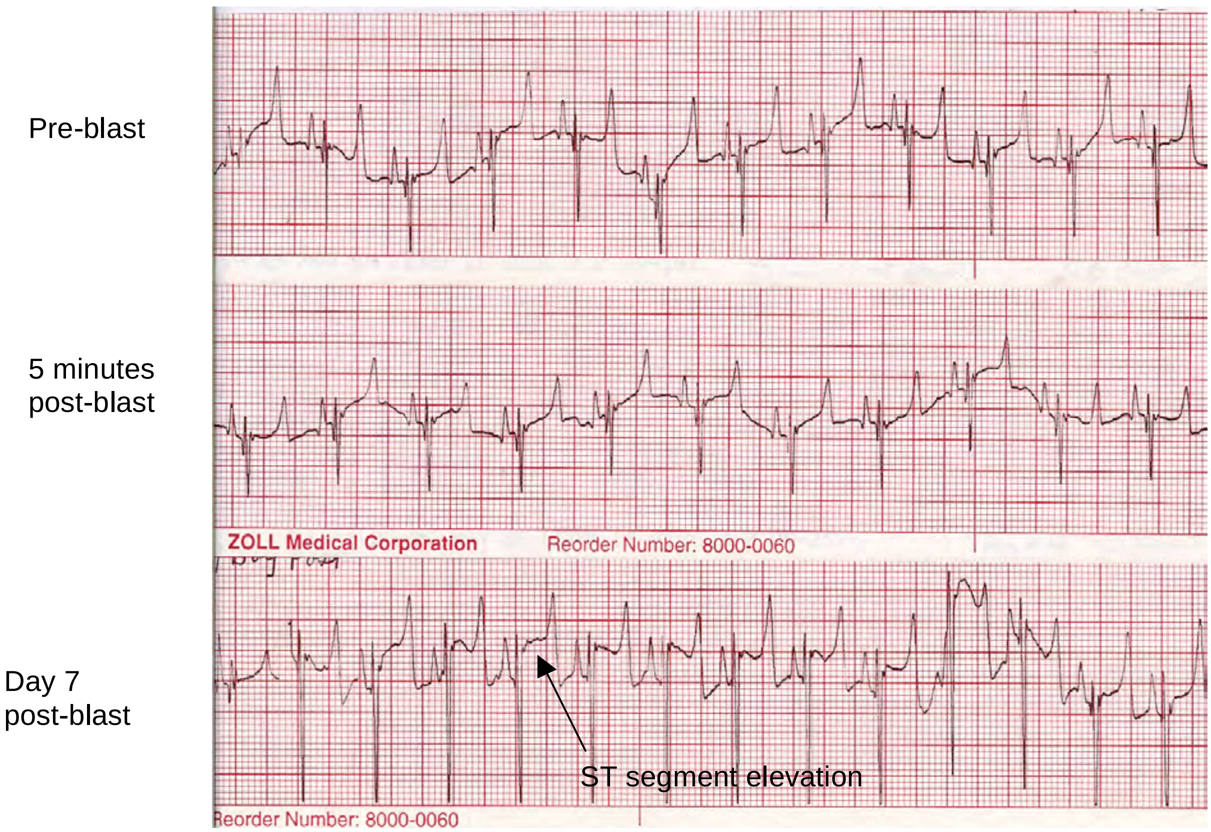


Figure 7B. Electrocardiograms, Lead II, Swine #4, 100 psi large gun, taken pre-blast, at 5 minutes and 7 days post-blast.



KEY RESEARCH ACCOMPLISHMENTS:

- Protocol submitted to IACUC and approved on 3/20/2008.
- Protocol submitted to DOD March 2008.
- ACURO approval received on July 2009.
- Calibration of two different size air guns at 5 pressures completed on May 2008.
- Eight swine and two sham control swine underwent blast-induced traumatic brain injury using two different size air guns at two different pressures.
- Behavioral/learning/memory testing was performed.
- Daily neurological assessment for one week following blast-induced traumatic brain injury.
- Swine brains perfused *in situ* and prepared for histology.

REPORTABLE OUTCOMES:

An abstract and data from the air gun calibration experiments was submitted to the Military Health Research Forum September 2009.

CONCLUSION:

The blast air gun calibrations showed linear and reproducible results that quantify known air velocities. Swine received blast induced-traumatic brain injury with air guns of two different sizes at two different pressures. Two of the three animals injured with the larger gun at the higher blast pressure,100-psi, had a slower recovery to normal, displaying circling behavior, increased startle reflex, hyperactivity, flailing, kicking, unsteady on feet, and vocalizing compared with the other animals in the study. Animals awoke sooner from anesthesia but took longer to stand with increasing blast pressure injuries. Two of the three animals injured with the larger gun at the higher blast pressure showed abnormal electrocardiogram changes on Day 7 following blast that indicate coronary ischemia or injury. The results of the study show that this swine model of blast induced traumatic brain injury will provide a needed tool for the development of treatment for traumatic head injuries, rehabilitation and improvements in military and civilian protective body gear.

Future directions: Transferring the model to miniature swine would allow the follow up period to be extended for months to evaluate treatments in the timeframe of human TBI and rehabilitation. The addition of complicating physiological conditions such as dehydration, stress, hyperthermia, other injuries, and multiple blasts can be useful in tailoring treatment for real world conditions.

REFERENCES:

None

APPENDICES:

None

SUPPORTING DATA:

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