SWEDISH DEFENCE
RESEARCH AGENCY

FOI-R--0756--SE
december 2002
ISSN 1650-1942
**Användarrapport**

# EVALUATION OF DIFFUSE BRAIN INJURY WITH MAGNETIC RESONANCE IMAGING

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| **Utgivare**<br>Totalförsvarets Forskningsinstitut - FOI<br>Försvarsmedicin<br>Experimentell Traumatologi<br>Södersjukhuset<br>118 83 Stockholm | **Rapportnummer, ISRN**<br>FOI-R--0756--SE<br>**Klassificering**<br>Användarrapport<br>**Forskningsområde**<br>8. Människan i totalförsvaret<br>**Månad, år**<br>December 2002<br>**Projektnummer**<br>EF002<br>**Verksamhetsgren**<br>5. Uppdragsfinansierad verksamhet<br>**Delområde**<br>82 Vapentraumatologi |
| **Författare/redaktör**<br>Mårten Risling | **Projektledare**<br>Mårten Risling<br>**Godkänd av**<br><br>**Uppdragsgivare/kundbeteckning**<br>Försvarsmakten<br>**Tekniskt och/eller vetenskapligt ansvarig**<br>Mårten Risling, Anders Suneson, Börje Bjelke |
| **Rapportens titel**<br>Luftburen explosionsinducerad stötvåg; undersökning av eventuell hjärnpåverkan med MR kamera | |

**Sammanfattning (högst 200 ord)**

Magnet resonans avbildning (MRI) kan ge detaljerad information om förändringar i hjärnan. Dock kan rutinmässiga MRI undersökningar vanligen ej ge tillräcklig information om skadeutbredning vid diffusa hjärnskador. Speciella avbildningstekniker som DWI (diffusion-weighted imaging) och ADC (apparent diffusion coefficient) kan ge bättre information om t.ex. skador på blod-hjärn barriären. I denna studie har vi undersökt om MRI med ADC beräkning kan användas för att detektera förändringar i hjärnan utlösta av explosionsinducerade luftburna stötvågor. Sövda vuxna försöksråttor exponerades i en specialbyggd testtub på en meters avstånd för sprängladdningar med en storlek på 0,5-2 g PETN. Djuren undersöktes med MRI såväl före exponeringen samt vid upprepade tillfällen under 3 veckor efter stötvågsexponeringen.

Varken T2 eller DWI undersökning tyder på några skillnader mellan kontrollldjur eller exponerade djur. ADC beräkning var förenlig med förändringar i vätskefördelningen hos vissa av de exponerade djuren. Dessa förändringar kan dock inte betraktas som säkerställda och det konkluderas att MRI undersökningar för närvarande inte förefaller att kunna ge säker information om skadeutbredning vid denna typ av hjärnpåverkan.

**Nyckelord**

Vapentraumatologi, sårbarhet, tryckvågseffekter, verkansvärdering, skallskador, hjärnan

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|---|---|
| **Övriga bibliografiska uppgifter** | **Språk** Engelska |
| **ISSN** 1650-1942 | **Antal sidor:** 7 s. |
| **Distribution enligt missiv** | **Pris: Enligt prislista** |

| **Issuing organization** | **Report number, ISRN** | **Report type** |
|---|---|---|
| FOI – Swedish Defence Research Agency<br>Defence Medecine<br>Experimental Traumatology<br>Söder Hospital<br>SE-118 83 Stockholm<br>Sweden | FOI-R--0756--SE | User report |
| | **Research area code**<br>8. Human Systems | |
| | **Month year**<br>December 2002 | **Project no.**<br>EF002 |
| | **Customers code**<br>5. Commissioned Research | |
| | **Sub area code**<br>82 Experimental Traumatology | |
| **Author/s (editor/s)**<br>Mårten Risling | **Project manager**<br>Mårten Risling | |
| | **Approved by** | |
| | **Sponsoring agency**<br>The Swedish Armed Forces | |
| | **Scientifically and technically responsible**<br>Mårten Risling, Anders Suneson, Börje Bjelke | |

**Report title (In translation)**

EVALUATION OF DIFFUSE BRAIN INJURY WITH MAGNETIC RESONANCE IMAGING

**Abstract (not more than 200 words)**

Magnetic resonance imaging (MRI) may provide detailed information about anatomical changes in the brain. However, the use of diagnostic routine MRI in patients with diffuse traumatic brain injury may underestimate the severity of injury. Diffusion-weighted imaging (DWI) has been useful in the diagnosis of stroke and ischaemia. In DWI and ADC (apparent diffusion coefficient) -mapping, the random movement of hydrogen atoms mainly in the water molecule is measured. In this study we have examined the effects on brain tissue of moderate air blast waves from explosions. A specially designed shock tube was used, in which anaesthetized rats were exposed to detonation of a charge consisting of the non-electric detonating cap with a 0,5 - 2 g PETN explosive at a distance of 1000 mm. The exposed animals were reanaesthetized at 6, 24 and 48 hours as well as 1, 2 and 3 weeks and after the injury and examined by MRI. The T2 and DWI did not reveal any lesions in the brain of the exposed animals. ADC calculation indicated an alteration in the values over time in cortex cerebri and white matter. These changes were, however, not consistent and it is concluded that more data is needed before MRI can be used as a diagnostic tool for the evaluation of patients exposed to air blast.

**Keywords**

weapon's traumatology, human vulnerability, shock wave effects, assessment of human effects, head injury

| **Further bibliographic information** | **Language** English |
|---|---|
| **ISSN** 1650-1942 | **Pages** 7 p. |
| | **Price acc. to pricelist** |

# EVALUATION OF DIFFUSE BRAIN INJURY WITH MAGNETIC RESONANCE IMAGING

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## INTRODUCTION

Magnetic resonance imaging (MRI) provides detailed information about anatomical changes in the brain. However, the use of diagnostic routine MRI in patients with diffuse traumatic brain injury may underestimate the severity of injury. Diffusion-weighted imaging (DWI) has been useful in the diagnosis of stroke and ischaemia, where it has been reported to be a more sensitive indicator of the early pathological changes. In DWI and ADC (apparent diffusion coefficient) -mapping, the random movement of hydrogen atoms mainly in the water molecule is measured. These measurements yield information about structure tissue quality and pathophysiology. Disruptions in the blood–brain barrier seem to be associated with vasogenic edema and changes in the ADC values (1).

In this study we have examined the effects on brain tissue of moderate air blast waves from explosions. A specially designed shock tube (Fig. 1 and 2) was used, in which anaesthetized rats were exposed to moderate air blast by the detonation of a charge consisting of the non-electric detonating cap with a 2 g PETN explosive at a distance of 1000 mm. This generated a shock wave with a duration of 1-2 ms. The exposed animals were re-anaesthetized at 24 and 48 hours as well as 1 week and 3weeks after the injury and examined by MRI.

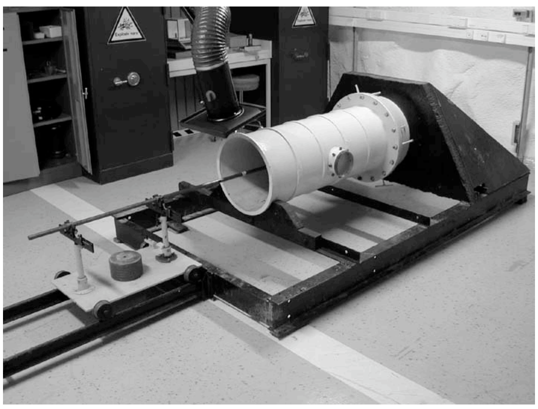

***Fig. 1. The open shock tube***

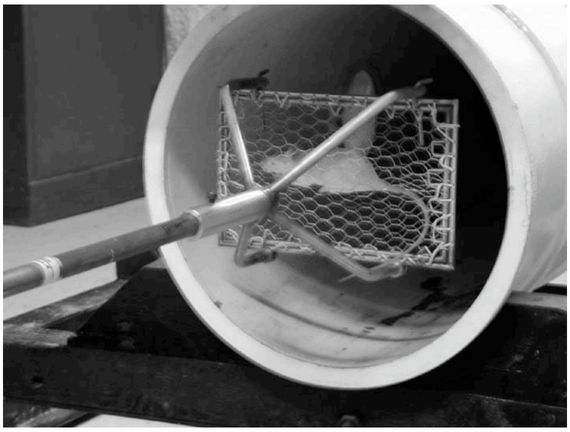

***Fig. 2. The tire netting cage for fixation of rat during blast exposure***

## MATERIAL AND METHODS

Male Sprague-Dawley rats were used (BK Universal, Stockholm Sweden), body weight 200 to 250 g, housed under standard conditions, lights on 07:00 am. and lights off 07:00 p.m., with free access to water and food pellets. All experiments were performed according to the ethical approval (Umeå Försöksdjursetiska nämnd A81-99).

The rats were delivered at the weight of 200 g, accommodated 5 per Macrolon IV cage. Animals were trained in beam walking during 2 days preceeding exposure to explosion. Thereafter their sensory-motor skill was recorded daily using the beam walking test. The study was performed on 10 rats.

For MR recordings a mixture of isoflurane and air was used with an concentration of 3.0% isoflurane at induction and the anesthesia was maintained with a concentration of 1.5%. For the blast or sham exposure the rats were anaesthetized by an intra-peritoneal injection of chloral hydrate (300 – 360 mg/kg body weight).

A wooden beam, 200 cm long, 15 or 10 mm wide, stabilized underneath by a U shaped metal profile preventing lateral vibrations, was used. On the first training day, the 15 mm wide beam was used and thereafter the 10 mm wide beam. The beam was rigidly fixed at each end, the lower end 40 cm above ground and the surface of the upper end flush with the top rim of the home cage creating an inclination of 10°. The middle part, 122 cm long, was marked at the start and end point and designated as the test distance (Fig. 3).

Training and testing sessions were conducted during the morning hours, in a quiet room with dim lighting, one cage at a time. The animals were handled only when transferred between cages or placed on the beam. After brief accommodation to the holding cage one animal was transferred back to the home cage, placed at the end of the beam, to start the training or testing session. After brief re-accommodation to the home cage the animal began 6 trials day one, each of which was separated by a brief (~60 sec) rest period. In the first trial, the animal was placed with its hind limbs straddling across the beam and the forelimbs inside the upper rim of the cage to familiarize it with getting down from the beam into the home cage. The second trial consisted of the animal being placed 20 cm from the end of the beam with the limbs straddling the beam. The third trial begun at the end point of the 122 cm test distance, the fourth at the midpoint of the test distance. The fifth and sixth begin at the start point. The second day of training consisted of 3 trials using the 10 mm wooden beam. The first trial begun at the end point of the testing distance, the second and third begun at the start point. Using this protocol, the animals traversed the beam with 2 or fewer hind limb foot-slips, well within 90 seconds. The animal was considered trained if it scored a "7" on two consecutive daily trials.

After the two training days, the rats were the following day exposed to a pressure characterized air blast (peak pressure ≈ 200 kPa, duration of first positive wave ≈ 2.4 ms) generated by 2.0 gram charge weights of pentaerythrittetranitrate at a distance of 1 m in an open detonation chamber (shock tube) or sham exposed. During blast or sham exposure the anaesthetized rat was placed between two pieces of wire netting modeled after the shape of the rat body in such a way that the body was kept in a horizontal position with the right side towards the explosion (Fig. 2). The fixation prevented undesirable movements due to the air blast that could cause tertiary blast injury but did not prevent normal spontaneous breathing movements of the animal.

24 hours after blast or sham exposure the testing began and the rat was placed just in front of the backboard with its nose at the start point. If the animal could stay balanced on the beam without assistance, the 90 seconds test session begun. The session ended when the impaired hind limb (contra-lateral to the brain lesion) passed the end point, when 90 seconds had elapsed, or when the animal fell off the beam. The rat was brought to its home cage at the end of the session regardless of how the session ended.

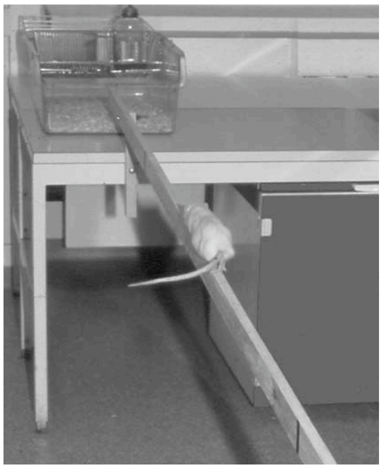

***Fig. 3. The walking beam model***

Motor function was measured using a 7 point rating scale. The rating was solely made on the hind limb contra-lateral to the brain lesion only since no effects on the sensory-motor performance was observed on the ipsi-lateral hind limb.

7 No more than 2 hind limb foot-slips contra-lateral to the lesioned cortex.
6 The contra-lateral hind limb was used for more than 50% of the test distance.
5 The limb was used less than 50% of the test distance.
4 The animal was able to get the affected limb up on the surface of the beam but could not push off the surface without slipping.
3 The animal was able to traverse the test distance without using the affected limb, unable to get the affected limb up on the surface of the beam.
2 The animal could not traverse the test distance within 90 seconds, but was able to stay balanced on the beam.
1 The animal fell off the beam.

Motor behavior was rated by two observers, of whome one was blinded to all treatment conditions. The sensory-motor functional

recovery was recorded daily up to 7 days after lesioning.
An additional parameter had to be added to the evaluation due to the very subtle sensory-motor impairment that was found. The time spent on the beam until the rat reached its home cage was measured and was considered as a measure of task performance skill.

The MRI measurements were performed on a 4.7 Tesla / 40 cm Bruker Biospec Avance spectrometer (Karlsruhe, Germany), equipped with a standard 12 cm self-shielded gradient system. Animals were imaged using an open semicircular purpose built resonator. The lesions were characterized using multi-slice MRI protocols at the following time points; 24 h, 3 and 7 days.

For conventional T2-weighted imaging 9 coronal slices were distributed through the brain. A repetition time = 3000 ms and an echo time = 112 ms were used with a RARE factor of 32, field of view 4 cm, slice thickness 1 mm, 4 averages and an acquisition and reconstruction matrix of 256x256. For T2 maps a multi slice multi echo protocol was used with the following parameters repetition time (TR) = 3000 ms and an echo time (TE) = 8, 16, 24, -…- 256 ms, 32 echos were used with a RARE factor of 8, field of view 4 cm, slice thickness 1 mm, 1 average and an acquisition and reconstruction matrix of 256x256. The proton density map is calculated from the T2-map as a sum of intensities giving the a value of the total number of protons in the analyzed volume i.e. whether an edema occur or not.For the standard spin-echo DWI protocol (TR = 2300 ms, TE = 29.8 ms) the gradients were as follows: small delta 8.5 ms, big delta 15.0 ms, gradient strength 200 mT/m; resulting in 5 b values = 0, 3653, 5592.8, 7683.8, 10000 cm2/s, field of view 4 cm, slice thickness 1 mm, 1 average and an acquisition matrix of 256 x 128 and reconstruction matrix of 256x256. The diffusion gradients were applied in transverse slice-gradient direction. The regional analysis in the T2-, PD- and ADC- maps was done bilateral in 10 different regions on each map (see fig 4).

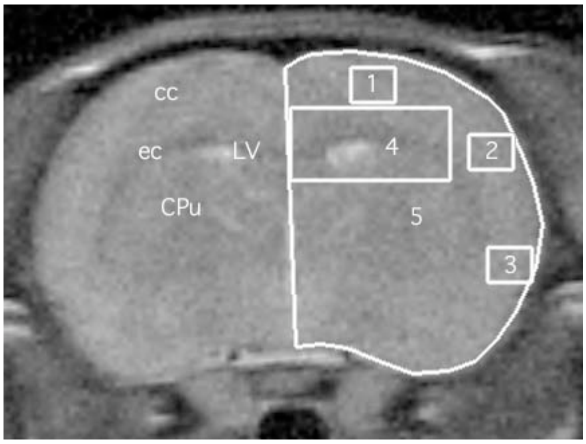


*Fig 4. Representative coronal sections showing the selected regions of interest (1-5) where the map values were selected and measured bilaterally.*

# RESULTS AND DISCUSSION

All rats surviving the blast exposure showed a normal behavior afterwards. On the beam walking test no significant difference was found between the groups regarding sensory-motor performance. The time spent on the beam was different between the two groups and the performance on the beam was different between the groups. The blast exposed animals spent longer time on the beam and was just sitting balanced in the beginning of the test distance unwilling to perform. This effect was most pronounced in the first days after the blast exposure (Fig. 5).

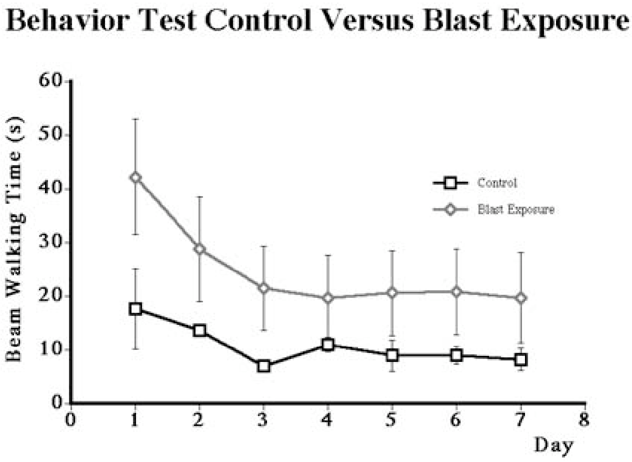


*Fig 5. Graph showing the time spent on the beam in the behavior test indicating a difference between the control (n=2) group and the blast exposured (n=8)*

In no one of the regions measured in the T2-, PD- or ADC- maps was it possible to find any statistical significant difference between the sides, between the groups or over time (Fig. 6).

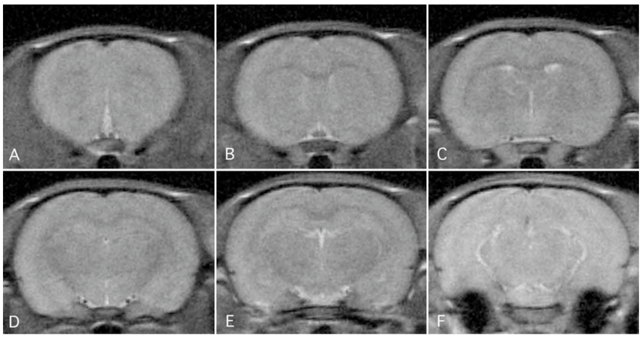

***Fig 6. Representative coronal sections from a $T_2$-weighted MRI recording of a explosion exposed rat. No morphological evidence is seen indicating any pathological changes.***

Säljö and co-workers have recently reported changes in distrubution of phosphorylated neurofilament subunits in neurons of the adult rat brain after exposure to similar blast waves (2). They have also reported activation of microglial cells (3) as well as induction of apoptosis (4). Magnetic resonance imaging (MRI) is now well established experimentally and clinically and provides detailed information about anatomy and biophysical processes. MRI, for example, has been use extensively in human stroke studies. Diffusionweighted imaging (DWI), in particular, has been useful in the diagnosis of stroke and ischemia, where it appears to be a sensitive indicator of early pathological changes. In DWI, the self-diffusion movement of water is measured. The use of standard MRI in patients with diffuse TBI may underestimate the severity of injury. However, DWI permits more accurate detection of focal and, particularly, diffuse tissue damage (1). Thus, investigators working with animal models of brain injury have recently applied DWI and/or standard MRI in studies involving TBI. Specifically, apparent diffusion coefficient (ADC) values have been used for measuring diffusion using diffusion-weighted MRI pulse sequences. These measurements yield information about structure and pathophysiology. For example, disruptions in the blood–brain barrier, found often in severe head trauma, seem to be associated with vasogenic edema and increased ADCs. Thus, the present results do not indicate any major changes in the vascular permeability in the central nervous system of rats exposed to blast waves as performed in the present study.

# CONCLUSIONS

The difference in performance in beam walking time but not concerning the score indicate that there probably was no focal damage in the brain. The longer time spent in beam-walking and the behavior (sitting on the beam) could be an indication of confusion and memory impairment after the blast exposure. It might as well be speculated if the blast pressure wave have affected the balance ability.

Even though the MRI mapping technique may detecting mild-moderate lesions in the brain could we not detect any significant changes after blastwave exposure, at the timepoints when MR analysis was performed. The results reported by Säljö should therefore be reevaluated using histological and histochemical techniques. Such studies are in progress.

# RECOMMENDATION:

Technical improvents in the MRI technique can be expected. With a better resolution of the images, MRI should be reevaluated as a tool for the evaluation for mild diffuse brain injuries.

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