

## Original Research

# Magnesium Attenuates Post-Traumatic Depression/Anxiety Following Diffuse Traumatic Brain Injury in Rats

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**Objective:** Magnesium (Mg) declines after traumatic brain injury (TBI), a decline believed associated with ensuing neuronal cell death and subsequent functional impairment. While Mg's effects on motor and cognitive deficits following TBI have been well studied, few studies have addressed post-traumatic depression as an outcome parameter, despite its being a major clinical problem with an incidence of between 6 and 77%. We investigated the incidence of post-traumatic depression/anxiety in an animal model of diffuse TBI, and explored the use of magnesium sulfate ( $\text{MgSO}_4$ ) as an interventional treatment.

**Methods:** Diffuse TBI was induced in 32 anesthetized, adult, male Sprague-Dawley rats, using the 2 m impact-acceleration model of injury. At 30 min after injury, half of the rats received 250  $\mu\text{mol/kg}$  i.v.  $\text{MgSO}_4$ ; the other half served as non-treated controls. Before and for 6 weeks after injury, the open-field, spontaneous activity test was used to determine post-traumatic depression/anxiety relative to pre-injury. In this test, animals are placed in a 1-meter square box with 100 squares marked on the base. The number of squares entered in a 5-min period is recorded. Incidence of post-traumatic depression/anxiety was defined as the number of animals demonstrating a reduction in spontaneous activity to less than 100 squares in 5 min. Prior to injury, rats typically entered a mean of  $201 \pm 12$  (SEM) squares over a 5 min observation period.

**Results:** At 1 week after injury, non-treated animals had a mean core of  $62 \pm 13$ . The incidence of post-traumatic depression/anxiety in these animals was 61%, which is similar to that observed clinically. In contrast, animals treated with  $\text{MgSO}_4$  had a mean activity score of  $144 \pm 23$  at 1 week after TBI and an incidence of depression/anxiety of less than 30%. The significant difference between groups persisted for the entire 6-week observation period.

**Conclusions:** The improvement in post-traumatic depression/anxiety conferred by Mg adds further weight to available evidence of Mg's benefit as a neuroprotective agent after TBI.

## INTRODUCTION

Neurologic depression that occurs following organic brain insult, such as trauma or infection, differs from endogenous, or idiopathic depression [1]. Among human survivors of traumatic brain injury (TBI), neurobehavioral disorders are among the most frequent long-term consequences. Such neurobehavioral changes may include cognitive or memory impairment, apathy, aggressiveness, and mood disorders [2]. Depression after TBI has a reported incidence that is highly variable and ranges from 6% to 77% of TBI patients [3–7]. This wide ranging incidence

is partly due to the fact that brain trauma is often associated with impaired self-awareness, which can obscure perceptions and affect the self-reporting of depressive symptoms [8]. Depressive disorder symptoms occurring immediately after TBI can be confused with symptoms of the TBI itself. Furthermore, emotional problems resulting from TBI can worsen over time [7], adding weight to the belief that TBI-induced depression presents a problem requiring long-term treatment, and which has potential to impinge on the patient's recovery from injury.

Depression after TBI and other neurological disease is now

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thought to be associated with bilateral dysfunction of the frontal and temporal lobes and disruption of deep diencephalic structures and networks. Disruption of normal neuronal networks and neurotransmitter levels in these anatomical areas is thought to be causative, and depression can be the expression of a final common pathway [9]. Contusional injuries from TBI may affect brain regions involved in the mediation of mood, especially along the temporal lobes and frontal cortex. Diffuse axonal injury, in addition to disrupting neuronal circuits directly, may also disrupt neurotransmitter systems such as noradrenaline, serotonin, dopamine and acetylcholine [10]. Such disruption is part of the secondary injury cascade that has been well described following TBI [11], and is thought to account for much of the mortality and morbidity after the traumatic insult. Another critical secondary injury factor is magnesium (Mg), decline of which has been associated with a decrease in both motor and cognitive function after TBI [12]. While a number of Mg's actions may account for this interrelationship between decline and functional outcome, the modulatory effects of Mg on neurotransmitter systems may be particularly significant.

Mg has been linked to the pathophysiology of psychiatric states and has, like other NMDA receptor antagonists, antidepressant-like effects in rodent screening tests [13]. Studies have also exploring the relationship between blood Mg levels in patients with major depression and other psychiatric illnesses. Blood Mg levels were significantly higher in nonmedicated patients with major depression as compared to healthy controls. Increased erythrocyte Mg was also associated with major depressive syndromes in many drug-free hospitalized male and female patients [14]. In addition, the most severely depressed patients had the highest intracellular Mg content, showing that intracellular Mg is related to the intensity of symptoms [15]. Widmer *et al.* [14] also observed that erythrocyte Mg concentrations tended to normalize in parallel with the clinical improvement of the patients. It has already been well-established in animals that lowering Mg increases central hyperexcitability due to disinhibition of the NMDA receptor channels and by its simultaneous activation of the GABA<sub>A</sub>-gated chloride channels [16]. Moreover, important relationships have been described between catecholamines and Mg metabolism, including a decrease in Mg with adrenaline perfusion [14]. Such observations lay the basis for a possible interaction between Mg levels and development of depression [14].

We hypothesize that the decrease in brain intracellular free Mg concentration observed after TBI may be associated with the development of post-traumatic depression/anxiety, and that administration of Mg after trauma may decrease its incidence. Accordingly, in the current experiment, we have examined the effects of MgSO<sub>4</sub> administration on post-traumatic depression/anxiety in rats subject to severe diffuse traumatic brain injury, using the spontaneous exploratory behavior open-field test as a measure of rodent depression/anxiety.

## MATERIALS AND METHODS

### Injury Induction

Male Sprague-Dawley rats (400 gm to 500 gm; n = 32) were anesthetized using a mixture of 2–3% isoflurane (Isoflo™, Abbott Australasia Pty Ltd, Kurnell, Australia) in oxygen (1L/min); anesthesia was reduced to 1% for maintenance. Animal temperature was maintained during surgery using a heating pad with the addition of a 60 W lamplight. Once adequate anesthesia was achieved, a midline scalp incision was performed and the temporalis muscles retracted to expose the skull and permit placement of a stainless steel disc (10 mm in diameter and 3 mm in depth) centrally between the lambda and bregma sutures using cyanoacrylate adhesive to fix the disc in place. Injury was then induced using the impact-acceleration model of TBI as described in detail elsewhere [17,18]. Briefly, a 450 g weight was dropped from a height of 2 m onto the metal disc fixed to the rat's skull. A 10 cm foam bed underneath the animal helped absorb the impact. After impact, the metal disc was removed and all wounds infused with topical 2% lignocaine hydrochloride, and closed with 2–3 surgical staples (Autoclip®, MikRon Precision Inc., Sparks, MD, USA). Povidone-iodine (10% w/v, Betadine®, Faulding Pharmaceuticals, Salisbury, SA) was applied undiluted following wound closure for post-operative surgical wound disinfection. Surgical staples were removed at one week post-injury, and wounds inspected to ensure complete healing had taken place.

### Open Field Test

Behavioral models, incorporating repeated exposure to stress have been widely used as experimental models for depression because stress is thought to play an important role in the etiology of depression [19]. The open field is a well-characterized model designed to reflect anxiety levels in rats based upon changes in their exploratory behaviour [20]. Several studies have shown it to measure anxiety and depression reliably in rodents [21,22], including after TBI [23]. The apparatus for the open field test consisted of a white, square open field (100 cm by 100 cm) made of white melamine and enclosed with 50 cm high walls placed under strong illumination. The arena was divided into 100 squares (10 by 10 cm) by means of black lines. Each rat was placed in the center of the field and its behavior recorded for five minutes [24]. The number of squares entered, defined by having at least the anterior paws in a square was scored by visual observation. The field was cleaned thoroughly with water containing a mild detergent before the introduction of each rat in order to reduce any lingering olfactory cues. Testing was performed in a temperature, noise and light controlled room. Each rat was transported to the testing room using the home cage with the animals being handled daily by the same two investigators until the completion of the study.

## Drug Administration

At 30 min after trauma, half of the animals were intravenously administered 250  $\mu\text{mol/kg}$   $\text{MgSO}_4$  (Sigma Chemical Co, St Louis, MO, USA), dose that has been determined to be optimal for improvement of functional outcome following TBI [18].

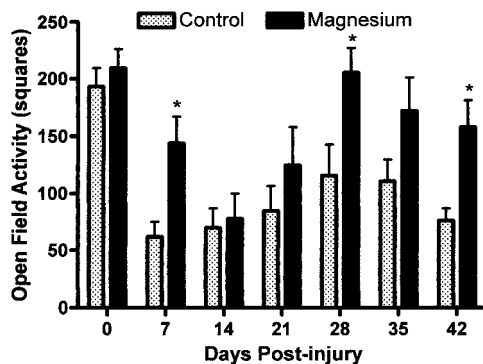
## Statistical Analysis

Continuous data are presented as means  $\pm$  SEM and were analyzed using analysis of variance (ANOVA) followed by Student Newman Keuls post-hoc tests. Ordinal data were analyzed by Kruskal Wallis ANOVA followed by individual Mann-Whitney U tests. A  $p < 0.05$  was considered significant.

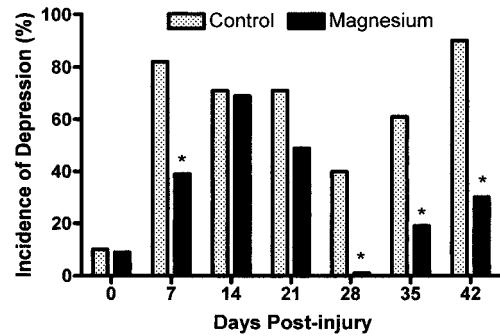
## RESULTS

The open-field test was performed on animals before induction of TBI and compared with the results of this test after injury. Prior to injury, spontaneous activity of all animals in the open field was  $201 \pm 12$ . There was no statistical difference among the treatment groups before injury. After injury, open field scores in the control group significantly decreased ( $p < 0.001$ ) to a mean score of  $62 \pm 13$  by day 7, which is less than 30% of pre-injury values (Fig. 1).

By day 42, the scores were still significantly lower than the pre-injury values, with the average activity being 43% of pre-injury level ( $p < 0.001$ ). In contrast, acute administration of  $\text{MgSO}_4$  30 minutes after injury resulted in a day 7 activity score of  $144 \pm 23$ , which was more than twice the score in control animals. By day 28, open field scores in Mg-treated rats were not significantly different from those obtained prior to injury in this group. At day 42, the Mg treatment had resulted in a 76% return of function relative to controls, representing a statistically significant difference between the treatment groups ( $p < 0.01$ ).



**Fig. 1.** Open field-testing over a six-week period following traumatic brain injury in rats. There was a significant decline ( $p < 0.001$ ) in open field activity after injury, with a return to pre-injury levels only observed in the magnesium treated group. \* =  $p < 0.05$  versus controls.



**Fig. 2.** Incidence of depression over a six-week period following traumatic brain injury in rats. There was a significant increase ( $p < 0.001$ ) in the incidence of depression after injury, with the magnesium treated animals having a decreased incidence relative to controls, particularly at later time points. \* =  $p < 0.05$  versus controls.

In order to assess the incidence of depressed behavioral activity, an ordinal scoring approach was adopted. Depressed behaviour was taken as an activity score of less than 100, and scored as zero, whilst normal activity was taken as an activity score of 150 and above, and scored as 2. An intermediate level of activity (101–149) was scored as 1. With this scoring approach, there was a mean incidence of depressed behavior following injury of 68% in control animals over the entire 42-day period of the study (Fig. 2). By day 42, 90% of control animals were classified as depressed. In contrast, Mg treated animals had a mean incidence rate for depression of 34% over the entire 42 day assessment period; an incidence of 30% was recorded on day 42.

## DISCUSSION

In the present study, we have demonstrated that long-term increase in stress/anxiety in rats occurs after traumatic brain injury, consistent with the development of a depressive disorder. Mg administration 30 min after TBI reduced the incidence of depression, and when present, reduced the severity. The incidence of depression following TBI has been difficult to establish conclusively in clinical studies and ranges between 6 to 77%. The animal model in this study used a controlled population and a consistent application of the open field test in order to identify the development of a depressive disorder. The actual incidence of post-traumatic depression was approximately 68%, which is at the upper end of the range of reported human depression. Our results emphasize the potential significance of this disorder, and the need for effective therapeutic intervention.

Depression in rats was assessed using the open field test, which is a well-characterized rodent model of anxiety and depression. Locomotion in an open field is considered to reflect general or exploratory activity, where a decrease in the overall mobility and ambulation are considered to represent increased

anxiety in the animal [25], which would indicate depressed behavior. Our results demonstrated a significant reduction in activity after TBI. However, reduced locomotor activity in the open field may also reflect individual motor deficits. On the basis of parallel studies performed in this laboratory, we know that motor deficits in this model are transient and that full recovery of motor function is achieved within 10 days in untreated animals, and in under a week in Mg treated animals [18]. Thus the reduction of spontaneous exploratory activity in present study cannot be attributed to the presence of persistent motor deficits. Given the close conceptual associations between stress, anxiety and depression which are intrinsic to most animal models and testing paradigms, and the fact that many antidepressants also serve as effective anxiolytics [26], a reasonable hypothesis emerges whereby Mg may be exerting its antidepressant effect through anxiolytic mechanisms. Depressive illness often represents a disorder that occurs after a traumatic event, but is seen by some as requiring a differential diagnosis to exclude or confirm comorbidity with post-traumatic stress disorder [27]. Whether Mg may be of benefit in post-traumatic stress disorder (PTSD) has not been previously investigated and no published literature exists describing the free blood Mg status in patients with PTSD. It would therefore be of interest to examine whether altered Mg status exists in PTSD patients, and whether subsequent treatment with Mg would in fact provide any benefit in this condition.

The acute administration of  $MgSO_4$  produced significant benefits in relation to behavioral function after TBI. Mg had almost an immediate effect on depressed activity, resulting in significant improvement as early as seven days after injury in comparison to controls. Behavioral function with  $MgSO_4$  treatment was restored to virtually pre-injury levels by 28 days post-injury. The mechanisms by which Mg produces these beneficial effects are unknown, although they are likely to involve actions on neurotransmitter systems, particularly glutamate. Mg is known to have modulatory effects, be they direct or indirect, upon glutamate release and glutamate receptor activity, and altered activity of the glutamine/glutamate cycle has been reported in patients with major depression [28]. Indeed, levels of glutamine and glutamate are elevated in plasma while high glutamine levels have been recorded in the cerebrospinal fluid of acutely depressed unmedicated patients [28]. Thus, actions on the glutamatergic neurotransmitter systems may play a major part in Mg's action on acute depression after TBI.

## REFERENCES

1. Rosenthal M, Christensen BK, Ross TP: Depression following traumatic brain injury. *Arch Phys Med Rehabil* 79:90–103, 1998.
2. Jorge RE, Robinson RG, Arndt SV: Are there symptoms that are specific for depressed mood in patients with traumatic brain injury? *J Nerv Ment Dis* 181:91–99, 1993.

3. Alexander MP: Mild traumatic brain injury: pathophysiology, natural history and clinical management. *Neurology* 45:1253–1260, 1995.
4. Bachman DL: The diagnosis and management of common neurologic sequelae of closed head injury. *J Head Trauma Rehabil* 7:50–59, 1992.
5. Gualtieri CT: Pharmacotherapy and the neurobehavioural sequelae of traumatic brain injury. *Brain Inj* 2:101–129, 1988.
6. Jorge RE, Robinson RG, Starkstein SE, Arndt SV: Depression and anxiety following traumatic brain injury. *J Neuropsychiatry Clin Neurosci* 5:369–374, 1993.
7. Morton MV, Wehman P: Psychosocial and emotional sequelae of individuals with traumatic brain injury: a literature review and recommendations. *Brain Inj* 9:81–92, 1995.
8. Kreuzer M: Brain injury: a comprehensive examination. *Brain Inj* 15:563–576.
9. Joseph AB, Wroblewski B: Depression, antidepressants, and traumatic brain injury. *J Head Trauma Rehabil* 10, 90–95, 1995.
10. Van Reekum R, Cohen T, Wong J: Can traumatic brain injury cause psychiatric disorders? *J Neuropsychiatry Clin Neurosci* 12: 316–327, 2000.
11. McIntosh TK: Neurochemical sequelae of traumatic brain injury. *Cereb Brain Metab Rev* 6:109–162, 1994.
12. Vink R, Cernak I: Regulation of brain intracellular free magnesium following traumatic injury to the central nervous system. *Front Biosci* 5:656–665, 2000.
13. Szewczyk B, Kata R, Nowak G: Rise in zinc affinity for the NMDA receptor evoked by chronic imipramine is species-specific. *Pol J Pharmacol* 53:641–645, 2001.
14. Widmer J, Henrotte J, Raffin Y, et al: Relationship between erythrocyte magnesium, plasma electrolytes and cortisol, and intensity of symptoms in major depressed patients. *J Affect Disord* 34:201–209, 1995.
15. Widmer J, Henrotte J, Raffin Y, et al: Relationship between blood magnesium and psychomotor retardation in drug-free patients with major depression. *Eur Psychiatry* 1:90–97, 1998.
16. Schwartz R, Wagner J, Yu X, Martin D: Bidirectional modulation of GABA-gated chloride channels by divalent cations: inhibition by  $Ca^{2+}$  and enhancement by  $Mg^{2+}$ . *J Neurochem* 62:916–922, 1994.
17. Foda MAA, Marmarou A: A new model of diffuse brain injury in rats; Part II: morphological characterization. *J Neurosurg* 80:301–313, 1994.
18. Heath DL, Vink R: Optimization of magnesium therapy following severe diffuse axonal brain injury in rats. *J Pharmacol Exp Ther* 288:1311–1316, 1999.
19. Izumi J, Washizuka M, Hayashi-Kuwabara Y, et al: Evidence for a depressive-like state induced by repeated saline injections in Fischer 344 rats. *Pharmacol Biochem Behav* 57:883–888, 1997.
20. Kulkarni S: Open Field Test: its status in psychopharmacology. *Ind J Pharmacol* 9:241–246, 1977.
21. Von Horston S, Exton M, Voge J, et al: Cyclosporine A affects open field behavior in DA rats. *Pharmacol Biochem Behav* 60:71–76, 1998.
22. Archer J: Tests for emotionality in rats in mice: A review. *Animal Behav* 21:205–235, 1973.
23. Vink R, O'Connor CA, Nimmo AJ, Heath DL: Magnesium attenuates persistent functional deficits following diffuse traumatic brain injury in rats. *Neurosci Lett* 336:41–44, 2003.

24. Suarez M, Molina S, Rivarola M, Perassi N: Effects of maternal deprivation on adrenal and behavioural responses in rats with anterodorsal thalami nuclei lesions. *Life Sci* 71:1125–1137, 2002.
25. Kontinen V, Kauppila T, Paananen S, et al: Behavioural measures of depression and anxiety in rats with spinal nerve ligation-induced neuropathy. *Pain* 80:341–346, 1999.
26. Mar A, Spreekmeester E, Rochford J: Fluoxetine-induced increases in open field habituation in the olfactory bulbectomized rat depend on test aversiveness but not on anxiety. *Pharmacol Biochem Behav* 73:703–712, 2002.
27. Ducrocq F, Vaiva G, Cottencin O, et al: Posttraumatic stress, post-traumatic depression and major depressive episode: literature review. *Encephale* 27:159–168, 2001.
28. Levine J, Panchalingam K, Rapoport A, et al: Increased cerebrospinal fluid glutamine levels in depressed patients. *Biol Psychiatry* 47:86–593, 2000.

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