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Glucose administration after traumatic brain injury exerts some benefits and no adverse effects on behavioral and histological outcomes

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Abstract

The impact of hyperglycemia after traumatic brain injury (TBI), and even the administration of glucose–containing solutions to head injured patients, remains controversial. In the current study adult male Sprague-Dawley rats were tested on behavioral tasks and then underwent surgery to induce sham injury or unilateral controlled cortical impact (CCI) injury followed by injections (i.p.) with either a 50% glucose solution (Glc; 2 g/kg) or an equivalent volume of either 0.9% or 8% saline (Sal) at 0, 1, 3 and 6 h post-injury. The type of saline treatment did not significantly affect any outcome measures, so these data were combined. Rats with CCI had significant deficits in beam-walking traversal time and rating scores (p's <0.001 versus sham) that recovered over test sessions from 1 to 13 days post-injury (p's <0.001), but these beam-walking deficits were not affected by Glc versus Sal treatments. Persistent post-CCI deficits in forelimb contraflexion scores

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and forelimb tactile placing ability were also not differentially affected by Glc or Sal treatments. However, deficits in latency to retract the right hind limb after limb extension were significantly attenuated in the CCI-Glc group ($p<0.05$ versus CCI-Sal). Both CCI groups were significantly impaired in a plus maze test of spatial working memory on days 4, 9 and 14 post-surgery ($p<0.001$ versus sham), and there was no effect of Glc versus Sal on this cognitive outcome measure. At 15 days post-surgery the loss of cortical tissue volume ($p<0.001$ versus sham) was significantly less in the CCI-Glc group (30.0%; $p<0.05$) compared to the CCI-Sal group (35.7%). Counts of surviving hippocampal hilar neurons revealed a significant (~40%) loss ipsilateral to CCI ($p<0.001$ versus sham), but neuronal loss in the hippocampus was not different in the CCI-Sal and CCI-Glc groups. Taken together, these results indicate that an early elevation of blood glucose may improve some neurological outcomes and, importantly, the induction of hyperglycemia after isolated TBI did not adversely affect any sensorimotor, cognitive or histological outcomes.

Keywords

beam-walking; cognition; controlled cortical impact; contusion volume; hippocampus; hyperglycemia; neurological exam; rat; spatial working memory

1. Introduction

It is well established that traumatic brain injury (TBI) results in an acute increase in cerebral energy demand, demonstrated as an increase in the cerebral metabolic rates of glucose (CMRGlc) and anaerobic glycolysis, that is followed by a more prolonged period of reduced CMRGlc and cerebral metabolic depression (Bergsneider et al., 1997, 2000; Hovda et al., 1991; Kato et al., 2007; Lee et al., 1999; Statler et al., 2003; Sutton et al., 1994; Yoshino et al., 1991). Patients with severe TBI frequently exhibit elevated blood glucose levels (hyperglycemia), but it remains uncertain if this simply reflects the severity of injury and the related stress response or if increased blood glucose levels exacerbate neurological injury (Van Beek et al., 2007; Young et al., 1989). During the early stages after TBI, low levels of cerebral extracellular glucose have been often reported in both experimental models (Chen et al., 2000; Fukushima et al., 2009; Krishnappa et al., 1999) and in TBI patients (Alessandri et al., 2000; Alves et al., 2005; Vespa et al., 2003). As the primary source for energy in brain cells is glucose, the prior findings suggest that endogenous fuel levels may be insufficient to meet the cerebral metabolic demands in the acute phase of TBI, resulting in an energy crisis (Vespa et al., 2003, 2005). Although changes in cerebral blood flow or mitochondrial functions may contribute to the metabolic dysfunctions and tissue damage after TBI (Harris et al., 2012; Jiang et al., 2000; Lifshitz et al., 2004; Sullivan et al., 2005), the “insufficient fuel” hypothesis is supported by multiple studies indicating that early administration of metabolic substrates after experimental TBI, including lactate (Alessandri et al., 2012; Chen et al 2000; Holloway et al., 2007; Rice et al., 2002), pyruvate (Fukushima et al., 2009; Moro and Sutton, 2010; Shi et al., 2015; Su et al., 2011; Zlotnik et al., 2008; 2012) and ketone bodies (Appelberg et al., 2009; Davis et al., 2008; Deng-Bryant et al., 2011; Prins et al., 2005; Prins and Hovda, 2009), can improve cerebral metabolic and behavioral outcomes and reduce histopathology.

Most likely due to the association of hyperglycemia with poor outcomes in patients with severe TBI (Griesdale et al., 2009; Jeremitsky et al., 2005; Lam et al., 1991; Liu-DeRyke et al., 2009; Rovlias and Kotsou, 2000; Young et al., 1989), the majority of studies on the effects of glucose after experimental TBI have administered doses sufficient to induce hyperglycemia. Early experiments in rats with closed head injury reported that infusion of large volumes of 5% dextrose in water lead to increased mortality, cerebral edema and worsened neurological scores within the first 48 h post-TBI, although there were no similar adverse effects after infusion of a 5% dextrose solution in 0.9% saline (Feldman et al., 1995; Gurevich et al., 1997; Shapira et al., 1992; Shapira et al., 1995; Talmor et al., 1998). Induction of hyperglycemia 20 min after a controlled cortical impact (CCI) injury in rats had no effects on contusion volume and neuronal loss in the hippocampus at 2 weeks post-injury (Cherian et al., 1998a), and a hyperglycemic dose of glucose injected 5 min after fluid percussion brain injury in rats was found to increase neutrophil infiltration, but not cortical injury volume, by 3 days post-injury (Kinoshita et al., 2002). Lower doses of glucose (100 mg/kg) administered from 1–10 days after fluid percussion brain injury did not alter cognitive outcome 11–15 post-injury (Kokiko-Cochran et al., 2008). More recently, single or multiple injections of hyperglycemic doses of glucose within the first 6 hours after CCI injury were found to significantly attenuate TBI-induced reductions in CMRGlc and reduce neuronal injury in cortex and hippocampus at 24 h post-injury (Moro et al., 2013).

Given the discrepant outcomes in the preceding studies and the sparse number of studies that have examined the effects of early post-injury glucose administration on behavioral recovery or sub-acute histopathology, the current study was conducted to determine the effects of multiple glucose injections (2 g/kg, i.p. at 0, 1, 3 and 6 h) on these outcomes in rats with moderate CCI injury. Recovery on sensorimotor and cognitive tasks were determined over a 2 week period, and cortical contusion volume and surviving neurons in the hilar region of the hippocampus were determined at 15 days post-CCI. Based on benefits seen with this glucose treatment regimen by 24 h post-CCI (Moro et al., 2013), we hypothesized that acute hyperglycemia would be beneficial for behavioral recovery and improve histological outcomes at 2 weeks post-injury.

2. Results

2.1 Beam-walking deficits were not altered by glucose treatments

Data for beam traversal time are illustrated in Fig. 1. It can be seen that groups did not differ prior to surgery. Post-surgical beam traversal times remained fairly constant in sham injury controls but increased most substantially in the first 2 test sessions after CCI injury and returned near sham levels thereafter. Repeated measures analysis of variance (ANOVA) for the post-injury data revealed a significant effect of Injury ($p<0.001$), Days ($p<0.001$) and for the Injury x Day interaction ($p<0.001$). There was no significant effect for glucose compared to saline treatment on beam traversal times as reflected by no significant effects of Treatment, the Injury x Treatment and the Day x Treatment interactions, or for the 3-way interaction.

Data for ratings of beam-walking ability are illustrated in Fig. 2. It can be seen that groups did not differ prior to surgery and beam-walking ability of sham injury controls remained

consistent across all testing days (rating of 7 indicating beam traversal with no more than 2 foot slips). Post-surgical beam-walking impairments were evident in rats with CCI injury (beam rating of 3 indicates ability to cross the beam without using the right hind limb to aid in forward locomotion), with gradual improvement from days 1 to 13 post-injury. Repeated measures ANOVA for the post-injury data revealed a significant effect of Injury ($p<0.001$), Days ($p<0.001$) and for the Injury x Day interaction ($p<0.001$). Although there were some mild improvements in beam-walk ratings of the CCI-Glc compared to CCI-Sal group in the second week of post-injury testing (Fig. 2), there were no significant effects of Treatment, Injury x Treatment and Day x Treatment interactions, or for the 3-way interaction.

2.2 Glucose treatments improved some neurological function

All animals had perfect scores (10 of 10) for tactile placing tests of both forelimbs prior to surgery. Sham injured animals continued to show perfect scores in both limbs after surgery, and animals in the CCI groups had no impairment of tactile placing ability in the left forelimb post-injury (data not shown). However, as illustrated in Fig. 3, severe deficits in placing of the right forelimb in response to tactile stimulation were observed after left hemisphere CCI injury. Although some improvement in tactile placing for CCI-Glc compared to CCI-Sal was evident on days 9 and 13 post-injury, the statistical analysis revealed a significant effect of Days ($p<0.001$), but no significant effects for Treatment or the Day x Treatment interaction.

All animals had normal extension of both forelimbs (0–15° angle between limbs) toward a countertop surface upon being lifted rapidly by the base of the tail prior to surgery, and this lack of contraflexion persisted in sham injury controls post-surgery (data not shown). After CCI injury rats exhibited contraflexion of the right forelimb (limb kept toward body surface rather than extending down toward countertop), thus increasing the angle formed between left and right forelimbs (see Fig. 4). Statistical analysis of the data for ratings of contraflexion angle post-CCI revealed a significant effect of Days ($p<0.05$), but no significant effects for Treatment or the Day x Treatment interaction.

All animals rapidly retracted the left and right hind limbs (<1 sec) during hind limb extension tests conducted prior to surgery. Sham injured animals continued to show perfect scores in both limbs after surgery, and there was no increased extension time for the left hind limb after CCI injury (data not shown). As illustrated in Fig. 5, after the left hemisphere CCI injury the latencies to retract the right hind limb after it was displaced lateral and posterior from the body was greatly increased in both CCI groups on day 1 post-injury. The duration of right hind limb extension gradually improved over subsequent tests (significant effect of Days, $p<0.001$), with more rapid retraction of the extended limb in CCI-Glc as compared to CCI-Sal rats (significant effect of Treatment, $p<0.05$). The Day x Treatment interaction was not significant.

2.3 Cognitive deficits were not altered by glucose treatments

Data for total number of arm entries during tests in a plus maze prior to surgery and on days 4, 9 and 14 post-injury are illustrated in Fig. 6. It can be seen that groups did not differ prior to surgery. Repeated measures ANOVA for the post-injury data revealed a significant effect

of Days ($p<0.05$), which post-hoc testing revealed as due to fewer arm entries on post-injury day 9 compared to day 4 ($p<0.01$). There were no significant effects of Injury or Treatment, and none of the 2-way or 3-way interactions were significant.

The percent four/five alternation measures of spatial working memory ability during the plus maze tests before and after surgery are shown in Fig. 7. Based on the stratified random assignment (see Experimental procedures), the pre-surgical, baseline percent four/five alternation scores did not differ for groups. As shown in Fig. 7, CCI rats exhibited lower alternation scores after surgery than did the animals in the sham injury groups. Repeated measures ANOVA for the post-injury data revealed a significant effect of Injury ($p<0.001$), with no significant effect for Days or Treatment. None of the 2-way or 3-way interactions were significant.

2.4 Glucose treatments reduced loss of cortical tissue but not loss of hilar neurons

Two-way ANOVA indicated no significant effects of Injury or Treatment on the volume of the contralateral cortex (n = 12/group) at 15 days post-surgery, so the percent tissue volume loss in the left/injured cortex was calculated [100-((Left/Right) × 100)]. Statistical analysis of these data (Fig. 8A) revealed no effect of Treatment, but a significant effect of Injury ($p<0.001$) and the Injury x Treatment interaction ($p<0.05$). Post-hoc testing indicated there was less cortical tissue volume loss in the CCI-Glc group as compared to CCI-Sal ($p<0.05$). The area measures used to calculate cortical volumes (Fig. 8B) showed that the glucose treatments reduced tissue loss at anterior aspects of the injury, as illustrated in representative animals from the CCI-Sal (Fig. 8C) and CCI-Glc (Fig. 8D) groups. Cell density counts were used to calculate neuronal loss in the left hilus as a percent of neurons in the right hilus [100-((Left/Right) × 100)] of the hippocampus, as illustrated in Fig. 8E. Statistical analysis of these data revealed both CCI groups had significant loss of hilar neurons compared to sham injury controls ($p<0.001$ for Injury effect), and there was no significant effect of Treatment or the Injury x Treatment interaction. Images of hilar regions included for the cell density counts in sham injury and CCI injury are shown in Fig. 8F–G.

3. Discussion

The current results indicate that injections of glucose (2 g/kg, i.p.) immediately and at 1, 3 and 6 h post-CCI had no significant effects on recovery of beam-walking, tactile placing, contraflexion, spatial working memory or on loss of hippocampal hilar neurons in the first two weeks post-injury. However, persistent hind limb extension deficits seen in saline-treated rats with CCI were significantly improved by this glucose treatment regimen. The multiple glucose injections also resulted in a significant prevention of cortical tissue loss at 15 days post-CCI. Because these doses of glucose increase blood glucose levels to between 17.0 and 19.5 mmol/L (Moro et al., 2013), the current results suggest that an acute period of hyperglycemia after experimental TBI may improve some neurological outcomes and, importantly, it exerts no detrimental effects in a rat model of TBI.

The mixed results found in the current study are consistent with the majority of studies on the effects of induced hyperglycemia after experimental TBI. Slow infusion of large volumes of solutions containing 5% or 25% glucose for 18 h after closed head injury did not

affect neurological outcomes (composite neuroscore at 1 or 18 h) or brain edema at 18 h post-TBI in rats (Shapira et al., 1992). Rapid infusion of 5% dextrose in water (a hypo-osmolar solution) in rats with closed head injury was found to increase mortality (Feldman et al., 1995; Shapira et al., 1995), increasing brain edema (at 2 or 48 h) in some studies (Shapira et al., 1995; Talmor et al., 1998) but not another (Feldman et al., 1995). The 5% dextrose in water solutions were also reported to transiently worsen neuroscores in rats with or without TBI (Feldman et al., 1995; Shapira et al., 1995). Using treatment with 5% dextrose in 0.9% saline (a hyper-osmolar solution) after closed head injury in rats, induction of hyperglycemia had either no effect on brain edema (Talmor et al., 1998) or was found to decrease brain edema (at 4 h) with no effects on mortality rates, neurological scores or injury volume at 18 h post-TBI (Gurevich et al., 1997). A hyperglycemic dose of dextrose administered 5 min (but not 4 or 24 h) after moderate fluid percussion injury in rats did not affect contusion volume 72 h post-TBI, albeit damage was increased in 1 of 8 cortical slices examined (Kinoshita et al., 2002). In contrast, we previously reported that the same glucose regimen employed in the current study was able to reduce neuronal loss in cortex and hippocampus as well as improve cerebral metabolic rates for glucose (CMRGIc) in cortical and subcortical brain regions at 1 day post-CCI (Moro et al., 2013). Interestingly, these early benefits of induced hyperglycemia appear to have only minimal beneficial impact on neurological functions based on results of our beam-walking and neurological tests conducted 1 day post-CCI (see Fig. 1 to 5), consistent with results of Gurevich et al. (1997).

Studies examining more delayed effects of post-injury hyperglycemia or glucose treatments in TBI models are rare. Contusion volume and hippocampal neuronal loss 2 weeks after moderate-severe CCI injury were not altered in rats when hyperglycemia (30 min glucose infusion) was induced 20 min post-TBI (Cherian et al., 1998a). These results are congruent with our current findings for hilar neurons, but contrast with our finding of reduced cortical tissue loss at 2 weeks after glucose treatments. Low dose glucose (100 mg/kg, i.p.) administered daily for 10 days beginning 24 h after fluid percussion brain injury in rats did not alter cognitive outcome 11–15 days post-injury as assessed using a Morris water maze (MWM) test (Kokiko-Cochran et al., 2008). The latter findings are consistent with our results showing no working memory improvements 4–14 days post-CCI injury after early post-injury administration of high dose glucose. Improved neurological outcomes and reduced lesion size one month after glucagon treatment in mice with closed head injury was attributed to glucagon-induced reductions of glutamate (Fanne et al., 2011), albeit blood glucose was also increased by glucagon administration. To our knowledge, the only adverse effects of post-injury glucose treatment on delayed outcomes in an animal model of TBI is that of Cherian et al (1998b), where a moderate CCI was followed by secondary ischemia (temporary bilateral carotid artery occlusion). This study found that induction of hyperglycemia 20 min after CCI injury (20 min prior to ischemia) increased mortality, contusion volume and neuronal damage in hippocampus by 2 weeks post-injury. These authors also reported that beam-balance, beam-walking and MWM performance during the post-injury study period was worsened in the glucose-treated group compared to saline controls (Cherian et al., 1998b). Given the discrepant outcomes reported for post-injury glucose elevations in animal models of TBI, further studies will be required to establish if

any dosage of acute or prolonged glucose treatments after TBI can consistently improve neurological, behavioral and histological outcomes.

As in the studies above where glucose was administered after the induction of TBI, a mixture of null, positive and adverse effects have been reported in studies where hyperglycemia is induced prior to experimental TBI. Pre-injury infusion of glucose did not alter intracellular pH, ATP or intracellular free magnesium levels or the cytosolic phosphorylation potential over a 4 h period after fluid percussion brain injury in rats, and composite neuroscores at 1 and 2 weeks post-injury were not significantly altered by this pre-injury glucose treatment (Vink et al., 1997). Induction of hyperglycemia prior to a moderate CCI injury in rats did not alter contusion volume or hippocampal neuronal loss 2 weeks post-injury (Cherian et al., 1997), although pre-injury glucose administration in a more severe CCI injury was found to increase contusion volume at 2 weeks compared to saline controls, with no effects on neuronal loss in the hippocampus (Cherian et al., 1998a). When a moderate CCI injury was followed by secondary ischemia (temporary bilateral carotid artery occlusion), pre-injury glucose administration was found to increase contusion volume and to decrease numbers of viable hippocampal neurons (Cherian et al., 1997). Most recently, induction of hyperglycemia 15 min prior to CCI injury in mice was reported to have no effects on balance beam or foot fault tasks in the first week post-CCI, minimal effects (some improved memory of platform location) on MWM performance 2–3 weeks post-injury, and no effect on contusion volume 1 month post-injury (Hill et al., 2010). This pre-injury glucose treatment in mice also had no effect on cerebral edema assessed at 2 days post-CCI. In contrast, rats with streptozotocin-induced diabetes subjected to CCI injury were found to have significantly reductions in cerebral edema 2 days post-CCI, and if these hyperglycemic rats were given insulin to attenuate blood glucose levels after CCI they had increased cerebral edema at 2 days post-injury (Hill et al., 2010). The latter effect is consistent with reports that post-TBI insulin treatment to lower glucose in humans can lead to detrimental hypoglycemia, increased cerebral metabolic crisis and peri-ischemic cortical depolarizations (Bilotta et al., 2008, Hopwood et al., 2005, Oddo et al., 2008, Vespa et al., 2006, 2012). However, a recent multi-center trial found no difference in neurological outcome (at 28 or 90 days) for patients sustaining severe TBI who were treated with insulin to target blood glucose between 5.5 to 9 mmol/L compared to those with a blood glucose target of 4.4 to 6 mmol/L (Cinotti et al., 2014).

We believe that the current findings that acute glucose treatment can preserve some cortical tissue and improve some sensorimotor outcomes are consistent with the hypothesis that endogenous levels of metabolic fuels are insufficient to meet increased energy demands in the injured brain. Lactate or pyruvate treatments attenuate TBI-induced reductions in extracellular brain glucose (Chen et al., 2000; Fukushima et al., 2009) and increased concentrations of systemic glucose are also reported to increase interstitial levels of brain glucose after TBI (Diaz-Parejo et al., 2003; Stover et al., 2002). Provision of exogenous metabolic fuel early after TBI may thus improve outcomes, as found here for supplemental glucose and as previously reported for administration of lactate, pyruvate or ketone bodies (Alessandri et al., 2012; Appelberg et al., 2009; Chen et al., 2000; Davis et al., 2008; Deng-Bryant et al., 2011; Fukushima et al., 2009; Holloway et al., 2007; Moro and Sutton, 2010; Prins et al., 2005; Prins and Hovda, 2009; Rice et al., 2002; Shi et al., 2015; Su et al., 2011;

Zlotnik et al., 2008; 2012). Although the mechanisms by which exogenous glucose improved outcomes are uncertain it is possible that increased blood levels of glucose, as well as increased lactate or pyruvate resulting from peripheral glucose metabolism, ensured that sufficient concentrations of these biofuels were available to meet the increased cerebral metabolic demands after TBI. This could include use of these fuels to meet energy demands within neurons and glia, or increased consumption of glucose by astrocytes to support glycolysis or pentose-phosphate pathway demands, with shuttling of lactate from astrocytes to neurons for use as fuel (Pellerin et al., 2007; Takahashi et al., 2012). Increased shunting of glucose to the pentose-phosphate pathway has been reported after TBI in rats (Bartnik et al., 2005, 2007) and humans (Dusick et al., 2007), and a neuroprotective activation of the pentose-phosphate pathway with increased glutathione production has been reported in mixed astrocyte-neuronal cultures exposed to high glucose levels (Takahashi et al., 2012). We are currently determining the effects of acute glucose treatments on pentose-phosphate pathway activation after experimental TBI.

The glucose treatment regimen employed in the current study was previously found to reduce neuronal loss in cortex and hippocampus at 24 h post-CCI (Moro et al., 2013) and the current results indicate that cortical tissue was preserved whereas loss of hilar neurons in the hippocampus was not affected at 15 days post-CCI. The reasons for this transient neuroprotection of polymorphic neurons of the hilus and the persisting preservation of cortical tissue after glucose treatment are not clear. It is worth noting that the greatest sparing of cortical tissue was in anterior tissue sections (see Fig. 8B), perhaps indicating that cortex and hippocampus under the center of the impact injury were too severely damaged to benefit from these short-term glucose treatments. However, it is known that secondary injury cascades and tissue damage after TBI are progressive (Bramlett and Dietrich, 2007) and changes in gene expression are also time-dependent (Shimamura et al., 2005), and these factors may have contributed to the delayed cell loss in the hippocampus. Transient or temporary decreases in cortical injury volume have also been reported in experimental TBI models after acute treatments with minocycline (Bye et al., 2007) or pyruvate (Moro and Sutton, 2010). Given that a prolonged period of cerebral hypometabolism is a characteristic response to TBI (Bergsneider et al., 1997, 2000; Hovda et al., 1991; Kato et al., 2007; Sutton et al., 1994; Yoshino et al., 1991), future studies using more chronic or delayed biofuel supplementation after TBI are needed to determine if these treatments can improve long-term outcomes. In this regard, it has been reported that administration of glucose or pyruvate prior to testing in the first two weeks post-TBI can improve cognitive outcomes (Kokiko-Cochran et al., 2008; Moro et al., 2011).

In summary, although interpretations of the current study are limited due to the lack of a dose response and the use of a single glucose treatment regimen, the results do indicate that glucose administration early after experimental TBI is not detrimental. Moreover, the neuroprotective effects of early post-injury glucose treatments observed in cortex acutely after CCI injury (Moro et al., 2013) persist to at least 2 weeks post-CCI, and these results may underlie the moderate functional gains achieved in the same subjects.

4. Experimental procedures

4.1 Subjects and housing conditions

A total of 48 young (366.7 ± 4.6 g at time of surgery) male Sprague Dawley rats (Charles River Breeding Labs, Hollister, CA) were used for the study. Animals were housed in rat shoebox cages (2 per cage) and acclimated to vivarium conditions (70–76°F, 30–70% humidity, room lights on 06:00 to 18:00) for 1 week before initiation of experiment. Food (Teklad 7904) and tap water were available *ad libitum* throughout the experiment. All experimental procedures and protocols were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals and were approved by the UCLA Chancellor's Animal Research Committee. Efforts were made to minimize the number of animals used and to reduce pain and discomfort to the animals.

4.2 Pre-surgical behavioral training and group assignments

All rats were gentled by daily handling (15 min/day) for 1 week prior to behavioral testing. During the next week they were trained over 5 days on a beam-walking task (Moro and Sutton, 2010; Sutton and Feeney, 1992). For this test of skilled locomotor function each animal was trained to traverse a long (140 cm), narrow (1.7 cm) wooden beam that was elevated (36 cm) above a lab bench and ended with a dark goal box (20 cm wide, 25 cm long and 17 cm high). A 60 W light source positioned over the starting point of the beam provided the only illumination in the room during test sessions, and white noise (50–60 dB) and "prodding" (index finger tapping of the tail) were used to encourage locomotion if rats stopped walking. With exception of the first pre-surgical testing day, animals were given a single beam-walking trial on assigned testing days. Beam-walking performance was rated using a 7-point rating scale (Feeney et al., 1982; Sutton and Feeney, 1992) and time to transverse the beam and enter the goal box was also recorded. A trial was ended if the animal had not entered the goal within 60 sec. All trials were scored/rated by two investigators, at least one of whom was blinded to the drug treatment conditions, and in cases of rating discrepancies the average of both scores was used.

Three neurological exams to assess forelimb or hind limb sensorimotor functions were administered to animals on the last 2 days of pre-surgical beam training. Forelimb tactile placing was tested by lightly touching the dorsum of the left and right forepaw on the vertical edge of a lab bench and recording each reflexive lifting and placement of the forepaw on the bench surface (10 trials/limb/test day). Forelimb contraflexion was tested by rapidly picking the animal up by the base of the tail, with the head facing down to a lab bench surface. The angle formed between the left and right forelimbs was recorded (average of 2 trials/test day) to the nearest 15° angle. For testing of hind limb extension, the latency (60 sec maximum) for the animal to retract the left or right hind limb to its normal posture under the body after the hind paw was pulled laterally and posterior was recorded (average of 2 trails/test day). As for the beam-walk testing, in cases of rating discrepancies between two investigators the average of both scores was used.

Spatial working memory was tested once during the week preceding surgery, using a four-arm plus maze as we have previously described (Moro and Sutton, 2010; Moro et al., 2011;

Taylor et al., 2008). Each rat was placed into the open central space (25-cm diameter) of the black Plexiglas maze (12 cm high walls) facing one of four symmetrical open arms (10 cm wide × 25 cm long) that meet the central space at 45° angles, and allowed to explore freely for 15 min. Most rats will normally spontaneously alternate between all four arms of the maze during exploration periods, using spatial working memory to retain knowledge of previously entered arms of the maze (McNay et al., 2000). The number of arm entries (both hind limbs crossing a line between the maze center and one arm entrance) and the sequence of entries into each arm (denoted A, B, C and D) during the 15 min test were recorded. Memory performance was measured using the percent four/five alternation criterion (McNay et al., 2000). An alternation was counted when the rat visited each arm of the maze within a span of five arm choices (e.g., when all four arms were included within entries 1–5, entries 2–6, and so on for all subsequent spans of five arm choices). No alternation was counted if the animal did not enter all four arms within five sequential arm choices. The total possible number of alternations in this test is equal to the total number of arm entries minus four. The rats' percent four/five alternation (performance) score was calculated as: [number of alternations/(total number of alternations − 4)] × 100. Chance performance on this task is 45–50%.

The pre-surgical, baseline percent four/five alternation scores served as the basis for stratified random assignment of animals to the four experimental treatment conditions (N = 12 in each Sham or CCI injury group treated with glucose or saline). However, 8 animals (2 per group) failing to achieve pre-surgical alternation scores ≥56% were not tested for spatial working memory post-surgically.

4.3 Surgery and cortical contusion injury

Surgery to induce CCI was performed as previously described (Fukushima et al., 2009; Moro and Sutton, 2010; Sutton et al., 1993; Taylor et al., 2008). Animals were placed under general anesthesia (4% isoflurane in oxygen at 1.5 L/min) and after securing the head in a stereotaxic frame the isoflurane was reduced and maintained at 2% during surgery. The core body temperature was monitored continuously by a rectal probe and maintained at 37.0±1.0 °C with a thermostatically controlled heating pad (Harvard Apparatus Limited, Edenbridge, KY). After making a midline incision and exposing the skull a 6 mm diameter circular craniotomy, centered −3 mm from Bregma and 3.5 mm lateral (left) from the midline, was made using a high-speed drill under a surgical microscope. The CCI injury was produced using a pneumatically-driven (20 psi; 2.30–2.40 m/sec velocity) dual-stroke piston with a 5 mm diameter flat-tipped impactor which compressed the exposed dura mater and underlying brain to a depth of 2 mm for 250 msec. Sham injury controls underwent similar anesthetic and surgical interventions, excluding the craniotomy and CCI. After administering the first post-injury glucose or saline treatment, the scalp was sutured closed, bupivacaine (0.1–0.14 mg/kg, s.c.) was injected around the incision site, and rats were placed in a heated (36.0 to 38.0 °C) recovery cage until ambulatory.

4.4 Glucose or saline administration

Animals assigned to Sham-GIc (N = 12) or CCI-GIc (N = 12) conditions were given injections (i.p.) of a 50% solution of glucose in sterile 0.9% saline (Glc, 2 g/kg) at 0,1, 3 and

6 h post-injury. The animals assigned to Sham-Sal (N = 12) or CCI-Sal (N = 12) groups were injected (i.p.) with comparable volumes of either 0.9% saline (N = 6 per injury condition, vehicle control) or 8% saline (N = 6 per injury condition, osmolality control; Sugimori et al., 1996) at 0,1, 3 and 6 h post-injury. All treatment solutions were sterilized by passing them through a 0.22 μm filter prior to injection.

4.5 Post-surgical behavioral testing

Post-surgical beam-walk testing was performed every other day, starting 1 day post-injury and continuing until day 13. Neurological exams were conducted on days 1, 5, 9 and 13 post-injury, just prior to the beam-walk test on these days. Tests of spatial working memory were conducted on days 4, 9 and 14 post-surgery. On day 9 the cognitive test was conducted ~1 h after conclusion of the beam-walk test.

4.6 Histology

At 15 days post-injury rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused transcardially with heparinized 0.1 M phosphate buffered saline (PBS, pH 7.4) followed by 400 mL of 4% paraformaldehyde in 0.1 M PBS. Brains were extracted and post-fixed for one h in the paraformaldehyde solution (4 °C) and then cryoprotected in graded sucrose solutions (10%, 20% in PBS, pH 7.4, 24 h each) at 4 °C. Brains were frozen, sectioned in the coronal plane (20 μm, saving 2 adjacent sections every 500 μm) and collected onto gelatinized microscope slides. Tissue sections were stained for thionin, cleared in Citrisolv (Fisher Scientific, Fair Lawn, NJ, USA), and cover-slipped using Cytoseal 60 (Richard Allan Scientific, Kalamazoo, MI, USA).

The area of the non-damaged cortical mantle lying dorsal to the rhinal sulcus and corpus callosum was measured bilaterally in 10 thionin-stained sections (every 1,000 μm) between +1.6 and −7.4 mm from Bregma using ImageJ software (version 1.42q; National Institutes of Health, Bethesda, MD). Cortical tissue volume in each hemisphere was calculated and, after verifying no significant group differences in the right/contralateral hemisphere, percent tissue volume loss in the left/injured cortex was calculated [100−((Left/Right) × 100)], as previously described (Fukushima et al., 2009; Moro and Sutton, 2010; Moro et al., 2011; Taylor et al., 2008).

Cell counts of surviving polymorphic neurons in the left and right dentate hilus of the dorsal hippocampus were performed on 3 tissue section (−2.8, −3.3 and −3.8 mm from Bregma) in 8 randomly selected rats from each experimental group. Counts were performed using bright-field illumination with a Leica microscope interfaced with a computer running StereoInvestigator software (MicroBrightField, Inc., Williston, VT). Contours for the dentate hilus on each tissue section included all tissue lying between the inner/outer blades of the dentate granule cells with exception of the $CA3_C$ pyramidal cell layer, as previously described (Moro and Sutton, 2010; Taylor et al., 2010). Cell density counts (cells/mm2) were made for all neurons lying within each left and right contour, summed for each animal, and these values were used to calculate neuronal loss in the left hilus as a percent of neurons in the right hilus [100−((Left/Right) × 100)].

4.7 Statistical analysis

All group data are expressed as the mean ± standard error of the mean (SEM), and all statistical analyses were performed using SPSS software (version 20: SPSS Inc., Chicago, IL, USA). Statistical significance was accepted with a 2-tailed $p<0.05$. The data were analyzed using analysis of variance (ANOVA), with repeated measures as appropriate. In cases of significant main or interaction effects for 2 × 2 ANOVAs, between group effects were further determined using planned comparisons.

Prior to analyzing data for glucose treatment effects, we conducted separate analyses to determine if treatments with 0.9% saline versus 8% saline differentially affected behavioral or histology outcomes in sham or CCI injury groups. No significant effects for the type of saline treatment were found for any dependent variable, so these data were combined for subsequent analyses of Treatment effects.

Acknowledgments

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Abbreviations

ANOVA	analysis of variance
ATP	adenosine triphosphate
CCI	controlled cortical impact
CMRGlc	cerebral metabolic rates of glucose
Glc	glucose (50%)
MWM	Morris water maze
PBS	phosphate buffered saline
Sal	saline
SEM	standard error of the mean
TBI	traumatic brain injury

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Highlights

- Four glucose treatments within 6 h after TBI improved hind limb extension latencies in rats.
- Glucose-treated rats had mild, non-significant, improvements in beam-walking and forelimb tactile placing ability after TBI.
- Glucose treatments after TBI did not affect forelimb contraflexion or spatial working memory.
- Four glucose treatments reduced cortical injury volume 15 days post-TBI.
- Glucose treatments after TBI did not alter loss of hilar neurons in the hippocampus.

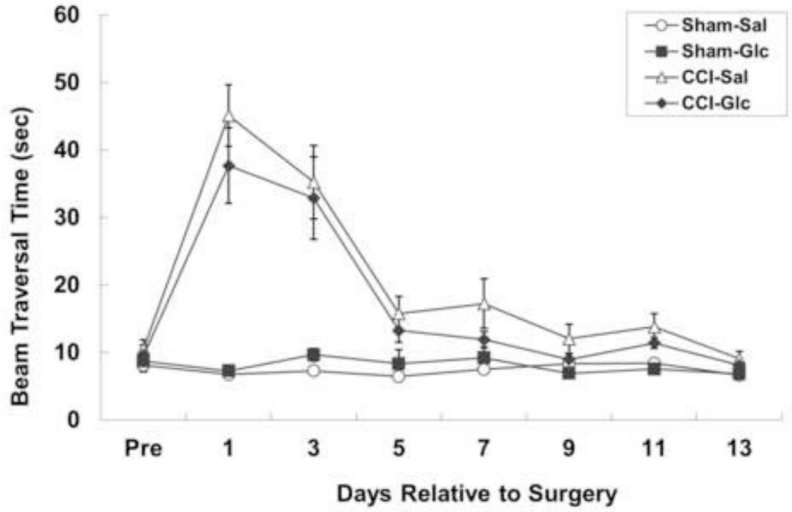

Fig. 1.

Mean (± SEM) beam traversal times for rats (n = 12/group) before (Pre) and after sham or controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. There was a significant effect of Injury ($p<0.001$), Days ($p<0.001$), and the Injury x Day interaction ($p<0.001$), with no effects for Treatment or Treatment interactions.

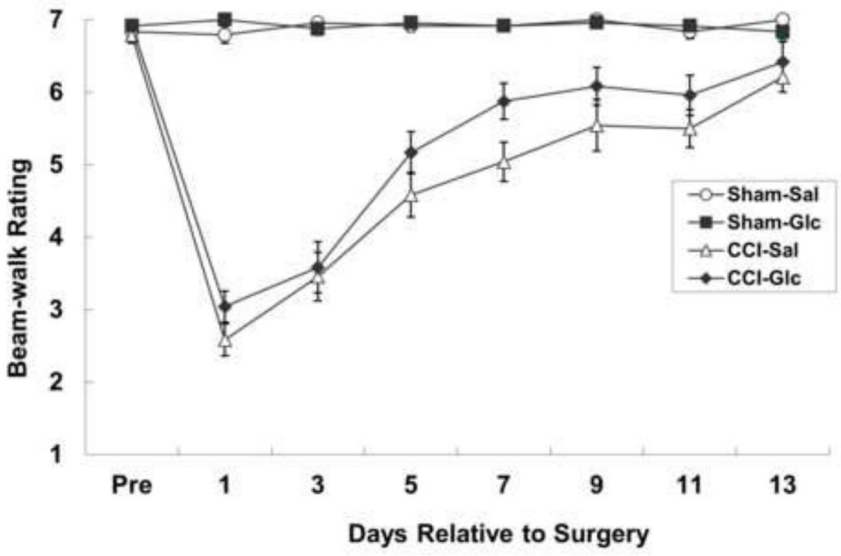

Fig. 2.

Mean (± SEM) beam-walk ratings for rats (n = 12/group) before (Pre) and after sham or controlled cortical impact (CCI) injury and treatments (at 0, 1, 3 and 6 h post-surgery) of either saline (Sal) or glucose (Glc). There was a significant effect of Injury ($p<0.001$), Days ($p<0.001$), and the Injury x Day interaction ($p<0.001$), with no effects for Treatment or Treatment interactions.

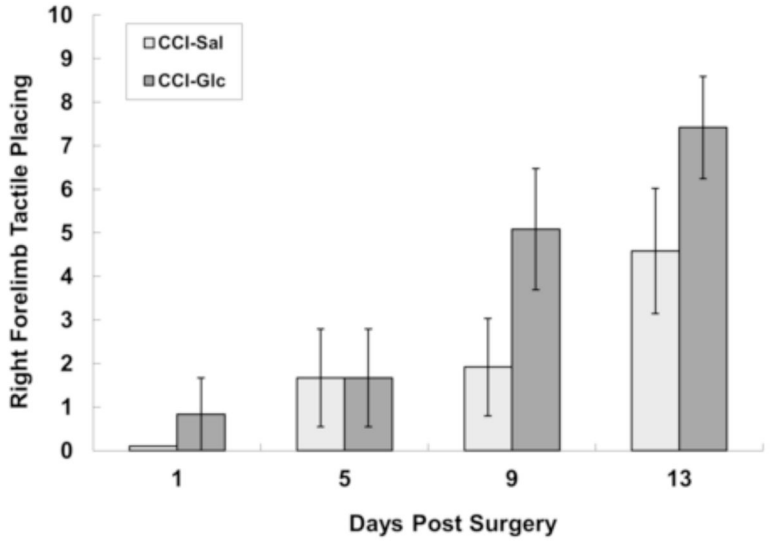

Fig. 3.

Mean (± SEM) tactile placing of the right forelimb for rats (n = 12/group) after controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. There was a significant effect of Days ($p<0.001$), but no significant effect for Treatment or the Day x Treatment interaction.

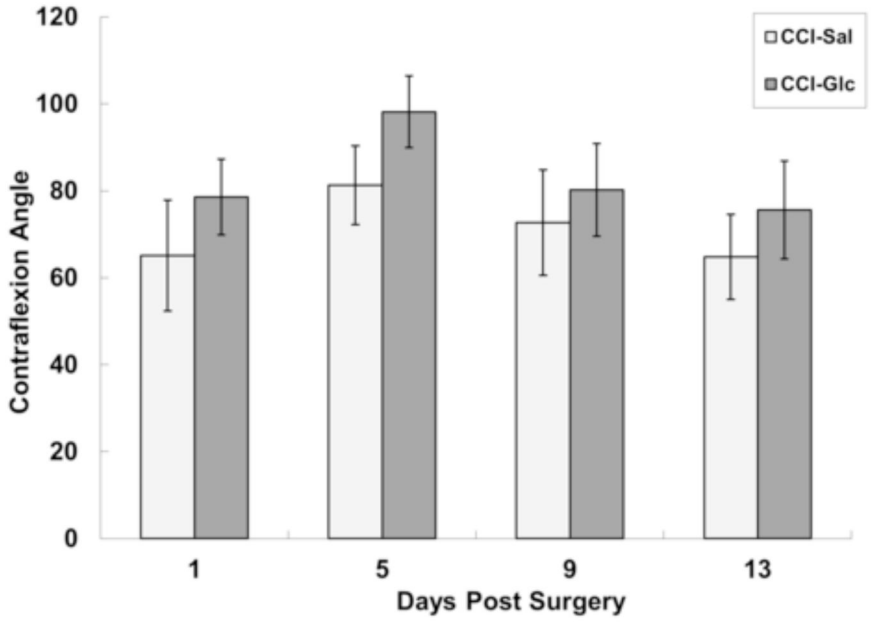

Fig. 4.

Mean (± SEM) ratings of contraflexion for rats (n = 12/group) after controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. There was a significant effect of Days ($p<0.05$), with no significant effects for Treatment or the Day x Treatment interaction.

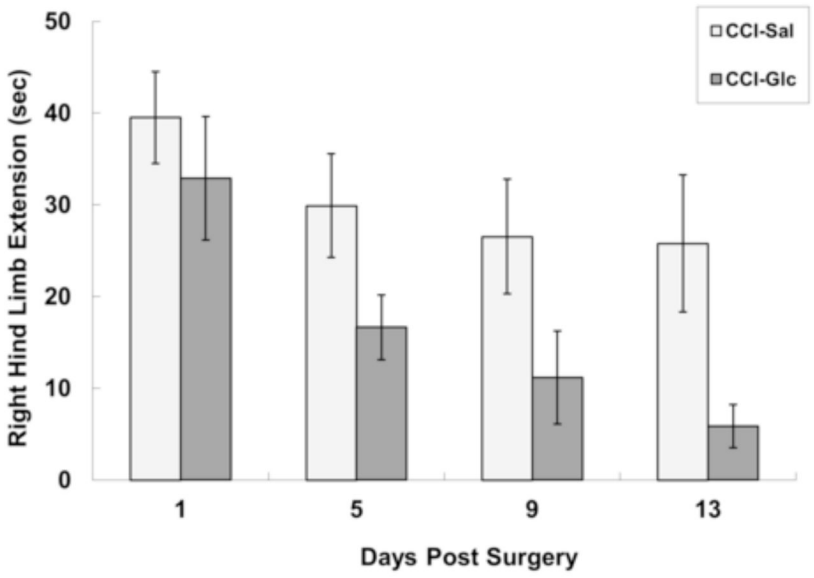

Fig. 5.

Mean (± SEM) duration to retract the right hind limb after lateral/posterior displacement in rats (n = 12/group) with controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. There was a significant effect of Days ($p<0.001$) and Treatment ($p<0.05$), with no significant Day x Treatment interaction.

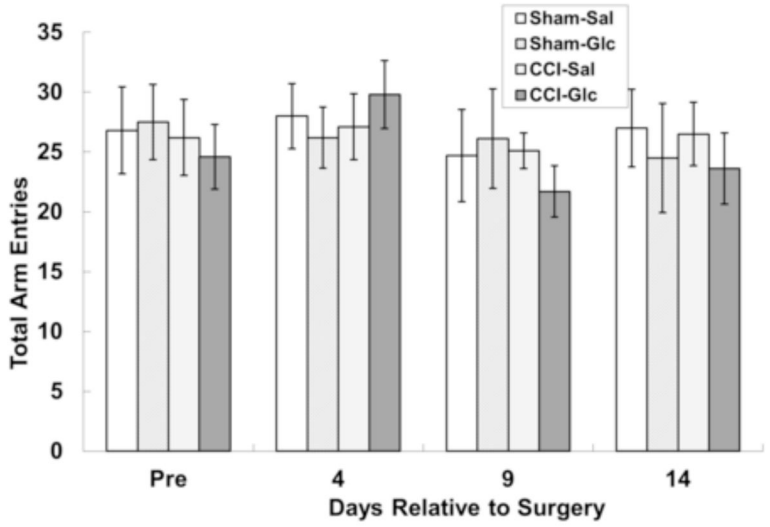

Fig. 6.

Mean (± SEM) number of arm entries during pre- and post-surgical tests of spatial working memory for rats (n = 10/group) with sham or controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. There was a significant effect of Days ($p<0.001$; day 9 less than day 4, $p<0.01$), with no effects of Injury or Treatment and no significant interactions.

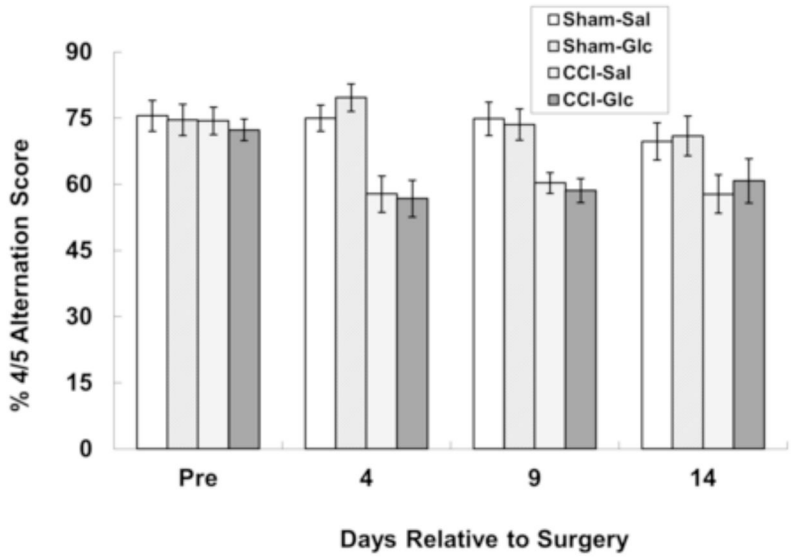

Fig. 7.

Mean (± SEM) percent four/five (% 4/5) alternation scores during pre- and post-surgical tests of spatial working memory for rats (n = 10/group) with sham or controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. There was a significant effect of Injury ($p<0.001$), but no significant effects for Days, Treatment, or for any interactions.

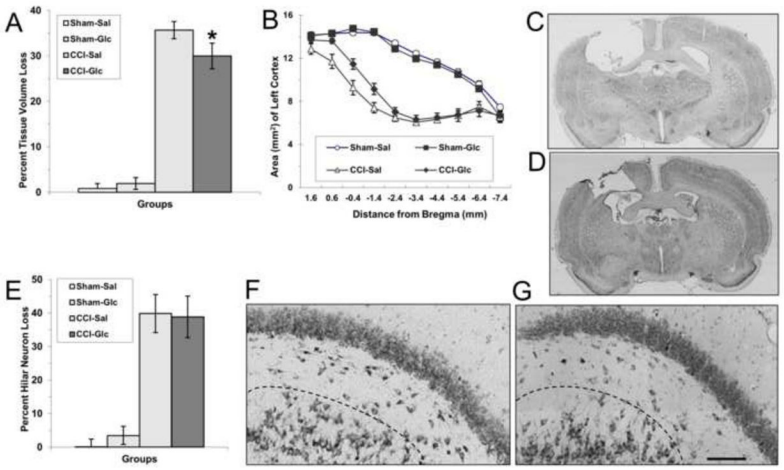

Fig. 8.

Mean (± SEM) percent tissue volume loss in the left/injured cortex (**A**) and mean (± SEM) cortical area at 10 equally spaced coronal sections (**B**) for rats (n = 12/group) with sham or controlled cortical impact (CCI) injury and treatments of either saline (Sal) or glucose (Glc) at 0, 1, 3 and 6 h post-surgery. Cortical tissue volume loss was reduced for CCI-Glc compared to CCI-Sal (* $p<0.05$). Photomicrographs from animals representing group means for cortical area measures at −1.4 mm from Bregma are shown for CCI-Sal (**C**) and CCI-Glc (**D**). The mean (± SEM) percent neuronal loss in the left hilus is shown for the four treatment groups (n = 8/group) in panel **E**. High magnification photomicrographs (scale bar of 100 μm) show polymorphic hilar neurons between the CA3c pyramidal cells (dashed line) and the inner blade of the dentate granule cells in sham injury (**F**) and CCI injury (**G**) conditions.