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Pyruvate treatment attenuates cerebral metabolic depression and neuronal loss after experimental traumatic brain injury

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Abstract

Experimental traumatic brain injury (TBI) is known to produce an acute increase in cerebral glucose utilization, followed rapidly by a generalized cerebral metabolic depression. The current studies determined effects of single or multiple treatments with sodium pyruvate (SP; 1000 mg/kg, i.p.) or ethyl pyruvate (EP; 40 mg/kg, i.p.) on cerebral glucose metabolism and neuronal injury in rats with unilateral controlled cortical impact (CCI) injury. In Experiment 1 a single treatment was given immediately after CCI. SP significantly improved glucose metabolism in 3 of 13 brain regions while EP improved metabolism in 7 regions compared to saline-treated controls at 24 h post-injury. Both SP and EP produced equivalent and significant reductions in dead/dying neurons in cortex and hippocampus at 24 h post-CCI. In Experiment 2 SP or EP were administered immediately (time 0) and at 1, 3 and 6 h post-CCI. Multiple SP treatments also significantly attenuated TBI-induced reductions in cerebral glucose metabolism (in 4 brain regions) 24 h post-CCI, as did multiple injections of EP (in 4 regions). The four pyruvate treatments produced significant neuroprotection in cortex and hippocampus 1 day after CCI, similar to that found with a single SP or EP treatment. Thus, early administration of pyruvate compounds enhanced cerebral glucose metabolism and neuronal survival, with 40 mg/kg of EP being as effective as 1000 mg/kg of SP, and multiple treatments within 6 h of injury did not improve upon outcomes seen following a single treatment.

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Keywords

^{14}C-2DG; Controlled cortical impact; Fluoro-Jade B; Pyruvate; Rat

1. Introduction

Traumatic brain injury (TBI) induces an immediate increase in neuronal depolarization and a concomitant increase in energy demand, which can persist for minutes to hours after experimental TBI. These effects are reflected in the elevated cerebral metabolic rates of glucose (CMRGlc) and anaerobic glycolysis (Katayama et al., 1990; Lee et al., 1999; Sutton et al., 1994; Yoshino et al., 1991) and by reduced levels of extracellular glucose and elevated concentrations of extracellular lactate (Chen et al., 2000; Fukushima et al., 2009; Krishnappa et al., 1999). This relatively brief period of hyperglycolysis is followed by a more prolonged (days to weeks) period of reduced CMRGlc (Dunn-Meynell and Levin, 1995; Moro et al., 2011; Prins and Hovda, 2009; Sutton et al., 1994; Yoshino et al., 1991) and increased shunting of glucose to the pentose-phosphate pathway occurs within hours of TBI (Bartnik et al., 2005, 2007). In TBI patients the durations of elevated CMRGlc, low levels of extracellular glucose with elevated lactate, and cerebral metabolic depression are generally more protracted than those observed in experimental TBI models (Bergsneider et al., 1997, 2000; Vespa et al., 2003).

The mismatch between cerebral metabolic or energy demands in the face of decreased levels of glucose, the primary energy source for brain cells, has led several investigators to consider early administration of supplemental fuels to meet the cerebral metabolic demands and/or to avoid “energy crisis” after TBI. Studies have now shown that providing experimental TBI subjects with exogenous 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) or ketone bodies (Appelberg et al., 2009; Deng-Bryant et al., 2011; Prins and Hovda, 2009; Prins et al., 2004) can improve various measures of cerebral metabolism, neuronal survival and/or neurological outcomes. Our group has also shown that early provision of high doses of glucose in rats can attenuate TBI-induced decreases in CMRGlc and reduce neuronal injury, while providing mild sensorimotor behavioral improvements (Moro et al., 2013; Shijo et al., 2015).

The current studies in rats with unilateral controlled cortical impact (CCI) injury were undertaken to determine the effects of acute administration of pyruvate compounds on CMRGlc and neuronal viability 24 h post-injury. These studies were conducted concomitantly with those to evaluate effects of glucose on these same measures (Moro et al., 2013), and the data for saline-treated controls here is the same as for those studies. Here we compare treatment with sodium pyruvate (SP; 1000 mg/kg, i.p.) with ethyl pyruvate (EP; 40 mg/kg, i.p.), a more lipophilic and electrically neutral derivative of pyruvic acid that is capable of entering cells without use of the monocarboxylate transporters (Kao and Fink, 2010; Tokumaru et al., 2009). We and others have previously shown that EP at 20-40 mg/kg is at least as neuroprotective as SP at doses of 500-1000 mg/kg (Kim et al., 2005; Lee et al., 2001; Moro and Sutton, 2010; Yu et al., 2005). In Experiment 1 we evaluated the effects of a

single treatment with SP or EP immediately following injury, to evaluate the hypothesis that supplemental pyruvate could meet the acute energy demands and attenuate neuronal injury after TBI. Experiment 2 was conducted to determine the effects of multiple SP or EP injections, given immediately (time 0) and then at 1, 3 and 6 h post-CCI. The multiple treatment protocol was employed based on evidence of CCI-induced energy demands persisting to at least 2 h post-injury (Lee et al., 1999), three acute SP treatments were needed to reduce contusion volume 2 weeks post-CCI (Fukushima et al., 2009), and post-TBI depolarization or neuronal hyperexcitability may prolong any mismatch between fuel supply and energy demands (Griesemer and Mautes, 2007; Hashemi et al., 2009; Lauritzen et al., 2011; Vespa et al., 2007; Hopwood et al., 2005).

2. Results

2.1 Experiment 1: single saline or pyruvate treatment

2.1.1. Physiological data—As shown in the data of Table 1, there was a significant loss in body weight within 24 hrs of injury for all CCI groups compared to Sham-SAL controls (p's < 0.05). The largest decline in body weight occurred in the CCI-SP group, where the weight change was significantly greater than that observed for CCI-SAL and CCI-EP groups (p's < 0.05). Also shown in Table 1 are the data for arterial blood gasses and plasma concentrations of glucose and lactate at 24 h post-injury, prior to the [^{14}C]2-deoxy-D-glucose (14C-2DG) injection. All of these physiological measures were within normal ranges and there were no significant differences between the 4 treatment groups.

2.1.2. Cerebral glucose utilization—As detailed in our prior publication (Moro et al., 2013), three shipments of ^{14}C-2DG that differed in their specific activities were used during these experiments, resulting in variable CMRGlc values (μmol/100 g/min) within each group. However, data analysis showed no effect of CCI injury on CMRGlc in right hemisphere structures in subsets of animals injected with the same lot number of 2DG, and treatment effects were similar for ipsilateral (left hemisphere) CMRGlc data and for CMRGlc asymmetry scores [(left − right/left + right) × 100] (Moro et al., 2013). Therefore, regional cerebral glucose utilization in the current experiments was analyzed based on calculated CMRGlc asymmetry scores. As illustrated in Fig. 1, these asymmetry scores for the Sham-SAL control group (n=10) were near zero in all brain regions at 24 h post-surgery, indicating the normally similar metabolism between hemispheres. The reductions in ipsilateral CMRGlc at 24 h after left hemisphere injury produced significantly negative asymmetry values in all brain regions in the three CCI groups compared to Sham-SAL (p's < 0.001). The magnitude of metabolic depression was generally larger in brain regions of CCI-SAL (n=9) compared to CCI-SP (n=10) or CCI-EP (n=9) groups. The single injection of either SP or EP after CCI injury significantly improved CMRGlc asymmetry scores in the midline peri-contusional cortex (p's < 0.05) and occipital cortex (p's < 0.05), as well as for the dorsal lateral geniculate (p's < 0.001). In addition, relative to CCI-SAL controls, improved (i.e., less negative) CMRGlc asymmetry scores by EP treatment after CCI were significant for the parietal ($p < 0.05$), auditory ($p < 0.05$), temporal ($p < 0.05$) and entorhinal cortex ($p < 0.01$), as well as for the amygdala ($p < 0.01$). Although glucose utilization was

improved relative to CCI-SAL in more regions after EP treatment, the CCI-SP and CCI-EP asymmetry scores did not differ significantly for any brain region.

2.1.3. Injury severity and neuronal injury in cortex and hippocampus—Ratings of tissue swelling at the contusion site post-injury were used as a measure of CCI injury severity. These ratings did not differ significantly ($p > 0.05$, 1-way ANOVA) for animals in the CCI-SAL (1.7 ± 0.15; n=9), CCI-SP (1.5 ± 0.13; n=10) or CCI-EP (1.7 ± 0.15; n=9) groups.

Fluoro-Jade B-positive (FJB+) neuronal profiles were counted in peri-contusional cortex and in the CA3 and hilus ipsilateral to injury (see Fig. 2). No FJB+ cells were detected in either the left cortex or hippocampus of Sham-SAL control rats. Numerous FJB+ neurons were apparent within cortex and hippocampus ipsilateral to CCI at 24 h post-injury. Cell density counts for FJB+ cells within the peri-contusional cortex and within the ipsilateral hilus and CA3 subsector of the hippocampus of CCI rats with single treatments (n=8/group) are shown in Fig. 3. Compared to the CCI-SAL group, FJB+ neurons were significantly reduced in the CCI-SP and CCI-EP groups for the peri-contusional cortex (p's < 0.05), hilus (p's <0.05) and CA3 (p's < 0.01). FJB+ neurons did not differ significantly between CCI-SP and CCI-EP groups for any of these brain regions.

2.2 Experiment 2: multiple saline or pyruvate treatments

2.2.1 Physiological data—As shown in the data of Table 2, Sham operates receiving injections of SAL, SP or EP at 0, 1, 3 and 6 h post-surgery lost an average of 5 to 7 g body weight within 24 h, an effect not seen with the single SAL injection (see Table 1). All CCI groups lost more body weight than their Sham injury counterparts, with CCI-SAL and CCI-EP rats losing almost twice that of Sham-SAL and Sham-EP controls (p's < 0.05). The largest decline in body weight occurred in the CCI-SP group, which lost almost three times the weight lost by Sham-SP controls ($p < 0.001$) and the weight change in the CCI-SP group was significantly greater than that observed for CCI-SAL ($p < 0.05$). Data for pH, arterial blood gasses and plasma concentrations of glucose and lactate at 24 h post-injury (Table 2) were all within normal ranges prior to the 24 h post-injury 14C-2DG injection. The whole blood pH was higher ($p < 0.05$) and the plasma glucose concentration was lower ($p < 0.05$) in CCI-SAL compared with Sham-SAL controls, while whole blood pO_2 was significantly higher ($p < 0.05$) in CCI-EP compared to the Sham-EP group.

2.2.2 Cerebral glucose utilization—The CMRGlc asymmetry scores of Sham-SAL (n=10), Sham-SP (n=8) and Sham-EP (n=8) controls were quite small in all brain regions, and they did not differ (p's > 0.05, 1-way ANOVA) between Sham control groups in any brain region (see Table 3). Therefore, data for the three Sham injury groups were pooled to form a single SHAM injury group (n=26), as illustrated in Fig. 4. Reductions in CMRGlc ipsilateral to CCI produced significantly negative asymmetry values in all brain regions relative to SHAM controls by 24 h post-injury (Fig. 4, p's < 0.001). As in Experiment 1, the CMRGlc asymmetry scores were generally larger in brain regions of CCI-SAL (n=10) compared to CCI-SP (n=10) or CCI-EP (n=10) groups. The four injections of either SP or EP after CCI injury significantly improved CMRGlc asymmetry scores in the auditory

cortex (p's < 0.05) and for the dorsal lateral geniculate (p's < 0.01) compared to CCI-SAL. In addition, relative to CCI-SAL controls, the improved CMRGlc asymmetry scores by EP treatments after CCI were significant for the parietal ($p < 0.05$) and temporal cortex ($p < 0.05$) and SP treatments improved metabolism in the ventral thalamus ($p = 0.51$). Reduced metabolic depression was also found in the dentate gyrus of CCI-SP rats compared to both the CCI-SAL ($p < 0.01$) and CCI-EP ($p < 0.05$) groups.

2.2.3 Injury severity and neuronal injury in cortex and hippocampus—Ratings of the tissue swelling immediately post-CCI did not differ significantly ($p > 0.05$, 1-way ANOVA) between the CCI-SAL (1.6 ± 0.05; n=10), CCI-SP (1.7 ± 0.11; n=10) and CCI-EP (1.5 ± 0.14; n=10) groups, suggesting equivalent injury severity.

FJB+ neuronal profiles were counted as in Experiment 1 (see Fig. 2). No FJB+ cells were detected in the left midline neocortex or in the left hippocampus of rats in the Sham-SAL, Sham-SP or Sham-EP groups. Cell density counts for FJB+ cells in the peri-contusional cortex and in the ipsilateral hilus and CA3 subsector of the hippocampus of CCI rats with multiple treatments (n=8/group) are shown in Fig. 5. Compared to the CCI-SAL group, FJB + neurons were significantly reduced in the CCI-SP and CCI-EP groups for the peri-contusional cortex (p's < 0.05), hilus (p's <0.05) and CA3 (p's < 0.01). FJB+ neuronal counts did not differ significantly between CCI-SP and CCI-EP groups for any of these brain regions.

3. Discussion

Key findings of Experiment 1 were that a single injection of SP (1000 mg/kg, i.p.) immediately after a lateral CCI injury significantly attenuated TBI-induced reductions in ipsilateral cerebral glucose utilization in 2 of 6 cortical regions and in 1 of 7 subcortical regions, whereas a single injection of EP (40 mg/kg, i.p.) increased cerebral glucose utilization in 5 cortical regions and in 2 subcortical regions at 24 h post-injury. Both of these pyruvate compounds exerted significant neuroprotection within the peri-contusional cortex, hilus, and CA3 at this time point. When 4 injections of pyruvate were administered at 0, 1, 3 and 6 h post-CCI in Experiment 2, SP was found to enhance glucose utilization in 1 of 6 ipsilateral cortical regions and in 3 of 7 subcortical regions while EP improved glucose utilization in 3 cortical regions and 1 subcortical region. The multiple pyruvate treatments were also found to exert significant neuroprotection in both cortex and hippocampus, similar to results found with single treatment. Overall, the results of the current studies with pyruvate and our prior work with glucose administration (Moro et al., 2013) indicate that provision of metabolic fuels during the early period of increased metabolic demands after TBI is sufficient to attenuate neuronal loss and to improve cerebral metabolism at 24 h post-CCI. A single administration of supplemental fuel early post-injury would appear sufficient for short-term neuronal survival and improved metabolism, as neither the pyruvate compounds nor glucose showed additional benefits of multiple treatments within the first 6 h post-CCI. However, the cortical neuroprotection provided 6 h post-CCI after a single injection of SP did not translate to reduced cortical contusion volume 2 weeks post-injury, and three SP treatments (at 5, 65 and 125 min) were required to reduce contusion volume 2 weeks post-CCI (Fukushima et al., 2009). Further research in timing and dosage of the

various biofuels currently under investigation for improving outcomes from TBI will be needed to find the optimal treatment conditions to improve chronic histopathology, metabolism and neurobehavioral outcomes.

The current findings that SP or EP treatment improved cerebral glucose metabolism are congruent with our prior report that these pyruvate compounds administered at 1, 12 and 24 h post-injury attenuated CCI-induced reductions in cerebral cytochrome oxidase activity, a measure of cerebral oxidative capacity, 3 days post-CCI (Moro and Sutton, 2010). These metabolic effects of pyruvate likely contribute to improved neurobehavioral outcomes reported for SP or EP treatments after TBI (Moro and Sutton, 2010; Shi et al., 2015; Su et al., 2011; Zlotnik et al., 2008, 2012), as functional recovery has been shown to parallel improvements in CMRGlc after TBI in rats (Dunn-Meynell and Levin, 1995; Moore et al., 2000; Prins and Hovda, 2001, 2009) and humans (Humayun et al., 1989; Nakashima et al., 2007; Nakayama et al., 2006) and increased cytochrome oxidase activity is associated with improved neurological or cognitive performance (Conejo et al., 2007; Hovda et al., 1987; Sutton et al., 2000; Wrubel et al., 2007). Other reported metabolic effects of exogenous pyruvate include improvements of mitochondrial redox states (NAD/NADH) which facilitate oxidative phosphorylation and the glutathione redox cycle (NADP/NADPH) for counteracting oxidative stress (Alvarez et al., 2003; Kashiwagi et al., 1997; Lee et al., 2004; Mongan et al., 2001, 2002; Sharma et al., 2003), stimulation of pyruvate dehydrogenase activity (Mongan et al., 2003; Sharma et al., 2009) and improvements in cell energy function and adenosine triphosphate production (Izumi and Zorumski, 2010; Zeng et al., 2007).

In addition to their metabolic effects, previously described anti-oxidant and anti-inflammatory properties of SP and EP likely contribute to the neuronal protection provided by administration of these pyruvate compounds (Das, 2006; Kao and Fink, 2010; Kim et al., 2005; Lee et al., 2004; Mongan et al., 2003; Moro and Sutton, 2010; Sharma et al., 2009; Shi et al., 2015; Su et al., 2011; Yu et al., 2005). Neural protection provided by SP has also been attributed to its ability to inhibit zinc accumulation after experimental transient ischemia (Lee et al., 2001) or kainate-induced seizures (Kim et al., 2007), as well as to ability to scavenge blood glutamate in a rodent model of TBI (Zlotnik et al., 2008, 2012). These combined, multifactorial properties of SP and EP make them interesting candidates for further studies in the treatment of TBI.

Although the current studies did not find significant differences between CCI-SP and CCI-EP groups for neuroprotection, and multiple SP treatments improved glucose utilization in the dentate gyrus compared to the multiple EP treatments, there is some indication that EP imparted more metabolic benefit by 24 h post-CCI than did SP. That is, SP improved cerebral glucose utilization in 3-4 of 13 brain regions after single or multiple treatments while EP improved metabolism in 7 regions after a single treatment and in 4 regions after multiple treatments. These indications of EP being superior to SP treatment are consistent with similar effects reported for EP-induced neuroprotection at 72 h and cognitive improvements at 1 week post-CCI compared to SP (Moro and Sutton, 2010). It is also worth noting that weight loss in CCI-SP rats was always greater than that of the CCI-EP groups. We believe this was due to the increased acidity of the SP compared to EP concentrations used, as we have observed a rapid drop in pH of SP at 400 mg/ml (but not EP at 16 mg/ml)

in phosphate buffered saline over the first few minutes after preparation (unpublished data). The data showing similar tissue swelling in all CCI groups suggest the greater weight loss after SP treatments was not due to differences in injury severity. These factors combined with reports that SP is not stable in aqueous solutions and EP is 10- to 100-fold more potent than pyruvate (Kao and Fink, 2010), EP is more effective than SP for reducing inflammatory markers in microglia (Yu et al., 2005) and for attenuating apoptosis and reductions in adenosine triphosphate and N-acetylaspartate in cortical slices exposed to hydrogen peroxide (Zeng et al., 2007), suggest that EP may be a more favorable candidate than SP for future TBI studies.

In summary, while both forms of pyruvate produced significant attenuation of cerebral metabolic depression and prevented cell loss after CCI injury, there were no added benefits in giving multiple treatments within 6 h of injury compared to the outcomes seen following a single treatment. The wider regional improvements in glucose metabolism indicate that EP treatment is marginally more efficacious compared to SP treatment.

4. Experimental procedures

4.1. Subjects

A total of 94 young adult male Sprague Dawley rats (289-422 g) from Charles River Breeding Labs (Hollister, CA) were used for the studies. Animals were pair-housed in rat shoebox cages and acclimated to vivarium conditions (temperature range 70-76°F; 30-70% humidity; 12:12 h light:dark cycle with lights on at 06:00 h) for 1 week before initiation of experiments, with food (Teklad 7904) and tap water available *ad libitum*. 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.

4.2 Surgery for CCI or sham injury

Surgery to induce CCI or sham injury was performed as previously described (Fukushima et al., 2009; Moro and Sutton, 2010; Sutton et al., 1993). After induction of general anesthesia (4% isoflurane in oxygen at 1.5 L/min) and shaving of the head, animals were secured in a stereotaxic frame and isoflurane was maintained at 2% during surgery. Core body (rectal) temperature was monitored continuously and maintained at 37.0±1.0 °C with a thermostatically controlled heating pad (Harvard Apparatus Limited, Edenbridge, KY). For animals randomized to CCI injury conditions a 6 mm diameter circular craniotomy (centered −3 mm from Bregma and 3.5 mm left of the midline) was made and injury was produced using a 5 mm diameter flat-tip impactor (20 psi; 2.32 m/sec velocity, 2 mm tissue compression for 250 msec). Sham injury controls underwent similar anesthetic and surgical interventions, excluding the craniotomy and CCI. After injury 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 recovery cage until ambulatory.

4.3. Drug preparation and experimental groups

All animals used in the experiments were randomly assigned to injury and drug treatment conditions, with Experiment 1 and Experiment 2 conducted sequentially. Control animals with sham or CCI injury received injection (i.p.) of 8% saline (SAL), as previously reported in our glucose treatment studies (Moro et al., 2013). All other animals in the studies were randomized to receive injection (i.p.) of either sodium pyruvate (SP; 1000 mg/kg) or ethyl pyruvate (EP; 40 mg/kg). The SP (P2256: Sigma Aldrich, Saint Louis, MO) was prepared at a concentration of 400 mg/ml and EP (E47808: Sigma Aldrich) was prepared at 16 mg/ml, each compound being dissolved in 0.1 M phosphate buffered saline and filtered (0.22 μm) just prior to injection.

Rats entered into the 4 groups for Experiment 1 (Sham-SAL, n=10; CCI-SAL, n=9; CCI-SP, n=10; CCI-EP, n=9) had a single injection of SAL, SP or EP administered immediately after induction of Sham or CCI injury.

Rats in the groups for Experiment 2 (Sham-SAL, n=10; Sham-SP, n=8; Sham-EP, n=8; CCI-SAL, n=10; CCI-SP, n=10; CCI-EP, n=10) were injected with SAL, SP or EP at 0, 1, 3 and 6 h after induction of Sham or CCI injury.

4.4. Cerebral glucose utilization

At 22 h post-injury animals were anesthetized with isoflurane and aseptic surgery was performed to place catheters (PE-50; Becton, Dickinson, NJ) in the right femoral vein and artery, the skin was sutured closed and the incision site was infiltrated with bupivacaine (0.1-0.14 mg/kg, s.c.). Each rat was then restrained on a cardboard plank to reduce hindlimb movements, and maintained in dim light and quiet room conditions for a 2 h period to allow recovery from anesthesia. At 24 h post-injury baseline arterial blood samples were collected for measures of blood gasses (using either a 238 pH/Blood Gas Analyzer, Ciba Corning Diagnostics Ltd, Halstead, UK or a Siemens Rapidpoint 340, Healthcare Diagnostics Inc, Plainfield, IN) and plasma glucose and lactate levels (2700 Select Biochemistry Analyzer). ^{14}C-2DG was then administered (120 μCi/kg, i.v.; American Radiolabeled Chemicals Inc, St. Louis, MO) over a 30 sec interval and 12 timed arterial blood samples were collected over the subsequent 45 min period. Plasma from these samples were assayed for ^{14}C activity on a scintillation counter (LS-6500; Beckman Coulter, Brea, CA) and for glucose levels. At 45 min after the ^{14}C-2DG infusion rats were given a lethal dose of sodium pentobarbital (100 mg/kg, i.v.) and their brains were rapidly removed and frozen in 2-methylbutane at −55 °C. Coronal brain tissue sections (20 μm) were cut (−20 °C) and collected onto glass coverslips that were exposed to Kodak Biomax film with 14C-methacrylate standards (Amersham, Arlington Heights, IL) for 2-3 days. Developed images were digitally captured with a flat-bed scanner (256 dpi, 8 bit gray scale). Image data were calibrated in ImageJ software (version 1.42q: National Institutes of Health, Bethesda, MD) using the brain standards and the plasma ^{14}C input curve using the equations of Sokoloff et al. (1977) so that the gray scale values were transformed to units of CMRGlc (μmol/100 g/min) for each brain region of interest (ROI), which were measured bilaterally from autoradiographs. For each ROI values were obtained from 5 tissue sections and averaged for each animal. After ensuring there were no significant effects of injury on the right

hemisphere ROIs, final data for cerebral glucose utilization were expressed as CMRGlc asymmetry scores [(left − right/left + right) × 100], where negative values reflect cerebral metabolic depression in the left/injured hemisphere (Hovda et al., 1991; Moro and Sutton, 2010; Moro et al., 2013).

4.5. Measures of injury severity and Fluoro-Jade B staining and analysis

At the injury level used in these studies tissue swelling in the craniotomy is observed in the first 1-2 minutes post-CCI and is routinely recorded on surgical records as mild, mild-to-moderate, moderate, moderate-to-severe, or severe. For the current experiments these descriptors were assigned rating scores of 0.5, 1.0, 1.5, 2.0 and 2.5, respectively. The average rating of tissue swelling for each CCI group was calculated as a measure of the initial injury severity.

Tissue sections (20 μm) adjacent to those saved for 2DG autoradiography were mounted onto glass slides and stored at −20 °C until staining for Fluoro-Jade B (FJB) (Schmued and Hopkins, 2000). On the day before staining these sections were brought to room temperature, fixed in 10% formalin overnight, then stained with FJB (0.0004% concentration of 2FJB; Histo-Chem Inc, Jefferson, AR) and 4′,6-diamidino-2-phenylindol dihydrochloride (DAPI; 0.0002%; D9542: Sigma Aldrich, St. Louis, MO). The stained sections were dried, cleared and cover-slipped as described previously (Moro and Sutton, 2010).

Dead/dying (FJB-positive; FJB+) cells within regions of the injured hemisphere were counted in 8 animals of each experimental group by an observer masked to the treatment conditions. Counts were performed using an epifluorescent microscope (480 nm excitation; Model DMRE: Leica Microsystems GmbH) interfaced with a computer running Stereo Investigator software (version 3.0: MicroBrightField Inc, Colchester, VT), using 20-40 X objectives to identify FJB+ cells with neuronal morphology. Total numbers of FJB+ neurons in the left midline peri-contusional cortex, dorsal to the corpus callosum and from midline to 1.5 mm laterally, were counted on 5 evenly spaced tissue sections from −0.8 to −4.8 mm posterior to Bregma. Total numbers of FJB+ neurons in the ipsilateral hippocampus were counted for 3 anterior sections (−2.8, −3.3 and −3.8 mm from Bregma) containing the CA3 subsector and the hilus, and from 3 tissue sections containing the ventral hilus (−4.8, −5.3 and −5.8 mm) and 2 sections containing the ventral CA3 (−4.8, −5.3 mm). The cell counts and counting areas within each region were summed for each animal, and the final cell density data were expressed as FJB+ cells per mm^2 of tissue area.

4.6. Data summary and analyses

All data are reported as the group average ± standard error of the mean (SEM), and were analyzed using IBM SPSS Statistics (v22). One-way or 2 × 3 analyses of variance (ANOVA) were carried out on physiological, CMRGlc (each ROI), injury severity and FJB+ neuronal count data. Significant main or interaction effects (2 × 3 ANOVA) were followed by planned comparisons, and after any significant group effects with 1-way ANOVA the individual group means were compared using the Tukey-Fisher least significant difference (LSD) criterion. Post-hoc or planned comparisons were assessed with α set at 0.05 (two-sided).

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Abbreviations

ANOVA	analysis of variance
CCI	controlled cortical impact
CMRGlc	cerebral metabolic rates of glucose
DAPI	4′,6-diamidino-2-phenylindol dihydrochloride
EP	ethyl pyruvate
FJB	Fluoro-Jade B
SAL	saline (8%)
SP	sodium pyruvate
SEM	standard error of the mean
TBI	traumatic brain injury

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Highlights

- One injection of sodium or ethyl pyruvate improved cerebral glucose utilization 24 h post-TBI.
- Neuronal injury in cortex and hippocampus were reduced by a single pyruvate treatment.
- Four sodium or ethyl pyruvate treatments also improved glucose utilization 24 h post-TBI.
- Multiple pyruvate treatments also reduced neuronal injury in cortex and hippocampus.
- Outcomes from one injection were not improved upon by use of multiple treatments.

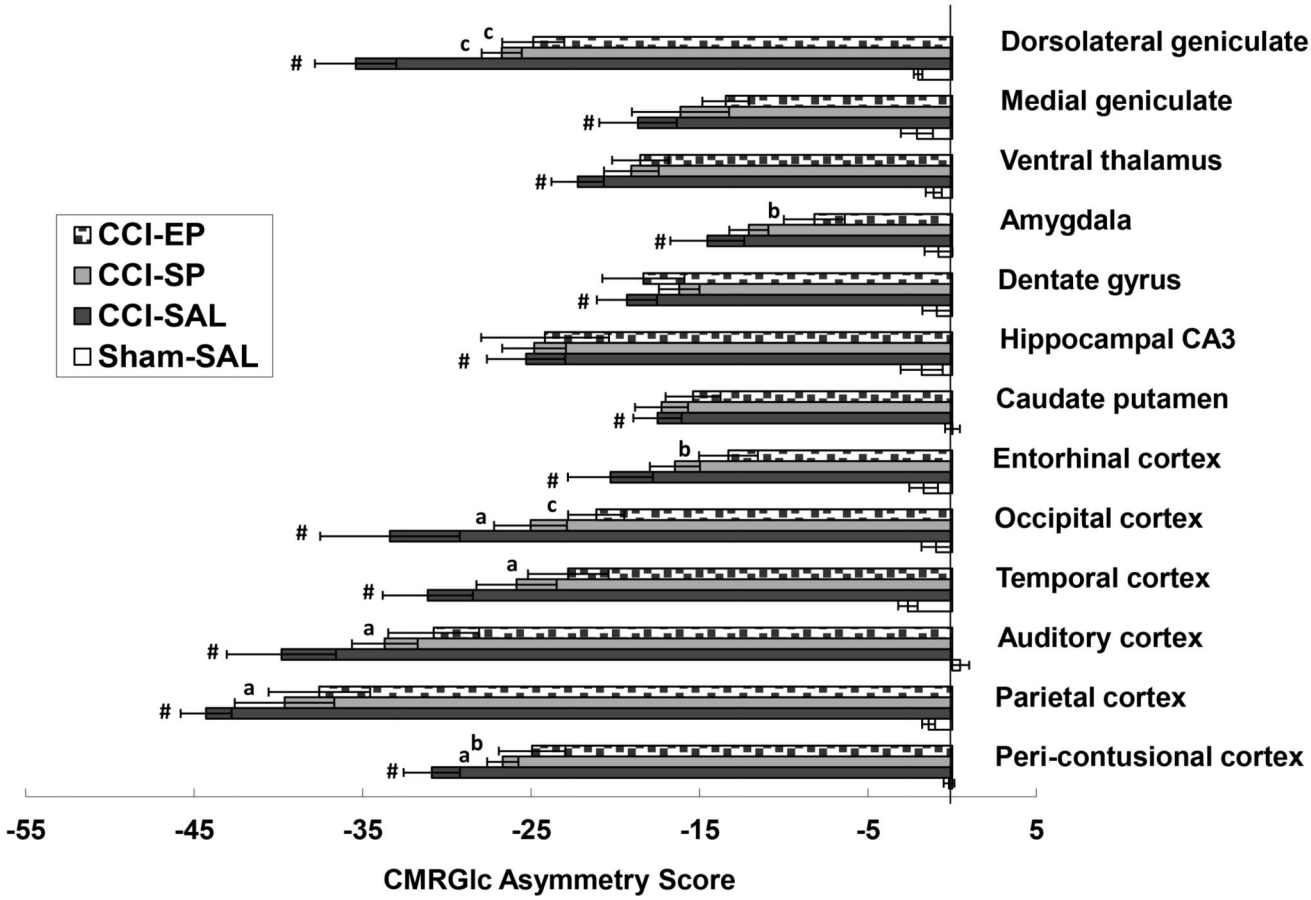

Fig. 1.

Mean (bars represent SEM) asymmetry scores [((L−R)/L+R)*100] for CMRGlc in brain regions 24 h after Sham or CCI injury and a single treatment of saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP). # $p < 0.001$ compared to Sham injury; [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ compared to CCI-SAL.

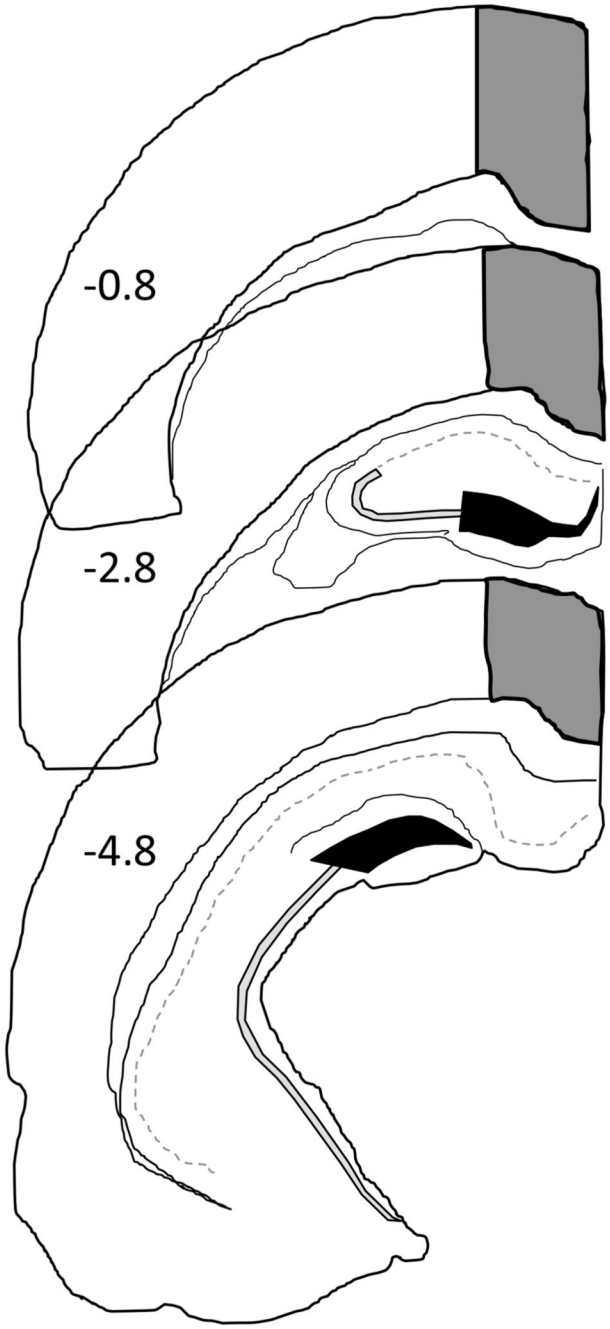

Fig. 2.

Diagram illustrating regions for counts of FJB+ neurons within the midline peri-contusional cortex (dark grey fill) and in the dorsal and ventral CA3 (light grey fill) and hilus (black fill). Numbers indicate the mm posterior to Bregma.

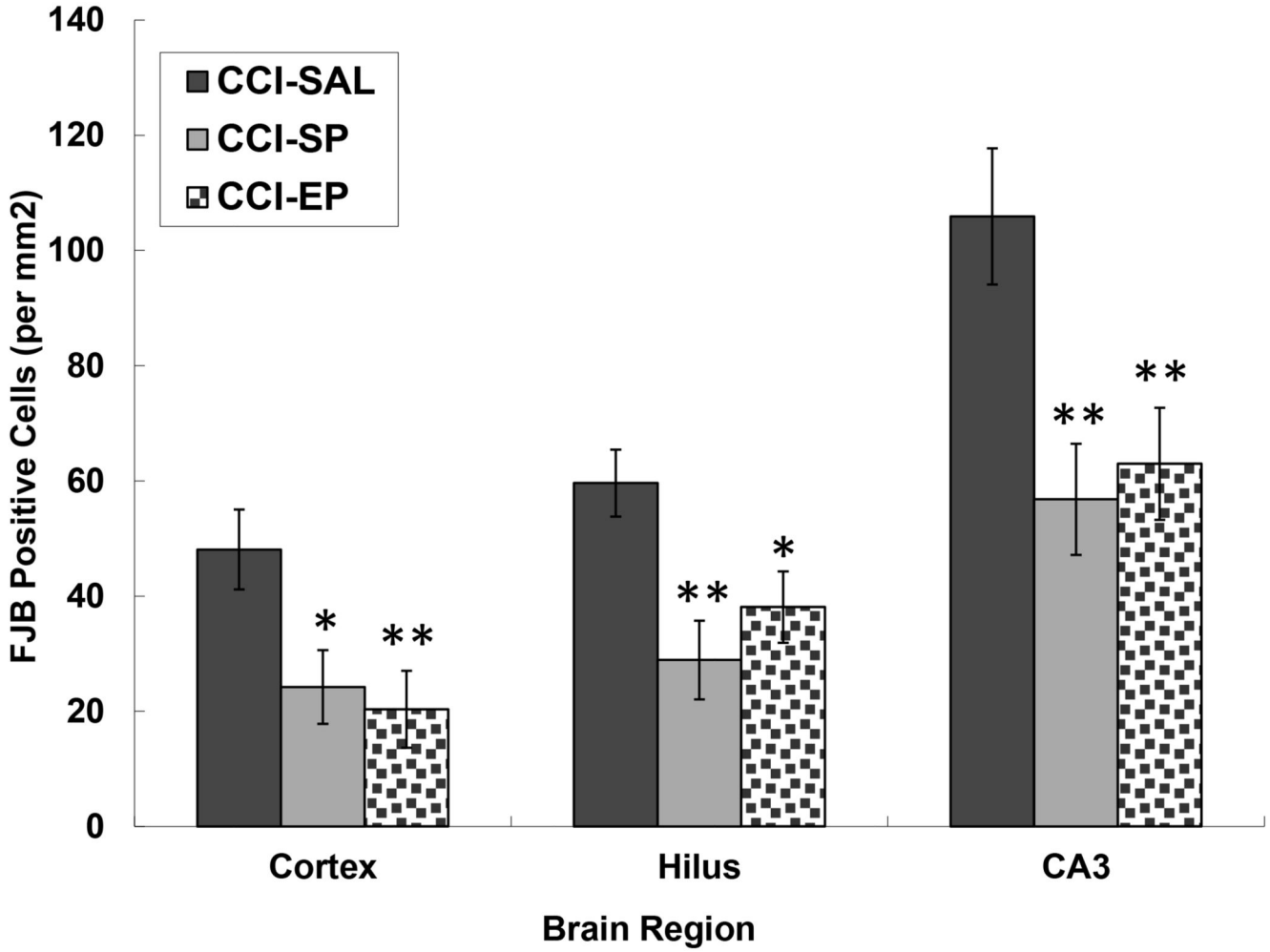

Fig. 3.

Shows the mean (bars represent SEM) cell densities for dead/dying neurons in the left peri-contusional cortex, hilus and CA3 subsector of the hippocampus 24 h after CCI injury and a single treatment of saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP). * $p \leq 0.05$, ** $p < 0.01$ compared to CCI-SAL.

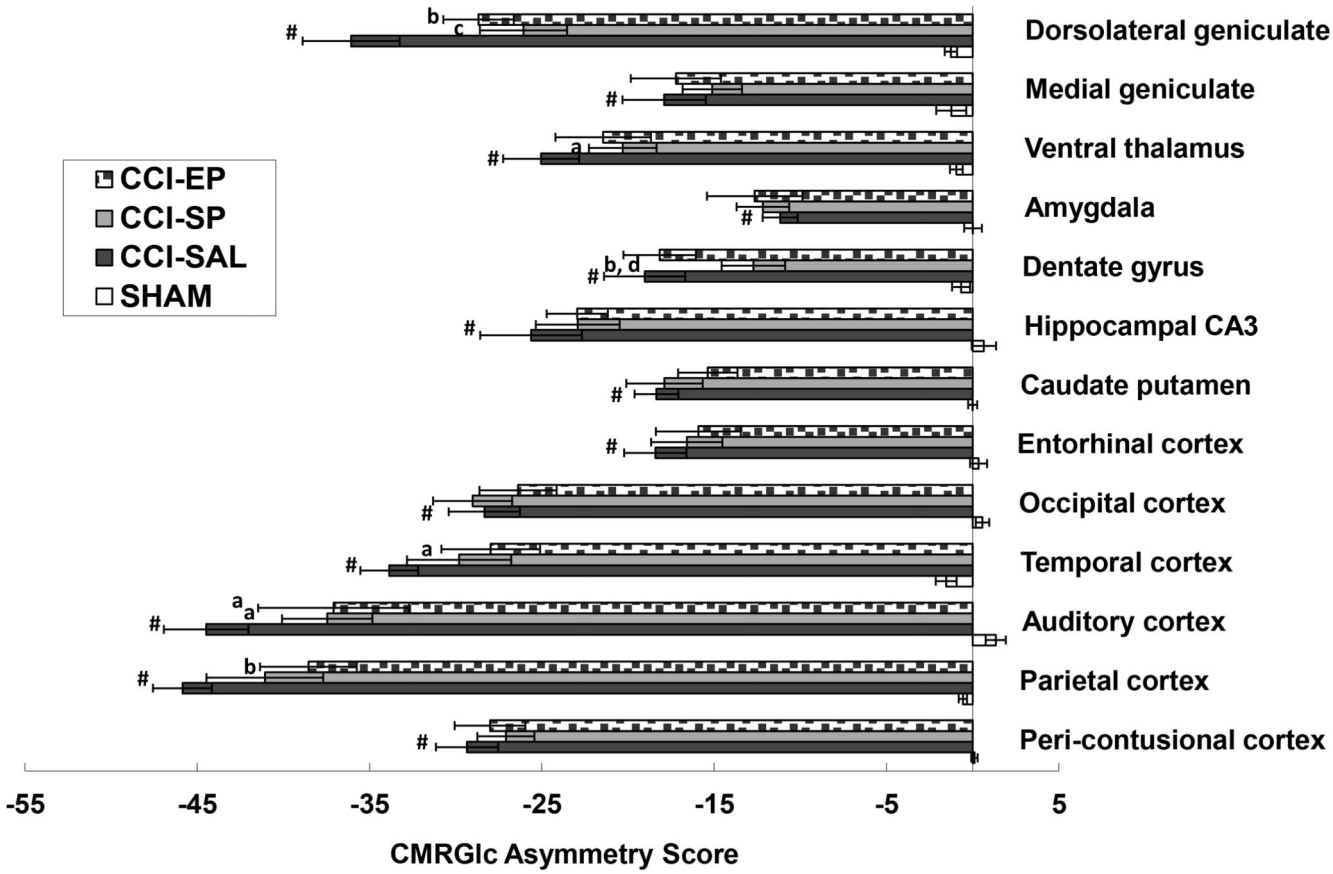

Fig. 4.

Mean (bars represent SEM**)** asymmetry scores [((L−R)/L+R)*100] for CMRGlc in brain regions 24 h after Sham or CCI injury and four treatments of saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP). SHAM denotes pooled data for Sham-SAL, Sham-SP and Sham-EP groups. # $p < 0.001$ compared to SHAM; [a] $p \leq 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ compared to CCI-SAL; [d] $p < 0.05$ compared to CCI-EP.

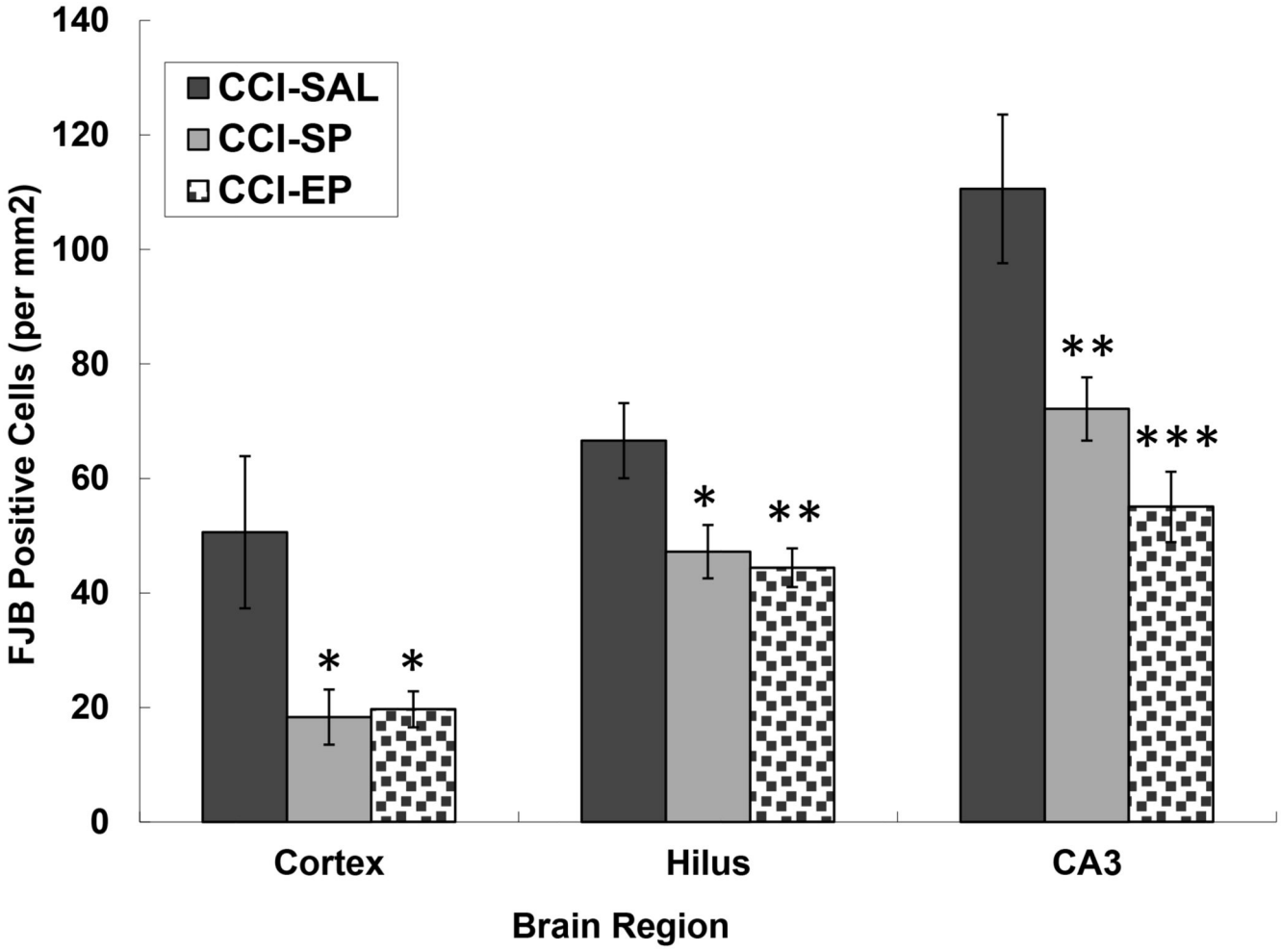

Fig. 5.

Mean (bars represent SEM) cell densities for dead/dying neurons in the left peri-contusional cortex, hilus and CA3 subsector of the hippocampus 24 h after CCI injury and four treatments of saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP). * $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$ compared to CCI-SAL.

Table 1

Mean (± SEM) change in body weight (in grams) one day post-injury, and the baseline arterial blood pH, gasses, and plasma glucose and lactate concentrations (mmol/L) prior to 2DG injection in Sham and CCI groups given one saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP) injection immediately after surgery.

	Sham-SAL	CCI-SAL	CCI-SP	CCI-EP
Sample size	n = 10	n = 9	n = 10	n = 9
Wt. change	−**0.7** ± *1.64*	−**8.9** ± *1.65* $^{*,\#}$	−**16.5** ± *3.64* ***	−**8.9** ± *2.11* $^{*,\#}$
pH	**7.42** ± *0.01*	**7.42** ± *0.01*	**7.42** ± *0.01*	**7.41** ± *0.01*
pCO_2 (mm Hg)	**39.5** ± *1.27*	**38.6** ± *0.58*	**37.9** ± *0.92*	**38.9** ± *0.99*
pO_2 (mm Hg)	**90.8** ± *3.62*	**84.2** ± *0.98*	**85.3** ± *2.39*	**82.6** ± *2.24*
HCO_3s	**26.1** ± *0.48*	**25.4** ± *0.60*	**24.9** ± *0.46*	**24.9** ± *0.24*
tCO_2	**26.9** ± *0.73*	**26.0** ± *0.72*	**25.8** ± *0.55*	**25.6** ± *0.39*
O_2Sat	**96.9** ± *0.35*	**96.4** ± *0.15*	**96.5** ± *0.35*	**96.0** ± *0.33*
Glucose	**9.5** ± *0.29*	**8.9** ± *0.36*	**8.8** ± *0.25*	**8.5** ± *0.27*
Lactate	**0.7** ± *0.08*	**0.8** ± *0.11*	**0.6** ± *0.05*	**0.6** ± *0.05*

* $p < 0.05$ compared to Sham-SAL.

*** $p < 0.001$ compared to Sham-SAL.

$^{\#}$ $p < 0.05$ compared to CCI-SP.

Table 2

Mean (± SEM) change in body weight (in grams) one day post-injury, and the baseline arterial blood pH, gasses, and plasma glucose and lactate concentrations (mmol/L) prior to 2DG injection in Sham and CCI groups given saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP) injections at 0, 1, 3 and 6 h after surgery.

	Sham-SAL	Sham-SP	Sham-EP	CCI-SAL	CCI-SP	CCI-EP
Sample size	**n = 10**	**n = 8**	**n = 8**	**n = 10**	**n = 10**	**n = 10**
Wt. change	**−5.1** ± *2.34*	**−6.0** ± *2.28*	**−6.8** ± *1.79*	**−11.1** ± *1.46* *	**−17.2** ± *2.30* ***,#	**−13.1** ± *1.84* *
pH	**7.45** ± *0.01*	**7.45** ± *0.01*	**7.45** ± *0.01*	**7.47** ± *0.01**	**7.45** ± *0.01*	**7.47** ± *0.01*
pCO_2 (mm Hg)	**41.3** ± *0.97*	**38.5** ± *1.14*	**40.4** ± *0.66*	**39.9** ± *1.12*	**39.8** ± *0.92*	**38.3** ± *0.44*
pO_2 (mm Hg)	**85.1** ± *2.28*	**87.8** ± *2.85*	**79.8** ± *1.80*	**82.8** ± *2.24*	**83.2** ± *2.64*	**86.9** ± *2.24* *
HCO_3s	**28.3** ± *0.48*	**27.4** ± *0.22*	**28.1** ± *0.28*	**28.7** ± *0.40*	**27.8** ± *0.55*	**27.7** ± *0.28*
tCO_2	**29.6** ± *0.48*	**28.6** ± *0.22*	**29.4** ± *0.31*	**29.9** ± *0.44*	**29.0** ± *0.57*	**28.9** ± *0.28*
O_2Sat	**96.7** ± *0.35*	**96.9** ± *0.33*	**96.1** ± *0.29*	**96.6** ± *0.37*	**96.6** ± *0.36*	**97.1** ± *0.21*
Glucose	**9.6** ± *0.21*	**9.5** ± *0.36*	**9.7** ± *0.36*	**8.5** ± *0.26* *	**9.3** ± *0.41*	**9.2** ± *0.34*
Lactate	**0.7** ± *0.08*	**0.6** ± *0.05*	**0.7** ± *0.09*	**0.6** ± *0.02*	**0.6** ± *0.03*	**0.6** ± *0.05*

* $p < 0.05$ compared to similarly treated Sham.

*** $p < 0.001$ compared to similarly treated Sham.

# $p < 0.05$ compared to CCI-SAL.

Table 3

Mean (± SEM) asymmetry scores [((L−R)/L+R)*100] for CMRGIc in brain regions 24 h after Sham injury and four treatments of saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP).

	Sham-SAL	**Sham-SP**	**Sham-EP**
Sample size	**n = 10**	**n = 8**	**n = 8**
Peri-contusional cortex	**−0.2** ± *0.3*	**0.2** ± *0.4*	**0.3** ± *0.3*
Parietal cortex	**−1.1** ± *0.3*	**0.0** ± *0.3*	**−0.5** ± *0.6*
Auditory cortex	**1.3** ± *0.9*	**1.1** ± *0.8*	**1.6** ± *1.4*
Temporal cortex	**−0.7** ± *0.8*	**−1.0** ± *0.8*	**−3.2** ± *1.4*
Occipital cortex	**−0.1** ± *0.6*	**1.0** ± *0.6*	**0.9** ± *0.8*
Entorhinal cortex	**−0.4** ± *0.7*	**1.1** ± *1.1*	**0.5** ± *0.9*
Caudate putamen	**−0.4** ± *0.5*	**−0.1** ± *0.4*	**0.5** ± *0.2*
Hippocampal CA3	**−0.4** ± *1.2*	**0.6** ± *1.3*	**2.0** ± *1.0*
Dentate Gyrus	**−1.5** ± *0.8*	**−0.6** ± *0.9*	**0.4** ± *0.9*
Amygdala	**−0.7** ± *0.9*	**−0.9** ± *0.7*	**1.9** ± *0.8*
Ventral thalamus	**−1.2** ± *0.4*	**−0.4** ± *1.0*	**−1.2** ± *0.6*
Medial geniculate	**−0.5** ± *1.9*	**−1.4** ± *0.6*	**−1.9** ± *1.4*
Dorsolateral geniculate	**−2.1** ± *0.6*	**−0.4** ± *0.6*	**−1.1** ± *0.5*