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RESEARCH

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# Decreased neuroinflammation and increased brain energy homeostasis following environmental enrichment after mild traumatic brain injury is associated with improvement in cognitive function

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## Abstract

**Background:** Persistent neuroinflammation and disruptions in brain energy metabolism is commonly seen in traumatic brain injury (TBI). Because of the lack of success of most TBI interventions and the documented benefits of environmental enrichment (EE) in enhancing brain plasticity, here we focused our study on use of EE in regulating injury-induced neuroinflammation and disruptions in energy metabolism in the prefrontal cortex and hippocampus. Adult male Wistar rats were used in the study and randomly assigned to receive either: mild TBI (mTBI) using the controlled cortical injury model or sham surgery. Following surgery, rats from each group were further randomized to either: EE housing or standard laboratory housing (CON). After 4 weeks of recovery, cognitive testing was performed using the non-matching-to-sample and delayed non-matching-to-sample tasks. After completion of behavioral testing, levels of the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  and the anti-inflammatory cytokine IL-10 were measured. In addition, levels of AMPK (adenosine monophosphate-activated protein kinase), phosphorylated AMPK and uMtCK (ubiquitous mitochondrial creatine kinase) were assessed as measures of brain energy homeostasis.

**Results:** Our results showed that EE: (1) decreased the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  and enhanced levels of the anti-inflammatory cytokine IL-10 after mTBI; (2) mitigated mTBI-induced cognitive impairment; and (3) attenuated mTBI-induced downregulation in pAMPK/AMPK ratio and uMtCK levels.

**Conclusions:** Our data demonstrated the potential of EE to modulate the persistent: (1) neuroinflammatory response seen following mTBI, and (2) persistent disturbance in brain energy homeostasis. It is possible that through the mechanism of modulating neuroinflammation, EE housing was able to restore the disruption in energy metabolism and enhanced functional recovery after mTBI.

**Keywords:** IL- $\beta$ , TNF- $\alpha$ , IL10, AMPK, Non-matching-to-sample task

## Background

Traumatic brain injury (TBI) is of major public health significance, affecting almost 1.7 million people in the United States each year [1]. Globally, the incidence of TBI is also increasing, particularly in developing countries where road traffic accidents are on the increase as a result

of widespread motor vehicle use. Mild traumatic brain injury (mTBI) comprises about 80% of all TBI cases and most individuals exhibit disabilities associated with cognitive problems [2]. Common cognitive impairments seen in mTBI include difficulties in attention, episodic memory, executive functions (such as higher-order planning, initiating and directing, monitoring, problem solving, and inhibitory control), working memory, information-processing speed, language functions, and visuospatial processing that can last for months or even years [3]. Large

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transient increases in excitatory neurotransmitter efflux [4] resulting in excitotoxicity, ionic imbalance, ATP depletion, proteolysis, and oxidative stress [5] happens in the acute phase of TBI. The complex array of responses to injury can result in energy crisis that compromises the capacity of the brain to cope with challenges, thus regulation of energy homeostasis after TBI is a critical step in maintaining brain function.

While the acute effects of mTBI can be devastating, increasing evidence suggest that mTBI also initiates long-term effects in some segment of survivors [6]. The acute effects of mTBI stemming from the primary insult can cause secondary injuries that can evolve over minutes to days and even months after the initial traumatic event. Secondary injury events such as neuroinflammation can also cause neurodegeneration and persistent cognitive impairment [7]. The neuroinflammatory process involves the movement of activated microglial cells to the site of injury in response to extracellular adenosine triphosphate (ATP) released by the injured tissue [8-10]. The microglial processes then fused to form an area of containment between healthy and injured tissues, suggesting that microglia may represent the first line of defense following TBI [11]. However, when microglia become over-activated or reactive they can induce detrimental neurotoxic effects by releasing multiple cytotoxic substances, such as pro-inflammatory cytokines (e.g. interleukin-1 beta and tumor necrosis factor-alpha) and arachidonic acid metabolites [12-14]. These reports suggest that clear beneficial effects can be achieved if neuroinflammation is controlled in a regulated manner and for a defined period of time. However, there is increasing number of evidence that chronic microglial activation is present in most cases of TBI leading to neuroinflammation that can persist for months or years after injury [14,15].

To date, most interventions that target a specific pathological consequence of mTBI have failed clinically. Thus, it is possible that using an intervention with known general effects on enhancing brain plasticity and resiliency may be beneficial in mTBI and one such strategy is environmental enrichment (EE). In general EE refers to conditions that provide increased social, cognitive, and physical stimulation [16,17]. Reports show that EE can exert positive effects on behavioral recovery after cerebral ischemia [18-21], mild TBI [22-25], and neurodegenerative disorders [26,27]. Even though the effects of EE in modulating neuroinflammation is just beginning to be explored [28], the impact of EE in modulating brain energy homeostasis after mTBI has not been studied. Here we focused our study on EE effects in regulating injury-induced neuroinflammation and disruptions in energy metabolism in the prefrontal cortex and hippocampus, as these are the brain regions commonly affected in mTBI. The main goal of the study was to determine the effects of EE in attenuating the

long-term consequences of mTBI relating to neuroinflammation, alterations in brain energy metabolism, and cognitive impairment. The rationale for examining long-term effects in the present was based on the dearth of information available in the persistent consequences of mTBI.

## Methods

### Animal model

Adult male Wistar rats were obtained from Harlan Laboratories (Madison, Wisconsin) weighing approximately 375 to 400 grams. Animals were housed in pairs in a pathogen-free vivarium under controlled condition (temperature  $22 \pm 1^\circ\text{C}$  and humidity  $70 \pm 5\%$ ) and a 14:10 hour light:dark cycle was maintained. All animals were housed in the same room so that temperature, humidity, and lighting conditions are similar for all groups. Animals had free access to food (regular rat chow) and water delivered through an automated and filtered system. Animals were also handled daily throughout the study so that they could get acclimated to the research personnel thereby decreasing stress. Experiments started one week after arrival of the animals from the breeder and all experimental protocols in this study were approved by the Institutional Animal Care and Use Committee (University of Illinois at Chicago) and in accordance with the National Institutes of Health guidelines. All efforts were made to minimize animal distress and to reduce the number of animals used.

### Mild traumatic brain injury

Following induction anesthesia with isoflurane inhalation (1%), animals were placed in a Cunningham stereotaxic frame (Stoelting, Wood Dale, IN) in a prone position and stabilized using the ear bars and incisor bar. The head was held in a horizontal plane with respect to the interaural line. Throughout the procedure, continuous isoflurane anesthesia was maintained at 2.5%, aseptic technique was used and the rats' body temperature was maintained at  $37^\circ \pm 1^\circ\text{C}$  using the feedback-regulated water heating pad. Once the animals were fully anesthetized and the head stabilized, a midline incision was made and the soft tissues were retracted. Then two 10 mm diameter craniotomies were made adjacent to the central suture, midway between lambda and bregma. Injury was induced in one of the craniotomies and the second one allowed for lateral movement of the brain during injury. The dura matter was kept intact over the cortex and injury was induced by striking the left or right (ipsilateral) cortex with a pneumatic piston with a 6 mm diameter tip at a rate of 4 m/sec, with 1.5 mm of compression. Velocity was measured using a linear velocity displacement transducer. Any bleeding from the skull during the craniotomy was controlled with bone wax. After the injury, the scalp was closed with 6-0 silk suture, anesthesia was discontinued, and the rat's temperature was maintained at  $37^\circ \pm 1^\circ\text{C}$  until recovery of

locomotion. Sham animals were subjected to the same anesthesia and craniotomy, but no cortical injury.

Animals were assessed every hour  $\times$  8 hours then daily  $\times$  one week, for postoperative complications such as excessive weight loss [ $>20\%$  of preoperative body weight], bleeding, seizures, and infection. Animals were euthanized immediately and excluded from the study when postoperative complications occurred ( $n = 0$ ). Liquid Tylenol was given orally with drinking water at 200 mg/Kg after surgery for 48 hours as postoperative analgesia. Sluggishness, extreme aversion to being touched, and weight loss were also assessed as indication of persistent pain ( $n = 0$ ).

### Animal housing

Immediately upon recovery from anesthesia, rats were randomly placed in either: enriched environment (EE) housing or paired housing (social controls). The rats remained in their assigned housing condition throughout the duration of the study. Animals in the EE group ( $n = 9$  mTBI and  $n = 8$  shams) were housed together in a sensory-rich living condition (wire cage measuring 2 m  $\times$  1 m  $\times$  1.65 m) consisting of a variety of objects as described previously [19,29]. In addition, these rats were placed each day in an open field (1.2  $\times$  1.2  $\times$  1.2 m) during the evening hours with a novel arrangement of toys and objects and allowed to explore for 30 minutes while the objects in the home cage were being changed. Objects in both EE housing and open field were changed daily to maintain novelty.

Animals assigned to the social control (CON) group ( $n = 9$  mTBI and  $n = 8$  shams) were housed in pairs in standard laboratory cages (16.5  $\times$  22.5  $\times$  13.5 cm). Although rats in this group were able to observe ongoing activity of the room, they did not receive any stimulation and contact was limited to daily handling and routine cage changing. Paired housing was used to control for the social interaction effect of the enriched environment.

### Non-matching-to-sample and delayed-non-matching-to-sample tasks

Behavioral testing was conducted 4 weeks after EE or control housing and approximately 2 hours prior to the onset of the dark cycle (this is close to the rats' active period) for a total of 9 days (including habituation) and performance was recorded using the Ethovision XT<sup>®</sup> v.8.5 video tracking program (Leesburg, VA). The non-matching-to-sample task consisted of a series of paired sample and test trials. At the beginning of each sample trial, either black or white cylinder was suspended directly above the submerged platform in the water maze. In subsequent test trials, both cylinders were present but the cylinder not present during the preceding sample trial was suspended over the platform and served as a cue for the location of the goal. Thus, if on a given sample trial, the black cylinder cued the platform,

and then on the succeeding test trial, the white cylinder was used to cue the platform. The black and white cylinders were selected as sample stimuli for each pair of trials according to a semi-random schedule that ensured each cylinder served as the sample stimulus on 50% of the trials over the phase of the experiment. For each test trial, the platform was moved to another quadrant with the non-sample cylinder located directly above it. The sample stimulus was moved to a different quadrant for each trial. Based on a random schedule, the position of the submerged platform was changed after each sample and test trial to eliminate the use of spatial cues. All quadrants were used equally for locating cues in the sample and test trials and the platform was positioned randomly. The day before testing, rats were allowed a habituation swim for 10 seconds without the submerged platform. The constant water temperature in the pool and habituation swim helped decrease animal stress associated with the task.

At the beginning of each sample trial, the rat was placed in the pool at the same location (in the center of the south-east quadrant), facing the wall of the pool, and allowed to swim to the submerged platform under the sample cylinder. The rat was allowed to remain on the platform for 10 seconds. If the rat failed to find the platform within 60 seconds, it was picked up and placed on the platform for 10 second then removed and placed under a heat lamp while the platform is moved and the cylinders put in position for the test trial. The heat lamp allowed for the rats to get dry before the test trial. Getting the cylinders and platform ready for the test trial took  $\approx 10$  seconds. Rats received five daily sessions and each session consisted of four pairs of sample and test trials. The day after completion of the non-matching-to-sample task, rats were tested in the delayed non-matching-to-sample task for three additional daily sessions. Each session consisted of three paired trials, with intervals of 60, 120, or 180 seconds between the sample and test trials (the intervals do not include the 10 seconds required for repositioning the cylinders and platform). The order of the delays varied each day according to a random schedule.

### Tissue preparation

Rats were euthanized using CO<sub>2</sub> asphyxiation the day after behavioral testing, the brains removed, and the prefrontal cortex and hippocampus were manually dissected then immediately placed in liquid nitrogen and kept frozen until processed. The ipsilateral regions were analyzed for cytokine levels (interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$ ) and measures of brain energy homeostasis such as AMPK (adenosine monophosphate-activated protein kinase) and uMtCK (ubiquitous mitochondrial creatine kinase).

### Cytokine protein quantification

The concentration of IL-1 $\beta$ , TNF- $\alpha$ , and IL-10 protein levels was determined using commercially available ELISA assays. Briefly, 0.5 g of frozen tissues were homogenized with a glass homogenizer in 1 ml buffer containing 1 mol/liter phenylmethylsulfonyl fluoride, 1 mg/liter pepstatin A, 1 mg/liter aprotinin, and 1 mg/liter leupeptin in PBS (pH 7.2) and centrifuged at 12,000  $\times$  g for 20 minutes at 4°C. The supernatant was collected and total protein was determined by bicinchoninic acid (BCA) protein assay reagent kit (PIERCE, Milwaukee, WI). Samples were used for ELISA to determine IL-1 $\beta$ , TNF- $\alpha$ , and IL-10 protein levels. The procedure was performed according to manufacturer's specification using the Quantikine rat-specific ELISA kits (R&D Systems, Minneapolis, MN) and the color reaction was detected using the chromogen tetramethylbenzidine. Color reaction was stopped by an equal volume of stop solution (provided by the manufacturer) and read in a microplate reader (Bio-Tek, Winooski, VT) at a wavelength of 450 nm (650-nm reference wavelength). The color change was proportional to the concentration of the cytokines measured and all samples measured were within the range of the standard curve. This ELISA system detects both natural and recombinant rat IL-1 $\beta$ , TNF- $\alpha$ , and IL-10. Assays were sensitive to 5 pg/ml for IL-1 $\beta$  and TNF- $\alpha$ , and 10 pg/ml for IL-10; the intra-assay and inter-assay coefficients of variation were <5% and <10% for TNF- $\alpha$  and IL-10, and <6% and <9% for IL-1 $\beta$ . Assays were performed in triplicates and measurements were averaged and used as one individual data point for statistical analysis.

### Western blot

To detect measures of brain energy homeostasis, 0.5 g of frozen tissues was used in the Western blot procedure. Tissues were homogenized and centrifuged at 25,000  $\times$  g for 20 minutes as previously described [18,30]. Aliquots from the supernatant were removed for protein determination. Protein concentration in samples was determined using the BCA-Protein assay (Pierce, Rockford, IL). Equal amounts of protein (40  $\mu$ g) from each rat were loaded and separated by SDS-PAGE gel electrophoresis in 8% - 16% acrylamide gradient gels. The protein bands were electrophoretically transferred to nitrocellulose membranes (Amersham, Piscataway, NJ) stained with 0.5% Ponceau Red to visualize total proteins, then destained. Non-specific binding sites were blocked then nitrocellulose membranes were incubated overnight at 4°C with gentle agitation in the following primary antibodies: (1) monoclonal mouse anti-AMPK (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA); (2) polyclonal rabbit anti-phosphorylated AMPK (1:1000, Cell Signaling, Billerica, MA); (3) monoclonal mouse anti-uMtCK (1:1000, Santa Cruz Biotechnology); (4) polyclonal rabbit anti- $\beta$ -actin (1:2000, Santa Cruz Biotechnology). The

secondary antibodies used were horseradish peroxidase-conjugated immunoglobulin (Sigma, St. Louis, MO) and the Super Signal chemiluminescence substrate kit (Pierce, Rockford, IL) was used to visualize immunoreactive bands. After visualization, the membranes were then stained with Amido-Black to qualitatively verify protein loading. Band visualization was obtained by exposure of membranes to autoradiographic film (Kodak Biomax™). Samples were analyzed in quadruplicates and measurements were averaged and used as one individual data point for statistical analysis. Quantification of differences in protein bands between samples was done using densitometric analysis (ImageJ software v.1.47). The internal control  $\beta$ -actin was used to standardize experimental values in densitometric analysis. Densitometric values were calculated as: density of sample band/density of background.

### Statistical analysis

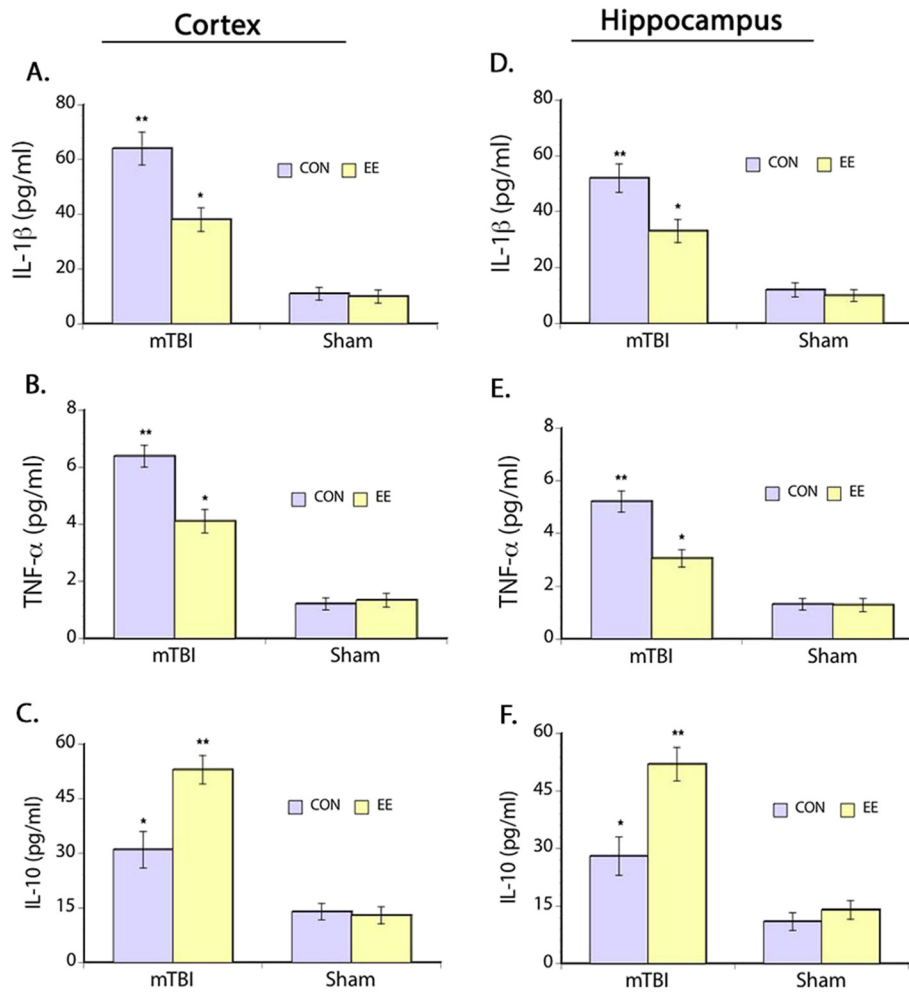
The SAS general linear model (SAS Institute, North Carolina) procedures for two-way analysis of variance (ANOVA) were used to examine effects of experimental conditions (mTBI vs. sham) and housing condition (enriched environment vs. control) on neuroinflammatory state (IL-1 $\beta$ , TNF- $\alpha$ , and IL-10) and brain energy homeostasis (AMPK, p-AMPK, and uMtCK). The SAS CONTRAST statement was used for planned comparisons when appropriate. Repeated measures ANOVA was used to analyze behavioral data. All error bars represent  $\pm$  standard error of the mean (SEM) of the sample size used in the study.

## Results

### EE effects on inflammatory state

We examined the expression of the pro-inflammatory cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF-1 $\alpha$ ) as well as expression of the anti-inflammatory cytokine interleukin-10 (IL-10). Our results showed significant main effects on IL-1 $\beta$  ( $F(3,30) = 9.21$ ,  $p < 0.05$ ) and TNF- $\alpha$  ( $F(3,30) = 9.67$ ,  $p < 0.05$ ) in the prefrontal cortex where mTBI led to the upregulation of these inflammatory cytokines even after more than a month of recovery from injury (Figure 1A and B). However, housing rats in EE immediately after brain injury significantly decreased levels of IL-1 $\beta$  and TNF- $\alpha$  in the prefrontal cortex to 36% and 32%, respectively, when compared to the mTBI animals housed in regular laboratory cages. No significant changes in IL-1 $\beta$  and TNF- $\alpha$  levels were seen in the sham groups. Similar pattern of significant effects of IL-1 $\beta$  ( $F(3,30) = 10.07$ ,  $p < 0.05$ ) and TNF- $\alpha$  ( $F(3,30) = 9.71$ ,  $p < 0.05$ ) were seen in the ipsilateral hippocampus (Figure 1D and E).

In addition, we also examined levels of IL-10 and found significant main effects of injury ( $F(3,30) = 10.99$ ,  $p < 0.05$ ) in IL-10 levels where a persistent downregulation of this anti-inflammatory cytokine was seen in the prefrontal cortex weeks after mTBI. But housing rats in EE immediately after



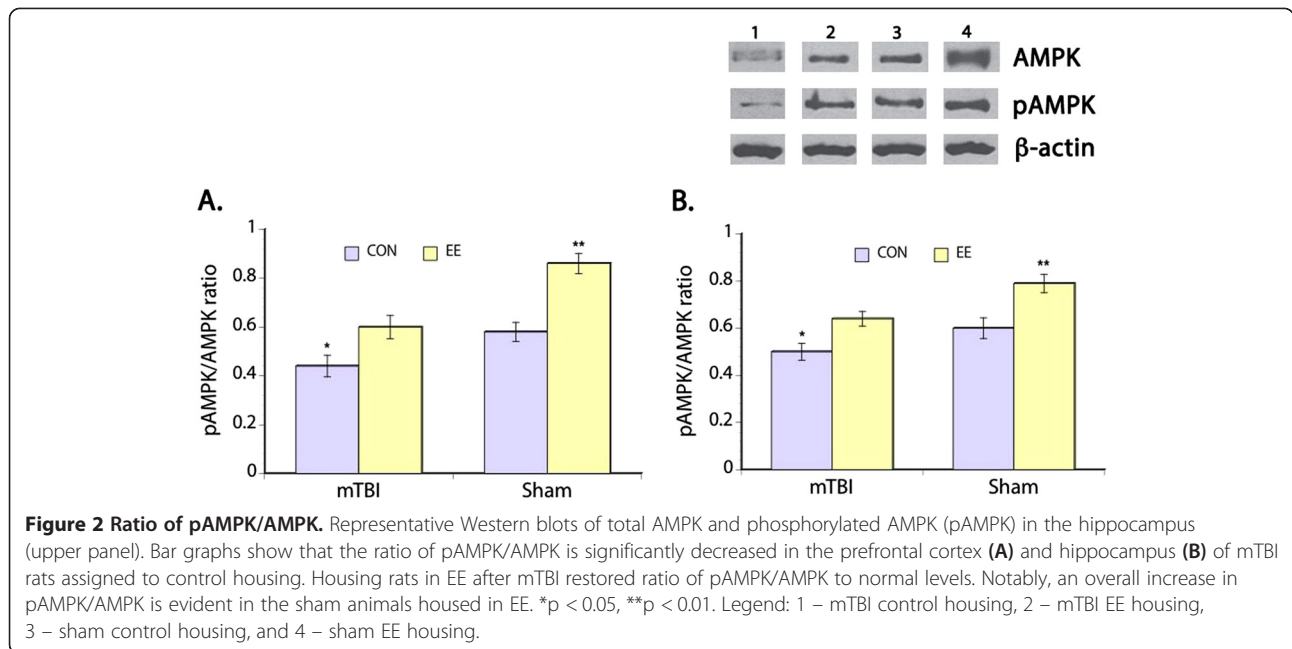
**Figure 1 Inflammatory markers.** ELISA results of IL-1 $\beta$  (A), TNF- $\alpha$  (B) and IL-10 (C) levels in the prefrontal cortex and hippocampus (D-F). Increased levels of IL-1 $\beta$  and TNF- $\alpha$  are evident in both prefrontal cortex and hippocampus of mTBI rats but housing animals in EE after injury modulated the upregulation in these pro-inflammatory cytokines. In contrast, levels of the anti-inflammatory cytokine IL-10 is decreased in mTBI rats assigned in the control environment compared to the mTBI rats housed in EE. Sham groups show minimal expression of both anti-inflammatory and pro-inflammatory cytokines. \* $p < 0.05$ , \*\* $p < 0.01$ .

mTBI significantly modulated the reduction of IL-10 levels ( $F(3,30) = 10.02$ ,  $p < 0.05$ ). IL-10 level in the mTBI rats housed in EE was 41% higher when compared to the mTBI animals housed in regular laboratory cages. No significant differences were seen in IL-10 levels in the sham rats (Figure 1C). Again, a similar pattern of significant effects of injury ( $F(3,30) = 11.05$ ,  $p < 0.05$ ) and EE housing ( $F(3,30) = 12.03$ ,  $p < 0.05$ ) were seen in the hippocampus (Figure 1F). Together, these results suggest that neuroinflammation can endure weeks after injury but housing rats in EE during recovery can modulate this persistent consequence of mTBI.

#### EE effects on pAMPK/AMPK ratio

We measured total AMPK and pAMPK to obtain the pAMPK/AMPK ratio as an indication of cellular energy

status [31,32]. We found significant main effects of injury ( $F(3,30) = 10.93$ ,  $p < 0.05$ ) and EE housing ( $F(3,30) = 10.48$ ,  $p < 0.05$ ) on pAMPK/AMPK ratio in the prefrontal cortex where mTBI resulted in significantly decrease pAMPK/AMPK ratio in comparison to the sham groups (Figure 2A). However, housing rats in EE after injury significantly lessened the effects of mTBI on pAMPK/AMPK ratio. Posthoc comparison showed that the pAMPK/AMPK ratio of mTBI rats housed in EE was not significantly different from the sham control group. Comparison of the sham groups also showed significant effects of EE housing on pAMPK/AMPK ratio where sham rats housed in EE had 33% higher ratio compared to the sham control animals. Examination of pAMPK/AMPK ratio in the hippocampus also showed significant main effects of



mTBI ( $F(3,30) = 11.33$ ,  $p < 0.05$ ) and housing condition ( $F(3,30) = 12.11$ ,  $p < 0.05$ ) with the pattern of changes similar to that seen in the prefrontal cortex (Figure 2B).

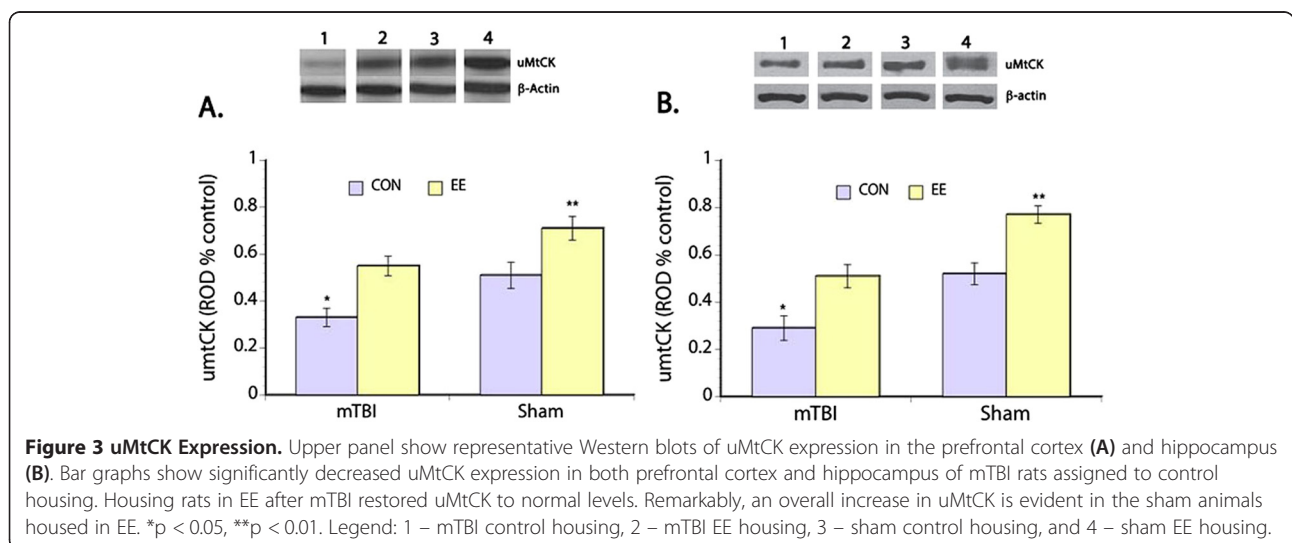
#### EE effects on uMtCK

Measurement of uMtCK was also done in the present study because of the involvement of this kinase in energy transduction [33]. Significantly decreased uMtCK expression was seen in the prefrontal cortex after mTBI ( $F(3,30) = 9.61$ ,  $p < 0.05$ ) compared to the sham groups; but housing rats in EE after injury mitigated the reduction in uMtCK levels ( $F(3,30) = 10.31$ ,  $p < 0.05$ ) induced by mTBI (Figure 3A). Posthoc comparison showed that the uMtCK levels of mTBI rats housed in EE was not

significantly different from the sham CON group. Comparison of the sham groups also showed significant effects of EE housing on uMtCK expression where sham rats housed in EE had 28% higher levels compared to the sham control animals. When uMtCK levels were examined in the hippocampus, significant main effects of mTBI ( $F(3,30) = 12.17$ ,  $p < 0.05$ ) and housing condition ( $F(3,30) = 11.22$ ,  $p < 0.05$ ) were also seen with expression levels similar to that seen in the prefrontal cortex (Figure 3B).

#### EE effects on mTBI-induced cognitive impairment

Rats were allowed to recover for 4 weeks in their assigned housing condition after injury before undergoing behavioral testing. All rats remained in their assigned



environment throughout the testing days until the end of the study. Our results showed that overall mTBI control rats demonstrated significantly increase swim latency ( $F(3,30) = 11.22, p < 0.05$ ) and made more errors ( $F(3,30) = 10.23, p < 0.05$ ) in the non-matching-to-sample task even after 4 weeks of recovery from injury (Figure 4). We also found that the number of errors made in the delayed non-matching-to-sample task increased with increasing time delay between the sample and test trials ( $F(3,30) = 10.14, p < 0.05$ ). Furthermore, performance in the delayed non-matching-to-sample tasks showed impairment in the mTBI control rats overall. Housing rats in EE after injury significantly mitigated the mTBI-induced cognitive impairment where their performance did not differ from those of the sham control group in both the non-matching-to-sample and delayed non-matching-to-sample tasks. Also, the sham EE groups demonstrated better performance overall in both the non-matching-to-sample and delayed non-matching-to-sample tasks.

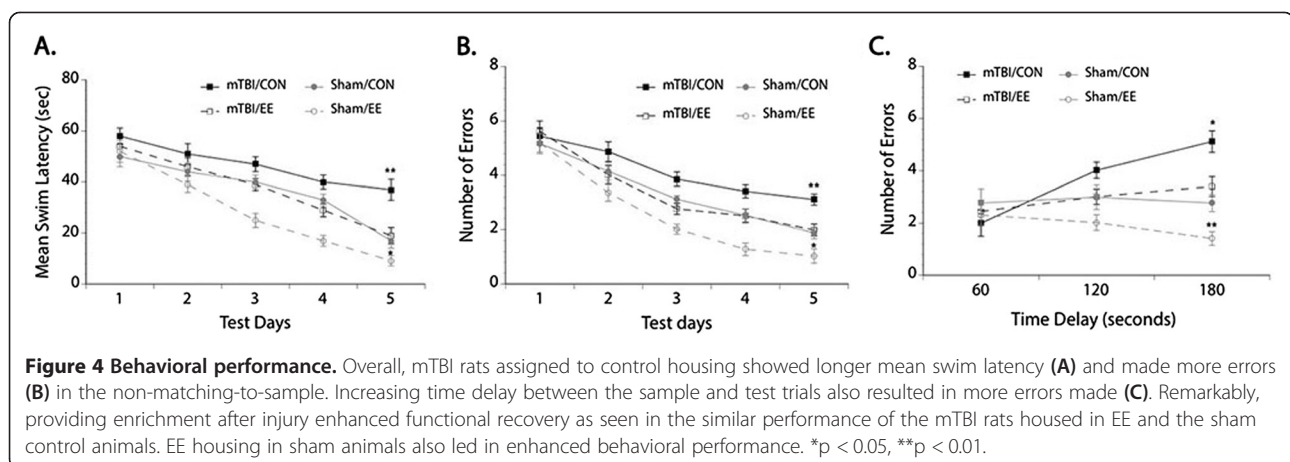
## Discussion

In the present study we show that mTBI-induced neuroinflammation, disruptions in brain energy metabolism, and behavioral impairment may be modifiable by enriched environment housing after injury. These findings are supported by our data that EE: (1) modulated mTBI-induced upregulation of the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  and enhanced levels of the anti-inflammatory cytokine IL-10; (2) mitigated mTBI-induced cognitive impairment; and (3) attenuated mTBI-induced downregulation in pAMPK/AMPK ratio and uMtCK levels. Ultimately, our findings extended the work of others [22-25,34-37] suggesting that EE housing after either mild or severe mTBI may be an effective therapeutic intervention that can optimize behavioral recovery after injury.

Here we show that the neuroinflammation induced by mTBI persisted for 4 weeks after the injury and that housing rats in EE immediately after injury significantly

reduced the inflammatory response. The persistent neuroinflammation seen in the mTBI control group is similar to reports from clinical and experimental studies that neuroinflammation resulting from mTBI can last from months to years after injury [14,38,39]. Neuroinflammation in the acute phase of mTBI triggers the release of pro-inflammatory cytokines essential for mounting a defense mechanism associated with neutralization of the insult and restoration of normal structure and function following injury [40]. However, if neuroinflammation is not regulated, it can result in a self-propagating and deleterious process [14]. This is evident in our data showing that the continuing neuroinflammation seen in mTBI rats assigned in control housing is associated with persistent cognitive impairment, downregulation of pAMPK/AMPK ratio, and uMtCK levels in the prefrontal cortex and hippocampus. The modulation of IL-1 $\beta$  and TNF- $\alpha$  expression in the mTBI rats housed in EE may be partially mediated by the increased release of the anti-inflammatory cytokine IL-10.

One of the contributing factors to cognitive impairment following mTBI is neuroinflammation. For example, the pro-inflammatory cytokine IL-1 $\beta$  in particular is reported to affect hippocampal-dependent memory tasks (reviewed in [41]). As well, increased pro-inflammatory cytokine TNF- $\alpha$  is reported to reduce synaptic plasticity and contribute to neurodegeneration [42-44]. Thus, it is logical to think that the modulation of IL-1 $\beta$  and TNF- $\alpha$  expression in the hippocampus and prefrontal cortex of mTBI rats housed in EE seen in this study led to the sparing of disruptions in cognitive function. This line of thinking is supported by our findings that the mTBI rats housed in EE performed as well as the sham control animals in both the non-matching-to-sample and delayed non-matching-to sample tasks. However, our behavioral findings are at odds with a recent study where only partial mitigation of cognitive impairment in mice housed in EE after Influenza-induced inflammation [28] is reported. Some possible reasons in the conflicting findings are: (1) different triggering





events for inflammation. wherein we used mTBI and the other study used Influenza virus. It is possible that Influenza also induced fatigue as part of the 'sickness behavior' commonly seen in peripheral inflammation [45], which affected the animals' performance in the water maze; (2) time of behavioral testing wherein we started the non-matching-to-sample test 4 weeks after mTBI while in the other study, mice were tested in the water maze 48 hours after inoculation with the Influenza virus. The possibility exists that our data may have been influenced by some degree of spontaneous recovery [46,47] reported to occur after brain injury. That is, in our study the rats were housed in EE for 4 weeks and throughout the behavioral testing period, thus spontaneous recovery may have been enhanced.

In this study we also show that mTBI resulted in persistent downregulation of pAMPK/AMPK ratio, which is tempered by EE housing suggesting that providing enrichment may initiate mechanisms that help conserve ATP levels that are depleted following injury. AMPK is an evolutionarily conserved signaling molecule that is considered one of the most important energy sensors in the body [48]. AMPK is activated via phosphorylation when ATP is depleted, which makes it a good marker for cellular energy [49]. The response of AMPK then to changes in neuronal energy status and metabolic stress makes the pAMPK/AMPK ratio a reasonable measure of neuronal responsiveness to changes in energy status. AMPK controls cellular energy through phosphorylation of metabolic enzymes that leads to the restoration of energy balance by activation of catabolic pathways that produce ATP and inhibition of anabolic pathways that use energy [50]. This role of AMPK in restoring energy balance is apparent in our data showing that the ratio of pAMPK/AMPK in the mTBI rats housed in EE is similar to the sham control groups. Furthermore, it is not surprising that in the current study pAMPK/AMPK ratio significantly increased in sham rats housed in EE in relation to the sham control group given the role of AMPK in maintaining energy homeostasis in concert with reports that EE housing can enhance synaptic plasticity, an energy consuming process.

In addition to its role in controlling cellular energy, AMPK also regulates a variety of transcription factors involved in learning and memory [51]. Accordingly, it is possible that EE housing may engage AMPK to prevent cognitive impairment resulting from mild energy depletion state such as that seen in mTBI. Indeed, this line of reasoning is supported by our data showing that the performance of mTBI rats housed of EE is similar to the sham control group. Furthermore, it is possible that the overall enhanced behavioral performance and upregulation of pAMPK/AMPK ratio seen in the sham EE housed rats in this study may be explained by increased activation of

AMPK linking intracellular energy levels and synthesis of proteins involved in learning and memory.

We also measured uMtCK levels in the present study since this molecule is highly expressed in hippocampal granule and pyramidal cells. UMtCK is functionally coupled to oxidative phosphorylation since mitochondrial-derived ATP is preferentially used by this molecule to transfer high-energy phosphates to creatine. The resultant phosphocreatine acts as the cytosolic transport and storage form of energy and used to regenerate ATP from cytosolic ADP, which is essential in maintaining the ATP supply during energy-consuming brain activity. Our data show that mTBI resulted in decreased uMtCK levels but housing rats in EE after injury regulated this injury-induced downregulation leading to levels similar to the sham control group. We also show that sham rats housed in EE have the highest levels of uMtCK overall. These expected findings suggest that EE restored the decreased levels of uMtCK following mTBI and intensified uMtCK expression in EE, a condition that requires high energy because of increased synaptic activity, thus providing a mechanism of energy transduction in which ATP synthesized in the mitochondria is transferred to sites of ATP utilization.

## Conclusions

Our data show the potential of EE to modulate the persistent: (1) neuroinflammatory response seen following mTBI, and (2) persistent disturbance in brain energy homeostasis. It is possible that through the mechanism of modulating neuroinflammation, EE housing is able to restore the disruption in energy metabolism induced by mTBI. Furthermore, the modulation of energy metabolism seen in the mTBI rats housed in EE seemed to correlate with functional recovery. Collectively, these results suggest that EE housing can be effective in providing neuroprotection after mTBI consistent with previous reports [22-25,34-37]. Our findings point to the importance of using therapeutic strategies directed at modulating the normal compensatory mechanism seen after CNS insult so that its harmful effects can be minimized.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

TLB conceived of the project, analyzed the data, and wrote the manuscript. JW performed the behavioral testing and ELISA procedures. MR performed the surgeries and Western blots. All authors read and approved the final manuscript.

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#### References

- Faul MD, Xu L, Wald MM, Coronado VG: *Traumatic brain injury in the United States: emergency department visits, hospitalizations and deaths 2002–2006*. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control; 2010.
- McAllister TW, Flashman LA, McDonald BC, Saykin AJ: **Mechanisms of working memory dysfunction after mild and moderate TBI: evidence from functional MRI and neurogenetics**. *J Neurotrauma* 2006, **23**:1450–1467.
- Kinnunen KM, Greenwood R, Powell JH, Leech R, Hawkins PC, Bonnelle V, Patel MC, Counsell SJ, Sharp DJ: **White matter damage and cognitive impairment after traumatic brain injury**. *Brain* 2011, **134**(Pt 2):449–463.
- Povlishock JT: **Pathophysiology of neural injury: therapeutic opportunities and challenges**. *Clin Neurosurg* 2000, **46**:113–126.
- Ansari MA, Roberts KN, Scheff SW: **Oxidative stress and modifications of synaptic proteins in hippocampus after traumatic brain injury**. *Free Radic Biol Med* 2008, **45**:443–452.
- Johnson VE, Stewart W, Smith DH: **Widespread tau and amyloid-beta pathology many years after a single TBI in humans**. *Brain Pathol* 2012, **22**:142–149.
- Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W: **Inflammation and white matter degeneration persist for years after a single traumatic brain injury**. *Brain* 2013, **136**:28–42.
- Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB: **ATP mediates rapid microglial response to local brain injury in vivo**. *Nat Neurosci* 2005, **8**:752–758.
- Israelsson C, Bengtsson H, Kylberg A, Kullander K, Lewen A, Hillered L, Ebendal T: **Distinct cellular patterns of upregulated chemokine expression supporting a prominent inflammatory role in traumatic brain injury**. *J Neurotrauma* 2008, **25**:959–974.
- Loane DJ, Byrnes KR: **Role of microglia in neurotrauma**. *Neurotherapeutics* 2010, **7**:366–377.
- Lynch MA: **The multifaceted profile of activated microglia**. *Mol Neurobiol* 2009, **40**:139–156.
- Block ML, Zecca L, Hong JS: **Microglia-mediated neurotoxicity: uncovering the molecular mechanisms**. *Nat Rev* 2007, **8**:57–69.
- Block ML, Hong JS: **Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism**. *Prog Neurobiol* 2005, **76**:77–98.
- Finnie JW: **Neuroinflammation: beneficial and detrimental effects after traumatic brain injury**. *Inflammopharmacology* 2013, **21**:309–320.
- Kumar A, Loane DJ: **Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention**. *Brain Behav Immun* 2012, **26**:1191–1201.
- Nithianantharajah J, Hannan AJ: **Enriched environment, experience-dependent plasticity and disorders of the nervous system**. *Nat Rev Neurosci* 2006, **7**:697–709.
- Will B, Galani R, Kelche C, Rosenzweig MR: **Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990–2002)**. *Prog Brain Res* 2004, **72**:167–182.
- Briones TL, Woods J, Wadowska M, Rogozinska M: **Amelioration of cognitive impairment and changes in microtubule-associated protein 2 after transient global cerebral ischemia are influenced by complex environment experience**. *Behav Brain Res* 2006, **168**(2):261–271.
- Briones TL, Woods J, Wadowska M, Rogozinska M, Nguyen M: **Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training**. *Behav Brain Res* 2006, **171**(1):17–25.
- Briones TL, Suh E, Hattar H, Wadowska M: **Dentate gyrus neurogenesis after cerebral ischemia and behavioral training**. *Biol Res Nurs* 2005, **6**:167–179.
- Briones TL, Therrien B, Metzger B: **Effects of environment on enhancing functional plasticity following cerebral ischemia**. *Biol Res Nurs* 2000, **1**:299–309.
- Johnson EM, Traver KL, Hoffman SW, Harrison CR, Herman JP: **Environmental enrichment protects against functional deficits caused by traumatic brain injury**. *Front Behav Neurosci* 2013, **7**. doi:10.3389/fnbeh.2013.00044.
- Kline AE, McAloon RL, Henderson KA, Bansal UK, Ganti BM, Ahmed RH, Gibbs RB, Sozda CN: **Evaluation of a combined therapeutic regimen of 8-OH-DPAT and environmental enrichment after experimental traumatic brain injury**. *J Neurotrauma* 2010, **27**:2021–2032.
- Matter AM, Folweiler KA, Curatolo LM, Kline AE: **Temporal effects of environmental enrichment-mediated functional improvement after experimental traumatic brain injury in rats**. *Neurorehabil Neural Repair* 2011, **25**:558–564.
- Maegele M, Lippert-Gruener M, Ester-Bode T, Garbe J, Bouillon B, Neugebauer E, Kulig N, Lefering R, Neiss WF, Angelov DN: **Multimodal early onset stimulation combined with enriched environment is associated with reduced CNS lesion volume and enhanced reversal of neuromotor dysfunction after traumatic brain injury**. *Eur J Neurol* 2005, **21**:2406–2418.
- Maesako M, Uemura K, Kubota M, Kuzuya A, Sasaki K, Asada M, Watanabe K, Hayashida N, Ihara M, Ito H, et al: **Environmental enrichment ameliorated high-fat diet-induced Abeta deposition and memory deficit in APP transgenic mice**. *Neurobiol Aging* 2012, **33**:e11–e23.
- Yao ZH, Zhang JJ, Xie XF: **Enriched environment prevents cognitive impairment and tau hyperphosphorylation after chronic cerebral hypoperfusion**. *Curr Neurovasc Res* 2012, **9**:176–184.
- Jurgens HA, Johnson RW: **Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection**. *Brain Behav Immun* 2012, **26**:1006–1016.
- Briones TL, Suh E, Jozsa L, Hattar H, Chai J, Wadowska M: **Behaviorally-induced ultrastructural plasticity in the hippocampal region after cerebral ischemia**. *Brain Res* 2004, **997**:137–146.
- Briones TL, Rogozinska M, Woods J: **Environmental experience modulates ischemia-induced amyloidogenesis and enhances functional recovery**. *J Neurotrauma* 2009, **24**:613–625.
- Fryer LGD, Fougelle F, Barnes K, Baldwin SA, Woods A, Carling D: **Characterization of the role of the AMP-activated protein kinase in the stimulation of glucose transport in skeletal muscle cells**. *Biochem J* 2002, **363**:167–174.
- Kleman AM, Yuan JY, Aja S, Ronnett GV, Landree LE: **Physiological glucose is critical for optimized neuronal viability and AMPK responsiveness in vitro**. *J Neurosci Methods* 2008, **167**:292–301.
- Boero J, Qin W, Cheng JP, Woolsey TA, Strauss AW, Khuchua Z: **Restricted neuronal expression of ubiquitous mitochondrial creatine kinase: changing patterns in development and with increased activity**. *Mol Cell Biochem* 2003, **244**:69–76.
- Kovesdi E, Gyorgy AB, Kwon SK, Wingo DL, Kamnakh A, Long JB, Kasper CE, Agoston DV: **The effect of enriched environment on the outcome of traumatic brain injury: a behavioral, proteomic, and histological study**. *Front Neurosci* 2011, **15**:42.
- Passineau MJ, Green EJ, Dietrich WD: **Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats**. *Exp Neurol* 2001, **168**:373–384.
- Monaco CM, Mattioli VV, Folweiler KA, Tay JK, Yelleswarapu NK, Curatolo LM, Matter AM, Cheng JP, Kline AE: **Environmental enrichments promotes robust functional and histological benefits in female rats after controlled cortical impact injury**. *Exp Neurol* 2013, **247**:410–418.
- Cheng JP, Shaw KE, Monaco CM, Hoffman AN, Sozda CN, Olsen AS, Kline AE: **A relatively brief exposure to environmental enrichment after experimental traumatic brain injury confers long-term cognitive benefits**. *J Neurotrauma* 2012, **29**:2684–2688.
- Wilson S, Raghupathi R, Saatman KE, Mackinnon MA, McIntosh TK, Graham DI: **Continued *in situ* DNA fragmentation of microglia/macrophages in white matter weeks and months after TBI**. *J Neurotrauma* 2004, **21**:239–250.
- Pierce JE, Smith DH, Trojanowski JQ, McIntosh TK: **Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats**. *Neuroscience* 1998, **87**:359–369.
- Ziebell JM, Morganti-Kossmann MC: **Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of TBI**. *Neurotherapeutics* 2010, **7**:22–30.
- Huang ZB, Sheng GQ: **Interleukin-1beta with learning and memory**. *Neurosci Bull* 2010, **26**:455–468.
- McAfoose J, Baune BT: **Evidence for a cytokine model of cognitive function**. *Neurosci Biobehav Rev* 2009, **33**:355–366.

43. Tonelli LH, Postolache TT, Sternberg EM: **Inflammatory genes and neural activity: involvement of immune genes in synaptic function and behavior.** *Front Biosci* 2005, **10**:675–680.
44. Smith JA, Das A, Ray SK, Banik NL: **Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases.** *Brain Res Bull* 2012, **87**:10–20.
45. Cunningham C, Sanderson DJ: **Malaise in the water maze: untangling the effects of LPS and IL-1beta on learning and memory.** *Brain Behav Immun* 2008, **22**:1117–1127.
46. Rijntjes M, Weiller C: **Recovery of motor and language abilities after stroke: the contribution of functional imaging.** *Prog Neurobiol* 2002, **66**:109–122.
47. Bach-y-Rita P: **Brain plasticity as a basis for recovery of function in humans.** *Neuropsychologia* 1990, **28**:547–554.
48. Kahn BB, Alquier T, Carling D, Hardie DG: **AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism.** *Cell Metab* 2005, **1**:15–25.
49. Carling D: **The AMP-activated protein kinase cascade - a unifying system for energy control.** *Trends Biochem Sci* 2004, **29**:18–24.
50. Manwani B, McCullough LD: **Function of the master energy regulator adenosine monophosphate-activated protein kinase in stroke.** *J Neurosci Res* 2013, **91**:1018–1029.
51. Carling D, Thornton C, Woods A, Sanders MJ: **AMP-activated protein kinase: new regulation, new roles?** *Biochem J* 2012, **445**:11–27.

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