Published in final edited form as:

J Neurosci Res. 2009 February 15; 87(3): 795–805. doi:10.1002/jnr.21893.

Controlled Contusion Injury Alters Molecular Systems Associated With Cognitive Performance

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Abstract

We investigated whether a learning impairment after a controlled cortical impact (CCI) injury was associated with alterations in molecules involved in synaptic plasticity and learning and memory. Adult male rats with moderate CCI to the left parietal cortex, tested in a Morris water maze (MWM) beginning at postinjury day 10, showed impaired cognitive performance compared with sham-treated rats. Tissue was extracted for mRNA analysis on postinjury day 21. The expression of brain-derived neurotrophic factor (BDNF), synapsin I, cyclic-AMP response element binding protein (CREB), and calcium-calmodulin-dependent protein kinase II (α -CAMKII) were all significantly decreased compared with sham injury levels within the ipsilateral hippocampus after CCI. No significant molecular level changes were found in the contralateral hippocampus. Decreased expression of BDNF and synapsin I was also found within the ipsilateral parietal cortex of CCI-injured rats compared with shams. However, BDNF and synapsin I expressions were significantly increased in the contralateral parietal cortex of the CCI rats. CREB expression was significantly decreased within the contralateral cortex of the CCI group. These findings show enduring reductions in the expression of BDNF, synapsin I, CREB, and α -CAMKII ipsilateral to a CCI injury, which seem associated with the spatial learning deficits observed in this injury model. In addition, the delayed increase in the expression of BDNF and synapsin I within the cortex contralateral to CCI may reflect restorative processes in areas homotypical to the injury.

Keywords

BDNF; injury; synapsin I; CREB; CAMKII; rat

Traumatic brain injury (TBI) is one of the leading causes of disability, strongly affecting the quality of life of individuals (Barth et al., 1983; Hellawell et al., 1999; McAllister et al., 2001; Rapoport et al., 2002; Vitaz et al., 2003; Ashman et al., 2006). Enduring cognitive deficits are a major factor in TBI-induced disabilities, and the hippocampus is among the most vulnerable structures after TBI in humans (Wilde et al., 2007). Experimental TBI in rodents, including fluid percussion injury (FPI) and controlled cortical impact (CCI), are valuable tools for modeling various aspects of human TBI because these experimental models produce hippocampal pathology as well as impairments in the ability to perform hippocampal-

dependent tasks (Smith et al., 1991; Hamm et al., 1992; Hicks et al., 1993; Colicos and Dash, 1996; Fujimoto et al., 2004). Thus, these models can be used to increase our understanding of the alterations of molecular systems that may be associated with the prevalent cognitive impairments that occur after TBI.

Brain injury leads to alterations in molecular substrates of synaptic plasticity that may contribute to the occurrence of cognitive impairments. In particular, the expression of brain-derived neurotrophic factor (BDNF) is acutely altered in different models of TBI (Yang et al., 1996; Hicks et al., 1998; Hellmich et al., 2005). The capacity of BDNF to react to tasks involving activity such as exercise is temporarily compromised after an FPI (Griesbach et al., 2002, 2004a, 2007). BDNF plays a crucial role in activity-dependent plasticity by facilitating the synapse (Tyler and Pozzo-Miller, 2001; Tyler et al., 2006) and enhancing neurotransmitter release (Levine et al., 1995, 1998; Takei et al., 1997; Albensi, 2001). Increases in hippocampal BDNF expression are associated with enhanced cognitive performance, and learning itself has also been shown to increase BDNF (Falkenberg et al., 1992; Kesslak et al., 1998). In turn, failure of the BDNF system through a particular polymorphism in the gene encoding BDNF has been linked with alterations in human hippocampal activation, memory, and reasoning abilities (Egan et al., 2003; Harris et al., 2006).

Synapsin I, cyclic-AMP response element binding protein (CREB), and calcium-calmodulin-dependent protein kinase II (CAMKII), all associated with the function of BDNF on cognition, can also be affected after TBI. Efficiency in synaptic function is facilitated through synapsin I. Synapsin I is downstream to BDNF and regulates synaptic transmission by controlling the amount of synaptic vesicles and consequentially regulating neurotransmitter release (Greengard et al., 1993). CREB is a transcriptional regulator that has been linked to long-term potentiation, a physiological correlate of learning and memory (Abel and Kandel, 1998; Silva et al., 1998). BDNF also modulates synaptic transmission by increasing CAMKII, which plays a vital role in the formation of long-term memories (Fukunaga and Miyamoto, 1999, 2000; Cammarota et al., 2002). Alterations in these molecules have been observed within the first 24 hr after TBI (Dash et al., 1995; Atkins et al., 2006; Folkerts et al., 2007).

Because cognitive impairments induced by experimental TBI are most frequently studied within the first 2- to 3-week period, the current study was conducted to determine the relationship between BDNF, synapsin I, α -CAMKII, and CREB and cognitive deficits during this postinjury time period. Rats with unilateral CCI to the parietal cortex were evaluated for spatial learning ability and after 10 days of testing molecular changes ipsilateral and contralateral to the injury were evaluated.

MATERIALS AND METHODS

Subjects

A total of 12 male Sprague Dawley rats (mean weight, 247 g) were used in these experiments. Rats underwent surgery to induce either sham injury ($n = 6$) or CCI injury ($n = 6$). All animals were monitored and cared for by Chancellor's Animal Research Committee approved veterinary care staff upon arrival to University of California Los Angeles (UCLA). During the experiments, rats were single housed in opaque plastic bins ($50.8 \times 25.4 \times 25.4$ cm), which were lined with bedding material. Rats had access to water and feed ad libitum. All procedures were performed in accordance with the United States National Institute of Health Guide for the Care and Use of Laboratory Animals, the principles presented in the Guidelines for the Use of Animal in Neuroscience Research, and were approved by the UCLA Chancellor's Animal Research Committee. The suffering and number of animals used was minimized.

CCI Injury

Animals were placed under inhalation anesthesia with isoflurane (4% for induction, 2.0% for maintenance, in 100% O₂ at 1.5 L/min). The level of anesthesia was monitored by level of respiration, muscular relaxation and the corneal and pedal reflexes. After loss of corneal and pedal reflexes the scalp was shaved. Animals were secured in a stereotactic head frame and the scalp was cleansed with ethanol and Betadine. Rectal temperature was monitored and maintained between 36.5°C and 38.0°C with a thermostatically controlling heating pad (Braintree Scientific, Braintree, MA). A midline sagittal incision was made, the scalp and temporal muscle were reflected and a 6-mm-diameter circular craniotomy was made over the left parietal cortex, centered at 3 mm posterior and 3.5 mm lateral to bregma. The bone flap was removed and the dura left intact in all animals to receive CCI. An electronically controlled pneumatic piston cylinder (Hydraulics Control, Emeryville, CA) mounted onto a stereotactic micromanipulator (Kopf Instruments, Tujunga, CA) was used to allow for precise localization and control of the impact (Sutton et al., 1993). The piston cylinder was angled 19° away from vertical to allow the flat (5 mm diameter) impactor tip to make contact perpendicular to the brain's surface. CCI was induced with a 2-mm compression of tissue under the exposed dura (250 msec, 1.9 m/sec velocity). After controlling for any mild bleeding after the injury, the scalp incision was sutured closed. Marcaine (0.15 mL) was injected into the margins of the scalp incision and triple antibiotic ointment was applied over the incision. Sham-injured animals underwent all surgical procedures, except for the craniotomies and CCI delivery. This injury model produces a regionally and qualitatively consistent cortical compression resulting in an ipsilateral cortical cavitation and hippocampal neuronal loss that has been previously categorized in the moderate range (Sutton et al., 1993; Taylor et al., 2008).

Behavioral Testing

Spatial navigation learning and memory were tested by a Morris water maze (MWM) task beginning on postinjury day 10. The water maze consisted of a 1.5-m-diameter, 0.6-m-high circular tank filled with white opaque organic paint (Stechler, Albuquerque, NM). The water level was kept at 2 cm above an escape platform (15 × 15 cm) and maintained at 20°C. The platform was 2 cm below the water surface and was fixed in position in the northwest quadrant of the tank for all trials. Rats received two training trials per each daily session for 10 sequential days, with an intertrial interval of 10 sec. On each trial animals were released from one of four predetermined points around the water maze in random order and were given 60 sec to locate the platform. It was ensured that each session included one long (south or east release point) and one short (north or west release point) trial swim. Once they reached the platform, they remained there for 10 sec before the second trial was initiated. If they failed to locate the platform, they were manually guided to it. Swimming behavior (path, distance, and latency to platform) was recorded with the SMART tracking system (San Diego Instruments, San Diego, CA).

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)

Rats were killed by decapitation on postinjury day 21, the brain was rapidly extracted, and the entire hippocampal formation and parietal cortex were dissected from the ipsilateral (left) and contralateral (right) hemispheres. The parietal cortex was selected because this was the region that surrounded the CCI site. Total RNA was isolated by the RNA STAT-60 (Tel-Test, Friendswood, TX). Briefly, after tissue homogenization (1 mL/50–100 mg tissue) 0.2 mL of chloroform per 1 mL of the RNA STAT-60 was added. Samples were centrifuged and the aqueous phase was mixed with isopropanol (0.5 mL/1 mL) before a second centrifugation. Supernatant was removed and the RNA pellet was washed with 75% ethanol, centrifuged, dried, and dissolved in water. RNA measurements were taken the next day.

Detection, quantification, and analysis of RNA were performed according to the TaqMan EZ RT-PCR kit specifications (Applied Biosystems, Foster City, CA), with the Applied Biosystems Prism Model 7700 sequence detection instrument. The system was based on the possibility to directly detect the reverse transcription polymerase chain reaction (RT-PCR) product with no downstream processing. This was accomplished with the monitoring of the increase in fluorescence of a dye-labeled DNA probe. Total RNA (100 ng) was converted into cDNA with TaqMan EZ RT-PCR Core reagents. Briefly, reagent mix was prepared with kit components and added to RNA (5 μ L RNA/20 μ L reagent mix). All samples were done in duplicate. Forward and reverse primers were sequenced with PrimerQuest software by Integrated DNA Technologies (Coralville, IA). An oligonucleotide probe (5'-CCGACTCTTGCCCTTCGAAC-3') probe specific for the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous control to standardize the amount of sample RNA. The GAPDH gene is a constitutively expressed housekeeping gene that has been shown to be suitable to correct variations in RNA quantity and quality (Medhurst et al., 2000). The following probes were used: BDNF (5'-AGTCATTTGCGCACAACCTTTAAAAGTCTGCATT-3'), BDNF forward (5'-GGACATATCCATGACCAGAAAGAAA-3'), BDNF reverse (5'-GCAACAAACCACAACATTATCGAG-3'), synapsin I (5'-CATGGCACGTAATGGAGACTACCGCA-3'), synapsin I forward (5'-CCGCCAGCTGCCTTC-3'), synapsin I reverse (5'-TGCAGCCCAATGACCAAA-3'), CREB (5'-CATGGCACGTAATGGAGACTACCGCA-3'), CREB forward (5'-CCGCCAGCATGCCTTC-3'), CREB reverse (5'-TGCAGCCCAATGACCAAA-3'), α -CAMKII (5'-CTCCACTGTGGCCTCCTGCATGC-3'), α -CAMKII forward (5'-AGCACCCCTGGATCTCGC-3'), α -CAMKII reverse (5'-TTCTTCAGGCAGTCCACGGT-3').

Statistical Analysis

Latency to reach the hidden platform (daily averages) was analyzed through a repeated-measures analysis of variance (ANOVA). Significant interaction effects were further analyzed by performing Bonferroni corrected comparisons. Data were also analyzed by acquisition of criteria that were defined as the ability to locate the platform in determined amounts of time for both trials within a session. Similar measures have previously been used to measure behavioral outcome after TBI (Prins and Hovda, 1998; Fineman et al., 2000; Giza et al., 2005; Gurkoff et al., 2006). Criterion times ranged from 4 sec or less to 10 sec or less to reach the platform. Different levels of criteria were used rather than arbitrarily choosing a single criterion score, which allowed us to assess the rate of MWM acquisition. Each criterion received a score, ranging from 1 to 11, by determining the session in which that criterion was reached. Whereas a score of 1 would indicate that no criterion was ever reached, a score of 11 was given if the criterion was reached on the first session of training (Table I). Criterion scores were analyzed with repeated measures ANOVA. Multiple comparisons were Bonferroni corrected. In addition, a final MWM criterion score was obtained by averaging the scores obtained for all criteria (4 sec or less to 10 sec or less). The final criterion score provided a single measure that could be used for regression analysis. Whereas swim latencies indicate performance across days of training, a high final MWM criterion score indicates that a rat is able to efficiently learn the task, by reaching a strict criterion, during the first days of training.

Duplicate values for gene expression data were averaged. Any values that fell 1 standard deviation away from the mean were eliminated. Quantification of mRNA was normalized with corresponding GAPDH values. Each hippocampal and cortical side was normalized to sham levels, thus allowing for comparisons between the left and right sides. The resulting corrected values were analyzed through a mixed-model ANOVA: [2 (Group) $\times$ 2 (Hemisphere)]. Regression analysis was performed to illustrate the association between MWM performance

(mean learning criterion scores) and mRNA expression. However, it should be noted that as a result of the limited number of subjects, data from sham and CCI rats were pooled.

RESULTS

Cognitive Performance

Rats with a CCI injury showed a significant learning deficit, given that the overall latency to reach the hidden platform was significantly longer in the CCI group compared with the sham group, as indicated by a significant Group main effect ($F_{1,90} = 5.35$, $P < 0.05$) and a significant Group $\times$ Time interaction ($F_{9,90} = 2.3$, $P < 0.05$). Individual session analysis revealed that CCI rats took longer to reach the platform on sessions 3, 5, 8, and 10 ($P < 0.05$). Overall performance in the MWM improved across time for both groups, as indicated by a decrease in swimming latency across days ($F_{9,90} = 12.26$, $P < 0.05$). The CCI rats showed more variability compared with shams. Besides the intrinsic variability of CCI injuries, this variability effect is likely to be accentuated by a floor effect in the shams' MWM performance (Fig. 1). Analysis of criteria also demonstrated group differences in MWM performance (Table II). Whereas all of the sham rats reached all the learning criteria, the CCI rats did not. None of the CCI rats were able to reach the platform in 4 sec or less for two consecutive trials within a session. Fifty percent of the injured rats were able to reach the criteria of 5, 6, and 7 sec or less. When the criterion was set at reaching the platform in 8, 9, and 10 sec or less, 83% of the injured rats were able to obtain this criterion. These findings were supported by a significant group effect for the MWM criterion scores ($F_{1,10} = 10.87$, $P < 0.01$). Multiple comparison analysis indicated that CCI rats had significantly lower scores for criteria set at 4, 5, 6, 7, and 8 sec ($P < 0.05$). These scores not only indicated that CCI rats reached fewer criteria, but also that those that were reached required more days of training compared with shams. No significant differences were observed in swim speed (cm/sec).

BDNF mRNA

Hippocampus—On the side ipsilateral to the CCI, BDNF expression was significantly decreased compared with shams ($F_{1,10} = 38.92$, $P < 0.0005$). Within the CCI group, BDNF was significantly lower in the ipsilateral side compared with the contralateral side ($F_{1,10} = 17.14$, $P < 0.005$). No differences were found in the levels of contralateral BDNF between CCI and sham rats (Fig. 2A). BDNF mRNA levels within the ipsilateral hippocampus showed a tendency to be correlated with MWM performance ($y = 3.53x + 66.2$, $R^2 = 0.27$, $P = 0.08$) (Fig. 2B). Because BDNF, within the contralateral hippocampus, was not altered in the CCI group, there was a tighter distribution within this side. Therefore, a correlation between BDNF and behavioral performance was not observed within the contralateral side.

Cortex—On the side ipsilateral to the CCI, BDNF expression was significantly decreased in the parietal cortex ($F_{1,10} = 20.41$, $P < 0.005$) compared with shams. On the side contralateral to the CCI, BDNF expression was significantly higher ($F_{1,10} = 8.4$, $P < 0.05$) compared with the shams. Within the CCI group, BDNF was significantly lower in the ipsilateral side compared with the contralateral side ($F_{1,10} = 32.85$, $P < 0.0005$) (Fig. 2A). A significant correlation was observed between BDNF mRNA, within the ipsilateral cortex and performance in the MWM test ($y = 4.296x + 65.38$, $R^2 = 0.51$, $P = 0.01$) (Fig. 2B).

Synapsin I mRNA

Hippocampus—On the side ipsilateral to the CCI, levels of synapsin I mRNA were significantly decreased ($F_{1,10} = 62.27$, $P < 0.0005$) compared with shams. Within the CCI group, synapsin I expression was significantly lower in the ipsilateral side compared with the contralateral side ($F_{1,10} = 51.62$, $P < 0.0005$). Further analysis indicated no differences in the levels of contralateral synapsin I between CCI and sham rats (Fig. 3A). A significant correlation

was observed between synapsin I mRNA, within the ipsilateral side and performance in the MWM test ($y = 5.663x + 53.7$, $R^2 = 0.61$, $P = 0.005$) (Fig. 3B). No significant correlation between contralateral synapsin I mRNA and behavioral performance was observed.

Cortex—On the side ipsilateral to the CCI, levels of synapsin I mRNA were significantly decreased in the parietal cortex ($F_{1,10} = 14.39$, $P < 0.005$), compared with shams. Cortical synapsin I expression was significantly higher in the side contralateral to the CCI compared with shams ($F_{1,10} = 39.59$, $P < 0.0005$). Within the CCI group, synapsin I was significantly lower in the ipsilateral side compared with contralateral side ($F_{1,10} = 131.49$, $P < 0.0005$) (Fig. 3A). Within the ipsilateral cortex, synapsin I mRNA levels had a tendency to be correlated with better learning in the MWM test ($y = 2.647x + 78.8$, $R^2 = 0.29$, $P = 0.07$) (Fig. 3B).

CREB mRNA

Hippocampus—On the side ipsilateral to the CCI, CREB expression was significantly decreased ($F_{1,10} = 18.8$, $P < 0.005$) compared with the shams. Within the CCI group, CREB was significantly lower in the ipsilateral side compared with the contralateral hippocampus ($F_{1,10} = 10.2$, $P < 0.05$) (Fig. 4A). A significant correlation was observed between CREB mRNA within the ipsilateral hippocampus and performance in the MWM test ($y = 4.224x + 65.13$, $R^2 = 0.43$, $P = 0.05$) (Fig. 4B). No significant correlation between contralateral CREB mRNA and behavioral performance was observed.

Cortex—CREB expression 21 days after CCI did not differ in the ipsilateral cortex compared with shams. However, in the cortex contralateral to the CCI, CREB expression was significantly lower compared with the shams ($F_{1,10} = 8.73$, $P < 0.05$). Within the CCI group, cortical CREB was significantly lower in the contralateral side compared with the ipsilateral side ($F_{1,10} = 8.53$, $P < 0.05$) (Fig. 4A). No significant correlations were observed between CREB mRNA and MWM performance within the cortex.

α -CAMKII mRNA

Hippocampus—On the side ipsilateral to the CCI, CAMKII mRNA levels were significantly decreased ($F_{1,10} = 67.83$, $P < 0.0005$) compared with the shams. Within the CCI group, CAMKII was significantly lower in the ipsilateral side compared with the contralateral side ($F_{1,10} = 23.54$, $P < 0.005$) (Fig. 5A). A significant correlation was observed between CAMKII mRNA, within the ipsilateral hippocampus and performance in the MWM test ($y = 4.19x + 62.22$, $R^2 = 0.36$, $P = 0.05$) (Fig. 5B). No significant correlation between contralateral CAMKII mRNA and behavioral performance was observed.

Cortex—CAMKII expression did not differ in the ipsilateral or contralateral cortex of the CCI rats compared with the shams. Likewise, no significant effects were observed between hemispheres within the CCI group. No significant correlations were observed between CAMKII mRNA and MWM performance within the cortex.

DISCUSSION

These findings indicate that cognitive deficits after an experimental model of “focal” brain injury are accompanied by alterations in the levels of expressions of molecules associated with synaptic plasticity and learning during the postacute period. In particular, the expressions of BDNF, synapsin I, CAMKII, and CREB were altered 21 days after CCI in a region dependent manner. The possibility of an association between molecular levels and behavioral performance was suggested for the hippocampus ipsilateral to the CCI.

Ipsilateral Effects

CCI injured rats showed cognitive deficits that were accompanied by ipsilateral, but not contralateral, decreases in the expression of hippocampal BDNF, synapsin I, CREB, and CAMKII. Decreases of these molecules within the hippocampus may weaken the molecular substrates that support synaptic function with potential implications for learning and memory capacity. TBI-induced cognitive impairments have been associated with hippocampal atrophy in humans (Himanen et al., 2005; Ariza et al., 2006; Ashman et al., 2006) and in animal models of TBI (Smith et al., 1991; Hamm et al., 1992; Hicks et al., 1993; Colicos and Dash, 1996; Fujimoto et al., 2004). Although decreases in the molecular systems studied within the hippocampus were only observed in the side ipsilateral to the injury, they may be sufficient to result in a learning impairment. Partial hippocampal damage has been demonstrated to impair the rate of learning (de Hoz et al., 2005). In effect, although their performance in the MWM was impaired, the CCI rats were still able to learn the location of the hidden platform over multiple trials.

It is possible that MWM training may have contributed to the levels of hippocampal BDNF observed in the sham animals as such training has been shown to increase BDNF (Kesslak et al., 1998). Although this may have contributed to group differences in MWM performance, it is likely that CCI rats had decreases in these molecules during MWM training that may have negatively affected learning. We have recently found significant decreases in levels of hippocampal synapsin I activation at 7 days after CCI injury (Griesbach et al., 2008). This same study has shown slight reductions in hippocampal BDNF protein levels. Other investigators have found a tendency for ipsilateral hippocampal BDNF mRNA to be decreased 4 weeks after CCI (Chen et al., 2005). Likewise, ipsilateral decreases in hippocampal levels of CAMKII have been observed 7 days after an FPI in mice (Schwarzbach et al., 2006). It is also likely that regulatory properties of CAMKII were affected as has been found after transient ischemia (Babcock et al., 1995, 2002). These changes in CAMKII activation could also impair memory.

These reported changes on BDNF differ from previous studies where hippocampal decreases in BDNF were not observed after FPI, at different postinjury times up to postinjury day 37 (Hicks et al., 2002; Griesbach et al., 2004a, 2004b, 2007). In contrast, here, with the CCI model, we show that ipsilateral hippocampal BDNF decreases at postinjury day 21. Because ipsilateral hippocampal cell loss is frequently reported in the CCI model of TBI (Smith et al., 1991; Colicos and Dash, 1996; Colicos et al., 1996; Baldwin et al., 1997), decreases in the observed molecules may be the result of neuronal degeneration. Neuronal degeneration, at least within the CA3 region of the hippocampus, does occur after CCI in the injury severity range used for the current study (Taylor et al., 2008).

Decreases in BDNF and synapsin I mRNAs were also found in the ipsilateral cortex after CCI injury and these may have contributed to the cognitive impairment found in the CCI rats. Although, spatial navigation and memory can largely be attributed to the hippocampus, cortical damage has also been shown to affect performance in the MWM task (DiMattia and Kesner, 1988; Dixon et al., 1991; Save and Moghaddam, 1996; Bramlett et al., 1997; Lindner et al., 1998).

It should be noted that although the observed molecules are linked to cognitive functioning, it is possible that many of the other biochemical and molecular systems reported to be altered after TBI contribute to the observed behavioral impairments (Laurer and McIntosh, 2001; Verbois et al., 2002; Thompson et al., 2005). CCI-induced neuronal degeneration, which also affects connectivity as a result of axonal damage (Hall et al., 2008), and vascular alterations (Lighthall et al., 1990; Verbois et al., 2002; Rafols et al., 2007) may also influence behavioral outcome.

Low levels of BDNF may have contributed to the hippocampal and cortical decreases in synapsin I as a result of the recognized BDNF effects on synapsin I activation (Jovanovic et al., 1996, 2000). Again, it should be noted that the current findings in the CCI model contrast with those from previous studies that use the FPI model, where no decreases in ipsilateral cortical BDNF or synapsin I protein were observed from 7 to 37 days after FPI (Griesbach et al., 2004a, 2007). These reported differences between injury models may be due to the larger cortical contusion associated with the CCI model (Sutton et al., 1993; Dixon et al., 1999). In turn, the FPI model induces an inertial loading on the brain resulting in a diffuse injury with less extensive cell death (Fujimoto et al., 2004; Gurkoff et al., 2006). Thus, the reduction in ipsilateral cortical BDNF and synapsin I mRNA in this CCI model may be the result of neuronal loss in the surviving tissue around the contusion site at 21 days after injury.

Contralateral Effects

Interestingly, the contralateral cortex, which experiences little or no cell loss compared with the ipsilateral side in our CCI model (Sutton et al., 1993; Taylor et al., 2008), had high levels of BDNF as well as synapsin I expression by 21 days after injury. Contralateral increases in BDNF have also been observed 4 weeks after CCI in areas remote to the injury site (Chen et al., 2005) as well as after FPI in developing rats (Griesbach et al., 2002). These increases may be indicative of a compensatory response in areas remote to the injured site. Reorganization after brain injury has been proposed as a mechanism to facilitate recovery (Stein and Hoffman, 2003; Dancause et al., 2005; Desmurget et al., 2007), and has been observed in humans (Weiller et al., 1992; Chu et al., 2000) and animals (Dunn-Meynell and Levin, 1995; Jansen and Low, 1996; Florence et al., 1998; Kozłowski and Schallert, 1998; Ip et al., 2002). Increases in BDNF (Hanover et al., 1999; Gorski et al., 2003; Tropea et al., 2003; Lauterborn et al., 2007) and synapsin I (Han and Greengard, 1994) may enhance synaptic remodeling and function, allowing for restorative processes to occur.

In contrast to BDNF and synapsin I, CREB expression was decreased in the contralateral cortex. This decrease could be associated to the action of the calcium-activated protease, calpain, which is altered after CCI. Calpain increases have been reported in the contralateral cortex after CCI injury (Hall et al., 2005). An increase in calpains may result in protein kinase A (PKA) dysregulation. Given that PKA is the major regulator of CREB (Tully et al., 2003; Liang et al., 2007), it is possible that the cortical decrease in CREB expression is associated with PKA dysregulation. Although calpain activation has been associated with neuronal damage frequently leading to cell death (Raghupathi, 2004), it has also been observed in “sublethally” stretched neurons (Arundine et al., 2004) and has been implicated in neuronal remodeling (Faddis et al., 1997; Huh et al., 2001). In addition, calpain activation has been shown to activate BDNF (Nagy et al., 2002), which has been linked to processes that underline neuroprotection (Alderson et al., 1990; Cheng and Mattson, 1994; Glazner and Mattson, 2000; Larsson et al., 2002).

These findings indicate that the CCI model of TBI results in delayed disruptions of molecular systems that play key roles in synaptic plasticity, and that these changes may be associated with reduced capacity for learning and memory. In addition, these results indicate that areas remote to the injury site show molecular changes that may foster regenerative processes. This information may help to define pharmacological targets to reduce cognitive impairment resulting from TBI.

Acknowledgments

Contract grant sponsor: NINDS; Contract grant numbers: NS048535, NS27544; Contract grant sponsor: UCLA Brain Injury Research Center.

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Fig. 1.

Swimming latency in the MWM task. Rats were tested for one session daily beginning on postinjury day 10. Time between sessions was 24 hr. A significant decrease in latency across sessions was observed in CCI and sham groups ($*P < 0.05$). The latency to reach the hidden platform, across all days, was significantly longer in rats that received a CCI injury. No significant differences in swimming speed were observed. Each value represents mean $\pm$ SEM.

Fig. 2.

Effects of CCI on BDNF mRNA at postinjury day 21. **A:** BDNF mRNA levels in the ipsilateral hippocampus and ipsilateral parietal cortex were significantly lower compared with shams. In contrast BDNF expression was significantly higher in the contralateral cortex compared with shams. Each value represents mean $\pm$ SEM (* $P < 0.05$). **B:** Correlation between BDNF and MWM performance. A higher final MWM criterion score indicates that learning criteria were reached earlier during training. It also indicates that stricter learning criteria were reached. Whereas cortical BDNF was significantly correlated with learning, the correlation with hippocampal BDNF only approached significance ($P = 0.08$).

Fig. 3.

Effects of CCI on synapsin I mRNA at postinjury day 21. **A:** Synapsin I mRNA levels in the ipsilateral hippocampus and ipsilateral parietal cortex were significantly lower compared with shams. In contrast, synapsin I expression was significantly higher in the contralateral cortex compared with shams. Each value represents mean $\pm$ SEM ($*P < 0.05$). **B:** Correlation between synapsin I and MWM performance. A higher final MWM criterion score indicates that learning criteria were reached earlier during training. It also indicates that stricter learning criteria were reached. Whereas hippocampal synapsin I was significantly correlated with learning, the correlation with cortical synapsin I only approached significance ($P = 0.07$).

Fig. 4.

Effects of CCI on CREB mRNA at postinjury day 21. **A:** CREB mRNA levels in the ipsilateral hippocampus and contralateral cortex were significantly lower compared with shams. Each value represents mean $\pm$ SEM ($*P < 0.05$). **B:** Correlation between CREB and MWM performance. A higher final MWM criterion score indicates that learning criteria were reached earlier during training. It also indicates that stricter learning criteria were reached. Hippocampal CREB was significantly correlated with learning.

Fig. 5. Effects of CCI on CAMKII mRNA at postinjury day 21. **A:** CAMKII mRNA levels in the ipsilateral hippocampus were significantly lower compared with shams. Each value represents mean $\pm$ SEM ($*P < 0.05$). **B:** Correlation between CAMKII and MWM performance. A higher final MWM criterion score indicates that learning criteria were reached earlier during training. It also indicates that stricter learning criteria were reached. Hippocampal CAMKII was significantly correlated with learning.

TABLE I
The Conversion From the MWM Session in that Criterion was Achieved to the Criterion Score*

Session	1	2	3	4	5	6	7	8	9	10	Never Reached
Criterion score	11	10	9	8	7	6	5	4	3	2	1

* Each criterion, ranging from 4 sec or less to 10 sec or less to reach the hidden platform, received a score that was determined by the session in which that criterion was obtained.

TABLE II
Individual Criterion Scores Obtained for Each Criterion Time*

Rat	4 sec or less	5 sec or less	6 sec or less	7 sec or less	8 sec or less	9 sec or less	10 sec or less
Sham	2	2	2	2	2	4	4
Sham	9	9	9	9	9	9	9
Sham	4	4	9	9	9	9	9
Sham	9	9	9	9	9	9	9
Sham	7	7	7	9	9	9	9
Sham	4	5	5	6	6	6	6
CCI	1	1	1	1	5	5	5
CCI	1	3	3	3	3	10	10
CCI	1	5	5	5	5	5	5
CCI	1	1	1	1	1	1	1
CCI	1	4	4	4	4	4	4
CCI	1	1	1	1	1	1	5

* Scores were averaged to obtain a final MWM criterion score. A score of 1 indicates that the criterion was never reached.