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A SATURATED-FAT DIET AGGRAVATES THE OUTCOME OF TRAUMATIC BRAIN INJURY ON HIPPOCAMPAL PLASTICITY AND COGNITIVE FUNCTION BY REDUCING BRAIN-DERIVED NEUROTROPHIC FACTOR

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Abstract—We have conducted studies to determine the potential of dietary factors to affect the capacity of the brain to compensate for insult. Rats were fed with a high-fat sucrose (HFS) diet, a popularly consumed diet in industrialized western societies, for 4 weeks before a mild fluid percussion injury (FPI) or sham surgery was performed. FPI impaired spatial learning capacity in the Morris water maze, and these effects were aggravated by previous exposure of the rats to the action of the HFS diet. Learning performance decreased according to levels of brain-derived neurotrophic factor (BDNF) in individual rats, such that rats with the worst learning efficacy showed the lowest levels of BDNF in the hippocampus. BDNF immunohistochemistry localized the decreases in BDNF to the CA3 and dentate gyrus of the hippocampal formation. BDNF has a strong effect on synaptic plasticity via the action of synapsin I and cAMP-response element-binding protein (CREB), therefore, we assessed changes in synapsin I and CREB in conjunction with BDNF. Levels of synapsin I and CREB decreased in relation to decreases in BDNF levels. The combination of FPI and the HFS diet had more dramatic effects on the active state (phosphorylated) of synapsin I and CREB. There were no signs of neurodegeneration in the hippocampus of any rat group assessed with Fluoro-Jade B staining. The results suggest that FPI and diet impose a risk factor to the molecular machinery in charge of maintaining neuronal function under homeostatic and challenging situations.

Key words: fluid percussion injury, neuroplasticity, cognition, BDNF, synapsin I, CREB.

Traumatic brain injury (TBI) is a major source of hardship for the American population, therefore individuals who survive the original insult remain with long-term neurological problems. Cognitive deficits including memory impairment are common neurological sequel of TBI, and their underlying mechanisms are poorly understood (Lowenstein et al., 1992; Hamm et al., 1993; Hicks et al., 1993; Sanders et al., 2000). It is becoming obvious that neuronal death may not account for the entire spectrum of deficiency (Lyeth et al., 1990; Scheff et al., 1997), such that it is crucial to learn more about molecular and functional changes occurring in the neurons that survive the primary injury. It is likely that molecular mechanisms underlying synaptic plasticity are disrupted following injury with profound consequences on neuronal function. Accordingly, we have performed studies focusing on the synapse to evaluate a molecular substrate of fundamental importance for the design of new treatments to reduce the effects of TBI.

Molecular events controlled by BDNF may play a critical role on shaping the capacity of the brain to endure the effects of TBI. Several studies using the concussion model, fluid percussion injury (FPI), have concluded that TBI alters levels of BDNF in rats (Hicks et al., 1999; Truettner et al., 1999). BDNF affects the function of molecules that modulate synaptic transmission and gene transcription, i.e. synapsin I and cAMP-response element binding protein (CREB). Synapsin I is a vesicle-associated phosphoprotein, that modulates neurotransmitter release under the influence of BDNF (Jovanovic et al., 1996). The transcription factor CREB, in a close interaction with BDNF (Nibuya et al., 1996; Tao et al., 1998; McAllister et al., 1999; Shaywitz and Greenberg, 1999; Conti et al., 2002; Ying et al., 2002), is involved in adaptive responses (Finkbeiner, 2000) and long-term memory (Yin and Tully, 1996; Tully, 1997). We have evaluated the possibility that BDNF and its downstream effectors synapsin I and CREB are involved with the chronic vulnerability of the injured brain, jeopardizing the capacity of cells to remain functional.

Although the type of nutrients ingested is a crucial mean to affect brain health on daily basis, the effects of dietary factors on the capacity of the brain to cope with insults have not been experimentally scrutinized. It has become recently known that a high-fat sucrose (HFS) diet, popularly consumed in most industrialized western societies, reduces levels of BDNF, leading to impairments in neuronal and behavioral plasticity (Molteni, et al., 2002). Based on the critical actions of BDNF and related systems in maintaining neural function, a failure of the BDNF system resulting from a HFS diet may increase susceptibility of the brain to insults. Accordingly, here we report the

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Abbreviations: BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP-response element-binding protein; ELISA, enzyme-linked immunosorbent assay; FPI, fluid percussion injury; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HFS, high-fat sucrose; p-CREB, phospho-CREB; p-synapsin I, phospho-synapsin I; RD, regular diet; RT-PCR, reverse transcription polymerase chain reaction; TBI, traumatic brain injury; UNG, uracil glycosylase.

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effects of a HFS diet on potentiating the deleterious effects of TBI on neuronal plasticity and cognitive function.

EXPERIMENTAL PROCEDURES

Experimental design and tissue preparation

Eighty male Sprague–Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing between 200 and 240 g were housed in cages (two rats per cage) and maintained in environmentally controlled rooms (22–24 °C) with a 12 h light/dark cycle. After acclimatization for 1 week on standard rat chow, the rats were randomly assigned to HFS diet or regular diet (RD) for 4 weeks. The diets, fed *ad libitum*, were provided in powder (TestDiet Inc., Richmond, IN) in large bowl and contained a standard vitamin and mineral mix with all essential nutrients, as described previously. The RD is low in saturated fat (13% of energy from fat) and contains complex carbohydrate (starch, 59% energy). The HFS diet is high in saturated and monounsaturated (primarily from lard plus a small amount of corn oil, ~39% energy) and high in refined-sugar (sucrose, ~40% energy). After 4 weeks of feeding with RD or HFS diet, the rats were exposed to mild FPI or just sham surgery. After 1 week maintained on the same diet post-injury, rats (n=5–8 within each group) were killed by decapitation, and the brain was rapidly dissected, frozen on dry ice, and stored at −70 °C until use for biochemical analyses. For Fluoro-Jade B and immunohistochemistry, the rats were deeply anesthetized (Nembutal, 75 mg/kg) and then transcardially perfused with 400 ml 4% paraformaldehyde and 100 ml 30% sucrose. The fixed brains were then removed and stored at −70 °C until use. All experiments were performed in accordance with the United States National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of California at Los Angeles Chancellor's Animal Research Committee. The suffering and number of animals used were minimized.

Fluid percussion injury

Rats were anesthetized with 1–1.5 ml/min of isofluorane using a gas mask. After reaching a surgical level of anesthesia, the head was placed in a stereotaxic frame where body temperature was monitored and maintained at 37 °C with a thermostatically controlled heating pad (Braintree Scientific Inc., Braintree, MA). All surgical areas were shaved and bathed with Betadine. A midline sagital incision was made. With the aid of a microscope (Wild, Heerburg, Switzerland) a 3.0-mm diameter craniotomy was made 3.0 mm posterior to bregma and 6.0 mm lateral (left) to the midline with a high-speed drill (Dremel, Racine, WI). A plastic injury cap was placed over the craniotomy with silicone adhesive and dental cement. When the dental cement hardened, the cap was filled with 0.9% saline solution. Anesthesia was discontinued and the injury cap was attached to the fluid percussion device. At the first sign of hind-limb withdrawal to a paw pinch, a mild fluid percussion pulse (1.5 atm) was administered. Apnea times were determined as the time from injury to the moment of spontaneous breathing. Sham animals underwent an identical preparation with the exception of the lesion. Immediately upon responding to a paw pinch, anesthesia was restored and the skull was sutured. Neomycin was applied on the suture and the rats were placed in a heated recovery chamber for approximately an hour before being returned to their cages.

Cognitive testing

To evaluate the effect of FPI and diet on cognitive function, all rats were tested in a Morris water maze before and after FPI or sham surgery. The swimming pool (130 cm diameter, 50 cm height) was divided into four quadrants as four zones. The quadrant where the escape platform (12 cm diameter) was located in a fixed position with 2 cm under the water surface was defined as target zone; the other three quadrants were left, right and opposite zone. The water (22±2 °C) was made opaque with white nontoxic biodegradable dye to prevent the rats from seeing the platform. The rats were trained in the water maze with 10 consecutive trials per day for 3 days. The rats were placed into the tank facing the wall from one of the equally spaced start locations that were randomly changed every trial. The spatial cues for reference around the pool were maintained constant throughout the duration of the experiment. Each trial lasted until the rat found the platform or for a max of 2 min. If the rat failed to find the platform in the allocated time, it was gently placed on the platform. At the end of each trial, the animals were allowed to rest on the platform for 1 min. The escape latencies to find the platform were recorded. In order to assess spatial memory retention, spatial probe trial was performed at 4 h after the last trial by removing the platform from the pool. The rats were allowed to swim for 1 min in the pool where escape platform was unavailable, and the percentage of time spent in each zone was calculated.

Real-time quantitative RT-PCR

Total RNA was isolated by using the RNA STAT-60 (TEL-TEST, Inc., 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 aqueous phase was mixed with isopropanol (0.5 ml/1 ml). Samples were centrifuged again. Supernatant was removed and RNA pellet was washed with 75% ethanol, centrifuged, dried and dissolved in water. The mRNAs for BDNF, Synapsin I, and CREB were measured by TaqMan real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using ABI PRISM 7700 Sequence detection system (Applied Biosystems, Foster City, CA). This system directly detects the RT-PCR product without downstream processing. This is accomplished with the monitoring of the increase in fluorescence of a dye-labeled DNA probe specific for BDNF, Synapsin I, or CREB plus a probe specific for the glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene used as an endogenous control for the assay. Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Perkin-Elmer, Branchburg, NJ). The sequences of probes, forward and reverse primers, designed by Integrated DNA Technologies (Coralville, IA) were: BDNF: (5'-AGTCATTTGCGCACAACTTTAAAAGTCTGCATT-3'), forward: (5'-GGACATATCCATGACCAGAAAGAAA-3'), reverse: (5'-GCAACAAACCACAACATTATCGAG-3'); Synapsin I: (5'-CATGGCACGTAATGGAGACTACCGCA-3'), forward: (5'-CCGCCAGCTGCCTTC-3'), reverse: (5'-TGCAGCCCAATGACCAAA-3'); CREB: (5'-CATGGCACGTAATGGAGACTACCGCA-3'), forward: (5'-CCGCCAGCATGCCTTC-3'), reverse: (5'-TGCAGCCCAATGACCAAA-3'). An oligonucleotide probe (5'-CCGACTCTTGCCCTTCGAAC-3') specific for the rat GAPDH gene was used as an endogenous control to standardize the amount of sample RNA. The GAPDH gene is a constitutively expressed housekeeping gene which has been shown to be suitable to correct variations in RNA quantity and quality (Medhurst et al., 2000). The RT-reaction conditions were 2 min at 50 °C as initial step to activate uracil glycosylase (UNG), followed by 30 min at 60 °C as reverse transcription and completed by UNG-deactivation at 95 °C for 5 min. The 40 cycles of two-step PCR reaction conditions were 20 sec at 94 °C and 1 min at 62 °C.

ELISA

Hippocampal tissue was homogenized in a lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged, the supernatants were collected and total protein concen-

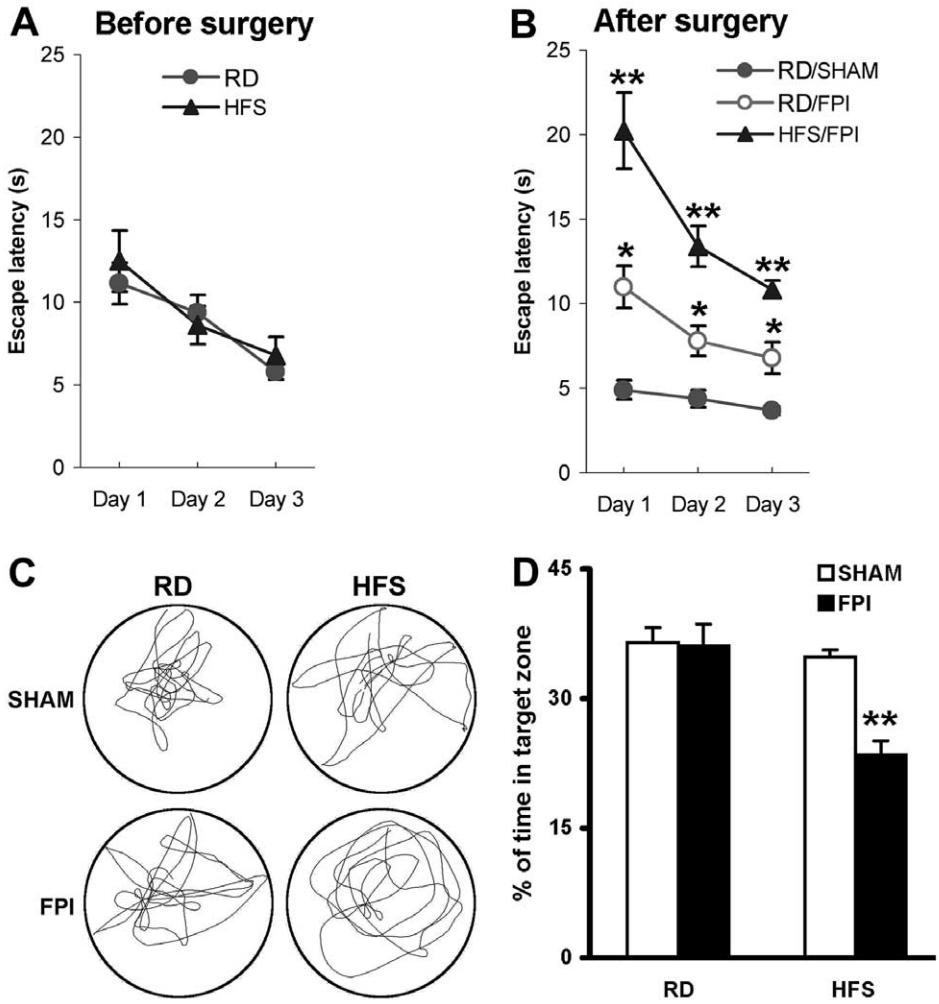

Fig. 1. Effects of diet and FPI on spatial learning and memory retention. A: The escape latency to find the platform was about the same in HFS *versus* RD intact rats. B: FPI resulted in longer escape latencies in RD and HFS rats compared with sham-operated animals, and the escape latency in HFS rats was longer than that in RD rats. C: Typical swim paths from the various groups indicated that the HFS-fed animals with lesions swam randomly, showing an obvious impairment to recognize the target quadrant compared with other groups. D: Probe trial showed that the HFS-fed rats with lesion spent less time in the target zone compared with other groups. Values represent mean±S.E.M. *$P<0.05$; **$P<0.01$.

tration was determined according to Micro BCA procedure (Pierce, Rockford, IL), using bovine serum albumin as standard. BDNF protein was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (BDNF Emax ImmunoAssay System kit, Promega Inc., Madison, WI) according to manufacturer's protocol.

Western blot

The total proteins from hippocampal tissue were extracted as described above. Synapsin I, phospho-synapsin I (p-synapsin I), CREB and phospho-CREB (p-CREB) were analyzed by western blot. Briefly, protein samples were separated by electrophoresis on an 8% polyacrilamide gel and electrotransferred to a nitrocellulose membrane. Non-specific binding sites were blocked in TBS, overnight at 4 °C, with 2% BSA and 0.1% Tween-20. Membranes were rinsed for 10 min in buffer (0.1% Tween-20 in TBS) and then incubated with anti-actin, anti-synapsin I, anti-phospho-synapsin I (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA), followed by anti-goat IgG horseradish peroxidase-conjugate (Santa Cruz Biotechnology); anti-CREB, anti-p-CREB (1:1000; Upstate, Waltham, MA), followed by anti-rabbit IgG horseradish peroxidase-conjugate (Santa Cruz Biotechnology). After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH Image software. Actin was used as an internal control for western blot.

Immunohistochemistry

Serial coronal sections (25 μm) were cut using a cryostat, mounted on gelatin-coated slides and processed for immunohistochemistry, as previously described (Gomez-Pinilla et al., 2001). A 1:1000 dilution was used for the rabbit polyclonal anti-BDNF (Chemicon International Inc., Temecula, CA). Immunohistochemistry controls were performed by omission of the primary antibody. The results of immunohistochemistry controls were negative as no staining was observed in cell structures.

Fluoro-Jade B staining

Fluoro-Jade B is an anionic tribasic fluorochrome that selectively stains degenerating neurons, popularly used for identifying neurodegeneration in response to insults (Schmued and Hopkins,

2000; Kubova et al., 2001). Staining was characterized by virtually no background labeling of neuropil or normal cells, while degenerating neurons and their processes stained conspicuously (Schmued and Hopkins, 2000). Coronal 25 μm sections were cut on a cryostat and stained with Fluoro-Jade B (Histo-Chem Inc., Jefferson, AR) using described protocol (Schmued and Hopkins, 2000; Kubova et al., 2001). The Fluoro-Jade B staining was performed on sections from sham or injured animals at 7 days post-injury. Briefly, sections were mounted from phosphate buffered saline (pH 7.4) onto gelatin-coated slides and dried at 37 °C overnight. Then they were immersed in absolute ethanol for 3 min, followed by 70% ethanol for 2 min, and distilled water for 2 min. The slides were then incubated in 0.06% potassium permanganate solution for 15 min. After rinsing with distilled water for 2 min, the slides were incubated for 30 min in 0.001% Fluoro-Jade B solution made in 0.1% acetic acid. Slides were rinsed in water three times, then dried at 37 °C, immersed in xylene, and coverslipped. Sections spanning the hippocampus were examined under a fluorescent microscope (I3 filter cube for FITC, excitation band 450–490 nm).

Statistical analysis

GAPDH and actin were employed as internal standards for real-time RT-PCR and Western blot respectively. The RD-fed rats with sham surgery were regarded as experimental controls for comparisons with other experimental groups. An analysis of variance (ANOVA) was performed on mean mRNA and protein values followed by post hoc Bonferroni *t*-test. The analysis of maze data and correlation was referred to our previous paper (Molteni, et al., 2002). Statistical differences were considered significant when $P<0.05$. The results were converted to percent of control for presentation in bar figures and represent the mean±S.E.M.

RESULTS

Spatial learning

Animals were maintained on HFS diet or RD for 4 weeks and some of them received a FPI and were killed 1 week later. We performed the Morris water maze test daily for three consecutive days before and after FPI to assess spatial memory function. The escape latency to find the platform was about the same in HFS *versus* RD intact rats (Fig. 1A). FPI resulted in longer escape latencies in RD and HFS rats compared with animals receiving sham surgery, and the latency of HFS rats was longer than that of RD rats (Fig. 1B). In order to evaluate memory retention, the spatial probe trial test was performed at 4 h after the last trial in the same maze (Fig. 1C, D). The rats were allowed to swim for 1 min in the same pool but without escape platform, and the percentage of time spent in each zone was calculated. In general, the animals showed a clear bias toward the platform quadrant (target zone) on the retention test before injury. The sham-operated groups still exhibited a clear preference for the target zone (Fig. 1C). The HFS-fed animals with lesion showed an obvious impairment to recognize the target quadrant (Fig. 1C), expressed as significantly less time spent in the target zone compared with other groups (Fig. 1D).

Morphological examination

Fluoro-Jade B is an anionic tribasic flurochrome that selectively stains degenerating neurons, commonly used for identifying neurodegeneration in response to insults (Schmued and Hopkins, 2000; Kubova et al., 2001). Fluoro-Jade B staining is normally characterized by virtually no background labeling of intact neuropil, while degenerating neurons and their processes stain conspicuously (Schmued and Hopkins, 2000). In our results, there were no Fluoro-Jade B-stained neurons in the hippocampus of any rat group receiving FPI (data not shown).

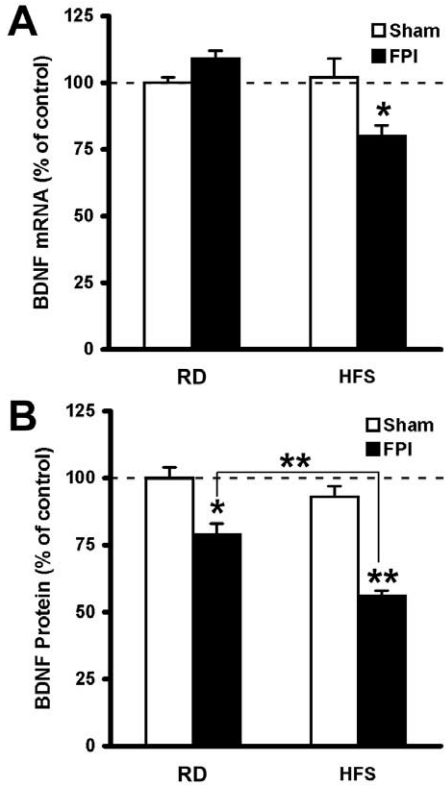

Fig. 2. Effects of FPI on BDNF mRNA and protein levels in the hippocampus of RD and HFS rats. A: The combination of FPI and HFS diet resulted in a significant decrease in BDNF mRNA. B: FPI reduced BDNF protein level in both RD- and HFS-fed rats with a greater reduction in injured HFS rats. Values represent mean±S.E.M. *$P<0.05$; **$P<0.01$.

BDNF mRNA and protein levels

We determined the mRNA and protein levels for BDNF by using real-time quantitative RT-PCR and ELISA, respectively. Real-time RT-PCR showed that HFS or FPI alone did not change levels of BDNF mRNA, while the combined effects of both resulted in a significant decrease in BDNF mRNA (Fig. 2A). ELISA revealed that FPI reduced levels of BDNF protein in both RD and HFS rats (Fig. 2B), and that this reduction was greater in injured HFS rats (Fig. 2B). BDNF immunohistochemistry was performed to determine the cellular localization for the reductions in BDNF protein (Fig. 3). Immunohistochemical analysis showed that BDNF labeling was predominantly distributed along the mossy fiber system that runs between CA3 and the dentate gyrus (DG), and in the molecular layer of the DG of the hippocampal formation. The pattern of BDNF immunostaining

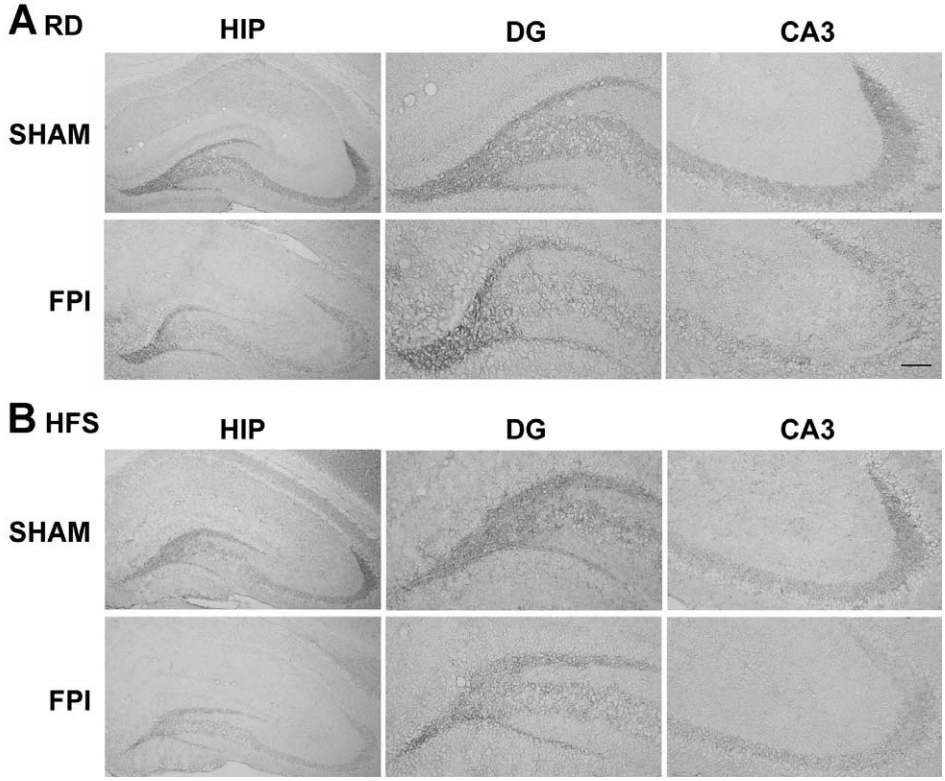

Fig. 3. Immunostaining for BDNF in the hippocampus of sham-operated and injured rats fed with RD (A) or HFS (B) diet. The BDNF labeling was predominantly distributed along the mossy fiber system that runs between CA3 and DG, and in the molecular layer of the DG of the hippocampal formation. FPI resulted in a qualitative reduction in BDNF-staining in the CA3 and DG of RD (A) and HFS (B) rats. Scale bar, 250 μm.

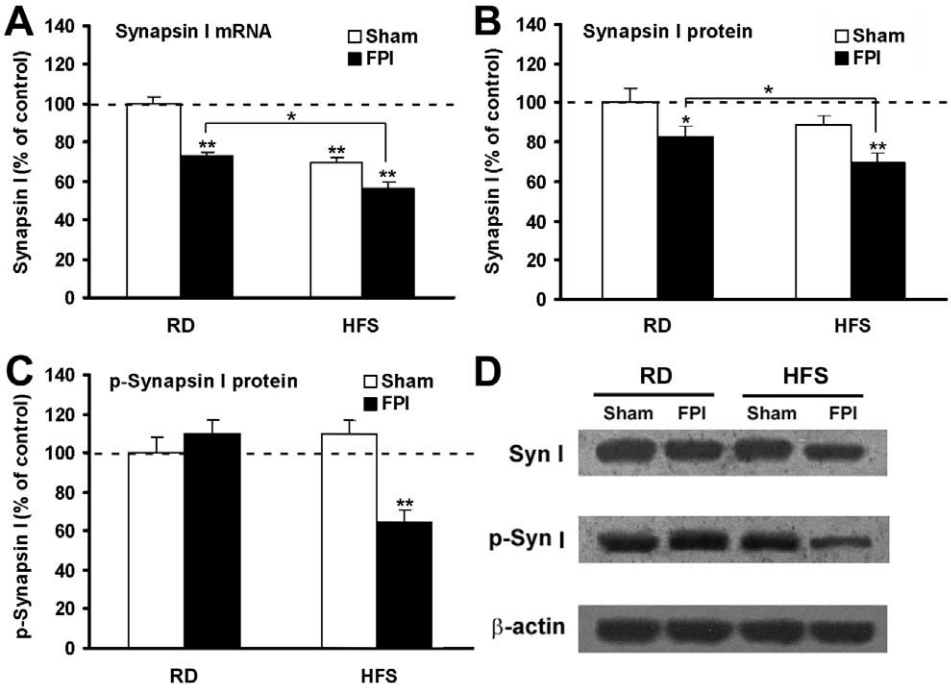

Fig. 4. Effects of diet and FPI on synapsin I mRNA and protein levels in the hippocampus. A: Both HFS and FPI reduced synapsin I mRNA, whereas FPI resulted in a greater reduction of synapsin I mRNA in HFS rats relative to RD rats. B: FPI decreased synapsin I protein level in both RD and HFS rats with a greater reduction in HFS rats. C: FPI led to a significant decrease of p-synapsin I in HFS-fed rats. D: Representative immunoblots for synapsin I and p-synapsin I in each group. Actin was used as an internal standard for Western blot. Values represent mean±S.E.M. *$P<0.05$; **$P<0.01$.

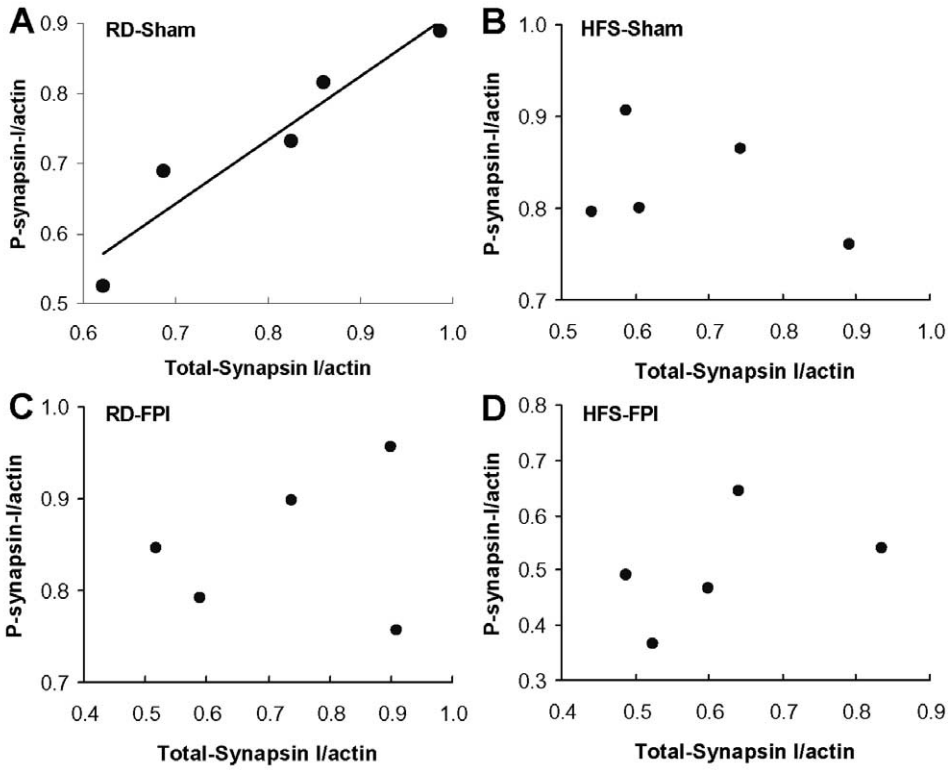

Fig. 5. Effects of diet and FPI on the relationship between total-synapsin I and p-synapsin I in the hippocampus. There was a significant and positive correlation between the levels of p-synapsin I and total synapsin I in sham rats fed RD (A). However, exposure to a HFS diet (B), FPI (C), or both together (D) disrupted such correlation.

is similar to previously published studies (Molteni et al., 2002). FPI resulted in a qualitative reduction in BDNF-staining in the CA3 and DG of RD (Fig. 3A) and HFS (Fig. 3B) rats.

Synapsin I mRNA and protein levels

We measured synapsin I mRNA and protein to evaluate how diet and TBI affect select molecular systems associated to the action of BDNF on synaptic plasticity. Real time RT-PCR revealed a significant reduction in synapsin I mRNA in the hippocampus of HFS rats compared with RD animals (Fig. 4A). FPI resulted in a reduction of synapsin I mRNA levels in both RD and HFS rats (Fig. 4A), with lower levels of synapsin I mRNA in HFS rats relative to RD rats (Fig. 4A).

Western blot analysis showed that the effects of the HFS diet and FPI were also expressed at the protein level of synapsin I, such that FPI decreased synapsin I in both RD and HFS rats with a greater reduction in HFS rats than in RD rats (Fig. 4B, D). To evaluate the effects of FPI and diet on the activational state of synapsin, we measured levels of the phospho-synapsin I. Results showed that FPI did not change the level of p-synapsin I in RD rats, but led to a significant decrease of p-synapsin I in rats fed HFS diet (Fig. 4C, D). In addition, to evaluate the possibility that FPI and diet might affect the process of activation of synapsin I, we performed a correlation analysis between levels of total and phosphorylated synapsin I (Fig. 5). There was a significant and positive correlation between levels of p-synapsin I and total synapsin I in sham rats fed RD ($r=0.95$, $P<0.05$; Fig. 5A). However, exposure to the HFS diet (Fig. 5B), execution of FPI (Fig. 5C), or both together (Fig. 5D) disrupted such correlation.

It is well documented that BDNF can affect synaptic plasticity by regulating synapsin I phosphorylation (Wang et al., 1995; Jovanovic et al., 1996). Therefore, in order to evaluate the effects of diet and FPI on the BDNF-synapsin interaction, we performed a correlation analysis between BDNF and p-synapsin I in all groups (Fig. 6). There was a positive and significant correlation for BDNF and p-synapsin I in RD rats with sham surgery ($r=0.95$, $P<0.05$; Fig. 6A). This correlation was disrupted by the HFS diet (Fig. 6B), FPI (Fig. 6C) or both simultaneously (Fig. 6D).

CREB mRNA and protein levels

To further evaluate how diet and TBI interact with molecular systems associated to the action of BDNF on synaptic plasticity, we assessed the expression of CREB in RD and HFS rats with or without FPI. Our results showed that FPI decreased CREB mRNA level in HFS and RD rats, although levels being much lower in HFS rats than that in RD rats (Fig. 7A). Western blot showed that CREB was reduced in RD rats after FPI with a greater reduction in HFS rats (Fig. 7B, D).

To evaluate the effects of FPI and diet on the active form of CREB, we measured p-CREB using Western blot

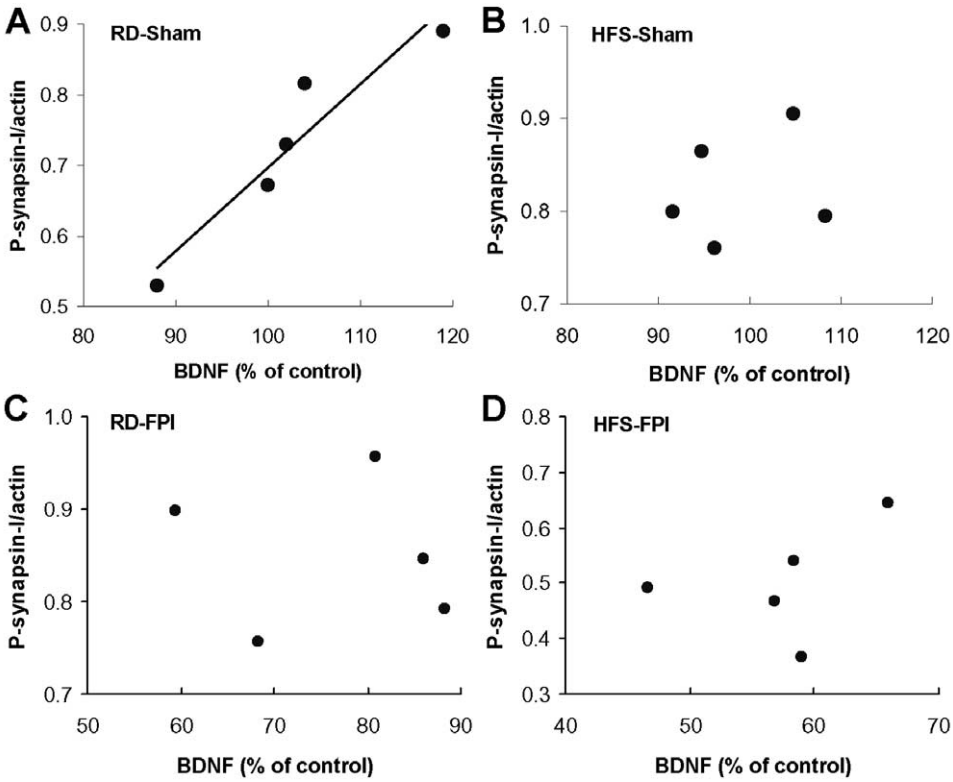

Fig. 6. Effects of diet and FPI on the relationship between BDNF and p-synapsin I in the hippocampus. There was a significant and positive correlation between the levels of BDNF and p-synapsin I in RD rats with sham surgery (A). However, such correlation was disrupted by the HFS diet (B), FPI (C), or both conditions together (D).

analysis. Results demonstrated that FPI did not alter p-CREB levels in RD rats, but caused significant decrease in p-CREB in rats fed HFS diet (Fig. 7C, D). In addition, to determine the possibility that FPI and diet might affect the activation process of CREB, we performed a correlation analysis between levels of total and p-CREB (Fig. 8). There was a significant and positive correlation between levels of p-CREB and total CREB in sham rats fed RD ($r=0.94$, $P<0.05$; Fig. 8A). However, exposure to a HFS diet (Fig. 8B), FPI (Fig. 8C), or both together (Fig. 8D) disrupted such correlation.

It is well known that BDNF regulates the phosphorylation of CREB (Finkbeiner, 2000; Ying et al., 2002), hence we evaluated the possibility that diet or FPI could affect such BDNF-CREB relationship (Fig. 9). The results revealed a positive and significant correlation of BDNF and p-CREB in RD rats with sham surgery ($r=0.90$, $P<0.05$; Fig. 9A), whereas this correlation was disrupted by the HFS diet (Fig. 9B), FPI (Fig. 9C), or both conditions together (Fig. 9D).

DISCUSSION

Current results demonstrate that FPI significantly impaired learning and memory performance in rats. Moreover, consumption of a HFS diet for 1 month before the injury, potentiated the deleterious effects of FPI on cognitive function and neuronal plasticity. BDNF and its downstream effectors on synaptic plasticity, synapsin I and CREB, appear to be involved with the effects of the HFS diet. The HFS diet and trauma reduce levels of CREB and synapsin I and the efficiency of crucial molecular mechanisms. Indeed, the HFS diet and FPI disturbed the association between BDNF and the activational state of synapsin I and CREB. These results propose a common molecular mechanism by which nutritional factors and trauma can affect the capacity of the brain to compensate for further insult.

HFS exacerbated TBI-induced impairment in cognitive function

Our results showed that FPI significantly impaired spatial learning in the Morris water maze, and that consumption of the HFS diet exacerbated the cognitive impairment induced by TBI. Animals were maintained on the HFS diet for 4 weeks, a time period under the minimum required for this diet to elicit detectable cognitive impairment (Molteni et al., 2002). These data imply that under our experimental conditions, the HFS diet acts as a risk factor to reduce the capacity of the brain to compensate for insults such as FPI. The results of fluoro-Jade B staining indicate that neuronal degeneration was not a crucial factor for our results, as no signs of hippocampal degeneration was found in any of the animal groups exposed to FPI. Abundant evidence indicates that spatial learning depends largely on proper hippocampal function (Korol et al., 1993). Although it has

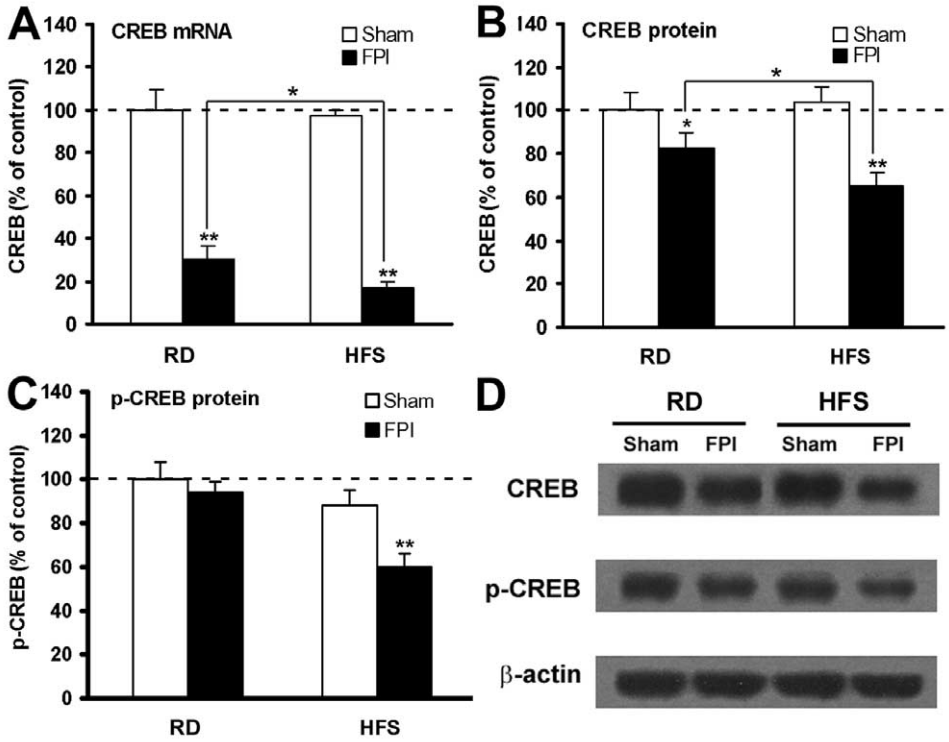

Fig. 7. Effects of diet and FPI on CREB mRNA and protein levels in the hippocampus. A: FPI decreased CREB mRNA level in HFS and RD rats with a much lower level in HFS rats relative to RD rats. B: FPI reduced CREB protein level in both RD and HFS rats with a greater reduction in HFS rats compared with RD rats. C: FPI led to a significant decrease of p-CREB in HFS rats. D: Representative immunoblots for CREB and p-CREB in each group. Actin was used as an internal standard for Western blot. Values represent mean±S.E.M. *$P<0.05$; **$P<0.01$.

been reported that neuronal death may contribute to TBI-induced cognitive deficits (Yakovlev et al., 1997; Conti et al., 1998; Keane et al., 2001), it is becoming accepted that neuronal damage cannot completely explain the chronic functional problems following TBI. For example, Lyeth et al. (1990) and Scheff et al. (1997) reported that TBI produced cognitive deficits in the rat without evidence of neuronal cell death in the hippocampus. Therefore, these data suggest that the cognitive impairment resulting from FPI can be secondary to alterations in select molecular systems in the hippocampus. The question is then, how TBI as well as a HFS diet can account for decreased cognition and neuroplasticity?

BDNF may act as a mediator for the reduced capacity of brain to compensate for induced cognitive deficit

According to the correlation analysis (Fig. 10), poor performance in the water maze test was associated to low levels of BDNF in individual animals. This condition holds for control and experimental animals, such that the group of rats more heavily affected, such as the HFS-FPI showed the lowest levels in BDNF. The correlation analysis also showed that the slope of the curve for HFS-FPI group was much more pronounced than in the control situation (RD-Sham). This suggests that decreases in BDNF in the HFS-FPI group may reach a pathological situation such that small BDNF decreases can result in dramatic cognitive impairment. Animals lacking BDNF show deficits in LTP and learning and memory (Korte et al., 1995), which can be amended by adding exogenous BDNF (Patterson et al., 1996; Linnarsson et al., 1997). Abundant evidence indicates that BDNF is a powerful synaptic facilitator; Thoenen, 1995; Kang and Schuman, 1996; Bolton et al., 2000), and that it may play a role on maintaining cognitive function (Linnarsson, et al., 1997; Croll et al., 1998; Hall et al., 2000; Gomez-Pinilla et al., 2001; Ying et al., 2002; Kovalchuk et al., 2002). For example, BDNF expression is increased in the hippocampus of animals that learn a spatial memory task (Kesslak et al., 1998; Mizuno et al., 2000; Gomez-Pinilla et al., 2001).

BDNF affects synaptic plasticity by modulating synapsin I and CREB

Results showed that FPI decreased BDNF protein levels and that the HFS diet potentiated this effect. We have evaluated the consequences of a decrease in BDNF on some of its downstream effectors on synaptic plasticity underlying cognition. BDNF facilitates synaptic transmission by regulating synapsin I phosphorylation through tyrosine kinase B (TrkB) receptor (Wang et al., 1995; Jovanovic et al., 1996). Synapsin I is a terminal specific protein involved with modulation of transmitter release (Baekelandt et al., 1994; Melloni et al., 1994; Jovanovic et al., 2000), formation and maintenance of the presynaptic structure (Takei et al., 1995), and axonal elongation (Akagi

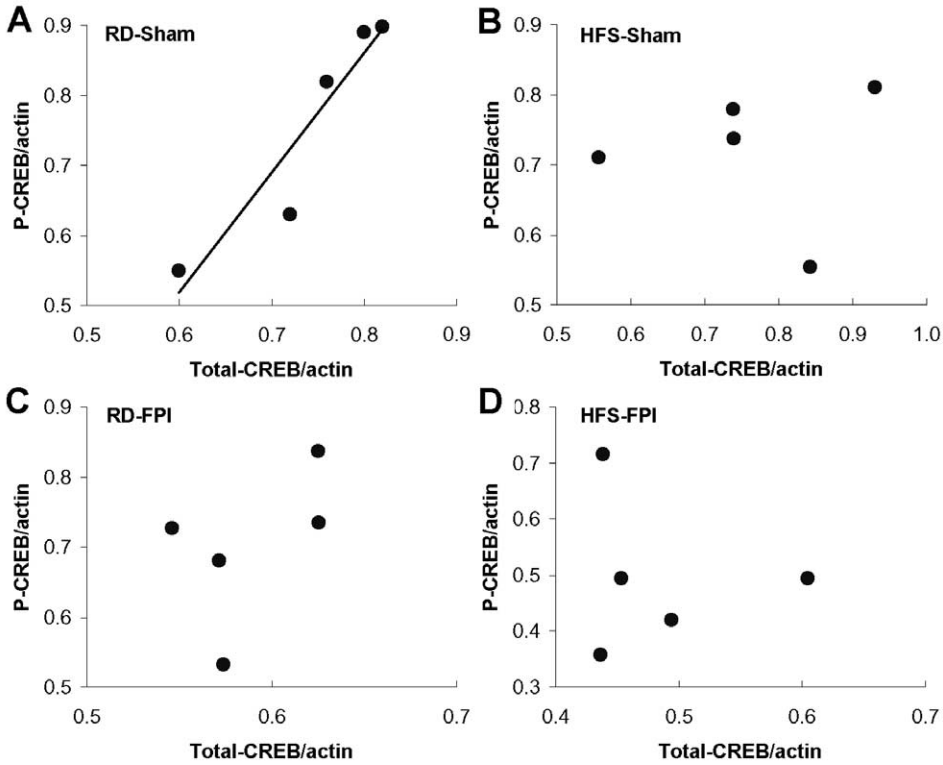

Fig. 8. Effects of diet and FPI on the relationship between total-CREB and p-CREB in the hippocampus. There was a significant and positive correlation between the levels of p-CREB and total CREB in sham rats fed RD (A). However, exposure to a HFS diet (B), FPI (C), or both together (D) disrupted such correlation.

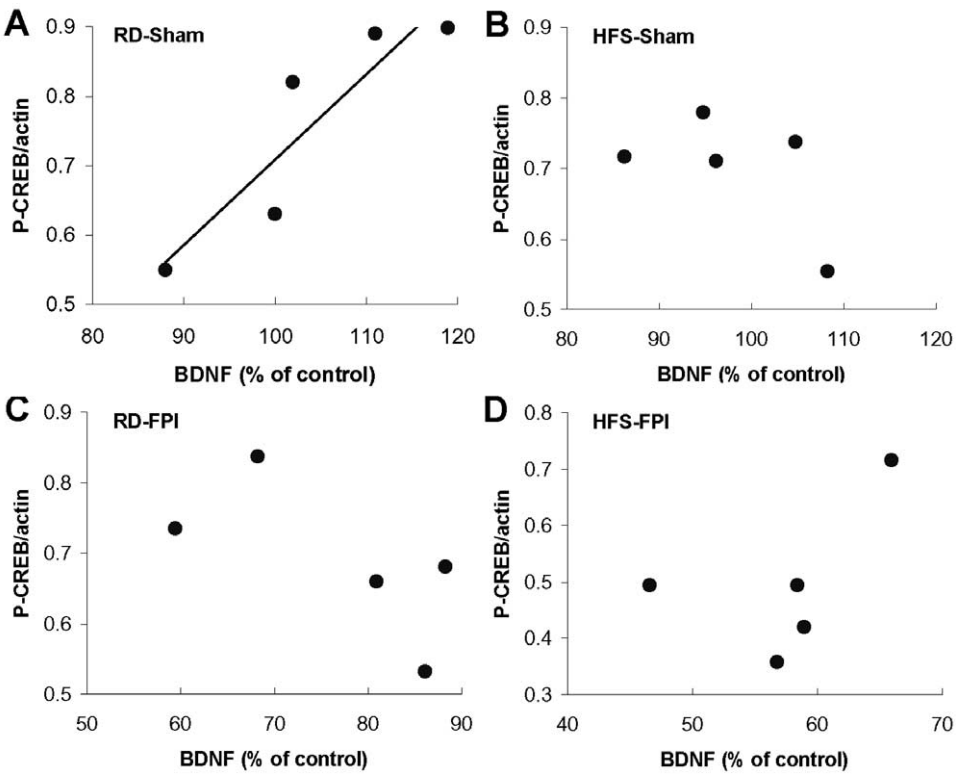

Fig. 9. Effects of diet and FPI on the relationship between BDNF and p-CREB in the hippocampus. There was a significant and positive correlation between the levels of BDNF and p-CREB in RD rats with sham surgery (A). However, this correlation was disrupted by the HFS diet (B), FPI (C), or both together (D).

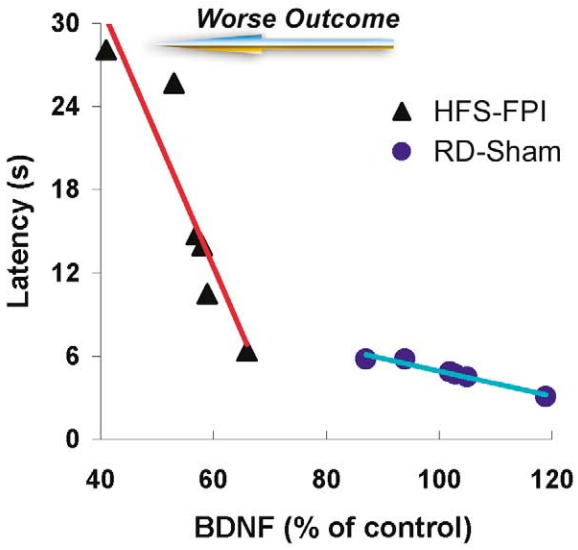

Fig. 10. There was a direct relationship between performance in the water maze and levels of hippocampal BDNF, such that animals with the lowest BDNF levels showed the poorest performance. The two extreme conditions, RD-sham group and the HFS-FPI, are shown for illustrative purpose. Note differences in the slope of the curve for the two rat groups depicted. The slope of the curve in the HFS-FPI group was much more pronounced than in the RD-sham group, and this may have serious implications for neuronal function.

et al., 1996). Our results showed that changes in synapsin I protein followed very closely changes in BDNF protein, and that synapsin I phosphorylation was associated to levels of BDNF. It is also significant that the relationship BDNF *versus* p-synapsin I was disrupted by the effects of the HFS diet and FPI, as shown by the correlation analysis.

CREB is one of the best-described stimulus-induced transcription factors involved in adaptive responses (Finkbeiner, 2000), neuronal resilience to insult (Dawson and Ginty, 2002; Lonze et al., 2002), and learning and memory (Yin and Tully, 1996; Tully, 1997). BDNF modulates the phosphorylation of CREB (McAllister, et al., 1999; Shaywitz and Greenberg, 1999; Finkbeiner, 2000; Ying et al., 2002). Conversely, CREB can regulate BDNF transcription (Nibuya et al., 1996; Tao et al., 1998; Conti et al., 2002). Similarly to synapsin I, changes in CREB protein paralleled changes in BDNF and this relationship was disrupted by the HFS diet and trauma. It is important that the combination of HFS and FPI affected more heavily the active states (phosphorylated) of both synapsin I and CREB, with obvious implications for the functions of these molecules. These results suggest that the HFS diet may impact synaptic function and cognition by affecting the functional relationship among BDNF, synapsin I and CREB.

How can HFS and trauma affect the brain?

Our results depict a possible mechanism by which diet and trauma can interact with a common molecular substrate with important effects on neuronal plasticity and cognition. These results suggest that the HFS decreases the capacity of the brain to compensate for insult, and may be a predictor of poor recovery after injury. The specific steps by which the action of dietary factors translates into changes of neurotrophins are inherently complex, but they likely involve the production of free radicals. Recent evidence suggests that high-sugar or high-fat diets can indeed increase free radical production (Faure et al., 1997; Beltowski et al., 2000), while antioxidant therapy can reduce these effects (Faure et al., 1997; Joseph et al., 1999). Further research will be necessary to determine the nature of the linkage between dietary factors and neurotrophins. BDNF, synapsin I, and CREB have a crucial role on maintaining neural function by stimulating neuronal excitability, synaptic transmission and providing neuroprotection. Therefore, present findings provide important insights into the understanding of possible molecular mechanisms by which lifestyle can affect the outcome of TBI, and may help development of new strategies to attenuate the deleterious effects of TBI.

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