Synaptogenesis in the Hippocampal CA1 Field following Traumatic Brain Injury

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ABSTRACT

Traumatic brain injury (TBI) results in both acute and chronic disruption of cognitive ability that may be mediated through a disruption of hippocampal circuitry. Experimental models of TBI have demonstrated that cortical contusion injuries can result in the loss of specific neurons in the CA3 subfield of the ipsilateral hippocampus, resulting in partial loss of afferents to the CA1 subfield. Numerous studies have documented the ability of the central nervous system to compensate for deafferentation by initiating a plasticity response capable of restoring lost synaptic contacts. The present study was designed to examine the time course of loss and replacement of synaptic contacts in stratum radiatum dendritic field of CA1. Young adult rats were subjected to a lateral cortical contusion injury and assayed for total synaptic numbers using unbiased stereology coupled with transmission electron microscopy. Injured animals demonstrated a 60% loss of synapses in CA1 at 2 days post-injury, followed by a reinnervation process that was apparent as early as 10 days post-injury. By 60 days post-injury, total synaptic numbers had approached pre-injury levels but were still significantly lower. Some animals were behaviorally tested for spatial memory in a Morris Water Maze at 15 and 30 days post-injury. While there was some improvement in spatial memory, injured animals continued to demonstrate a significant deficit in acquisition. These results show that the hippocampus ipsilateral to the cortical contusion is capable of a significant plasticity response but that synapse replacement in this area does not necessarily result in significant improvement in spatial learning.

Key words: behavior; hippocampus; plasticity; stereology; synapse

INTRODUCTION

Numerous studies have indicated that traumatic brain injury (TBI) in laboratory rats results in significant alterations in both motor skills and cognition (Lyeth et al., 1990; Smith et al., 1991; Hamm et al., 1992a,b; Hoffman et al., 1994; Smith et al., 1994; Dash et al., 1995; Scheff et al., 1997). TBI-related cognitive deficits related to spatial learning can persist as long as 1 year post-injury in these animal models (Pierce et al., 1998; Dixon et al., 1999), whereas significant motor skills deficits are more transient. These results are supported by human studies that also report persistent cognitive dysfunction after injury (Russell and Smith, 1961; Gronwall and Wrightson, 1974; Levin et al., 1979, 1990; Lewin et al., 1979; Rimel et al., 1981; McLean et al., 1983; Binder, 1986; McAllister, 1992).
Following cortical contusion, there is substantial neuronal damage in the ipsilateral hippocampal formation disrupting the normal neuronal circuitry (Baldwin et al., 1997; Grady et al., 2003; Anderson et al., 2005). This injury significantly disrupts the CA3 pyramidal neurons, which gives rise to the Schaffer collaterals (Witter and Amaral, 2004), resulting in a partial deafferentation of CA1 region superior dendritic field. CA1 long-term potentiation (LTP), a major synaptic event implicated in learning and memory (Bliss and Collingridge, 1993; Bear and Malenka, 1994; Reeves et al., 1995), undergoes significant changes following TBI. Rodent models have reported significant changes in hippocampal synaptic transmission lasting as long as 15 days following injury (Miyazaki et al., 1992; Reeves et al., 1995; D’Ambrosio et al., 1998; Sick et al., 1998; Albensi et al., 2000; Sanders et al., 2001) and suggest that these alterations may underlie some of the learning and memory deficits observed following TBI.

CNS injury, resulting in partial loss of neuronal inputs to a particular region, can signal undamaged residual afferents in the immediate area to sprout and replace lost synaptic connections (Cotman et al., 1981). The hippocampal formation has been a model system used for the study of reactive synaptogenesis following partial denervation (Cotman and Lynch, 1976; Scheff, 1989). While prior studies have demonstrated alterations in granule cell mossy fibers projects following TBI (Santhakumar et al., 2001), little is known about hippocampal synaptogenesis following TBI. The present study was undertaken to quantitatively assess the loss and replacement of synaptic contacts in the hippocampus following TBI.

**MATERIALS AND METHODS**

Adult Sprague-Dawley rats (274–300 g; Harlan Laboratories, IN; n = 42) were used in these experiments. Animals were housed in group cages (4/group) on a 12-h light/dark cycle with free access to water and food. All animal procedures were approved by the institutional Animal Care and Use Committee.

**Surgical Procedures**

The apparatus and surgical procedures used for administration of cortical contusion were as previously described (Baldwin et al., 1997; Scheff et al., 1997). Briefly, all rats were anesthetized with isoflurane (2%) and placed in a stereotaxic frame prior to TBI. The head was positioned in the horizontal plane with the nose bar set at −5. Following a 6-mm craniotomy performed with a handheld Michele trephine (Miltex, Lake Success, NY), the exposed cortex was injured using a pneumatically controlled impactor device (Baldwin et al., 1997). The impactor rod had a beveled tip with a 5-mm diameter that was used to compress the cortex to a depth of 2 mm at 3.5 m/sec. The injury of the cortex was placed lateral to midline and equidistant between lambda and bregma. Following injury, Surgicel (Johnson & Johnson, Arlington, TX) was laid on the dura, and the skull cap was replaced and sealed with a thin layer of dental acrylic that was allowed to dry before closing the wound with staples (Stoelting, Wood Dale, IL). Core body temperature was monitored continuously throughout the surgical/recovery procedure and maintained at 36–37°C with a heating pad.

Injured animals were allowed to survive for 2, 10, 15, 30, or 60 days (n = 6/group) post-injury. Sham-operated animals (n = 6/group) received the craniotomy alone with no cortical injury and survived for 15 or 30 days post-surgery. At the designated time, each subject was perfused transcardially with physiological saline followed by a mixture of 4% paraformaldehyde, 1% glutaraldehyde, 0.54% d-glucose in 0.1M sodium phosphate buffer (pH 7.4). Perfusate was delivered using a continuous infusion pump. The brains were removed and placed in cold perfusate for an additional 24 h. Only the 15-day post-surgery time point for the sham animals was evaluated ultrastructurally.

The brains were embedded in agar and sectioned at 100 µm in the coronal plane with a vibratome (Vibratome Co., St. Louis, MO). Individual sections throughout the rostral-caudal extent of the hippocampal formation were collected in 0.1M sodium phosphate buffer. For most brains, this resulted in approximately 48 sections.

**Delineation of Sections for Ultrastructural Analysis**

A systematic sampling scheme with a random starting point was used to assess total number of synaptic contacts in stratum radiatum (str. rad) of the hippocampal CA1 dendritic field. For practical reasons, the total number of sections obtained from each brain was divided into six rostral-caudal regions, with approximately eight sections per region. Each set of eight sections was further divided into two subsets (A and B) consisting of four sections. A die was rolled to determine which sections from each of these subsets of four sections would be embedded for ultrastructural analysis (e.g., a 3 on the die would indicate that section 3 from subset A and section 3 of subset B would be embedded). A total of 12 sections were embedded for ultrastructural analysis, with two sections representing each of the six regions. The designated sections were subsequently placed in cold (4°C) 0.1M phosphate buffer, and the entire hippocampal formation was
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dissected free from the cortical and subcortical structures with the aid of a dissecting microscope. Each hippocampal tissue section was rinsed in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide (OsO₄) in 0.1M sodium phosphate buffer, and stained with a 5% uranyl acetate. The samples were dehydrated in a graded series of ethanol, treated with propylene oxide, and infiltrated with epoxy resin overnight with continuous agitation. Sections were subsequently flat embedded in circular molds (Ted Pella, Redding, CA) using resin. All samples were cured at 60°C for 24 h. Blocks were coded at this time so that all subsequent analysis could be done blind with respect to experimental group.

Reference Volume

Sections immediately adjacent to those used for ultrastructural analysis (total of 12 sections) were designated for determination of the reference volume (V(ref)). Because the process used for electron microscopy requires embedding the tissue in a plastic resin and this histological process could result in tissue shrinkage, these sections were treated in an identical fashion to those for ultrastructural analysis, with the exception that the hippocampal formation was left attached to the cortical and subcortical structures. Each 100-µm vibratome section used for V(ref) was postfixed in osmium tetroxide, dehydrated in ethanol, treated with propylene oxide, infused with embedding resin, flat embedded on glass microscope slides held in rubber molds (Ted Pella, Redding, CA), and covered-slipped using the embedding resin as the mounting medium prior to curing. This protocol allows for increased resolution in the exact determination of regio superior area boundaries using a light microscope. All slides were cured at 60°C for 24 h. The V(ref) of CA1 str rad was determined with the Cavalieri method (Michel and Cruz-Orive, 1988). Using an image analysis system (BIOQUANT, Nashville, TN), the str rad region on each of the 12 sections was obtained using previously described anatomical landmarks (Witter and Amaral, 2004), and the value was applied to the following formula: 

\[ V_{\text{ref}} = \Sigma_{t=1}^{12} (t \cdot A_{\text{str rad}}) \]

where \( t \) is the distance between sections and \( A_{\text{str rad}} \) is the str rad area of the individual section. The average tissue section thickness used for the determination of \( t \) was derived from measurements of the width of the semi-thin sections used for the ultrastructural analysis.

Prior to sectioning this tissue with the vibratome, three naive fixed brains were sectioned at 100 µm, and random free floating sections were stained with cresyl violet and mounted with an aqueous medium. The thickness of each section was assessed with a Z-axis microcater to confirm that the vibratome was sectioning at the desired thickness. The vibratome used to section the tissue in the present study had less than 3% error in thickness between sections.

Determination of the Fields for Ultrastructural Analysis

One of the primary objectives of the dissector method is that every object of interest has an equal opportunity of being sampled. The total linear length of the CA1 region was determined during the estimation of the V(ref) and this value used in determining which portions of CA1 would be sampled. Because the length of the CA1 pyramidal cell region varied between sections, with those from the septal end (Paxinos 2.8) having less CA1 length than some of the more temporal end (Paxinos 5.8) (Paxinos and Watson, 1982), it was important that the sampling be proportionate to the length. Because only one block from each of the six initial subregions of the hippocampus was sampled, due to practical reasons, the longest length of the CA1 region found in an individual section was arbitrarily divided into six parts. The length of one of these six parts became the unit of measurement for all the other sections. Thus, the section that represented the most anterior portion of the hippocampus (A1) had a CA1 region divided into only two parts while the section from the middle region (M1) had a CA1 region divided into four parts. In this fashion, the sampling of CA1 was proportionate throughout each of the representative sections. No area of CA1 had a greater probability of being sampled and it ensured that every synapse had an equal probability of being sampled. A die was thrown to determine which part of the CA1 region for each section would be sampled.

Blocks were trimmed to the appropriate region and semi-thin (2 µm thick) sections containing the full width of regio superior were taken to accurately determine the boundaries of str rad and to aid in further trimming the blocks. These sections were stained with toluidine blue. On stained semi-thin sections, the str rad region was divided into three equal zones (proximal, middle, distal) with reference to the pyramidal cell layer, and a die was thrown to determine from which subzone ultrathin sections would be taken. After determining the subzone of str rad to assess, blocks were further trimmed and ribbons of 6–8 ultrathin sections (silver-gold interface range) were obtained and collected on formvar coated, carbon stabilized slot grids (1 × 2 mm slot size). The ultrathin sections were stained with an aqueous solution of uranyl acetate (3%, 20 min), then rinsed and stained with Reynold’s lead citrate (Reynolds, 1963) for 4–5 min. A die was thrown to identify the first of a pair of consecutive sections in the series. The identical area on both sections was identified. Two electron micrographs were taken on each section at an initial magnification of

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Determination of Synaptic Density per Unit Volume

Synapse Identification and Density Determination

The stereological physical disector technique (Sterio, 1984; Gundersen, 1986) was used to count and estimate the numerical density of synapses per unit volume (\(N_v\)). Every synaptic profile on each micrograph was labeled. Synaptic profiles were defined by the presence of the postsynaptic density (PSD) in association with the postsynaptic element and synaptic vesicles in a presynaptic terminal. After all micrographs had been assessed and all synapses labeled, adjacent disector pairs of micrographs were identified with one micrograph assigned as a reference section and the other as a look-up section (Calverley and Jones, 1990). An unbiased counting frame (Gundersen et al., 1988) was randomly superimposed over the reference section, with the stipulation that the edge of the micrograph and the counting frame were separated by a distance which was greater than the dimension of a synaptic profile. Those synapses located either entirely or partially within the counting frame and not intersecting the forbidden edges or extension lines were counted. Discontinuous or perforated synapses were treated as a single synapse. Only those synaptic profiles that were observed on the reference micrograph within the counting frame and not on the look-up micrograph were counted. To increase efficiency, the look-up and reference sections were reversed and the counting frame was again applied in a random fashion.

Determination of Ultrathin Section Thickness

The thickness of the ultrathin sections was estimated with the Small’s method of minimal folds (Weibel, 1979), in which small folds in the tissue are photographed at extremely high magnification (50,000×) and photographically enlarged to 120,000×. These folds represent twice the thickness of the section. A few sections in each series did not have folds, and the mean section thickness for all of the sections for that animal was used.

Determination of Synaptic Density per Unit Volume and Total Number of Synapses

The numerical density of synapses per unit volume, \(N_v\), was calculated using the formula: \(N_v = Q^-/V(\text{dis})\), where \(Q^-\) is the mean number of synapses counted in each disector and \(V(\text{dis})\) is the mean disector volume. The total number of synapses, \(N(\text{syn})\), was calculated for each animal using the formula: \(N(\text{syn}) = \bar{N}_v \cdot V(\text{ref})\).

Morris Water Maze Performance

At either 15 or 30 days post surgery, animals were tested for their ability to acquire a previously validated single day Morris Water Maze (MWM) task that has been shown to be sensitive to TBI (Kraemer and Randall, 1995; Scheff et al., 1997). Injured animals were compared to Sham groups with the same post surgical time point. All testing occurred during the light period of the animal’s light-dark cycle. The featureless pool was made of galvanized steel painted flat black and was 127 cm in diameter and 56 cm in depth and placed in a room 3 m × 3 m with numerous extra-maze cues. The pool was filled with water (26 ± 2°C) to a depth of 30 cm and contained a removable circular metal platform 13.5 cm in diameter that was 28 cm in height (i.e., 2 cm below the water’s surface) and used as the goal platform. This platform was positioned approximately 30 cm from the pool wall. Non-toxic black powdered paint was added to the water to obscure the visual appearance of the platform. A video camera was placed directly above the center of the pool to record swimming during the trials. Each video record was processed by a video motion analyzer (Videomax V, Columbus Instruments, Columbus, OH). This device computes the time each subject spent in each of the four pool quadrants, the number of crossing of the correct or other platform positions (annulus crossings), and swim speed.

Beginning at 15 or 30 days after surgery, rats in these survival groups were given 12 trials to navigate to the hidden platform followed by a probe trial to measure place memory. A single block of trials with a visible platform (protruding 1 cm above the water surface) was administered to control for nonspecific deficits. The entire training occurred in a single day and consisted of three blocks of four trials each, with each block separated by 1 h. Trials within each block were separated by 5 min. On each trial, the subject was released into the water facing the wall from one of four compass points (N, S, E, W). The order of the starting location was randomized. Each subject was allowed to swim until either it climbed onto the submerged platform or 60 sec had elapsed. If the platform was not located during this period, the subject was removed from the water and placed immediately onto the platform. All rats spent 15 sec on the platform at the end of each trial before being placed in a heated recovery cage. The probe trial consisted of removing the platform and allowing
the subject to swim freely and using the visual tracking system to measure the percent time the animal swam in the maze quadrant that previously contained the platform. The percent time each subject spent searching the quadrant that previously contained the escape platform was computed (percentage search time). Because rats abandon their search in the target quadrant and begin searching other areas after 30 sec (Kramer and Randall, 1995; Scheff et al., 1997), percentage search times in this study are computations of only the first 30 sec during the probe trial. The total number of annulus crossings in each of the quadrants was obtained, and the crossings that occurred in the appropriate quadrant were divided by the total number of crossings (percentage relevant target visits). This is a more accurate measurement of spatial memory than the percentage search time and factors out problems associated with a particular animal, which may be a slow swimmer and unable to make as many annulus crossings as a faster swimming animal (Schenck and Morris, 1985).

Statistical Analysis

For the morphological data, the coefficient of error (CE) was calculated for intra-animal variation and coefficient of variation (CV) for inter-animal variation (Gundersen and Jensen, 1987). The StatView® version 5.0 statistical software (SAS Institute, Cary, NC) was used for the present analysis. A one-way ANOVA was performed for each of the various dependent variables as a function of days post injury. A Student-Newman Keuls ($p < 0.05$) procedure for multiple comparisons was applied when indicated by the ANOVA. The behavioral data were ana-

FIG. 1. Coronal sections of the hippocampus stained with cresyl violet showing no injury to the CA3 pyramidal layer in a sham-operated animal (A) and a loss of CA3 in an injured animal at 15 days (15d) post-surgery (C). Lower magnification micrographs showing the extent of the cortical involvement in the sham-operated animal (B) and an injured animal (D). All animals undergoing a cortical contusion showed an unequivocal loss of CA3 neurons with virtually no involvement of the CA1 pyramidal cell layer. Bar = 1500 μm (A,C), 1000 μm (B,D).
lyzed with a two-way repeated measure ANOVA (Groups × Trial Blocks) followed by a Student Newman-Keuls post hoc test ($p < 0.05$) when appropriate.

RESULTS

All injured animals showed obvious cortical damage (Fig. 1) and loss of neurons in the hippocampal CA3 pyramidal cell subfield. This field displayed numerous intermittent acellular regions. A well defined cortical cavity had formed by 10 days post injury and appeared to remain unchanged at the 60-day survival time. Sham-operated animals failed to demonstrate any obvious cortical or hippocampal damage. This injury results in unequivocal signs of degeneration of presynaptic sites still in contact with postsynaptic sites at 2 days post-injury (Fig. 2).

**CA1 Stratum Radiatum Volume**

The volume of the str rad region of the CA1 apical dendritic field demonstrated a significant (5–14%) reduction as a function of the time post injury ($F = 21.689$, df 5,30, $p < 0.0001$) (Fig. 3). Post hoc comparisons revealed that the injured reference volume was significantly reduced ($p < 0.01$) beginning at day 10 post-injury and at all subsequent post injury time points. The analysis also revealed that the post-injury reference volumes did not undergo any further ($p > 0.1$) change after 10 days post-injury. Although there did appear to be a small (5%) diminution of volume at 2 days post-trauma, it was not significantly different from sham-operated controls ($p > 0.05$). However, there was a significant difference between 2 and 10 days post-injury.

**Total Number of CA1 Synapses**

A one-way ANOVA demonstrated a significant change in the total number of synapses in str rad as a function of time post injury [$F = 34.162$, df 5,30, $p < 0.0001$]. Post hoc comparisons revealed that at 2 days post-injury there was a significant 58% loss in normal appearing synapses compared to sham-operated controls. By 60 days post-injury, the total number of synapses had increased by 168% compared to the 2-day post-injury group, but was still significantly lower (30%) than the sham-operated controls (Fig. 3).

**Coefficient of Error and Coefficient of Variance**

The procedures for calculating the coefficient of error (CE) and the coefficient of variance (CV) were identical to that used previously (Baldwin et al., 1997). The mean of the CE for all animals in the present study is $0.038 ± 0.005$. The mean CV among all of the animal groups is $0.125 ± 0.062$. The observed CV is a combination of the inherent biological interanimal coefficient of variation. The ratio $CE^2/CV^2$ is about 0.09, indicating that the precision of the estimate observed with this sampling scheme meets the criterion for optimal sampling (West et al., 1991).

![Figure 2](image-url)
Packing Density of CA1 Synapses

The average number of synapses per unit volume changed dramatically as a function of time post-injury (F = 21.815, df 5,30, p < 0.001). At 2 days post-trauma, the packing density was 44% of control levels but had increased to 86% by 30 days post-injury but was significantly different from sham controls (p > 0.05). There was no significant difference in synaptic packing density between 30 and 60 days post-injury (p > 0.1), indicating that the synaptogenic response had possibly reached a plateau (Fig. 3).

Synapse Size

The mean length of the synaptic contacts for all groups was 0.376 ± 0.037 μm. A one-way ANOVA demonstrated no significant change in the size of the synapses in str rad as a function of time post injury (F = 1.757, df 5,30, p > 0.1), indicating that the loss of synapses was not se-

FIG. 3. The number of normal appearing synaptic contacts in the hippocampal CA1 stratum radiatum progressively increased over time following the unilateral cortical contusion. Both the synaptic packing density (A) and the total number of synapses (C) declined within 2 days following the injury and approached pre-injury levels by 60 days post-injury. The packing density showed greater recovery following the injury but was significantly influenced by the decline in the stratum radiatum volume (B). Points represent individual animal values. Horizontal line represents group mean.
lective for a given synaptic size and the reinnervation process did not include enlargement of synaptic contacts.

**Morris Water Maze**

All rats swam normally using the natural adult swimming posture and were able to climb onto the escape platform. Upon reaching the platform, all rats remained there until removed by the experimenter.

A repeated measure ANOVA (Group × Trial Blocks) revealed a significant main Group effect \([F = 10.029, \text{df} 3,40, p < 0.0005]\) (99.5% power) and a main effect for Trial Blocks \([F(2,40) = 57.107, p < 0.0001]\) (100% power), as well as a Group × Trial Blocks interaction \([F = 2.378, \text{df} 6,40, p < 0.05]\) (84.9% power). Mean latencies to locate the submerged platform were not significantly different during the first block of trials \([F = 1.682, \text{df} 3,20, p > 0.2]\) (36.5% power) but were significantly different for the second \([F = 8.469, \text{df} 3,20, p < 0.0005]\) (98.4% power) and third \([F = 13.561, \text{df} 3,20, p < 0.0001]\) (100% power) trial blocks (Fig. 4). Post hoc

![Morris Water Maze](image)

**FIG. 4.** Morris water maze behavioral analysis at 15 days (15d) and 30 days (30d) following a moderate unilateral cortical contusion. Injured animals were significantly worse than sham-operated controls in their ability to acquire the water maze task (A). All of the animals were able to locate the platform when it was visible above the water, indicating that visual deficits were not responsible for the deficits. Although both injured groups demonstrated some learning ability in trial block 3, they required significantly longer latency compared to sham-operated controls. Spatial memory, as measured by search time (B) and relative target visits (C), was significantly disrupted by the injury during the transfer test that immediately followed the acquisition phase of the behavioral testing. The horizontal dotted line in B and C represents chance performance on these two tasks. Bars represent group means (±SD). \(*p < 0.05\) compared to sham-operated control at same survival time; \(#p < 0.05\) compared to 30-day injured group.
TBI-RELATED SYNAPTOGENESIS

The present study is the first to report a quantitative evaluation of total synaptic numbers in the hippocampal CA1 str rad subfield following a controlled cortical impact (CCI). These findings indicate that the hippocampal formation is significantly denervated following the CCI trauma and subsequently capable of initiating a reactive synaptogenic response. The reinnervation process is initiated relatively quickly following the trauma and persists for an extended period of time.

Models of TBI not only disrupt normal cortical morphology but also alter hippocampal circuitry (Cortez et al., 1989; Dietrich et al., 1994; Goodman et al., 1994; Hicks et al., 1996; Santhakumar et al., 2001; Sato et al., 2001; Grady et al., 2003; Anderson et al., 2005). We previously reported that moderate to severe cortical contusions result in a time dependent loss of hippocampal CA3 pyramidal cells ipsilateral to the site of impact (Baldwin et al., 1997). The loss of CA3 neurons proceeds very quickly, with a 60% decline occurring in the first 24–48h post injury, with no additional significant loss at later time points. This neuronal damage results in significant denervation of a major portion of the apical dendrites of the hippocampal CA1 pyramidal cells, thus impeding the normal functioning of this important structure. Early ultrastructural studies have monitored synaptic replacement in denervated zones of the hippocampus (Matthews et al., 1976; Lee et al., 1977; Steward and Vinsant, 1988; Scheff, 1989; Marrone et al., 2004), including the CA1 dendritic field (Goldowitz et al., 1979; Nadler et al., 1980; Anderson et al., 1986). All of these studies reported a time-dependent increase in synaptic numbers indicative of a robust injury-induced compensatory response, regardless of the mechanism underlying the initial denervation. The present results report a similar restorative response following TBI.

In the present study, TBI resulted in a significant reduction in total number of synapses in str rad of the CA1 apical dendritic field. This is the first TBI study to use unbiased stereological methods to study the reinnervation time course in this hippocampal subfield. By 60 days post-injury, the total number of normal appearing synapses had increased to almost 75% of pre-injury levels indicative of a significant compensatory response. Previous non-TBI investigations of hippocampal injury-induced reactive synaptic replacement have reported a loss and reestablishment of areal or numerical synaptic density, a value that can be altered by a simple changes in the hippocampal reference volume (Coggleshall and Lekan, 1996). In several of these studies, the reinnervation density recovered to pre-injury levels by 60 days post-injury (Matthews et al., 1976; Goldowitz et al., 1979; Nadler et al., 1980). Increased packing density of synaptic contacts may be attributed to shrinkage that occurs in the denervated zone (Scheff, 1989). While many studies have ignored this important variable, the collapse of a denervated neuropil can occur as early as 5 days post-injury (Lynch et al., 1975). In the present study, there was a highly significant (14%) decrease in the reference volume of the str rad that affected the packing density. One of the advantages of using unbiased stereology to estimate total synaptic number is that it is unaffected by this change in reference volume (Coggleshall and Lekan, 1996). Unbiased sampling and stereologic methods provided us with the means to estimate the total number of synapses ($5.77 \times 10^9$) in the str rad subregion of the CA1 dendritic field. This value is very close to that obtained by Geinisman et al. (2004), who reported $5.1 \times 10^9$ synapses in this same region for male Long Evans rats. Our data is the first to report total synaptic numbers in str rad of young adult male Sprague-Dawley rats using unbiased stereology.

Changes in neuropil volume could have important implications on regulating the amount of synaptic plasticity. With relatively few exceptions (Matthews et al., 1976; Goldowitz and Cotman, 1980), past studies have demonstrated that following partial denervation, CNS structures replace lost synaptic contacts to a packing density level that does not exceed that observed in the naive animal. This suggests that signals regulating reactive synaptogenesis are influenced in part by the packing density of synaptic contacts. In the present study, reactive synaptogenesis resulted in a relatively rapid and significant replacement of contacts that followed a time-de-
that injury-induced plasticity might underlie functional
tial deafferentation, several studies have explored the idea
the robust plasticity in the hippocampus following par-
jecting to these neurons can be compromised. Because of
vated since not only target cells but also afferents pro-
Recovery of function following brain injury is compli-
remained a deficit in the acquisition of the MWM. In the present set of
experiments, there was no significant difference between
30 and 60 days post-injury, although other studies have
reported continued synaptic replacement following
denervation with longer recovery paradigms (Matthews et al., 1976; Hoff et al., 1982).

The sources of the afferents providing the replacement
terminals in the denervated region are unclear at the pre-
ent time. The most obvious candidates would be resid-
Schaeffer collaterals (Ishizuka et al., 1990; Li et al., 1994) from the CA3 pyramidal cells that are highly col-
lateralized fibers that distribute information to both ipsi-
lateral and contralateral hippocampi. These collaterals
normally synapse on the apical and basal dendrites of the
CA1 pyramidal neurons in regions known as str rad and
stratum oriens, respectively. Previous studies by our lab-
report that a severe CCI injury results in a 60% loss of neurons in the ipsilateral CA3 hippocampal sub-
field by 24 h post-injury (Baldwin et al., 1997), leaving
projections from the remaining neurons intact. These
fibers would provide a homotypic input and supply the
denervated region with the most similar pre-injury input.
It is unknown if this homotypic input would have a com-
petitive advantage over other possible afferent sources,
although homotypic plasticity is the most prevalent fol-
owing partial denervation of the hippocampal formation
(Cotman et al., 1981; Cotman and Anderson, 1988).

Other afferent systems projecting to this CA1 terminal
field include commissural fibers arising from the con-
tralateral CA3 pyramidal neurons (Swanson et al., 1978),
cholinergic septal neurons (Nyakas et al., 1987), and
other regions such as the amygdala and dopaminergic cell
groups (Gasbarri et al., 1994, 1996; Pitkanen et al., 2000).
The present results revealed that, although the CCI in-
jury produced a significant loss of synaptic connectivity
and replacement in the CA1 region of the hippocampus,
there remained a deficit in the acquisition of the MWM.
Recovery of function following brain injury is compi-
cated since not only target cells but also afferents pro-
jecting to these neurons can be compromised. Because of
the robust plasticity in the hippocampus following par-
tial deafferentation, several studies have explored the idea
that injury-induced plasticity might underlie functional
recovery. Early studies assessing axonal sprouting and
changes in behavior (spontaneous or reinforced alternation)
were controversial (Loesche and Steward, 1977; Scheff and Cotman, 1977; Olton et al., 1982; Ramirez
and Stein, 1984). Subsequent studies using a variety of
behavioral tasks have failed to show a clear relation-
ship between injury-induced plasticity and functional recov-
er (Ramirez, 2001). A novel study combining TBI and
removal of the entorhinal cortex, resulting in alterations
of the normal hippocampal plasticity response, demon-
strated a disruption in the recovery of MWM learning be-
havior (Phillips et al., 1994). Synaptophysin staining
within the hippocampal dentate gyrus was qualitatively
different in animals with a combined injury. These re-
results and those of subsequent studies (Phillips et al., 1997;
Reeves et al., 1997) suggest that TBI can disrupt multi-
ple pathways that may play an important role in a sub-
sequent recovery process, including a loss of compen-
satory synaptic plasticity.

Because there were no significant differences between
groups in the present study on the visible platform trials,
differences in MWM learning could not be related to al-
terations in visual acuity. On the third trial block (Fig.
4), the 30-day post-injury animals performed significa-
cantly better than the 15-day injured animals but were
still significantly different from sham-operated controls.
Whether or not the difference between the two injury
groups is the result of synaptic replacement in CA1 is
unclear at the present time. It is intriguing to consider
that the 30 days post-injury levels represent the maxi-
mum morphological plasticity response for this region
following a CCI injury but not the maximum functional
output. Perhaps animals tested at 60 days post-injury
would manifest greater acquisition ability because the
system would have had greater opportunity to stabilize
this synaptic field.

Therapeutic interventions that rely on normal synaptic
numbers and function for pharmacologic uptake might
not benefit from early intervention strategies. The max-
imum loss of synapses in the CA1 terminal field appeared
within the initial 48 h following the insult. Although a
substantial compensatory response had been initiated by
10 days post-injury, the total number of synapses at 15
days post-injury was still very low. Comparing the be-
havioral data of animals tested at 15 days in the MWM
with the morphological data, it is surprising that the an-
imals performed as well as they did if proper function-
ing of hippocampal circuitry is required for this task. The
damage sustained to the CA3 field resulted in a bilateral
denervation of the CA1 dendritic field because the in-
jured CA3 pyramidal cells are also the source of the com-
missural afferents to str rad (Swanson et al., 1978). A re-
cent study employing silver staining following TBI
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shows significant degeneration in the contralateral hippocampus CA1 and dentate gyrus not only early after the trauma but persisting even at 7 days post-injury (Hall et al., 2005). Animals demonstrating significant recovery in MWM task initiated early following TBI injury may be using alternative behavioral strategies or alternate CNS circuitry than sham injured subjects. Dependence on hippocampal circuitry ipsilateral to the CA3 damage is probably not the region involved. Even animals with a 30-day recovery period with significant synaptogenesis were markedly worse than controls in the MWM task. Spatial learning, such as that involved in the MWM task, involves integrated activity of multiple systems working in concert for both acquisition and recall. Electrophysiological studies (Miyazaki et al., 1992; D’Ambrosio et al., 1998; Sick et al., 1998; Albensi et al., 2000) have reported significant suppression of LTP, thought to be a correlate of memory, even at extended periods following the injury (Reeves et al., 1995). However, there is an early report that the emergence of LTP, can occur during the early phase of the reinnervation process even though synaptic complexes may not be structurally mature (Reeves and Steward, 1986). Therapeutic interventions then should be designed to either spare CA3 neurons resulting in a diminution of degeneration in CA1, or attempt to significantly enhance the reactive synaptogenic process by increasing not only the number of synapses necessary for hippocampal-related activities but also the time course of the compensatory process.

Although previous experiments have detailed the pathophysiology following experimental TBI, this is the first study to quantitatively study the loss and reacquisition of synaptic contacts in the hippocampus following this type of insult and how these relate to cognitive improvement. The data presented in this study represent the first account of the time course of an active structural and functional compensatory response by the brain following TBI leading to a reorganization of synaptic circuitry.

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