Enhanced Hippocampal Neurogenesis by Intraventricular S100B Infusion Is Associated with Improved Cognitive Recovery after Traumatic Brain Injury

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ABSTRACT

Evidence of injury-induced neurogenesis in the adult hippocampus suggests that an endogenous repair mechanism exists for cognitive dysfunction following traumatic brain injury (TBI). One factor that may be associated with this restoration is S100B, a neurotrophic/mitogenic protein produced by astrocytes, which has been shown to improve memory function. Therefore, we examined whether an intraventricular S100B infusion enhances neurogenesis within the hippocampus following experimental TBI and whether the biological response can be associated with a measurable cognitive improvement. Following lateral fluid percussion or sham injury in male rats (n = 60), we infused S100B (50 ng/h) or vehicle into the lateral ventricle for 7 days using an osmotic micro-pump. Cell proliferation was assessed by injecting the mitotic marker bromodeoxyuridine (BrdU) on day 2 post-injury. Quantification of BrdU-immunoreactive cells in the dentate gyrus revealed an S100B-enhanced proliferation as assessed on day 5 post-injury (p < 0.05), persisting up to 5 weeks (p < 0.05). Using cell-specific markers, we determined the relative numbers of these progenitor cells that became neurons or glia and found that S100B profoundly increased hippocampal neurogenesis 5 weeks after TBI (p < 0.05). Furthermore, spatial learning ability, as assessed by the Morris water maze on day 30–34 post-injury, revealed an improved cognitive performance after S100B infusion (p < 0.05). Collectively, our findings indicate that an intraventricular S100B infusion induces neurogenesis within the hippocampus, which can be associated with an enhanced cognitive function following experimental TBI. These observations provide compelling evidence for the therapeutic potential of S100B in improving functional recovery following TBI.

Key words: differentiation; hippocampus; neurotrophic; proliferation; regeneration; S100B; trauma

INTRODUCTION

Cognitive impairment is one of the most disabling features of traumatic brain injury (TBI) and has been linked to the hippocampus, a region critical for learning and memory, which displays an increased susceptibility to injury (Hicks et al., 1993). Recent findings of neurogenesis within the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus in adult mammals suggest that these regions could provide a possible source to
replace lost neurons (Eriksson et al., 1998; Gould and Gross, 2002; Kornack and Rakic, 1999). Indeed, neuronal progenitor cells within these regions undergo increased cellular proliferation and subsequent neuron formation following TBI (Chirumamilla et al., 2002; Dash et al., 2001).

It has been shown that this endogenous response to insult can be associated with the up-regulation of mitogenic and neurotrophic factors within the injured brain. Moreover, an exogenous application of growth factors enhances hippocampal neurogenesis with subsequent improved cognitive function following TBI (Gould et al., 1999; Nilsson et al., 1999; van Praag et al., 2002). Hippocampal astrocytes are a cellular source for these mitogenic/neurotrophic factors, and have been found to actively regulate adult neurogenesis both by instructing neuronal fate commitment and by promoting proliferation of adult neural stem cells (Song et al., 2002).

One factor actively secreted by astrocytes is S100B, a low-molecular-weight (9–13 kD) calcium-binding, neurotrophic protein (Shashoua et al., 1984; Van Eldik and Zimmer, 1987; Whitaker-Azmitia et al., 1990). Intracellularly, S100B is involved in signal transduction, regulation of enzyme activity and calcium homeostasis (Donato, 2001; Heizmann et al., 2002), which can ultimately regulate cell morphology (Nishi et al., 1997) and prevent apoptosis (Brewton et al., 2001). Additionally, in vitro studies show that S100B exerts mitogenic properties (Goncalves et al., 2000; Klein et al., 1989; Rustandi et al., 1998; Selinfrud et al., 1991; Wilder et al., 1998). S100B has been implicated in developmental plasticity, lesion-induced reactive synaptogenesis (McAdory et al., 1998), as well as in cell processes thought to be involved in learning and memory, such as long-term potentiation (Fazeli et al., 1990). Collectively, these studies demonstrate the crucial role of S100B in normal brain development and hippocampal function.

Furthermore, S100B has been shown to be released following a variety of brain insults (Herrmann et al., 2001; Pleines et al., 2001; Raabe and Seifert, 2000). Since we had previously demonstrated an improvement in cognitive function resulting from an intaventricular S100B infusion following lateral fluid percussion injury (Kleindienst et al., 2004), we sought to determine whether any enhanced hippocampal neurogenesis after S100B infusion contributed to the functional integration of neurons, by correlating the histological data with the cognitive performance assessed by the Morris water maze.

**MATERIALS AND METHODS**

**Animals and Surgical Procedure**

The studies were conducted under approval of the Institutional Animal Care and Use Committee and NIH guidelines. Experiments were carried out on 250–300-g adult male Sprague-Dawley rats (n = 60; Harlan, Indianapolis, IN). Rats were housed with a 12:12-h light/dark cycle, and at 22 ± 1°C with 60% humidity, pellet food, and water ad libitum. Surgery was performed after intubation under isoflurane anaesthesia and controlled ventilation (3% isoflurane in 70% N2 and 30% O2). End-tidal CO2 was monitored throughout anesthesia and kept at 37 ± 0.2°C using a heating blanket. A 4.9-mm craniotomy was trephined half way between lambda and bregma over the left hemisphere for fluid percussion injury. Two holes were made rostral and lateral to the craniotomy. Screws were inserted into the holes for stability, and a Luer-Loc hub, which was made from the 3-mm plastic end of a 20-gauge needle, was cemented into the craniotomy with dental cement.

A fluid percussion pulse of 2.09 ± 0.05 atm was administered through the craniotomy onto the intact dura by a fluid percussion injury device (Dixon et al., 1987). Sham animals received all procedures except the percussion pulse. After the percussion pulse or sham procedure, the Luer-loc hub was removed, and the animals were placed in a stereotactic frame and fitted with a brain infusion cannula (Alzet brain infusion kit 3–5 mm, Durect Corp., Cupertino, CA). The cannula was implanted according to the atlas of Paxinos and Watson (1986) with the tip inserted into the lumen of the left lateral ventricle (stereotactic coordinates 0.8 mm behind bregma, 1.5 mm lateral to midline, 3–4 mm beneath the surface of the skull). The correct placement of the infusion cannula was verified by the measurement of the intracranial pressure, demonstrating a drop by the passage from the brain parenchyma into the ventricle. The cannula was secured with dental cement to two stainless steel screws inserted into the holes made before the percussion pulse. A micro-osmotic pump (Alzet model 1007D, Durect Corp.), filled with 90-μl infusion volume kept at 37°C, was implanted subcutaneously in the neck and connected to the
infusion cannula. After sutures were completed, anaesthesia was turned off and the animals returned to the animal facility. Infusion cannula and osmotic pump were removed under anaesthesia once the original volume of S100B or vehicle solution was delivered.

Study Protocol

The objective of these experiments was to assess the effect of an intraventricular S100B infusion on brain progenitor cell proliferation and differentiation, and to correlate this biological response to recovery of cognitive deficits after fluid percussion injury. The experimental design is demonstrated in Figure 1. The animals were randomly assigned to an intraventricular S100B or vehicle infusion group, following injury or sham procedure. Purified bovine S100B protein (Calbiochem, La Jolla, CA) was added to a vehicle solution containing phosphate buffered saline (PBS) and 0.1 mg of rat serum albumin per ml. We infused S100B intraventricularly at a rate of 0.5 μL per hour (2.5 nM S100B infusion per hour) up to day 7 in order to reach a S100B CSF concentration of approximately 10 nM (Kleindienst et al., 2004). We choose this dose because it has been found that this concentration of S100B causes a proliferative response by astrocytes (Goncalves et al., 2000) and is within the range (below 50 nM) shown to cause an increased proliferative activity on melanoma cell lines (Klein et al., 1989) and rat C6 glioma cells (Selinfreund et al., 1991). The animals were sacrificed by an overdose of pentobarbital either on day 5 or after the water maze testing was completed on day 34.

Histological Assessment

To assess the proliferative response and differentiation of progenitor cells following TBI and S100B treatment, animals were injected intraperitoneally with the mitotic marker 5-bromo-2′-deoxyuridine-5′-monophosphate (BrdU, 150 mg/kg body weight, Roche Molecular Biochemicals, Indianapolis, IN), which was given at three injections with a 4-h interval between injections. These BrdU injections were initiated on day 2 post-injury because this time point represents the early peak period of the injury-induced proliferation (Dash et al., 2001; Rice et al., 2003), ensuring the majority of dividing cells to be labeled. A stock solution of 150 mg/mL of BrdU dissolved in sterile saline was made fresh daily. The animals were sacrificed and transcardially perfused with 4% paraformaldehyde (Electron Microscopy Sciences, Ft. Washington, PA) in 0.1 M phosphate buffer (pH 7.25). The brains were then removed, blocked for coronal sections, and paraffin embedded for microtome sectioning or stored in saline at 4°C for vibratome sectioning.

Immunohistochemistry

To assess progenitor cell proliferation using BrdU immunocytochemistry, 60-μm vibratome sections were collected from the most rostral point of the lateral ventricles through to the rostral edge of the hippocampus (to assess proliferation in the SVZ) and from the habenular commissure to the dorsal blade of the DG (to assess proliferation in the hippocampus). Free-floating sections were subjected to 50% formamide (Sigma-Aldrich, St. Louis, MO) in 2× SSC (0.3M sodium chloride, 0.03M sodium citrate) at 65°C for 1 h to denature the DNA. After rinsing in 2× SSC, sections were incubated in 2 N HCl at 37°C for 30 min. Sections were washed twice in PBS (pH 7.3) and then inhibited for endogenous peroxidase activity with 3% H2O2. Subsequently, sections were incubated in PBS with 0.1% triton X-100 and blocked with a solu-

![FIG. 1. Experimental design. Rats were subjected to a lateral fluid percussion or sham injury and an intraventricular S100B or vehicle infusion for up to 7 days, then given BrdU on day 2, and sacrificed either on day 5 or after 5 weeks. Progenitor cell proliferation was assessed at the earlier time point (day 5), while survival and differentiation was assessed at the later time point (day 34) after the Morris water maze for test of cognitive function was completed. i.c.v., intra-cerebro-ventricular.](image-url)
tion of 10% horse serum in PBS. Sections were incubated with a primary antibody against BrdU (mouse anti-BrdU, 1:100, DAKO, Carpinteria, CA) in PBS for 48 h at 4°C. Sections were then washed with PBS and incubated with a peroxidase-conjugated secondary antibody overnight (goat anti-mouse, 1:200, Chemicon, Temecula, CA). Stained cells were visualized with a peroxidase reaction solution (0.25 mg/mL diaminobenzidine, 0.01% H2O2, 0.04% NiCl2), counterstained with cresyl-violet, and subsequently quantified as described below. For double-labeling experiments to assess the differentiation of the progenitor cells within the hippocampus, 5-μm sections were taken using a rotary microtome at the level of the DG (see coordinates above), and two adjacent sections were collected every 100 μm. Sections were deparaffinized and rehydrated, then subjected to 0.1% trypsin in tillased H2O and incubation in 2 N HCl at 37°C for 25 min. This was followed by a dip in ice cold distilled H2O and incubation in 2 N HCl at 37°C for 25 min. Sections were washed twice in PBS, then blocked with PBS plus 0.3% H2O2 and incubated in a solution of PBS with 3% horse serum, 0.5% triton X-100, and primary antibodies overnight at 4°C. Primary antibody were rat anti-BrdU (1:100, Oxford Biotech, St. Louis, MO), and either mouse anti-neuronal nuclear antigen (NeuN, 1:100, Chemicon, Temecula CA) or rabbit anti-glial fibrillary acidic protein (GFAP, 1:1000, DAKO). The following day, sections were washed with PBS plus 0.1% Tween 20 (PBST) and incubated with a secondary antibody. These antibodies were detected with goat anti-rat secondary antibody conjugated to Alexa-Fluor 488, or either goat anti-mouse or goat anti-rabbit, both conjugated to Alexa-Fluor 568 (Molecular Probes, Eugene, OR). They were visualized using a FITC or Texas Red filter set. Confocal pictures were captured using an Olympus BX61WI upright laser scanning confocal microscope (Leitz Technical Instruments) with an argon laser for excitation at 488 nm, a green helium neon laser at 543 nm, and a red helium neon laser at 633 nm. Images were acquired using Fluoview (FV 300) image acquisition and analysis software.

Stereological Analysis and Quantification

Unbiased stereological quantification of BrdU-immunoreactive cells in both the SVZ and DG was performed using the optical fractionator method and the C.A.S.T. stereology software program (Olympus, Denmark). In this method, all particles in a known fraction of an object are counted, and the total number of particles in the object is extrapolated by multiplying the counted particles by the reciprocal of the fraction sampled. The number of BrdU-immunoreactive cells residing in the SVZ, granular cell layer (GCL), or subgranular zone (SGZ) was determined in every fourth section (section sampling fraction, ssf = 1/4) in a series of 60-μm coronal sections throughout the rostro-caudal extent of these regions. The sections to be sampled were selected using a systematic random sampling scheme and resulted in the sampling of approximately 10 sections for each region.

In every section sampled, the contour of each region (SVZ, GCL, SGZ) was delineated for counting using the tracing function of the C.A.S.T. system. Following this, the program randomly overlays a grid of equidistant counting frames over the region, and the BrdU-immunoreactive cells falling within these counting frames are counted using a 60× oil immersion objective lens. For quantification of BrdU-immunoreactive cells in the SVZ, the fraction of area quantified was equal to the area of the counting frames sampled in the region divided by the total area of the region (the area sampling fraction, asf = 305/22500). Due to the relatively low numbers of BrdU-immunoreactive cells in the DG, BrdU-immunoreactive cells in the entire area of the region were quantified (asf = 1).

For these studies, 60-μm-thick sections were cut throughout the SVZ and hippocampus using a vibratome. Following tissue processing (BrdU immunocytochemistry, mounting and coverslipping sections), measurement of the thickness of these sections along the Z-axis using a micrometer revealed that the average thickness of sections was reduced to 23 μm. Quantification of BrdU-immunoreactive cells was performed in the middle 15 μm of the total tissue height, resulting in guards zones of 4 μm thickness on either side (height sampling fraction, hsf = 15/23). The optical fractionator formula \( N = \text{no. counted} \ast \frac{1}{\text{ssf}} \ast \frac{1}{\text{asf}} \ast \frac{1}{\text{hsf}} \) was used to estimate the total number of BrdU-immunoreactive cells in each of the regions quantified by multiplying the counted particles by the reciprocal of the fraction sampled. The assessment of cell counts was done by the same investigator who was blinded to the experimental groups.

In order to identify the differentiation of recently divided cells into astrocytes or neurons, co-localization of NeuN and GFAP immunoreactivity with BrdU immunoreactivity was assessed in the DG on day 34. Within multiple sections (seven to eight) for each animal, both the total number of BrdU-immunoreactive cells and the percentage of BrdU-immunoreactive cells that co-localized with NeuN and GFAP were determined using confocal microscopy.

Assessment of Cognitive Deficits

On day 30–34 post-injury, cognitive performance of the animals was tested in the Morris water maze (Hamm
FIG. 2. Effect of an intraventricular S100B infusion on progenitor cell proliferation in the subependymal zone of the lateral ventricles on day 5 following TBI or sham injury. (A,B) Photomicrographs showing cells labeled with BrdU at the level of the ipsilateral ventricle counterstained with cresyl-violet. (A) Lower magnification, arrow points to the track of the infusion needle. (B) Higher magnification of the ipsilateral ventricle showing BrdU-immunoreactive cells. (C) Quantification of BrdU-immunoreactive cells (mean ± SEM) on day 5 revealed a significant proliferative response in TBI as compared to sham animals, but not additional effect by an intraventricular S100B infusion.

FIG. 3. Effect of an intraventricular S100B infusion on the fate of injury-induced progenitor cell proliferation in the hippocampus. (A–C) Photomicrographs showing the distribution of BrdU-immunoreactive cells within the ipsilateral DG in S100B- or vehicle-infused animals at different time points following lateral fluid percussion injury. (A) Lower magnification of the ipsilateral DG at day 5 post-injury. (B) Higher magnification showing that BrdU-immunoreactive cells on day 5 post-injury are predominantly identified in the area of their origin, the SGZ. The arrow points to a typical cluster of cells. (C) At 5 weeks post-injury, some BrdU-immunoreactive cells are now located in the GCL, suggesting their migration from the SGZ. (D) Graph showing the total numbers of BrdU-immunoreactive cells found in two regions of the DG, the SGZ and GCL, in S100B- or vehicle-infused animals at different time points post-injury. Data for sham, uninjured animals are shown as a shaded box at the bottom of the graph and are used to establish a baseline of normal levels of progenitor cells demonstrating a minor decline in number over the 5-week period following BrdU injection on day 2. Quantification of the TBI-induced proliferative response in the DG of the hippocampus are given as the number of BrdU-immunoreactive cells (mean ± SEM) in the region of progenitor cell generation (SGZ) and the region of their migration destiny (GCL). Quantification of progenitor cells revealed a TBI-induced proliferative response on day 5 after injury over sham animals (* p < 0.05), which was significantly enhanced by an intraventricular S100B infusion as compared to vehicle-infused animals († p < 0.05). The TBI-induced newly generated progenitor cells did not persist in vehicle-infused animals by 5 weeks post-injury, but did persist in S100B-infused animals compared to sham animals (* p < 0.05). Furthermore, in S100B-infused animals, an enhanced relative percentage of progenitor cells was found in the GCL (p = 0.076), suggesting their successful migration.
The maze consisted of a 180-cm-diameter pool filled with opaque white water in a room with extra-maze visual cues. The water temperature was 23–26°C. The goal platform was hidden 2 cm below the water surface in a fixed location. The assessment consisted of four swim trials per day for 5 consecutive days. The animal was lowered into the water at a randomly chosen location (north, west, south, east) for each trial and allowed up to 120 sec to locate the platform. A computerized video tracking system (Polytrack, San Diego Instruments, San Diego, CA) was used to track the animal’s swim pattern, total distance swum, and time (latency) to reach the platform. At the end of each trial, the animal remained on the platform for 30 sec.

Statistical Analysis

SPSS software was used for statistical analysis. The maze goal latency (sec, mean ± SEM), swim speed (cm/sec, mean ± SEM), and the cell counts (mean ± SEM) were analysed by an ANOVA for group variations followed by a Duncan post-hoc analysis. Significance was accepted at $p < 0.05$.

RESULTS

All animals tolerated the operative procedure without obvious side effects or infections, and no morbidity or mortality occurred.

Proliferation of Progenitor Cells following TBI and Intraventricular S100B Infusion

Using BrdU-immunoreactivity to quantify the total number of proliferating cells within the ipsilateral SVZ (Fig. 2), we found on day 5 a significant mitogenic response following TBI (70,197 ± 4900 vs. 114,043 ± 1078; $p = 0.013$), which was pronounced by intraventricular S100B infusion (86,828 ± 1659 vs. 128,442 ± 589; $p = 0.000$). In the DG (Fig. 3), we found on day 5 a significant mitogenic response following TBI (1894 ± 319 vs. 3590 ± 420; $p = 0.054$), which was profoundly increased by an intraventricular S100B infusion (1718 ± 187 vs. 5821 ± 387; $p = 0.000$). Thus, we demonstrated in the hippocampus a significant proliferative/mitogenic effect of S100B following TBI ($p = 0.012$).

To determine the fate of the proliferating cell population generated in the DG following TBI and intraventricular S100B infusion, we quantified BrdU immunoreactivity 5 weeks post-injury (Fig. 3). In non-injured animals, intraventricular S100B infusion did not increase the number of progenitor cells in the DG, both on day 5 and 34. Following TBI, both in vehicle- and S100B-infused animals, the total number of BrdU-immunoreactive cells decreased over time. In particular, in vehicle-infused animals following TBI, the number of BrdU-immunoreactive cells returned to normal values by week 5 (1590 ± 146 vs. 1890 ± 279; n.s.). In contrast, with S100B treatment, a significantly larger number of BrdU-immunoreactive cells were present in the DG by week 5 post-injury (1563 ± 113.83 vs. 2690 ± 219; $p = 0.033$).

By determining the precise location of BrdU-immunoreactive cells within the subregions of the DG following TBI, we found that, in S100B-infused animals, the SGZ contributed significantly to the injury-induced proliferative response on day 5 compared to vehicle-infused animals (2838 ± 355 vs. 4523 ± 295; $p = 0.026$). Specifically, of the total population of BrdU-immunoreactive cells in the DG following TBI, approximately 70% were found within the SGZ, with 30% located in the GCL. In vehicle-infused animals, the percentage of these cells within the two regions remained unchanged over the 5-week period. Following S100B treatment on the other hand, the percentage of BrdU-immunoreactive cells increased in the GCL by 5 weeks post-injury compared to vehicle-infused animals (419 ± 66 vs. 792 ± 9; $p = 0.076$), which is consistent with the notion of cell migration.

Differentiation of Progenitor Cells following TBI and Intraventricular S100B Infusion

To determine the differentiation of the hippocampal progenitor cell population described above at 5 weeks post-injury, co-localization of BrdU-immunoreactive cells with the neuronal or glial-specific antibodies, NeuN and GFAP, was examined by confocal microscopy (Fig. 4). Following TBI and intraventricular S100B or vehicle infusion, the percentage of BrdU-immunoreactive cells co-expressing GFAP, remained the same in both groups (35.5 ± 6.1% vs. 40.5 ± 6.5%; n.s.). However, following TBI and S100B treatment, the percentage of BrdU-immunoreactive cells co-expressing NeuN was significantly increased as compared to vehicle infusion (23.6 ± 4.4% vs. 46.7 ± 2.5%; $p = 0.033$).

Cognitive Performance following TBI and Intraventricular S100B Infusion

To assess the extent to which an S100B infusion affects cognitive performance following TBI, animals were subjected to a spatial learning ability test on days 30–34 post-injury (Fig. 5). No differences in swim pattern between the experimental groups were found, and an ANOVA of the swim speed excluded an injury-induced motor deficit ($F_{3,37} = 0.200$). The group × day split-plot
ANOVA of maze goal latencies yielded a significant main effect of groups ($F_{3,39} = 3.93, p = 0.039$). The Duncan post-hoc analysis revealed, in animals treated with an intra-ventricular S100B infusion, a significantly improved average cognitive performance as compared to vehicle-infused animals as assessed by the Morris water maze at week 5, both after injury or sham procedure ($p < 0.05$). By correlating the water maze performance to hippocampal neurogenesis as quantified by the percentage of BrdU-immunoreactive cells co-expressing NeuN, we found a strong correlation between an improved cognitive recovery and the number of newly generated neurons in the DG following TBI and our S100B treatment ($r = 0.86$).

**DISCUSSION**

A number of studies have characterized the spatial and temporal profile of the known neurogenic response of the hippocampus following TBI (Braun et al., 2002; Chen et al., 2003). Further, studies have begun to link this endogenous response of the hippocampus to an up-regulation of growth factors that have been shown to enhance stem/progenitor cell proliferation (Yoshimura et al., 2001, 2003). With this in mind, treatments that aim to replace cells lost from TBI have utilized an exogenous application of growth factors to the brain to enhance this proliferative response following TBI. To what extent the newly generated neurons aid in functional recovery is unclear. However, there is evidence for functional consequences of a therapeutically enhanced neurogenesis (Lu et al., 2003, 2004; Mahmood et al., 2003, 2004). In our study, we demonstrated a significant increase in neurogenesis within the hippocampus by an intraventricular S100B infusion following TBI, which was associated with a significant improvement in cognitive function. These observations are consistent with the notion that the newly generated neurons are appropriately integrating into hippocampal circuits.

**Effect of S100B on Brain Stem/Progenitor Cell Proliferation**

In the current study, the mechanisms by which an S100B infusion aids in cognitive recovery were examined by uncovering the extent to which proliferation, differentiation, and cell fate were affected in the hippocampus following TBI. With regard to proliferation, we found an injury-induced proliferative response in the
DG on day 5, which was enhanced nearly twofold by an intraventricular S100B infusion. Additionally, in vehicle-infused animals, the number of BrdU-immunoreactive cells returned to control values by 5 weeks post-injury, while in S100B-infused animals, the number of BrdU-immunoreactive cells was still significantly increased at this time point. This observation can be attributed to an enhanced persistence of injury-induced progenitor cells in the DG via the known neuroprotective properties of S100B (Ahlemeyer et al., 2000; Barger et al., 1995; Landar et al., 1996; Zimmer and Van Eldik, 1986). In sham non-injured animals, no proliferative effect of an intraventricular S100B infusion was demonstrated, and the total number of BrdU-immunoreactive cells decreased minimally over time, which is in agreement with other studies (Kempermann et al., 2002; Takasawa et al., 2002; Yagita et al., 2001). It should be noted that our reported numbers of BrdU-positive cells in the control animals were a quarter of that reported by others (Cameron and McKay, 2001; Gould and Gross, 2002). This is, we believe, in part because we gave BrdU at half the reported dose (150 versus 300 mg/kg) so as to avoid BrdU toxicity. In addition, we administered BrdU over a shorter time period (12 h versus 24 h). Although we cannot exclude that the BrdU incorporation occurred in part as a result of a DNA repair mechanism, it has been well documented by others that BrdU labeling is specific for progenitor cell division after TBI (Dash et al., 2001; Rice et al., 2003; Sharp et al., 2002). It should also be noted that, since an intraventricular S100B infusion did not cause cell proliferation in the hippocampus of our sham non-injured animals, it is likely that S100B may not aid in neurogenesis under normal physiological conditions. However, under pathological circumstances like TBI, when S100B is administered to the injured brain, a neurogenic response is evoked. The mechanism underlying this proliferative response is unknown, but it is likely that S100B may act in concert with other mitogenic factors so as to elevate the overall proliferative response to injury.

Since the SVZ is the largest germinal zone in the mammalian brain (Doetsch and Alvarez-Buylla, 1996), we also examined the proliferative effect of an intraventricular S100B infusion in this region and found that proliferation was slightly enhanced, both in sham and injured animals. As our study focuses on the recovery of hippocampal function, and subventricular progenitor cells are known to predominantly migrate anteriorly to the olfactory bulb (Kornack and Rakic, 2001) and not to the hippocampus, we did not investigate further the fate of these progenitor cells.

Differentiation Fate of the S100B-Induced Cell Population in the Dentate Gyrus

To unambiguously identify newly generated cells as neurons or astrocytes in the DG, we used the cell-type specific markers NeuN and GFAP in addition to BrdU immunodetection. NeuN, which is a transcription factor that is expressed in the nucleus and cytoplasm of mature neurons (Magavi et al., 2000; Mullen et al., 1992), is universally used as a neuron-specific marker. Following injury, we found the percentage of progenitor cells, which differentiated into neurons in the DG, to be significantly increased by an intraventricular S100B infusion (25% in vehicle versus 50% in S100B). Whether the above demonstrated mitogenic effects or known neurotrophic properties of S100B (Brewton et al., 2001; Iwasaki et al., 1997; Nishi et al., 1997; Reeves et al., 1994) contributed to this enhanced neuronal differentiation following TBI has to be elucidated by further studies. In addition, we found a similar sizable percentage of progenitor cells in the DG to express the glial marker, GFAP, at 5 weeks post-injury in both vehicle and S100B groups. Whether the above demonstrated mitogenic effects or known neurotrophic properties of S100B (Brewton et al., 2001; Iwasaki et al., 1997; Nishi et al., 1997; Reeves et al., 1994) contributed to this enhanced neuronal differentiation following TBI has to be elucidated by further studies. In addition, we found a similar sizable percentage of progenitor cells in the DG to express the glial marker, GFAP, at 5 weeks post-injury in both vehicle and S100B groups. This gliogenesis following TBI is in general agreement with that reported by other groups, which have shown that 5–25% of the newborn cells in the GCL became astrocytes (Dash et al., 2001; Komitova et al., 2002; Liu et al., 1998; Takasawa et al., 2002).

Although gliogenesis within the hippocampus has been reported in transgenic mice expressing increased levels of S100B (Reeves et al., 1994), we did not find that an S100B infusion increased gliogenesis after injury, as compared to control animals.
Consequence of Neurogenesis on Hippocampal Function

Although we have now demonstrated that an S100B infusion can greatly increase neurogenesis in the hippocampus following injury, we have not directly measured the extent to which these cells integrate into local hippocampal circuits and facilitate learning. Nevertheless, we have found that animals infused with S100B showed a significant improvement in cognitive function over that observed in vehicle-infused animals. This observation implies that the significantly increased number of newly generated neurons by S100B infusion have been functionally integrated into hippocampal circuits. This notion is in agreement with others, who have demonstrated the generation of functionally mature neurons (Kawai et al., 2004; Schmidt-Hieber et al., 2004) and that newly generated neurons facilitate learning (Nottebohm, 2002; Shors et al., 2001). Furthermore, light- and electron-microscopic studies are consistent with the hypothesis that S100B plays a role in lesion-induced synaptogenesis (Huttunen et al., 2000; Li et al., 1998; McAdory et al., 1998; Rickmann et al., 1995) and S100B has been found to improve memory function (Mello e Souza et al., 2000; O’Dowd et al., 1997).

In summary, we have made three new findings regarding a method to augment the reparative response of the brain to injury. Firstly, we have demonstrated that S100B increases proliferation and survival of hippocampal progenitor cells after experimental TBI. Secondly, we have shown that S100B enhances the neuronal differentiation of hippocampal progenitor cells after TBI. Thirdly, we have correlated this increased neurogenesis by S100B with an improved cognitive performance. Collectively, we demonstrated a beneficial effect of S100B on neurogenesis and cognitive function following TBI. Thus, S100B may offer a treatment option to augment important innate repair mechanisms of the brain, and promote memory consolidation after TBI.

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