The effects of combined fluid percussion traumatic brain injury and unilateral entorhinal deafferentation on the juvenile rat brain

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Abstract

The current study was designed to address the effects of traumatic brain injury (TBI) on plasticity and reorganization in the juvenile brain. Given that two of the major pathological sequelae of TBI involve a generalized neuroexcitation insult and diffuse axonal injury, we have employed models of these pathologies, delivered either independently or in combination, to examine their effects on injury-induced synaptic reorganization of the dentate gyrus in the developing rat. Postnatal day 28 rats received either sham, central fluid percussion traumatic brain injury (TBI), unilateral entorhinal cortical lesion (UEC), or TBI+UEC (TUEC) injury. Cognitive performance was assessed in the Morris water maze (MWM) between 11 and 15 days post-injury and the brains were processed for synaptophysin immunohistochemistry and routine electron microscopy. The MWM results revealed that TBI or UEC lesions delivered independently do not produce significant morbidity in P28 rats. However, when these injuries are combined, they reveal significant deficits in the MWM, accompanied by measurable changes in the distribution of presynaptic synaptophysin immunoreactivity over the deafferented dentate molecular layer. These observations are further supported by qualitative ultrastructural alterations in synaptic architecture in the same subregions of the dentate neuropil. The present findings show that the resilience of the immature brain following TBI is reduced when neuroexcitatory insult is combined with deafferentation. Moreover, when deafferented tissue is assessed morphologically, evidence exists for aberrant plasticity and abnormal synaptic reorganization in the juvenile brain.

Keywords: Traumatic brain injury; Entorhinal lesion; Synaptic plasticity; Development; Deafferentation; Synaptic reorganization

1. Introduction

Traumatic brain injury (TBI) is the leading cause of injury related death and disability among children and young adults in the United States, with the age of greatest incidence falling between 15 and 19 years of age [16]. Despite these epidemiological findings, most TBI research has focused on adult animal models. Because of this, our concept of how the younger brain responds to TBI is much less clear than our current understanding of the same injuries in the adult brain. Only in the last 5 years have injury models for the developing brain emerged [1,2,4,9,10,32]. From these studies, it is clear that the more immature brain shows remarkable recovery when challenged by a variety of independent forms of trauma, however, it is not well understood how the more complex pathologies of TBI affect this developmental resilience. For example, it is common for human head injury to include a combination of neuroexcitation [3] and diffuse axonal injury [19,20,26,35]. This resultant pathology is initiated by a severe excitotoxic insult, compounded by axotomy-induced deafferentation which evolves with time [26–28]. While there are TBI models that generate both neuroexcitation [37] and/or axonal injury [26], they do so in a diffuse fashion, making it difficult to localize and assess consequent synaptic changes. For this reason, we have utilized a unique combined insult model [22,23], specifically designed to address the effects of acute TBI neuroexcitation on long-term synaptic recovery. The
combination of fluid percussion-induced neuroexcitation with targeted hippocampal deafferentation produced by entorhinal cortical lesion permits the study of reactive synaptogenesis in a well-characterized brain circuitry [5,18,21,22,34]. In the adult form of this model, it has been shown that these combined pathologies produce maladaptive reorganization of synaptic input to the dentate gyrus and persistent severe cognitive deficits [23]. However, it is unknown how the recovery mechanisms affecting reorganization and synaptic plasticity in the developing brain respond to this type of combination injury.

In the present study we test the hypothesis that combining neuroexcitatory insult with deafferentation in the juvenile brain will reduce the capacity for functional and structural reorganization which may be observed following either injury paradigm alone. Postnatal day 28 (P28) rats were selected for these studies because they are post-weaned and they retain behavioral and metabolic plasticity in response to independently applied percussive TBI [30,31]. Moreover, given the current views on inter-species age estimations [29], P28 rats would be a reasonable choice to represent the juvenile human population (10–15 years of age) which we wish to assess in this study. P28 rats were subjected to either sham injury, central fluid percussion (TBI), unilateral entorhinal cortical lesion (UEC), or TBI+UEC injury (TUEC). Cognitive performance was assessed in the Morris water maze (MWM) on post-injury days 11–15, and then quantitative synaptophysin (SYN) immunohistochemistry (IHC) was performed on day 15 hippocampi from the same cases to assess how regenerative afferent patterns correlated with inspired gas anesthesia (4% isoflurane in 70% N₂O/30% O₂) formed on day 15 hippocampi from the same cases to Loesche and Steward method [17]. Under nose-cone topophysin (SYN) immunohistochemistry (IHC) was performed on day 15 hippocampi from the same cases to determine how synaptic reorganization of the hippocampus in each of the post-injury days 11–15, and then quantitative synaptic outgrowth at 15 days postinjury was performed to determine how changes in synaptic morphology were spatially and temporally correlated with behavioral and synaptophysin outcome measures. The overall goal of this approach is to establish a TBI model in which plasticity and synaptic reorganization could be more systematically examined in the younger brain, potentially identifying novel therapeutic interventions for juvenile humans suffering from brain injury.

2. Materials and methods

2.1. Subjects

A total of 33 postnatal day 28 (P28) male Sprague-Dawley rats were used to assess behavioral recovery and synaptic reorganization of the hippocampus in each of the following groups: (i) central fluid percussion injury (TBI, n=7), (ii) unilateral entorhinal lesion (UEC, n=6), (iii) TBI+UEC (TUEC) (n=7), and (iv) sham-injury (n=7). All protocols received prior approval by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

2.2. Fluid percussion injury

The procedure for induction of central fluid percussion (FP) brain injury in younger rats was the same as previously described for adults [23]. Briefly, under gas anesthesia (4% isoflurane in 70% N₂O/30% O₂) rats were placed in a stereotaxic frame and the 2.0–2.1 atm). The levels of injury used in this study do not result in cerebral contusion or cortical cavitation. Overt histopathology is minimal, with limited tissue damage in the parietal cortex and only modest cell loss in the dentate hilus and CA3 subfield of the hippocampus.

2.3. Entorhinal cortical lesion

UEC lesion was performed by a modification of the Loesche and Steward method [17]. Under nose-cone inspired gas anesthesia (4% isoflurane in 70% N₂O/30% O₂) rats were placed in a stereotaxic frame and the entorhinal cortex exposed for the passage of a 0.2-mm Teflon-insulated wire electrode. Electrolytic lesions were made by passing a 1.5-mA current (30-s duration) through the electrode at a total of eight stereotaxic placements at the lambda suture. Two steel screws were placed 1 mm rostral to bregma and 1 mm caudal to lambda. A modified syringe hub with a 2.6 mm inside diameter was placed over the exposed dura, bonded to the skull with cyanoacrylate adhesive. Dental acrylic was applied around the hub and steel screws to secure implant rigidity. Then 24 h later, animals were connected to the injury device and subjected to a moderate TBI (2.0–2.1 atm). The levels of injury used in this study do not result in cerebral contusion or cortical cavitation. Overt histopathology is minimal, with limited tissue damage in the parietal cortex and only modest cell loss in the dentate hilus and CA3 subfield of the hippocampus.
to confirm a complete deafferentation of affected hippocampal sites following entorhinal cortical lesion [21]. With survival intervals of 7–14 days, elevations of AChE histochemical staining are diagnostic of the deafferented neuropil. To verify this pattern of AChE activity, coronal sections (40 μm) of the hippocampus were collected from those prepared for synaptophysin immunohistochemistry (SYN IHC), and subjected to a modification of the El Badawi and Schenk method for AChE histochemistry [6,7], using acetylthiocholine as a substrate [5]. Any cases exhibiting evidence of incomplete lesioning by AChE methods were removed from the study.

As a second analysis of lesion extent, damaged cortical regions from each case subjected to UEC surgery were documented in horizontal brain sections (100 μm) placed on a flatbed scanner (Umax Scanner). Using image analysis software (Photoshop), the extent of lesion was determined over a range of dorsoventral brain levels relative to bregma. As in our adult lesion studies, any case with lesions extending past the hippocampal/subiculum interface were excluded from the study.

2.4. Behavioral outcome assessment

Based on the time-course of motor recovery in adults and the time-course of sprouting and reinnervation following a UEC lesion (7–14 days postlesion), Morris water maze (MWM) testing was initiated on post-injury day 11 and conducted through day 15 to assess long-term cognitive deficits following brain injury. The MWM tank (180 cm diameter, 60 cm high) was filled with water to 28 cm, maintained between 23 and 28 °C, and made opaque with white latex paint. A clear plexiglass platform (10 cm diameter, 26 cm high) was hidden 2 cm below the water’s surface and remained in a fixed location in the southwest quadrant of the tank. Rats were given four trials per day with an intertrial period of 4 min for 5 consecutive days. For each trial the animal was released from each of the four release points in a random order. Animals were allowed 120 s to find the platform, after which they were manually guided to it and remained on it for 30 s before returning to a warmed cage. The duration of the escape latency was timed and the escape latency results were analyzed with a one-way ANOVA for each day.

2.5. Synaptophysin immunohistochemistry

Synaptophysin immunohistochemistry (SYN IHC) was performed on day 15 to evaluate change in presynaptic input within the dentate gyrus, and to determine whether an atypical synaptic reorganization might be correlated with any observed long-term behavioral morbidity. Rats were perfused (0.9% saline/4% paraformaldehyde) and 40-μm serial coronal vibratome sections containing both hippocampi were collected. Sections were washed in phosphate-buffered saline (PBS: 0.15 M NaCl, 0.1 M Na phosphate, 0.5% CaCl₂, pH 7.2), permeabilized in methanol and H₂O₂ and then preincubated for 1 h in 10% normal serum (in PBS) of the secondary antibody species. Sections were incubated overnight in primary antibody to SYN (Sigma, St. Louis, MO) diluted in PBS to 1:500. Sections were washed rigorously in three changes of PBS and the primary antibody visualized with avidin–biotin–horseradish peroxidase complex (Vector Labs).

Sections stained for SYN immunoreactivity (IR) were quantitatively analyzed using the sectional density analysis method previously described [19]. SYN stained sections were first digitally captured (Nikon CoolPix 990 camera). NIH Image was used to obtain optical densities (ROD× area) within each of the three molecular layer subregions defined by SYN IR. Each measure was then normalized to the optical density of the adjacent corpus callosum as an internal IHC control. Because the width of the dentate molecular layer changes as a function of postlesion interval in adults, we also measured the height of each SYN IR subregion and the width of the entire molecular layer for all cases. The percent reduction in molecular layer width was determined and used to normalize measures of SYN IR optical density for each molecular layer subregion. Adjusted SYN IR densitometry measures and molecular layer height/width changes were then analyzed with a one-way analysis of variance.

2.6. Qualitative electron microscopic analysis

In order to determine basic changes in cytoarchitecture underlying different patterns of SYN distribution after injury, we applied routine EM analysis to tissue sections from a subset of animals within each group, focusing on the deafferented dentate molecular layer. Because of its correlative nature, the present analysis was limited to qualitative assessment, using the well-described patterns of synapo-dendritic reorganization after UEC lesion as a guideline [34]. Alternate sections were harvested from the same 15-day postinjury cases prepared for SYN IHC and processed for ultrastructural (EM) analysis using a modification of Yaghmai and Povlishock [36]. Sections underwent osmication and were flat embedded on plastic slides sandwiched between two glass slides. After the plastic had cured, the glass slides were removed and sample regions of mid-dorsal hippocampus containing the CA1 and dentate gyrus were excised from the plastic embedded material. These regions were mounted onto chucks and a series of thick and thin sections cut using an ultramicrotome. The thin sections were collected on membrane-coated slotted grids and observed with a Jeol 1200 electron microscope. Regions containing proximal, middle and distal dendrites within the dentate molecular layer were photographed at 10,000× magnification.
3. Results

3.1. Entorhinal cortical lesion in P28 rats

Sectional analysis of average UEC lesion in P28 animals showed that the lesion destroyed the entorhinal cortex and extended into the presubiculum, parasubiculum and subiculum. Examples of lesion extent in both UEC and TBI+UEC animals are shown in Fig. 1A. The maximal extent of lesion reached the hippocampal/subiculum interface, the same criterion utilized for entorhinal lesion in adult rats [23]. Similarly, P28 AChE postlesion staining in the dentate gyrus showed a pattern identical to that previously reported following adult UEC lesion [21]. Specifically, the dendritic laminae deafferented by UEC ablation were marked by an increase in AChE histochemical staining during periods of reactive synaptogenesis (Fig. 1B). Ipsilateral to the lesion, the side

Fig. 1. Entorhinal lesion injury extent documented with degenerative profile and AChE histochemistry. (A) Minimal (hatched) and maximal (black) extents of the lesion following unilateral entorhinal cortical ablation (left) and combined TUEC injury in P28 rats (right). The insert shows a 200-μm horizontal section through the lesion site from a representative combined injured animal. (B) Hippocampi stained bilaterally for AChE verify that elevations in the enzyme are produced over deafferented dentate molecular layer of both TUEC and UEC cases ipsilateral to the lesion (arrows). This pattern of AChE staining known to be induced by UEC was observed in all reported cases for UEC and combined TUEC insults. Bar represents 200 μm.
conducting the majority of entorhinal projections to the hippocampus, showed strong AchE staining over the deafferented dentate molecular layer dendrites. Taken together, these results confirm that our method for producing P28 UEC lesion produced complete entorhinal cortical lesions consistent with those generated in adult rats.

3.2. Morris water maze performance

There was no significant weight gain differences between any groups ($F(5,37)=2.13, P=0.09$) and no difference in righting times between TBI ($308.0\pm52.7$ s) and TUEC ($304.3\pm42.0$ s) groups ($F(2,20)=0.002, P=1.0$).

The average escape latencies for P28 sham, TBI injured, UEC and TUEC rats are shown in Fig. 2. All animals showed improved performance with training from day 11 to 15. Comparison between groups on each day of MWM training revealed no significant difference between groups on day 11 ($F(3,23)=2.48, P=0.09$), 12 ($F(3,23)=2.57, P=0.08$), or 13 ($F(3,23)=1.55, P=0.23$). On days 14 and 15 TUEC animals were significantly impaired compared to sham and UEC injured animals ($F(3,23)=5.01, P=0.01; F(3,23)=5.19, P=0.001$).

3.3. Synaptophysin immunohistochemistry

The distribution of the presynaptic terminal marker SYN in P28 sham animals exhibited the same trilaminar pattern within the dentate molecular layer as previously described for adult rats [19,23] (Fig. 3A). A higher level of SYN IR was found over proximal and distal segments of granule cell dendrites, with the mid-portion of the dendritic arbor containing fewer IR sites (see arrows in Fig. 3A). A reduction of SYN distribution in the distal granule cell dendrites following UEC lesion has been correlated with the deafferentation-induced loss of presynaptic input, while the subsequent reemergence of SYN trilaminar organization has been temporally linked with the process of reactive synaptogenesis [19]. Given that synaptic reorganization is part of the recovery process we are exploring, we have assessed SYN distribution following each insult in our study.

3.3.1. Qualitative findings

Animals subjected to TBI alone also showed a trilaminar SYN pattern, although the intensity of the IR in each layer was less than that observed for sham animals (Fig. 3B). This reduction in SYN intensity, without apparent change in molecular layer width or relative proportion of each zone, has been reported following the same TBI insult in adult rats [23]. By contrast, UEC and TUEC animals showed distinct differences in SYN IR suggesting a redistribution of presynaptic input. We observed the greatest intensity of SYN IR over the proximal granule cell dendrites, a zone which occupied a larger proportion of the total dendritic length. Notably, however, SYN labeling was now retracted to a thin layer over the distal margin of the molecular layer dendrites (Fig. 3C,D; see arrows). In addition, loss of a distinct interface between SYN laminae at the mid to distal dendritic zones was observed, along with what appeared to be a reduction of overall molecular layer width. These findings for SYN distribution are generally consistent with those observed after deafferentation in the adult rat [19].

3.3.2. Quantitative findings

We quantified SYN distribution in different portions of the deafferented granule cell dendrites as a function of the distinct laminae revealed by SYN IR. Density of IR was assessed for each layer, as well as the height of each region relative to the entire molecular layer width. In the UEC and combined TUEC cases, each IR density measure was corrected for total shrinkage of molecular layer width prior to data analysis. Results showed that for the inner IR region (inner molecular layer, IML) there was no significant difference in SYN optical density between groups ($F(3,20)=1.20, P=0.34$) (Fig. 4B). However, there was a significant increase in the height of the inner SYN region among UEC and combined injured animals relative to sham and TBI alone cases ($F(3,23)=37.98, P<0.001$) (Fig. 4A).

Quantitative analysis of the middle IR region also showed no difference in the optical density between groups.
Fig. 3. Light microscopy immunohistochemistry for synaptophysin in the P28 dentate gyrus of sham, TBI, UEC and combined TUEC animals. Upper panels show cases after sham-injury (A) and percussive TBI alone (B). A section from a UEC case is shown in (C) and a TUEC case in (D). Note the similar trilaminar pattern of SYN distribution in the dentate molecular layer (arrows) of both sham controls and TBI alone. After UEC and the combined injury SYN distribution is altered in its relative distribution between these layers. The outer (deafferented) molecular layer shows a highly attenuated SYN localization over the distal dendrites, while SYN binding over the inner molecular layer is increased. G, granule cell layer. Bar represents 40 μm.

\[(F(3,20)=2.48, P=0.096) (\text{Fig. } 4B)\]. In contrast to what was observed for the inner IR region, there was a significant decrease in the height of the middle SYN region when compared with sham and TBI alone animals \((F(3,23)=7.92, P<0.001) (\text{Fig. } 4A)\).

When the outer IR laminae were subjected to quantitative analysis, significant changes in both optical density \((F(3,20)=3.84, P<0.029)\) and lamina height \((F(3,23)=13.99, P<0.001)\) were detected. The optical density of SYN labeling was significantly decreased in the combined injured animals relative to sham and TBI (Fig. 4B). Similarly, the height of the outer IR zone showed a significant reduction in the UEC and TUEC groups relative to sham and TBI alone cases (Fig. 4A).

3.4. Electron microscopy

Ultrastructural analysis of TBI, UEC and TUEC cases revealed qualitative differences in the extent of synaptodendritic recovery at 15 days postinjury. While these effects were apparent in all layers, the outermost subregion of the molecular layer displayed the most robust changes. Relative to sham-injured controls, the dentate molecular layer of rats subjected to TBI alone showed little pathology, with typical dendritic cytoarchitecture and synaptic profiles (compare A and B in Fig. 5). By contrast, UEC lesion resulted in an injury-induced reinnervation of the outer molecular layer, however, the dendritic cytoarchitecture did not fully match that seen in comparable control sections (compare Fig. 6A to Fig. 5A). Relatively few sites
Fig. 4. Quantitative assessment of SYN immunobinding in the dentate molecular layer of P28 sham, TBI, UEC and combined TUEC animals. (A) Heights of each SYN stained lamina relative to the whole width of the molecular layer for sham, TBI, UEC and combined injured animals (*$P<0.05$ relative to sham and TBI). Note that reductions in SYN layer heights for both UEC and combined insult cases were detected in the outer and middle zones. By contrast, an increase in the height of the inner SYN zone was observed when deafferentation was involved. (B) Optical densities of each SYN lamina in the molecular layer for sham, TBI, UEC and combined injured animals (values normalized to corpus callosum staining density; †$P<0.05$ relative to sham). The only significant effect of injury on SYN density was identified in the combined insult cases over the outer molecular layer. IML, inner molecular layer; MML, middle molecular layer; OML, outer molecular layer.

of degenerative debris were visible in the dennervated neuropil at 15 days after UEC, and the inner molecular layer, a zone not subjected to deafferentation, showed normal synapto-dendritic morphology (Fig. 6C). In the combined TUEC cases, striking differences from TBI and UEC alone were observed in both the outer and inner molecular layers. While substantive regeneration of synapses had occurred, multiple sites of tissue damage
4. Discussion

The current study compared the effects of combining neuroexcitatory insult with deafferentation on cognitive function and synaptic reorganization in the postnatal day 28 rat brain. Our findings demonstrate that when the juvenile rat brain is independently subjected to either central fluid percussion injury or unilateral entorhinal lesion (UEC) lesion, cognitive deficits are not detectable between 11 and 15 days post-injury. However, when presented in combination, these injuries produce significant deficits in spatial memory function, accompanied by measurable changes in the distribution of hippocampal synaptophysin, a marker protein for presynaptic terminals. Ultrastructural analysis suggests that changes in distribution of synaptophysin following the combined TBI and UEC insult are correlated with qualitative differences in synaptic architecture. These findings suggest that, although the P28 brain is capable of enhanced functional and structural recovery relative to the adult brain following TBI alone, the resilience of the immature brain is significantly compromised when TBI neuroexcitatory insult is combined with targeted hippocampal deafferentation.

4.1. Behavioral recovery in P28 versus adult rats

Overall, the current study shows that P28 rats experience less cognitive deficits after either TBI or UEC lesion alone, attaining a higher index of recovery over the first 2 weeks after injury than adult rats. P28 MWM latency to platform measures for both TBI and UEC are approximately half of the latencies for adults (20–25 s for juveniles vs. 40–50 s for adults on day 15 of assessment) [23]. Notably, the younger rats subjected to TBI or UEC perform equivalent to sham-injured animals. Similar scores for P28 and adult sham cases also indicate that age is not a confounding factor in the MWM task discrimination. The present results for central percussive TBI alone are also consistent with those previously reported following lateral fluid percussion injury, where P28 rats exhibited escape latency deficits only during the first 7 days postinjury, after which none were detected [30,31]. In contrast, adult TBI cases show MWM deficits for up to 30 days after injury.
Fig. 6. Qualitative electron microscopy of the outer and inner subregions of the dentate molecular layer at 15 days following UEC alone and combined TBI and UEC injuries. The outer molecular layer of the dentate gyrus after UEC lesion (A) shows structural recovery in the denervated neuropil, with relatively few sites of enlarged, disorganized dendrites (arrows). Normal synaptic profiles are visible (arrowheads), however the associated postsynaptic densities (psds) were often less electron dense than typically observed in control animals. By contrast, the TUEC cases (B) revealed a reorganized neuropil with distinct differences from sham-injured (Fig. 5A) and UEC lesioned rats. The combined injury exhibited persistent sites of tissue damage (arrows) and synapses with thick, electron dense psds (arrowheads). Differences between UEC and TUEC were also observed in the inner molecular layer of the dentate neuropil. (C) The UEC cases showed intact cytoarchitecture in the large proximal dendrites (arrows), zones not deafferented by the lesion. As in the distal, deafferented outer molecular layer, the proximal dendritic zones of the TUEC rats (D) also showed sites of tissue degeneration (arrows) and the same thickened, electron dense psds (arrowheads). Bar represents 1 μm.
Such differences in P28 cognitive performance are most likely due to an increased level of synaptic plasticity generally observed in younger, developing animals [12–15]. While the exact mechanism(s) underlying such age-dependent effects on recovery are not well established, it is possible that the greater potential for synaptic plasticity in the younger brain enables the surviving circuits to re-innervate more effectively than those in adult animals. Interestingly, the time course of cognitive recovery in juvenile rats subjected to TBI parallels the recovery from injury-induced deficits in glucose metabolism [31], an index which appears to be correlated with functionally active synapses [11,33].

In contrast to TBI and UEC alone, we found that combining TBI and UEC lesion in P28 rats significantly increased the level of cognitive deficit assessed with the MWM. This differs from the adult response to this insult, where combined injured animals showed latency scores equivalent to those for either injury alone [23–25]. While the extent of cognitive deficit achieved for P28 and adult TUEC cases was quite similar over the 11–15-day period of testing (latencies of ~70–90 s), it is important to keep in mind that the separate injuries induced more profound deficits in adult rats [23,25]. Thus, it appears that the juvenile rats can better tolerate the effects of independent TBI neuroexcitatory or UEC deafferentation insult, however, when the two are presented in combination, the younger animals show an enhanced vulnerability and a significant increase in long-term cognitive deficits. This finding may have clinical relevance for brain injured juveniles with complex injuries involving both concussive excitatory damage and axonal injury with deafferentation.

4.2. Synaptic recovery following juvenile brain injury

Our results with SYN immunohistochemistry show that P28 animals subjected to either TBI or UEC lesion alone exhibit a similar distribution of this presynaptic protein as has been reported for adults following UEC lesion [19]. In the dentate molecular layer, the typical pattern of high SYN content in proximal and distal dendritic subregions is reproduced for both the P28 sham-injured and percussive TBI groups, with only slight changes in density detected after TBI. After UEC lesion, P28 rats show the same shift in SYN labeling previously reported during reactive synaptogenesis in adult rats [19]. Specifically, the findings show a loss of detectable protein in distal dendrites where presynaptic terminals are removed by the lesion and an increase in the extent of SYN along the more proximal primary dendrites which retain intact presynaptic input. However, P28 rats failed to show the full re-emergence of SYN expression in the dendritic zones undergoing synaptogenesis, a pattern consistently reported for adults at 15 days after lesion [25]. Interestingly, we found that SYN distribution in the juvenile TUEC cases closely matched that of the UEC alone group. Despite this similarity, we were able to detect a significant reduction in the density of SYN immunobinding over the distal dendrites after the combined insult. Although such effects on SYN cannot fully explain the significant difference in cognitive function between these two groups, a difference in the extent of reinnervation of these distal dendrites would be consistent with the poorer behavioral performance observed after TUEC. Indeed, we have reported that the combined percussive TBI and bilateral entorhinal lesion (BEC) in adult rats produces profound long-term cognitive deficits and a dramatic shift of SYN distribution during reinnervation reduced in proximal and distal dendrites and elevated in medial dendritic zones [23]. Collectively, these results suggest that reactive synaptogenesis in P28 rats may have a delayed time course and different spatial reorganization relative to the same process in lesioned adults. While the current study only documents synaptic changes in the dentate gyrus, there is published evidence that TBI can inhibit environment enrichment-induced plasticity within the cortex of P19 rats [8]. Such cortical synaptic changes may also contribute to the acute cognitive impairments seen in juvenile rats during the first week after TBI. Even with these observed differences, SYN distribution alone is not sufficient to directly associate behavioral effects with aberrant reactive synaptogenesis. A detailed ultrastructural analysis of the neuropil in the dentate molecular layer is required to confirm this link.

Routine electron microscopic analysis of the P28 groups in this study shows that TBI alone induces only modest pathology in the dentate molecular layer, which by 15 days postinjury appears structurally equivalent to sham-injured control cases. By contrast, the UEC and combined TUEC groups generated more severe pathology and significant levels of reactive synaptogenesis at 15 days survival. Qualitatively, the UEC cases exhibited widespread recovery of cytoarchitecture and synaptic replacement in the distal deafferented dendrites. A similar level of ultrastructural recovery in adult UEC cases has been directly correlated with elevations in SYN protein over the deafferented dendrites in the outer third of the molecular layer [20]. By contrast, the current study shows that synaptic reorganization in the same denervated zone of juvenile rats is not matched by SYN distribution, suggesting that the pre-synaptic protein may not mark sprouting terminals which reinnervate the molecular layer in the P28 brain. Moreover, ultrastructural analysis of the combined TUEC insult in juvenile rats revealed clear qualitative differences in the profile of post synaptic densities, which were observed to be thicker and more electron dense than those present after UEC alone. Such profiles were found throughout the molecular layer, but were particularly notable in the proximal dendrites. As with the UEC cases, changes in synaptic ultrastructure did not directly correlate with the pattern of SYN immunoreactivity over the TUEC molecular layer. Taken together with our behavioral and SYN results, these qualitative EM observations suggest
that cognitive recovery after brain injury in P28 rats may not necessarily be associated with the re-emergence of normal patterns of pre-synaptic input, and that atypical postsynaptic structure may contribute to maladaptive recovery processes when combined neuroexcitation and deafferentation are present. A more detailed quantitative study of these ultrastructural observations will be required to establish significant injury-induced effects on synaptic reorganization in juvenile rats sustaining head injury.

5. Conclusions

The present study shows that recovery from concussive brain injury in the juvenile rat can be measurably reduced when secondary deafferentation insult is present. Exacerbation of behavioral deficits and attenuated synaptic plasticity are observed. Future studies using juvenile TBI models should examine the molecular mechanisms underlying these differences in recovery and seek to identify new targets for postinjury therapy in young individuals suffering from brain trauma.

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