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Postinjury Administration of L-Deprenyl Improves Cognitive Function and Enhances Neuroplasticity after Traumatic Brain Injury

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The rat model of combined central fluid percussion traumatic brain injury (TBI) and bilateral entorhinal cortical lesion (BEC) produces profound, persistent cognitive deficits, sequelae associated with human TBI. In contrast to percussive TBI alone, this combined injury induces maladaptive hippocampal plasticity. Recent reports suggest a potential role for dopamine in CNS plasticity after trauma. We have examined the effect of the dopamine enhancer L-deprenyl on cognitive function and neuroplasticity following TBI. Rats received fluid percussion TBI, BEC alone, or combined TBI + BEC lesion and were treated once daily for 7 days with L-deprenyl, beginning 24 h after TBI alone and 15 min after BEC or TBI + BEC. Postinjury motor assessment showed no effect of L-deprenyl treatment. Cognitive performance was assessed on days 11–15 postinjury and brains from the same cases examined for dopamine β-hydroxylase immunoreactivity (DBH-IR) and acetylcholinesterase (AChE) histochemistry. Significant cognitive improvement relative to untreated injured cases was observed in both TBI groups following L-deprenyl treatment; however, no drug effects were seen with BEC alone. L-Deprenyl attenuated injury-induced loss in DBH-IR over CA1 and CA3 after TBI alone. However, after combined TBI + BEC, L-deprenyl was only effective in protecting CA1 DBH-IR. AChE histostaining in CA3 was significantly elevated with L-deprenyl in both injury models. After TBI + BEC, L-deprenyl also increased AChE in the dentate molecular layer relative to untreated injured cases. These results suggest that dopaminergic/noradrenergic enhancement facilitates cognitive recovery after brain injury and that noradrenergic fiber integrity is correlated with enhanced synaptic plasticity in the injured hippocampus. © 2000 Academic Press

Key Words: **neuroexcitation; deafferentation; traumatic brain injury; L-deprenyl; spatial memory; plasticity.**

INTRODUCTION

Excessive neuroexcitation (18, 34) and diffuse axotomy (72) are two of the primary pathological sequelae of traumatic brain injury (TBI). Until recently, most of the experimental models of TBI investigated these two pathobiological processes separately (23), with little known about the relative contributions and potential interactions between the neuroexcitation and deafferentation induced by TBI. Even less is known about how the brain remodels synaptic architecture and neural circuitry following TBI when both neuroexcitatory insult and the secondary deafferentation are present. Recently, we have utilized an animal model combining neuroexcitatory injury by central fluid percussion and subsequent focal deafferentation by bilateral entorhinal cortical lesion (BEC) (65). Since this model produces both significant long-term spatial memory deficits analogous to those seen after human TBI and a maladaptive regenerative plasticity in the brain, it provides the opportunity to dissect interactions between neuroexcitation and deafferentation pathologies and determine how such interactions might influence synaptic recovery. Further, our initial studies with this combined injury model revealed differences in functional and structural response to pharmacological manipulations depending upon which of the individual injury components is targeted (66, 67).

Neurochemical disturbances following TBI involve multiple neurotransmitter systems which may respond differently depending upon brain region examined (34, 54, 55). While a large portion of the published evidence supports glutamate and acetylcholine receptor activation after brain trauma, effects on dopaminergic neurotransmission are also documented (19, 54, 73, 74). The first animal studies to examine regional dopamine (DA) concentration after TBI reported cortical decreases as early as 1 h after injury, with a persistent attenuation for 2 weeks (19, 54). In a subsequent study of cortical impact insult (74), regional levels of norepi-

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nephrine declined during acute postinjury intervals (30 min to 24 h) and were significantly decreased in the cortex ipsilateral to the insult. Interestingly, some of these earlier studies showed that glutamate and dopamine could be pharmacologically manipulated, resulting in the amelioration of persistent functional deficits (32, 75, 94). More recent reports, also utilizing the controlled cortical impact model, have better defined the potential role of DA in recovery processes following TBI. These studies show that cognitive performance is enhanced following chronic postinjury treatment with the dopamine agonist amantadine (15) and the dopamine-associated CNS stimulant methylphenidate (45). These controlled laboratory findings are important since several clinical studies addressing dopaminergic influence upon recovery after brain trauma suggest that amantadine (47, 59), methylphenidate (29, 42, 70), and the D2 agonist bromocriptine (53) all positively affect cognitive outcome, including measures of attention, concentration, orientation, and prefrontal executive function.

One potential mechanism for the action of DA in TBI recovery processes would be that it serves to regulate excitation/inhibition homeostasis. This idea is supported by recent evidence showing that the activity of NMDA glutamate receptors can be modulated by DA (22). Similarly, the expression of different DA receptor genes in the hippocampus appears to be regulated, in part, by glutamate via activity at the NMDA receptor (35). Perhaps more pertinent to TBI outcome are the results of memory consolidation studies which suggest that both NMDA and DA receptors must be actively associated for proper integration of hippocampal and cortical circuits (10, 82). This transmitter interaction is further supported by *in vitro* electrophysiology characterizing excitatory synaptic transmission in the CA1 region of the hippocampus, where very low concentrations of DA reduce the excitatory response of pyramidal neurons, possibly through presynaptic D2 receptors (41). This response was documented as an increase in magnitude of paired-pulse facilitation, a phenomenon attributed to increased transmitter release in response to a second stimulus. Such a mechanism would be consistent with our observations that CA1 neuronal excitability is depressed after TBI (76) and that hippocampal paired-pulse facilitation is enhanced following TBI (78). While these lines of evidence suggest that DA plays an important role in the maintenance of excitation/inhibition homeostasis after CNS injury, it remains unclear how DA systems might affect long-term structural and functional outcome. We now report the results of our first studies utilizing L-deprenyl, a DA-enhancing compound historically applied in Parkinsonian therapy, to test whether manipulation of the DA system can influence TBI behavioral outcome and hippocampal plasticity.

L-Deprenyl is a selective and irreversible monoamine oxidase-B (MAO-B) inhibitor used clinically in the treatment of Parkinson's disease (44, 46). In addition to MAO-B inhibition, experimental studies have also demonstrated that L-deprenyl may alter DA through increasing the expression of the DA transporter system *in vivo* (48). L-Deprenyl also protects neurons from a variety of toxins [MPTP (1-methyl-4-phenyl-1,2,3,6 terahydropyridine), 6-hydroxydopamine, AF64A (methyl-β-acetoxyethyl-2-chloroethylamine, ethylcholine mustard aziridinium)] which induce neurodegeneration, as well as acts to reduce hippocampal pyramidal cell death following ischemic damage (46). While the mechanism for such protection is not established, it is postulated that L-deprenyl may offer protection through blocking the generation of toxic substances induced by injury (i.e., free radicals, H_2O_2, and endotoxins), stabilizing the uptake and turnover of catecholamines or stimulating the synthesis of protective molecules such as superoxide dismutase (SOD) or neurotrophins (i.e., NGF, BDNF, EGF) (27).

In the present study, the effect of chronic postinjury L-deprenyl administration on cognitive performance was assessed utilizing the Morris water maze task. Since dopamine β-hydroxylase (DBH) and acetylcholinesterase (AChE) histochemistry are useful markers of noradrenergic fiber integrity and hippocampal neuroplastic fiber remodeling, respectively, we also utilized these endpoints to assess L-deprenyl treated cases. While the systemic administration of L-deprenyl certainly has the potential to affect a variety of CNS loci, in the present study we restricted our analysis to hippocampal regions for two reasons: (i) our principal cognitive assessments with the Morris water maze test spatial memory which is known to be highly dependent upon the integrity of hippocampal circuitry (57), a site also selectively vulnerable to TBI (34), and (ii) the deafferenting EC lesions used in our injury models directly target the dentate gyrus and CA1 subsectors of the hippocampal formation (65). Thus, we believe that the most appropriate point to begin our structural analysis is within the hippocampus, where a comparison of DBH, AChE, and behavioral endpoints for each injury condition will permit a direct correlation of structural neurochemical distribution with functional recovery. We report that postinjury treatment with L-deprenyl enhances both the cognitive function and the integrity of impaired hippocampal circuitry following either fluid percussion TBI alone or in combination with BEC lesion.

MATERIALS AND METHODS

Subjects and Experimental Conditions

Sixty-four male Sprague–Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, PA; 300–350 g) were used in

the study. Animals were individually housed with free access to food and water in a 12-h dark–light cycle at 22°C. All protocols for injury and use of animals were approved by the Institutional Animal Care and Use Committee.

All animals were trained to criterion on motor tasks prior to surgery and randomly assigned to five groups: sham-injured group (Sham, $n = 10$); fluid percussion traumatic brain injury group (TBI, $n = 20$); combined TBI with BEC lesion group (TBI + BEC, $n = 20$); and BEC lesion alone group (BEC, $n = 14$). L-Deprenyl or vehicle was administered to the experimental rats (1 mg/kg in saline, ip) at 24 h after TBI or sham injury ($n = 10$, 5 respectively) and 15 min after BEC lesion in the combined injury group ($n = 10$) and BEC alone group ($n = 7$). A daily dose of L-deprenyl (1 mg/kg in saline) was given for 7 days postinjury. Motor tasks were assessed from day 1 to day 5 postinjury and Morris water maze performance was determined on days 11 to 15 postinjury. All behavioral assessments were made in a blinded fashion.

Surgical Preparation

Male Sprague–Dawley rats (300–350g) were anesthetized with sodium pentobarbital (60 mg/kg, ip) and were placed in a stereotaxic frame. A 4.8-mm-diameter skull trephine opening was prepared centrally over the sagittal suture between bregma and lambda. Two screws were placed 1 mm rostral to bregma and 1 mm caudal to lambda and a Leur-Loc syringe hub was secured on the skull with cyanoacrylate. Dental acrylic was next applied to the syringe hub and two small screws for implant rigidity. The implant site was then packed with Gelfoam and the scalp was sutured closed. Bacitracin was applied to the surgical site and each subject was monitored for full recovery from anesthesia before returning to its home cage.

Fluid percussion traumatic brain injury. The fluid percussion device used to produce experimental TBI was identical to that described in detail by Dixon *et al.* (17). The device consisted of a 60-cm-long and 4.5 cm-diameter Plexiglas cylinder with a rubber-covered O-ring-fitted Plexiglas piston at one end and, on the opposite end of the cylinder, a 2-cm-long metal housing mounted with an extracranial pressure transducer (Entran Devices, Inc., Model EPN-0300*-100A). This metal housing attaches to a 5-mm tube with a 2.6-mm-i.d. which connects with the surgically implanted female Leur-Loc fitting at the time of injury. The entire system is then filled with distilled water and the injury is produced by a metal pendulum which is lowered to strike the piston of the injury device. A small volume of distilled water is injected into the closed cranial cavity to produce a brief displacement and deformation of brain tissue. The magnitude of injury is controlled by varying the height from which the pendulum is released.

Twenty-four hours after surgical preparation, at the time of injury, the rats were anesthetized by breathing 4.0% isoflurane with 70% N_2O: 30% O_2 mixture for 4 min. The surgical site was then exposed and the animal was connected to the fluid percussion device. The injury magnitude administered was between 2.0 ± 0.5 atm, equivalent to a moderate-level brain injury, and was recorded by the in-line transducer connected to a storage oscilloscope (Tektronix 5111; Beaverton, OR). Sham-injured controls received the same surgical preparation, anesthesia, and connection to the injury device; however, no injury was delivered. All animals were immediately ventilated with room air until spontaneous breathing resumed, tested for suppression of righting reflex, and monitored for 2–3 h before returning to their home cages. As routinely observed with righting response, fluid percussion injury suppressed consciousness in all injured groups [$F(4, 45) = 35.651$, $P < 0.0001$, data not shown]. No significant difference in duration of unconsciousness between any of the injured groups was found ($P > 0.05$), indicating that all injured groups received comparable severity of fluid percussion injury.

BEC and combined fluid percussion + BEC lesion. Rats were subjected to BEC by a modification of the method of Loesche and Steward (see 65). Briefly, all animals were first anesthetized by gas inhalation (70% N_2O, 30% O_2, 4% isoflurane) for 4 min and then intubated and continuous anesthesia was administered by ventilation of mixed gases with 70% N_2O, 30% O_2, 2% isoflurane. Intubated rats were placed in a stereotaxic head holder and the dorsal aspect of the entorhinal cortex was exposed bilaterally. A 0.2-mm Teflon-insulated wire electrode was lowered into the cortex and a lesion current was passed at a total of nine stereotaxic sites (1.5 mA, 30 s duration for each site; 3, 4, and 5 mm lateral to midline, at 2, 4, and 6 mm ventral to the cortical surface). All sites were 1.5 mm anterior to the transverse sinus and the electrode was positioned at a 10° angle away from the midline. After lesions were completed, all rats were extubated and monitored for recovery of righting reflexes before returning to their home cages. Rats subjected to a combined injury procedure were first surgically prepared and received a moderate (2.0 ± 0.05 atm) fluid percussion brain injury as descried above. Animals were returned to their home cages for a 24-h interval, and subsequently bilateral enthorhinal cortical lesion was performed as indicated.

Drug/Vehicle Preparation

L-Deprenyl [*R*(−)-deprenyl hydrochloride, Research Biochemicals International] was first dissolved in an

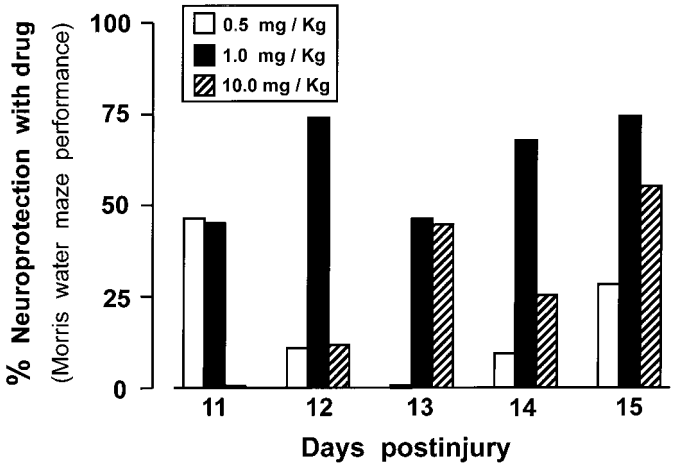

FIG. 1. Neuroprotection with L-deprenyl on Morris water maze performance after central fluid percussion TBI. 0.5 ($n = 5$), 1.0 ($n = 10$), or 10.0 ($n = 6$) mg/kg was administered ip for 7 days after injury (see Materials and Methods for details) and goal latency was expressed as a percentage of the sham control ($n = 8$) performance. During learning of the task (days 11–13) mixed effects of dosage were observed, but on days 14–15, where spatial memory was specifically tested, a clear dose–response difference was seen. Based on these observations, the 1.0 mg/kg dose was selected for further use in this study.

isotonic saline solution. Each individual injection was administered at a 1 mg/kg dose, ip, and animals in the injury + vehicle groups received an equal volume of saline ip. This dosage and route of administration was determined from our preliminary studies in the fluid percussion model (see Fig. 1). When a range of L-deprenyl doses were tested (0.5–10 mg/kg), optimal attenuation of injury-induced behavioral deficits (expressed as percentage of neuroprotection) was observed with the 1 mg/kg dosage. Notably, a clear dose–response relationship was found on days 14 and 15 of cognitive testing, the period of memory assessment in our paradigm which is independent of task acquisition (days 11–13). Based upon these data, we selected the 1 mg/kg dose of L-depreynl for the present study.

Outcome Measures

Choice of postinjury intervals for motor (days 1–5) and cognitive (days 11–15) assessment were based upon previous studies in these trauma models. Motor deficits have been shown to be maximal the first 2 days after injury (17, 65) and gradually recover over the subsequent 3 days so that by day 5, motor deficits are no longer present. Our selection of days 11–15 for cognitive assessment is also based on previous data (65) which documents differences in spatial memory recovery as a function of the individual trauma models used in this study.

The beam-balance, beam-walking, and rota-rod tasks were used to assess motor function in all experimental groups as previously described (17, 65, 66). Briefly, the beam-balance task, used to assess integrity of the vestibulomotor systems, was tested by placing the rat on a suspended narrow wooden beam (1.5 cm wide) and measuring the duration for which the rat remained on the beam (maximum limit was set at 60 s). Fine motor coordination was assessed with the beam-walking task, where rats were trained to escape a bright light and loud white noise by traversing an elevated 100-cm-long, 2.5-cm-wide wooden beam to enter a darkened goal. Performance was assessed in all experimental groups by recording the latency of the animal to traverse the beam. The data for each daily session consisted of the mean of three trials which was subjected to statistical analysis. Rota-rod task was also used to assess fine motor coordination (31). The animals were required to traverse the rungs of the device (Gearmotor, Bodine Electric Company) as the frame of rota-rod rotated. Rotational speeds ranged from 0 to a maximum speed of 30 rpm. During assessment, the animal was placed on the device with the frame remaining stationary for 10 s. The speed was slowly increased to 3 rpm at 10 s and then steadily rose 3 rpm/10 s until a maximum speed of 30 rpm was achieved. Animals remained on the device for a maximum of 120 s (20 s at 30 rpm) and were given two trials per day, after which the mean duration of each daily block of two trials was recorded.

The Morris water maze (MWM) (57) used to assess cognitive function as previously described (65–67). For assessment, rats were given four trials per day for 5 consecutive days including days 11 to 15 after injury. For each daily block of four trials, rats were placed in the pool by the experimenter, facing the wall. Rats started a trial once from each of the start locations (north, east, south, and west). The order of starting locations was randomized and the goal platform was positioned 45 cm from the outside wall, remaining constant for all trials. Rats were given a maximum of 120 s to find the hidden platform. If the rats fail to find the platform after 120 s, they were placed on the platform by the experimenter. All rats remained on the platform for 30 s before being placed in a heated incubator between trials. There was a 4-min intertrial interval. A mean daily latency to find the goal platform during MWM testing on days 11–15 postinjury was computed for each group. To ensure that the latency measure of maze performance was not caused by noncognitive effects of injury or L-deprenyl treatment, swim speeds were calculated for each animal. Swim speeds were not found to be significantly different when all groups were compared [$F(4, 45) = 1.093$, $P > 0.05$ data not shown], indicating that differences in latency were not confounded by injury-induced or L-deprenyl-induced motor impairment.

Histological Evaluation

After completing MWM assessment at day 15 postinjury, five cases from each experimental group were randomly chosen and processed for histological evaluation. Groups consisted of sham-injured (n = 5, 2 received saline, 3 received drug); TBI and combined TBI + BEC lesion groups injected with either saline (n = 5/each injury); or 1 mg/kg of L-deprenyl for 7 days (n = 5/each injury). (Based on MWM results shown below, L-deprenyl did not affect cognitive performance when administered following BEC lesion alone; therefore, no BEC cases were processed for histological evaluation.) Animals were anesthetized with lethal dose of sodium pentobarbital and killed via transcardiac perfusion with 4% paraformaldehyde + 0.2% picric acid (in 0.1 M phosphate buffer, pH 7.2, in 30 min). Before aldehyde perfusion, the vascular bed was flushed with isotonic saline (0.9% NaCl). The brains were then removed, blocked, and postfixed in the same solution overnight at 4°C. Serial 35-μm sections containing dorsal hippocampus were cut with a Vibratome and collected in 0.1 M phosphate buffer for free-floating DBH or AChE staining.

DBH immunohistochemistry. DBH immunohistochemistry was carried out using methods described previously (77). Briefly, after three 10-min washes in 0.1 M phosphate buffer (with agitation), sections were placed in 10% normal serum contain 0.3% Triton X-100 in 0.01 M phosphate-buffered saline (PBS) solution for 1 h in 37°C to block nonspecific binding, followed by a 10-min wash in PBS. The sections were then incubated in anti-DBH antibody (Chemicon Inc., 1:6000), first for 1 h at 37°C and then switched to 4°C for an additional 24–36 h. After primary antibody was removed, the sections were washed vigorously in five changes of PBS over a period of 1.5 h. Visualization of primary antibody binding was accomplished using the avidin–biotin–horseradish peroxidase complex and diaminobenzidine (Vectastain standard kit, Vector Laboratories, Burlingame, CA) and subsequently enhanced by the modified glucose oxidase–3,3′-diaminobenzidine dihydrochloride (DAB)–nickel (GDN) method. Briefly, after Vectastain visualization was completed, sections were washed twice in PBS and once in 0.1 M Tris-buffered saline (TBS) and then incubated in GDN solution which included 0.05% DAB, 0.2% nickel ammonium sulfate, 2% β-D-glucose, 0.04% ammonium chloride, and 0.005% glucose oxidase (Sigma, type VII) in Tris buffer (pH 7.6). The GDN reaction was stopped by transferring the sections into TBS for two washes with agitation, each 10 min. The sections were mounted on gelatin–chrome alum-coated glass slides, allowed to dry overnight at room temperature, dehydrated, and coverslipped with Permount. The immunolabeled products were visible as fine diffuse blue staining under the light microscope. Immunobinding controls were performed for each case and included: (i) incubation with diluted normal serum without the primary antibody and (ii) substitution of PBS alone for biotinylated IgG (secondary antibody) incubation.

AChE histochemistry. A modified histochemistry method (93) for AChE was used to examine cholinergic sprouting in the neuropil after brain injury. Briefly, brain sections were washed with three changes of 0.1 M maleate buffer (maleic acid–NaOH buffer, pH 6.0) and then incubated for 60 min at room temperature with a maleate–NaOH buffer solution containing 0.18 mM acetylthiocholine iodide, 0.5 mM sodium citrate, 0.3 mM cupric sulfate, 0.5 mM potassium ferricyanide, 0.4 mM iso-OMPA (an inhibitor of butyrylcholinesterase). Sections were rinsed in 0.1 M maleate buffer for 5 min, five changes (5 min each) of 0.1 M Tris–HCl buffer (pH 7.6), and then incubated for 5 min with DAB–nickel–H_2O_2 (0.05% diaminobenzidine, 0.2% nickel ammonium sulfate, and 0.003% H_2O_2 in THB) at room temperature. Sections were next washed with 0.1 M maleate–NaOH buffer (pH 6.0) for 5 min and then with two changes of 0.1 M Tris–HCl buffer (pH 7.6) 10 min for each, mounted on gelatin–chrome alum-coated glass slides, air-dried, dehydrated, and coverslipped. Methodological controls included: (i) replacing acetylthiocholine iodide with maleate–NaOH buffer or (ii) pretreatment of the reactive solution with 1 mM BW284c51 [1,5-bis-(4-allyldimethyl-ammoniumphenyl)pentan-3-one dibromide, a specific acetylcholinesterase inhibitor, Sigma].

Image Analysis

A microcomputer imaging device system (MCID; Imaging Research, Inc., Canada) was used to measure the relative differences in DBH immunobinding and AChE histochemistry (103). DBH reactivity was observed in cellular processes attributable to noradrenergic axons projecting to the hippocampal laminae (102). As previously documented (102), we measured immunopositive areas occupied by DBH-positive neuronal processes in selected subregions of the hippocampal formation. Relative optic density (ROD) measurement was used to evaluate the differences in AchE-positive zones of the hippocampus (7). Because shrinkage of dentate molecular layer dendritic regions occurs following deafferentation lesion, an average percentage reduction in dendritic laminar width was determined for each data set and the final DBH area/AChE ROD density measurements were corrected for shrinkage prior to grouping for statistical analysis (9).

Biostatistical Analysis

Group mean data for all motor assessments were analyzed by a single-factor analysis of variance

(ANOVA). If a significant effect was observed with the ANOVA, a post hoc analysis was made with Duncan's multiple range test. The MWM latency data was analyzed by a split-plot ANOVA (group × day). *F* ratios for overall main effects and for group differences at individual testing days were computed using a mixed-design algorithm (43, 51). Group data for DBH immunolabeling (expressed as mean percentage of total sample areas) and AChE RODs (group means) were subjected to a single-factor ANOVA. If a significant DBH or AChE effect was observed with ANOVA, a post hoc assessment was made with the Bonferroni test. All analyses were implemented in SPSS v.6.0 (60), and a significance level of $P < 0.05$ was used for all tests.

RESULTS

Behavioral Outcome: Motor and Cognitive Performance

As previously reported for fluid percussion injury (17, 31, 32), beam-balance, beam-walk and rota-rod task performances each showed significant deficits on days 1 to 5 postinjury [$F(4, 45) = 3.906$, $P < 0.008$; $F(4, 45) = 3.756$, $P < 0.01$; $F(4, 45) = 5.319$, $P<0.001$, respectively]; however, no significant difference between individual injured vehicle groups and injured L-deprenyl-treated groups was detected. Collectively, these data suggest that chronic 1 mg/kg L-deprenyl treatment postinjury did not significantly affect motor performance.

Chronic postinjury dosage of L-deprenyl reduced injury-induced cognitive deficits both in the fluid percussion injury model and in the combined fluid percussion and BEC lesion insult [overall ANOVA $F(4, 45) = 19.897$, $P < 0.0001$]. Notably, the effect of DA enhancement on cognitive performance was greater in the isolated fluid percussion injury. In the TBI alone cases, daily treatment with L-deprenyl significantly reduced the latency required to find the hidden platform on days 11–15 postinjury when compared with the vehicle treated TBI group [$F(1, 18) = 4.603$, $P < 0.05$] (Fig. 2A). The results also indicated that latencies in the sham-injured group were not significantly different from the TBI group treated with L-deprenyl [$F(1,18) = 3.54$, $P > 0.05$]. It was also concluded that the present L-deprenyl administration did not affect MWM performance through mechanisms unrelated to injury since we observed no significant difference in latencies or swim speeds between sham-injured animals treated with L-deprenyl or vehicle [$F(1, 8) = 0.151$, $P > 0.05$; $F(4, 45) = 1.093$, $P > 0.05$, respectively].

In the combined TBI + BEC lesion group, daily treatment with L-deprenyl also produced significant reduction in latency on days 11–15 postinjury relative to the combined injury + vehicle group [$F(1,$

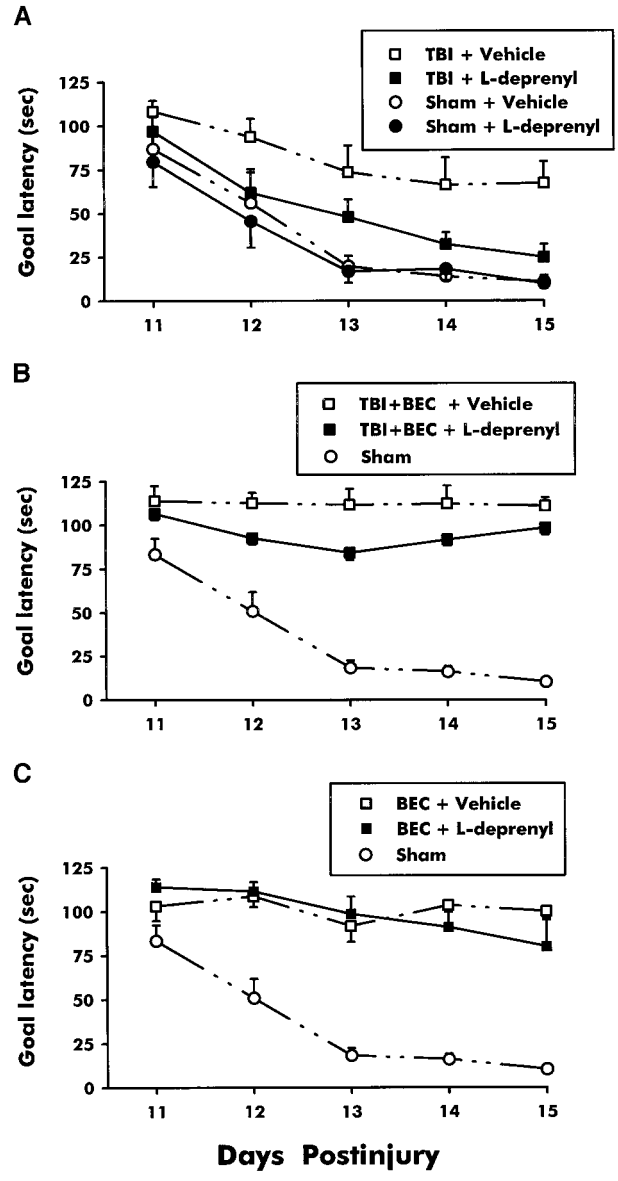

FIG. 2. Morris water maze spatial memory deficits on days 11–15 after chronic L-deprenyl treatment of (A) fluid percussion TBI, (B) combined TBI + BEC lesion, and (C) BEC lesion alone. In (A) a 1 mg/kg/day dose of L-deprenyl significantly reduced latency to find the hidden platform relative to the saline-treated TBI group ($P < 0.05$; $n = 10$). Notably, the performance of L-deprenyl-treated TBI animals was not different from the treated sham group and latencies of sham-injured animals treated daily with L-deprenyl were not different from the sham cases treated with saline. As expected, vehicle-treated TBI cases were significantly impaired relative to the sham + vehicle group ($P < 0.05$; $n = 5$). In (B) the cognitive performance of combined TBI + BEC animals was significantly improved with L-deprenyl relative to the vehicle-treated TBI + BEC group ($P < 0.05$; $n = 10$); however, animals given L-deprenyl showed persistent cognitive impairment relative to sham controls ($P < 0.01$). In the BEC lesion group (C), maze performance was not significantly improved with L-deprenyl treatment compared to saline-treated lesioned rats, both groups showing cognitive impairment relative to the treated sham group ($P < 0.01$; $n = 7$).

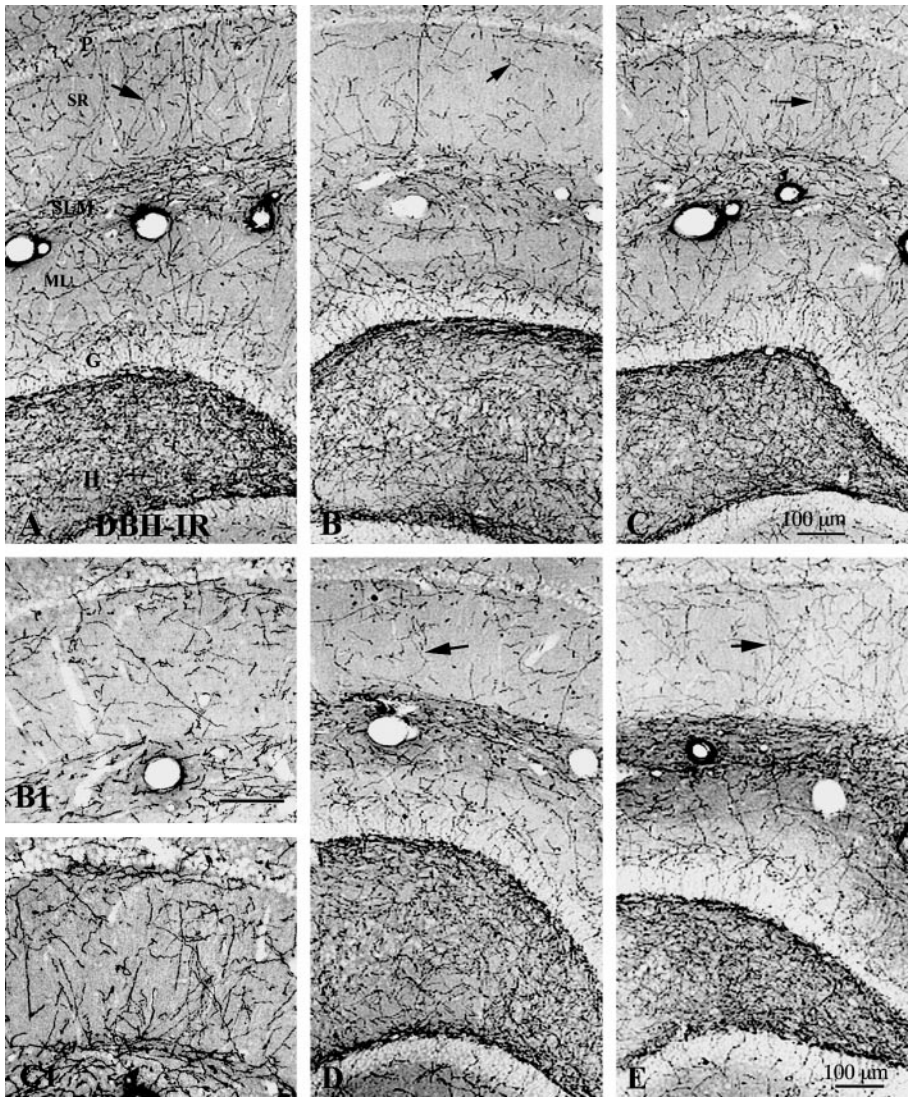

FIG. 3. Changes in CA1 DBH-IR following TBI alone or TBI + BEC lesion with L-deprenyl treatment at 15 days postinjury. In (A) DBH-IR fibers were observed throughout the hippocampus of sham control animals, with the greatest DBH-IR over the SLM and hilus of the dentate gyrus. In fluid percussion TBI cases receiving vehicle (B and B1) an injury-induced loss in DBH-IR was seen over CA1 SR. L-Deprenyl treatment restored DBH-IR in SR following TBI alone (C and C1). Similarly, in TBI + BEC + saline cases DBH-IR in SR of CA1 was reduced (D). A partial restoration of DBH-IR in the SR was observed in the L-deprenyl-treated TBI + BEC group (E). Bar, 100 μm. Abbreviations: SR, stratum radiatum of CA1; SLM, stratum lacunosum-moleculare; ML, the molecular layer of dentate gyrus; H, hilus of dentate gyrus; P, pyramidal cell layer; G, granule cell layer.

18) =7.651, $P < 0.05$] (Fig. 2B). In contrast to TBI alone animals, however, combined insult cases treated with L-deprenyl continued to show significantly longer goal latencies than did sham-injured rats [$F(1, 18) = 266.422$, $P < 0.001$] (Fig. 2B). These data indicate that chronic postinjury L-deprenyl treatment only partially improves MWM performance following TBI + BEC brain injury.

Following BEC lesion alone, L-deprenyl administration failed to affect MWM performance when compared to vehicle treated BEC-lesioned rats [$F(1, 12) = 0.113$, $P > 0.05$] (Fig. 2C).

DBH Immunoreactivity

DBH-immunoreactivity (IR) was observed in a highly collateralized plexiform network of fibers and terminals within the hippocampus (see sham-injured case in Fig. 3A). DBH-IR was most dense in variecosities along axonal processes (arrows, Fig. 3A), usually exhibiting lighter IR within thin intervening segments. The highest density of DBH-IR was seen in the stratum lacunosum molecular layer of CA1, the stratum radiatum of CA3, and the dentate gyrus as a whole. There was no evidence that L-deprenyl affected the

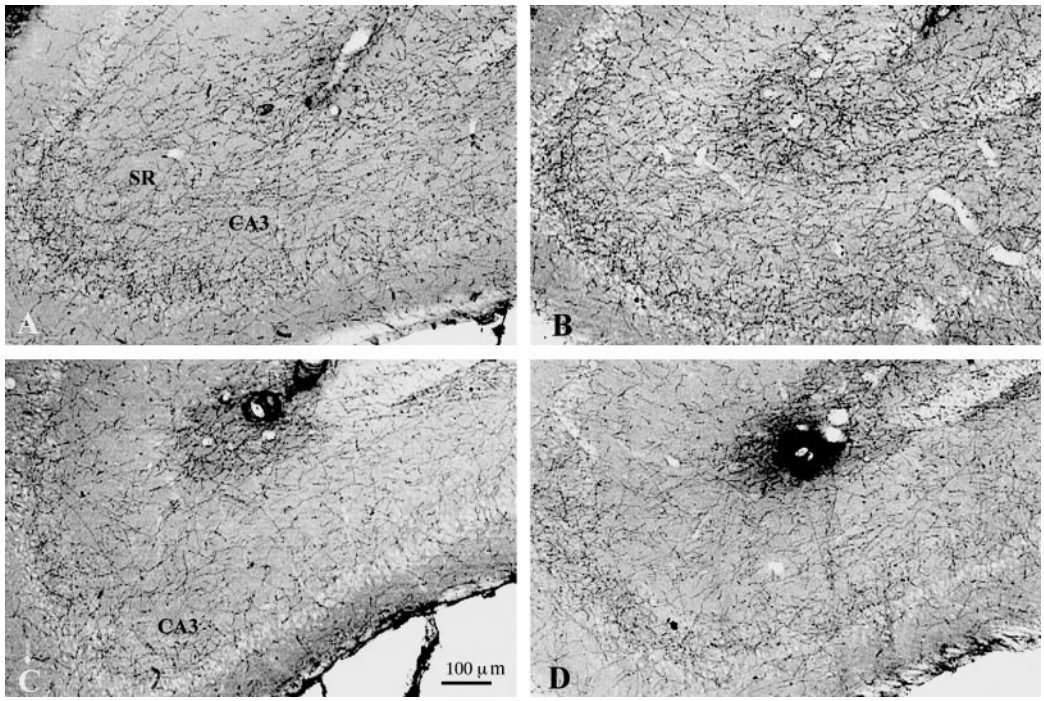

FIG. 4. Changes in CA3 DBH-IR following TBI alone or TBI + BEC with L-deprenyl treatment at 15 days postinjury. In TBI + vehicle cases (A) loss of DBH-IR was observed in the SR of CA3. This DBH-IR loss after TBI was reversed in CA3 with L-deprenyl treatment (B). As in CA1, a similar loss of DBH-IR in CA3 was seen after the combined TBI + BEC lesion insult (C). Unlike TBI alone, L-deprenyl failed to increase DBH-IR over CA3 SR in the TBI + BEC cases (D). Bar, 100 μm. Abbreviations: SR, stratum radiatum of CA1; SLM, stratum lacunosum-moleculare; ML, the molecular layer of dentate gyrus; H, hilus of dentate gyrus; P, pyramidal cell layer; G, granule cell layer.

distribution or cellular appearance of DBH-IR in sham-injured animals or BEC alone cases (data not shown).

At 15 days postinjury, both TBI alone and TBI + BEC insult induced a loss of DBH-IR fibers and terminals, predominantly within the stratum radiatum (SR) of CA1 and CA3 subregions (Figs. 3B, 3B1, 3D, 4A, and 4C). Chronic treatment with L-deprenyl (1 mg/kg/day, for 7 days) reversed the injury-induced loss of DBH-IR in CA1 and CA3 subregions seen after TBI alone (Figs. 3C, 3C1, and 4B). In the TBI + BEC lesion group, the apparent protective effect of L-deprenyl on DBH-IR was less robust in the CA1 subregion (Fig. 3E) and absent altogether in CA3 subregion (Fig. 4D). No additional qualitative differences between DBH-IR were observed in other regions of the hippocampus for the experimental groups.

Quantitative assessment of DBH IR at 15 days postinjury (expressed as percentage sample area) showed an overall significant effect of L-deprenyl treatment on injury within the CA1 and CA3 subregions of the hippocampus [$F(4, 20) = 19.302$, $P < 0.001$ in CA1; and $F(4, 20) = 15.688$, $P < 0.001$ in CA3]. Post hoc analysis, however, revealed a differential response between TBI alone and combined TBI + BEC insult. Specifically, the relative area of DBH-IR for the treated TBI alone group was significantly larger compared to the TBI + vehicle group within both CA1 and CA3 ($P < 0.01$; see Fig. 5A). Notably, DBH-IR areas in the TBI cases treated with L-deprenyl were equivalent to control values (not significantly different from sham-injured cases). By contrast, TBI + BEC lesion animals treated with L-deprenyl showed significant increases of DBH-IR only in the CA1 region when compared to injured + vehicle group ($P < 0.01$; Fig. 5B). L-Deprenyl treatment of the combined insult did not affect DBH-IR in CA3 when compared to the untreated injured cases. No changes were observed after drug treatment in the dentate outer molecular layer (OML) for either injury model.

Further comparison between injured untreated cases and sham-injured animals confirmed histological observations that both injury models induced a decrease in DBH-IR within the CA1 and CA3 subregions of the hippocampus and in the dentate outer molecular layer or OML ($P < 0.001$ for CA1 and CA3; $P < 0.05$ for OML after TBI alone; $P < 0.01$ for OML after TBI + BEC lesion; see again Fig. 5).

AChE Histochemistry

In all regions of the hippocampus, AChE histochemistry stained fine fibers, appearing most dense in the stratum oriens, pyramidal cell, and radiatum subfields CA1 and CA3 (see sham-injured case in Fig. 6A). In-

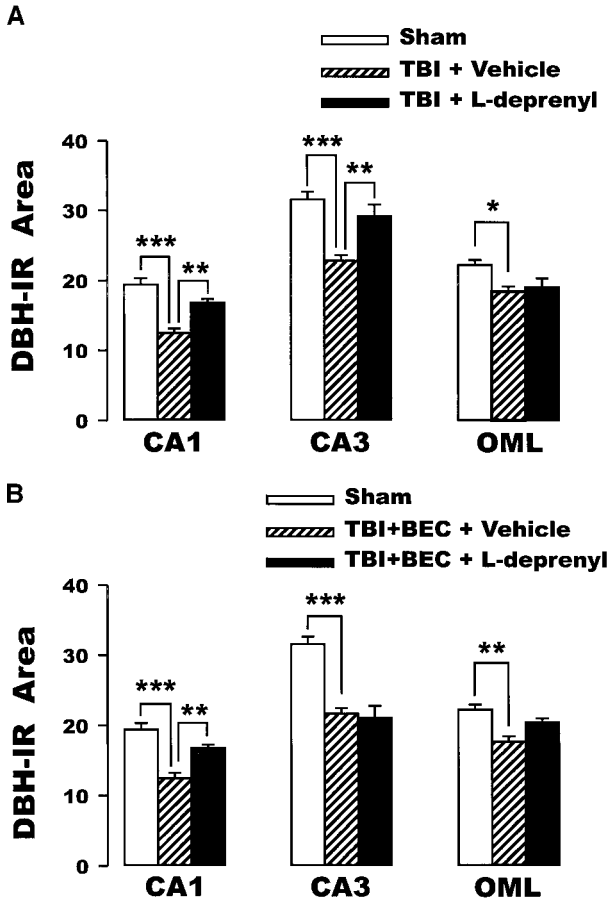

FIG. 5. The effects of L-deprenyl treatment on the cross-sectional area occupied by DBH-IR fibers at 15 days postinjury. The DBH fiber area in SR of CA1, CA3, and the outer molecular layer (OML) of the dentate gyrus are plotted as the mean DBH-IR area ± SEM. In TBI + vehicle cases the area occupied by DBH-positive fibers was significantly reduced in CA1 and CA3 (***$P < 0.001$) and in the OML (*$P < 0.05$) compared to sham-injured rats (A). L-Deprenyl treatment significantly attenuated the loss of DBH-IR in SR of CA1 and CA3 compared to saline-treated group (**$P < 0.01$). Notably, the area of DBH-IR after L-deprenyl treatment was not significantly different from sham cases in any of the sampled regions. Similarly, TBI + BEC lesion rats treated with vehicle (B) showed a significant reduction in the cross-sectional area of DBH-IR fibers in both CA1 and CA3 (***$P < 0.001$) and in the OML (**$P < 0.01$) compared to sham controls. In contrast to the TBI alone cases, L-deprenyl treatment attenuated the loss in area of DBH fibers only in CA1 when compared to the injured + vehicle group (**$P < 0.01$) and failed to affect the injury-induced loss of DBH-IR in CA3. L-Deprenyl did not change the DBH cross-sectional area in the OML after either injury; however, a trend toward increased DBH was observed in the combined insult cases.

tense staining was observed throughout the dentate gyrus, prominently in the molecular layer (ML) and dentate hilar (H) regions. In addition, punctate AChE staining was also seen in the regions of primary afferent terminals (arrows, Fig. 6D). At 15 days postinjury, TBI alone did not affect AChE distribution; however, the injury did increase staining density (Fig. 6E). By contrast, TBI + BEC insult induced a selective increase AChE staining in the OML of the dentate gyrus (Fig. 6G). When the injured cases were treated with L-deprenyl, both showed a visible enhancement of AChE staining relative to untreated injured and sham controls. In the TBI alone group which received the drug, AChE labeling was greater in all hippocampal pyramidal layers, but most prominently over CA3, as well as increases within the dentate OML (Figs. 6B and 6F). The combined TBI + BEC insult also induced AChE staining elevations; however, in this case the increases were much greater and were highly selective for CA3 subregion and the OML of the dentate gyrus (Figs. 6C and 6I). As was observed for DBH-IR, there was no evidence that L-deprenyl affected the distribution of AChE labeling in sham-injured animals or BEC alone cases (data not shown).

Quantitative assessment of AChE staining density (expressed as ROD) showed a overall significant effect of L-deprenyl treatment on injury within the CA3 and dentate OML subregions [$F(4, 20) = 98.84$, $P < 0.001$ in OML; and $F(4, 20) = 93.42$, $P < 0.001$ in CA3; Fig. 7]. In the TBI alone group, treatment with L-deprenyl significantly increased AChE density in both the CA3 and the OML subregions compared to injured saline rats (see Fig. 7A; $P < 0.01$ and $P < 0.001$, respectively). Similarly, when the combined TBI + BEC lesion cases treated with L-deprenyl were compared with injured + vehicle animals, AChE staining was significantly increased in CA3 and the dentate OML (see Fig. 7B; $P < 0.001$ for both regions). Morphometric analysis also confirmed that there was an increase in AChE ROD within both CA3 and the OML as a function of either injury model alone (see also Figs. 7A and 7B; $P < 0.001$ and $P < 0.01$, respectively). No significant change in AChE staining was observed for the SLM of CA1 in any of the experimental groups.

DISCUSSION

The present study examined whether the pharmacological enhancement of DA with L-deprenyl during the first 7 days following TBI affects cognitive outcome and the integrity of hippocampal circuitry at 2 weeks after injury. Two models of TBI were examined, representing neuroexcitation insult with ensuing adaptive recovery and the combined model of neuroexcitation and focal hippocampal deafferentation, which induces a more severe pathology with maladaptive recovery. Utilizing the MAO inhibitor L-deprenyl, administered systemically in a daily dose of 1 mg/kg, we show that cognitive dysfunction associated with both central fluid percussion TBI and combined TBI with BEC lesion can be significantly attenuated when treatment is applied over the first 7 days following injury. Further, the L-deprenyl-induced recovery of MWM performance is

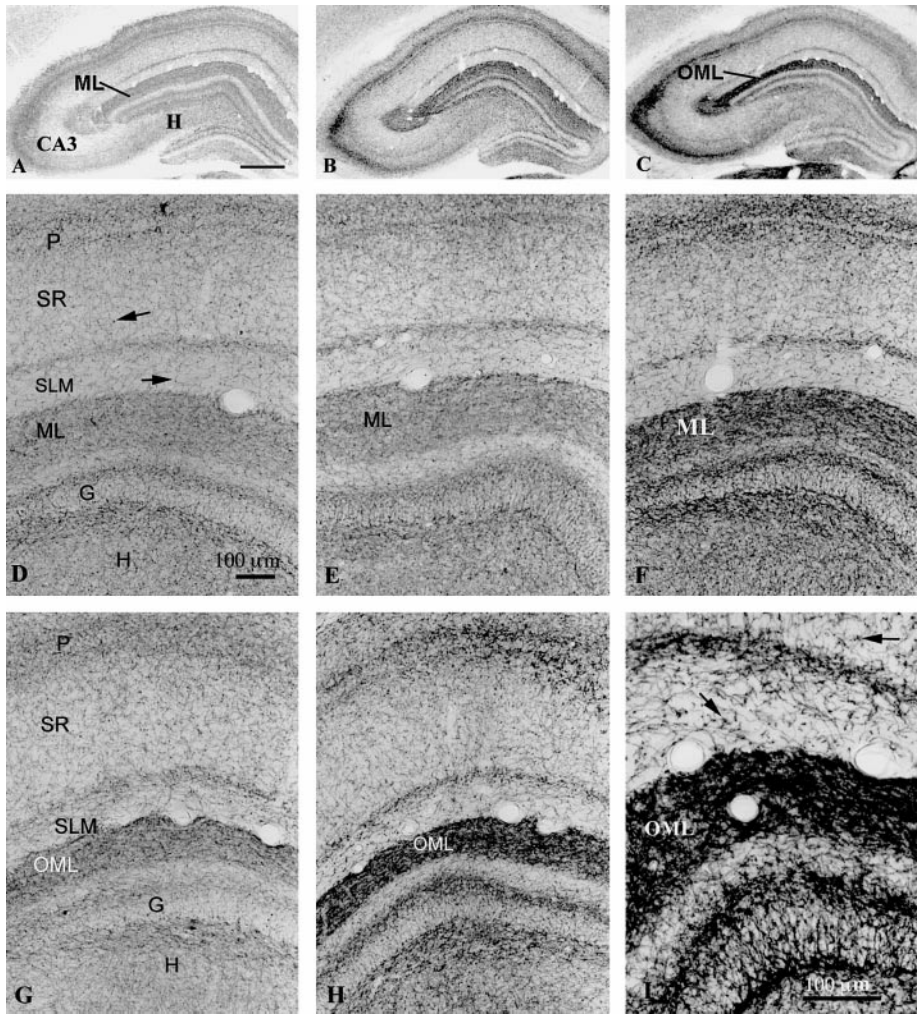

FIG. 6. The effect of L-deprenyl treatment on AChE histochemical staining at 15 days postinjury. AChE shows a laminar pattern of distribution in sham-injured cases (A, D), the heaviest staining in dentate ML, with notable fiber staining in SLM and SR of CA1 (arrows). L-Deprenyl treatment after TBI enhanced AChE within CA3 and the dentate ML (B, F) relative to TBI + vehicle-treated cases (E). L-deprenyl treatment of TBI + BEC increased AChE intensity in CA3 and the OML of the dentate gyrus (C, H) compared to vehicle-treated combined insult rats (G). Higher magnification of TBI + BEC after L-deprenyl treatment (I) shows intensity of ML AChE stain, including fibers of SLM and SR (arrows). Bars, 100 μm. Abbreviations: SR, stratum radiatum of CA1; SLM, stratum lacunosum-moleculare; ML, the molecular layer of dentate gyrus; OML, outer ML; H, hilus of dentate gyrus; P, pyramidal cell layer; G, granule cell layer.

greater in the TBI rats than in animals subjected to TBI + BEC insult. Structural analysis with histochemical methods revealed that L-deprenyl treatment exerts a neuroprotective effect on injury-induced DBH-IR fiber loss and produces an enhancement of AChE staining in the rat hippocampus at 15 days following TBI. Collectively, these findings support the hypothesis that dopaminergic systems are active participants in the recovery processes initiated following TBI, potentially influencing cellular mechanisms responsible for both structural and functional reorganization.

Cognitive Outcome and L-Deprenyl

In terms of the postinjury treatment, perhaps the most important finding from the present study is that chronic L-deprenyl administration during early phases of injury-induced plasticity can significantly reduce the cognitive impairment caused by either traumatically induced neuroexcitatory insult alone or in combination with focal deafferentation. Our finding that enhancing DA can positively influence recovery following neurodegeneration is consistent with published studies of L-deprenyl treatment in aged rats, which have reported a significant improvement in cognitive performance using the same 1 mg/kg dosage (6, 8). For example, the studies of Brandeis and his colleagues (8) showed that daily oral administration of L-deprenyl (in 1.25, 2.5, or 5 mg/kg doses) for 10 days reversed progression of cognitive deficits in aging rats. The same group also reported that higher doses of L-deprenyl were required

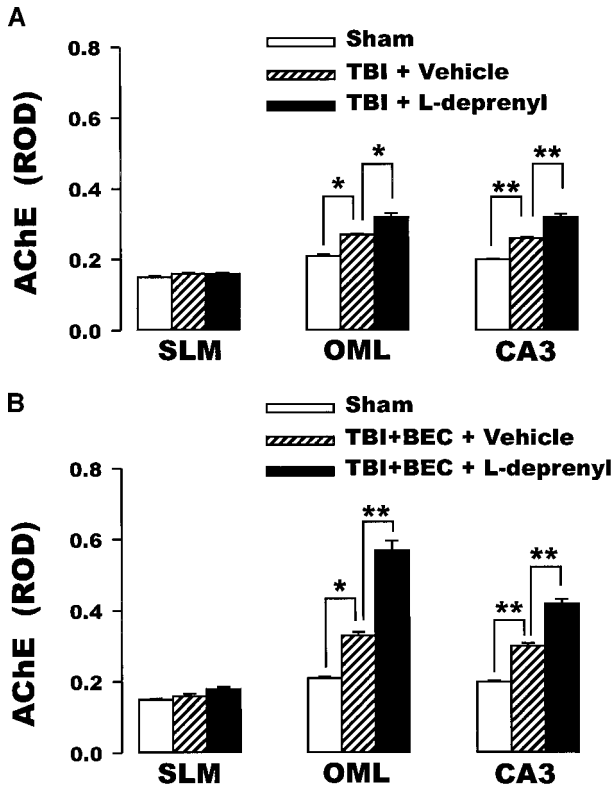

FIG. 7. The effects of L-deprenyl treatment on the relative optical density (ROD) of AChE histochemical staining at 15 days postinjury. Values are expressed as mean ROD ± SEM. After TBI alone (A), L-deprenyl significantly increased AChE density in both OML and CA3 when compared to vehicle-treated injured cases (*$P < 0.001$ and **$P < 0.01$, respectively). TBI alone induced a significant increase in AChE density in these same two laminae (*$P < 0.001$ for the OML and **$P < 0.01$ for CA3). There was no significant difference in AChE ROD between saline-treated and L-deprenyl-treated groups over the SLM after TBI alone. L-Deprenyl given after TBI + BEC significantly enhanced AChE density in both the OML and the CA3 compared to injured + vehicle cases (**$P < 0.001$). TBI + BEC lesion rats administered the vehicle showed an injury-induced increase of AChE ROD in dentate OML (*$P < 0.001$) and CA3 (**$P < 0.01$) when compared to sham-injured cases.

to affect motor function. Our application of L-deprenyl in TBI seems to follow this same pattern since the apparently protective 1 mg/kg dose failed to affect motor function. In previous studies, not reported here, we also found that a higher dosage of L-deprenyl (10 mg/kg/day for 7 days) produced motor hyperactivity and failed to alter cognitive performance after TBI (L. L. Phillips, unpublished data; Fig. 1). More recent studies in aged animals have identified the D1 population of DA receptors as having a prominent role in cognitive performance (2, 36, 37). In both aged monkeys and aged rats, the partial D1 agonist SKF-38393 improved cognitive outcome when delivered acutely, just prior to the testing paradigm. In the rat study, Hersi and colleagues (37) found a significant reduction in latency to find the hidden platform after D1 agonism, suggesting dopaminergic modulation of cognitive function. Our present results using L-deprenyl are consistent with these findings. Moreover, further studies in our laboratory utilizing SKF-38393 show that chronic treatment for 7 days postinjury can selectively improve MWM performance in the combined TBI + BEC model (L. L. Phillips, unpublished data). The importance of DA as modulator of cognitive function has previously been documented with the radial arm maze, again pointing to D1 receptors as mediators of hippocampal–cortical processing in spatial memory function (82). The association of DA with memory circuitry in the CNS can also be linked with specific NMDA-induced changes in cognitive function (10, 86). From the results of these studies, it was shown that DA receptor agonists attenuated MK-801-induced cognitive dysfunction (10) and that concurrent activation of NMDA and D1 receptors is required for gene transcriptional changes in the limbic striatal circuitry following morphine-induced memory impairment (86). These findings are consistent with the known vulnerability of both NMDA glutamate and DA transmitter systems in the rat hippocampus following TBI (19, 34, 55). More importantly, when taken with our present results, they support the hypothesis that a chronic DA agonism during periods of postinjury synaptic plasticity can improve cognitive function in brain-injured rats.

Our data also show that brain injury models exhibiting different types of pathology and different degrees of synaptic reorganization can respond differentially to DA enhancement with L-deprenyl. Importantly, cases with combined neuroexcitation and deafferentation insult displayed significantly less cognitive recovery following L-deprenyl treatment than did rats subjected to the neuroexcitation pathology of TBI alone, the latter performing better than uninjured sham controls. Moreover, BEC deafferentation alone showed no effect of L-deprenyl treatment. This response is in line with the relative amounts of synaptic damage and behavioral recovery in these three models: TBI alone having the least damage and greatest recovery, BEC alone showing significant synaptic damage and only modest recovery (72, 99), and the combined insult producing extensive, persistent synaptic pathology with no recovery. Because such targeted hippocampal deafferentation and maladaptive synaptic rearrangement occurs after combined TBI and BEC lesion (65), and since these hippocampal circuits are known to be critical for spatial learning (58), we believe that, over the first 2 weeks postinjury, the interaction between neuroexcitation and deafferentation in the combined insult cases results in a dysfunctional circuitry whose response to L-deprenyl therapeutic intervention is appreciably reduced (27, 28, 64, 67). This is consistent with our observation that TBI alone + L-deprenyl cases exhibited

continued improvement in performance over the testing interval, while the treated TBI + BEC animals had persistently poor performance, failing to show cognitive improvement after day 13 (repeated measures ANOVA for days 13 and 15; $F(1, 9) = 2.99$, $P > 0.05$). While we cannot rule out the possibility that L-deprenyl acts solely on the TBI component in our combined insult model, the fact that the drug failed to elicit cognitive improvement following BEC lesion also suggests that the two pathologies interact to produce synaptic pathology which is distinct from either injury alone. Based upon more recent findings, we believe that DA circuits involved in the plasticity and cognitive recovery after TBI +BEC insults may require the activation of specific receptor subpopulation(s). For example, when we administer the D1 agonist SKF-38393 for 7 days postinjury in rats with combined TBI + BEC insult, we find a much greater improvement in cognitive performance than with L-deprenyl (L. L. Phillips, unpublished data). To fully assess the different role(s) of DA in recovery processes as a function of injury pathology we must test other DA receptor subtypes, utilizing selective agonists and antagonists in the different injury models. Although not completely understood, DA appears to have an important role in the recovery of cognitive function after TBI.

Noradrenergic Fiber Integrity and L-Deprenyl

The present study has provided direct evidence that DBH-positive axons in the CA1 and CA3 subregions of the hippocampal formation are significantly decreased after trauma. Since DBH is a membrane-bound enzyme which converts dopamine to norepinephrine, it is often used as a marker to investigate the structural integrity of noradrenergic (NA) axons (20) as well as to indirectly assess the DA level in these axons. With L-deprenyl treatment, DBH-IR restored in hippocampal pyramidal cell laminae; however, in rats subjected to combined TBI + BEC, DA enhancement provided protection of DBH-IR only in the CA1 region. These data suggest that DA/NA systems in CA1 and CA3 are selectively vulnerable to the neuroexcitatory injury produced by fluid percussion TBI. Further, they imply that manipulation of DA levels (or other pathological components affected by L-deprenyl, see discussion below) can ameliorate both structural and functional damage induced by excessive neuroexcitation. Our findings also suggest that when secondary deafferentation occurs after TBI neuroexcitation injury, some of these vulnerable sites (e.g., the CA3 subregion of hippocampus) are subject to further injury such that the L-deprenyl treatment cannot induce DBH-IR recovery. It is interesting that our cognitive results parallel this difference between the two models, with only a modest improvement in MWM performance for the treated combined insult cases in comparison to treated TBI cases, where enhanced DBH-positive fibers correlated with a highly significant behavioral recovery. Notably, the lack of L-deprenyl effect on the BEC alone cases for DBH IR and very modest cognitive improvement is also consistent with this interpretation.

The exact mechanism of DBH loss and recovery following TBI remains to be identified. It is well known that primary axodendritic damage occurs with trauma impact and may be the result of direct mechanical forces producing contusions and lacerations, diffuse axonal injury, and intracranial hemorrhage (54, 55, 72). Secondary damage (including ischemia, edema, hypoxia, and endogenous neurochemical alterations) may also induce delayed nonmechanical damage to axons and dendrites of the injured brain. Since dopamine β-hydroxylase is a molecular-oxygen-dependent enzyme (24), and the neurons of the dentate gyrus and CA3 are highly vulnerable to damage in severe hypoxia models (56), the vascular pathology produced by both primary and secondary TBI may selectively damage DBH fibers in the dentate gyrus and CA3 through hypoxic-induced mechansims. The conditions which might stimulate an increase in hippocampal DBH-positive fibers have been addressed by Peterson (63), who demonstrated that the degree of noradrenergic sprouting (as measured by DBH-IR) was a function of the specific transmitter pathways injured and their combined pathologies. Although Peterson's work does not address models with neuroexcitatory pathology, his results do suggest that model-related differences in DBH-IR observed in our study may be attributable to interactive effects between distinct pathologies.

AChE, Synaptic Plasticity, and L-Deprenyl

AChE has been traditionally considered as a marker of cholinergic innervation in the central nervous system (90). Pronounced increase in AChE staining has been repeatedly observed in the outer molecular layer after EC lesion (3, 14) and is known to be both spatially and temporally associated with the sprouting of cholinergic septohippocampal fibers (90). In the present study, L-deprenyl treatment produced an increase the staining density of AChE over the molecular layer of the dentate gyrus and the CA3 lamina of the hippocampus both in TBI alone and in TBI + BEC rats. Our results are similar to the study of Ricci *et al.* (79), where oral administration of L-deprenyl daily for 4 weeks fully reversed neurotoxin AF64A induced loss of ChAT-IR neurons in the septum and enhanced the AChE staining in the CA1 and CA3 of the hippocampus. The same study also showed that L-deprenyl both inhibited MAO-B activity and increased the density of M_2 receptors in the hippocampus. This effect of L-deprenyl treatment is not unique to neurotoxin-induced

pathology since the drug has also been shown to delay loss of cholinergic motor neurons after facial nerve axotomy (1). These findings have relevance to our studies since TBI induces a loss of ChAT-IR, fimbria-fornix-induced septal degeneration and reduces scopolamine induced ACh release, all indicating a hypofunction of the cholinergic system (16, 49, 50, 68, 69). Moreover, a variety of therapeutic interventions aimed at enhancing cholinergic function after TBI have been shown to significantly improve cognitive performance (16, 68, 69, 87).

Recently, noncholinergic AChE functions in plasticity have also been proposed based on the fact that increased AChE staining following EC lesion did not directly correspond to changes in muscarinic receptor binding density or ChAT activity in the same tissue regions (3). Such results support the idea that increases in AChE at sites of synaptic plasticity may represent a noncholinergic role. Interestingly, some of these studies provide evidence that AChE may play a cell adhesive role (85) and proteolytic function (88), each of which are associated with neurite outgrowth or synaptic plasticity (62, 81). Based on these findings, it is possible that AChE histochemistry may represent change in more generalized components of the sprouting process. Whatever the role of AChE, its increase after trauma is associated with tissue sites of enhanced sprouting and plasticity in both the BEC alone and the TBI + BEC cases. It must be noted, however, that our data also show the greatest L-deprenyl-induced enhancement of AChE in the combined insult cases where cognitive recovery was only moderately improved and no effect of L-deprenyl on AChE in the BEC-lesioned rats whose behavior was least affected. Such results clearly suggest that other mechanisms detrimental to successful plasticity are involved. Consistent with our observation of elevated AChE during maladaptive plasticity response, Chen and colleagues (13) recently observed that persistent AChE mRNA overexpression in a transgenic mouse model can impair recovery after closed head injury. Such results support the possibility that mechanisms which promote synaptic recovery may require selective control as a function of postinjury recovery interval and injury type.

L-Deprenyl: Mechanism of Action in TBI

The exact cellular mechanism(s) underlying L-deprenyl-induced improvement in cognitive function and axodendritic integrity after TBI remains unclear. Four mechanisms of action have been proposed based on both *in vivo* and *in vitro* studies. The most common action is MAO inhibition, supported by documentation that low doses of L-deprenyl (0.05–0.25 mg/kg in the rat; 1 mg/kg in humans) selectively reduces MAO-B enzyme activity, enhances DA levels, and improves cognitive function (6, 8, 92). Second, L-deprenyl is proposed to reduce oxidative free radicals (via increasing expression of scavenging systems such as SOD), but only when present at concentrations insufficient to inhibit MAO-B activity (10^{-11} M) (97). Third, L-deprenyl appears to protect oxidative metabolism in cultured PC-12 cells through a stabilization of the mitochondrial membrane potential (97). Finally, L-deprenyl is associated with enhanced neurotrophic activity (e.g., increased expression of bFGF and CNTF) and protection against neuronal cell death after brain insults or degeneration (1, 4, 5, 24).

Given that each of the proposed actions of L-deprenyl can be directly associated with known TBI pathologies, the mechanism(s) underlying the beneficial effects of L-deprenyl in our study may well include multiple components. For example, studies with the major TBI models of cortical ablation (18), fluid percussion TBI (54), and controlled cortical impact (73) all suggest that DA/NE metabolism is altered after injury. In addition, it has been shown that other transmitter systems which can be directly modulated by DA, including NMDA glutamate (89), acetylcholine (36), and GABA (33), are all known to be linked to the excessive neuroexcitation induced by TBI (34). Other TBI studies have also implicated the generation of free radicals (superoxide and hydroxyl) with trauma (26, 52, 71) as well as demonstrated injury-induced increases in endogenous radical scavenging molecules such as SOD (11, 12, 21). Mitochondrial pathology has also been recognized as part of TBI, with earlier studies showing indirect effects on oxidative metabolism (39,40) and direct structural degeneration of axonal mitochondria (64). Recently, clear evidence of energy production failure after TBI has been shown using *in vitro* assays of mitochondrial function (99). In the cortical impact model of TBI, the latter study reported a rapid (within 1 h) decrease in State 3 respiratory rates, respiratory control indices and P/O ratios. The same group further demonstrated that these *in vitro* reductions in mitochondrial oxidative metabolism were dependent upon the presence of Ca^{2+} (100) and that N-type Ca^{2+} channel antagonists can also block the TBI-induced mitochondrial dysfunction (96). Given this selective vulnerability of mitochondria following TBI, it is of interest that, in other models of mitochondrial dysfunction, L-deprenyl is capable of stabilizing mitochondrial membrane potential, a requirement for the proper function of mitochondrial oxidative chain enzymes producing ATP (97). It is possible that one mechanism of action for L-deprenyl in our study may be through stabilizing mitochondrial membrane potential and energy production.

Finally, L-deprenyl can induce NGF, CNTF, and bFGF expression (80, 83, 84) as well as rescue axotomized axons in a manner similar to that of CNTF and

BDNF treatment (84). Several of these growth factors have already been associated with postinjury recovery periods following TBI (38, 61, 95, 101). To date, we cannot confirm the relative contribution of these different cellular mechanisms in producing the observed effects of L-deprenyl treatment; however, we do know from additional studies with the selective D1 receptor agonist SKF-38393 that cognitive performance in the combined TBI + BEC insult model can be enhanced to a greater degree than with L-deprenyl alone (L. L. Phillips, unpublished data). This suggests that a prominent feature of the L-deprenyl effects observed here may be a direct function of DA agonism at the synapse.

It is clear from these varied studies that the dose of L-deprenyl and time intervals of administration can influence which mechanisms of action might predominate. For example, in the rat hippocampus, MAO-A, the enzyme which converts DA to NE, is thought to be more common, making the MAO-B effective doses of 0.05–0.25 mg/kg less likely to cause elevations in DA. However, when L-deprenyl doses of 1–2 mg/kg are used (as in the present study), inhibition of hippocampal MAO-A is achieved and DA levels affected (44). Thus, it is likely that our experimental paradigm induced, at least in part, an elevation of DA in the limbic circuitry important to spatial memory function and synaptic plasticity. Nevertheless, other mechanisms of action related to L-deprenyl dosage must be considered. For example, chronic dosing comparable to that in the present study can induce the expression of free-radical-scavenging molecules such as SOD in rat brain (44). Since free radical generation is a known pathological consequence of TBI (26, 30), the highly beneficial effects of L-deprenyl in TBI models may also be due to the increased expression of SOD in these animals. Given that single dosing of L-deprenyl does not appear to induce change in SOD expression (44), future studies might examine single dosage paradigms to resolve the issue of whether reduction in free radical pathology is part of L-deprenyl's mechanism of action following brain injury.

CONCLUSION

The present results demonstrate that L-deprenyl, a MAO-B inhibitor, at the dose of 1 mg/kg/day, can significantly reduce cognitive deficits induced by fluid percussion TBI and combined fluid percussion TBI with BEC lesion. Restoration of DBH-IR and enhancement of AChE staining by L-deprenyl treatment were spatially and temporally correlated with improvement of cognitive performance, suggesting that inhibition of MAO-B activity after TBI is linked with protection of hippocampal NA or DA function and the promotion of AchE-associated synaptic plasticity.

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REFERENCES

1. Ansari, K. S., P. H. Yu, T. P. Kruck, and W. G. Tatton. 1993. Rescue of axotomized immature rat facial motoneurons by R(−)-deprenyl: Stereospecificity and independence from monoamine oxidase inhibition. *J. Neurosci.* **13:** 4042–4053.
2. Arnsten, A. F., J. X. Cai, B. L. Murphy, and P. S. Goldman-Rakic. 1994. Dopamine D1 receptor mechanism in the cognitive performance of young adult and aged monkeys. *Psychopharmacology* **116:** 143–151.
3. Aubert, I., J. Poirier, S. Gauthier, and R. Quirion. 1994. Multiple cholinergic markers are unexpectedly not altered in the rat dentate gyrus following entorhinal cortex lesions. *J. Neurosci.* **14:** 2476–2484.
4. Biagini, G., A. Frasoldati, K. Fuxe, and L. F. Agnati. 1994. The concept of astrocyte-kinetic drug in the treatment of neurodegenerative diseases: Evidence for L-deprenyl-induced activation of reactive astrocytes. *Neurochem. Int.* **25:** 17–22.
5. Biagini, G., M. Zoli, K. Fuxe, and L. F. Agnati. 1993. L-Deprenyl increases GFAP immunoreactivity selectively in activated astrocytes in rat brain. *NeuroReport* **4:** 955–958.
6. Bickford, P. C., C. E. Adams, S. J. Boyson, P. Curella, G. A. Gerhardt, C. Heron, G. O. Ivy, A. M. Lin, M. P. Murphy, and K. Poth. 1997. Long-term treatment of male F344 rats with deprenyl: Assessment of effects on longevity, behavior, and brain function. *Neurobiol. Aging* **18:** 309–318.
7. Biegon, A., and M. Wolff. 1986. Quantitative histochemistry of acetylcholinesterase in rat and human brain postmortem. *J. Neurosci. Methods* **16:** 39–45.
8. Brandeis, R., M. Sapir, Y. Kapon, G. Borelli, S. Cadel, and B. Valsecchi. 1991. Improvement of cognitive function by MAO-B inhibitor L-deprenyl in aged rats. *Pharmacol. Biochem. Behav.* **39:** 297–304.
9. Caceres, A., and O. Steward. 1983. Dendritic reorganization in the denervated dentate gyrus of the rat following entorhinal cortical lesions: A golgi and electron microscopic analysis. *J. Comp. Neurol.* **214:** 387–403.
10. Cestari, V., and C. Castellano. 1997. MK-801 potentiates morphine-induced impairment of memory consolidation in mice: Involvement of dopaminergic mechanisms. *Psychopharmacology* **133:** 1–6.
11. Chan, P. K. 1992. Antioxidant-dependent amelioration of brain injury: Role of CuZn-superoxide dismutase. *J. Neurotrauma* **9:** S417–S423.
12. Chan, P. K., C. J. Epstein, T. T. Huang, E. Carlson, H. Kinouchi, G. Yang, H. Kamii, S. Mikawa, T. Kondo, J.-C. Copin, S. F. Chen, T. Chan, J. Gafni, G. Gobbel, and E. Reola. 1995. Transgenic mice and knockout mutants in the study of oxidative stress in brain injury. *J. Neurotrauma* **12:** 815–824.
13. Chen, Y., R. Beeri, H. Soreq, and E. Shohami. 1997. Overexpression of acetylcholinesterase is a risk factor for impaired recovery after closed head injury. *J Neurotrauma* **14:** 796.
14. Chen, L. L., G. W. van Hoesen, C. L. Barnes, and J. R. West. 1983. Enhanced acetylcholinesterase staining in the hip-

pocampal perforant pathway zone after combined lesions of the septum and entorhinal cortex. *Brain Res.* **272:** 354–359.

15. Dixon, C. E., M. F. Kraus, A. E. Kline, M. Xiecheng, H. Q. Yan, R. G. Griffith, B. M. Wolfson, and D. W. Marion,. 1999. Amantadine improves water maze performance without affecting motor behavior following traumatic brain injury in rats. *Rest. Neurol. Neurosci.* **14:** 285–294.
16. Dixon, C. E., P. Flinn, J. L. Bao, R. Venya, and R. L. Hayes. 1997. Nerve growth factor attenuates cholinergic deficits following traumatic brain injury in rats. *Exp. Neurol.* **146:** 479–490.
17. Dixon, C. E., B. G. Lyeth, J. T. Povlishock, R. L. Findling, R. J. Hamm, A. Marmarou, H. F. Young, and R. L. Hayes. 1987. A fluid percussion model of experimental brain injury in the rat. *J. Neurosurg.* **67:** 110–119.
18. Faden, A. I., P. Demediuk, S. S. Panter, and R. Vink. 1989. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* **244:** 798–800.
19. Feeney, D. M., and V. S. Westerberg. 1990. Norepinephrine and brain damage: Alpha noradrenergic pharmacology alters functional recovery after cortical trauma. *Can. J. Psychol.* **44:** 233–252.
20. Fritschy, J. M., M. Geffard, and R. Grzanna. 1990. The response of noradrenergic axons to systemically administered DSP-4 in the rat: An immunohistochemical study using antibodies to noradrenaline and dopamine-beta-hydroxylase. *J. Chem. Neuroanat.* **3:** 309–321.
21. Fukuhara, T., S. Nishio, Y. Ono, M. Kawauchi, S. Asari, and T. Ohmoto. 1994. Induction of Cu, Zn-superoxide dismutase after cortical contusion injury during hypothermia. *Brain Res.* **657:** 333–336.
22. Gandolfi, O., and R. Dall'Olio. 1996. Modulatory role of dopamine on excitatory amino acid receptors. *Prog. Neuropsych. Pharmacol. Biol. Psych.* **20:** 659–671.
23. Gennarelli, T. A. 1994. Animate models of human head injury. *J. Neurotrauma* **11:** 357–368.
24. Gerlach, M., P. Riederer, and M. B. Youdim. 1992. The molecular pharmacology of L-deprenyl. *Eur. J. Pharmacol.* **226:** 97–108.
25. Gibson, G. E., W. Pulsinelli, J. P. Blass, and T. E. Duffy. 1981. Brain dysfunction in mild to moderate hypoxia. *Am. J. Med.* **70:** 1247–1254.
26. Globus, M. Y., O. Alonso, W. D. Dietrich, R. Busto, and M. D. Ginsberg. 1995. Glutamate release and free radical production following brain injury: Effects of posttraumatic hypothermia. *J. Neurochem.* **65:** 1704–1711.
27. Glover, V., and M. Sandler. 1993. Neurotoxins and monamine oxidase B inhibitors: Possible mechanisms for the neuroprotective effect of (−)-deprenyl. In *Inhibitors of Monoamine Oxidase B: Pharmacology and Clinical Use in Neurodegenerative Disorders* (I. Szelenyi, Ed.), pp. 169–181. Birkhauser Verlag, Basel.
28. Gordon, D. E, L. L. Phillips, and J. T. Povlishock. 1996. The interation of neuroexcitation and target deafferentation in the pathobiology of traumatic brain injury (TBI): An assessment of disordered recovery in the rat dentate gyrus. *Soc. Neurosci. Abstr.* **22:** 2153.
29. Gualtieri, C. T., and R. W. Evans. 1988. Stimulant treatment for the neurobehavioural sequelae of traumatic brain injury. *Brain Inj.* **2:** 273–290.
30. Hall, E. 1989. Free radicals and CNS injury. *Crit. Care Clin.* **5:** 793–805.
31. Hamm, R. J., B. R. Pike, D. M. O'Dell, B. G. Lyeth, and L. W. Jenkins. 1994. The rotarod test: An evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. *J. Neurotrauma* **11:** 187–196.
32. Hamm, R. J., B. G. Lyeth, L. W. Jenkins, D. M. O'Dell, and B. R. Pike. 1993. Selective cognitive impairment following traumatic brain injury in rats. *Behav. Brain Res.* **59:** 169–173.
33. Harsing, L. G., Jr., and M. J. Zigmond. 1997. Influence of dopamine on GABA release in striatum: Evidence for D1–D2 interactions and non-synaptic influences. *Neuroscience* **77:** 419–429.
34. Hayes, R. L., L. W. Jenkins, and B. G. Lyeth. 1992. Neurotransmitter-mediated mechanisms of traumatic brain injury: Acetylcholine and excitatory amino acids. *J. Neurotrauma* **9:** S173–S187.
35. Healy, D. A., and J. H. Meador-Woodruff. 1996. Dopamine receptor gene expression in hippocampus is differentially regulated by the NMDA receptor antagonist MK-801. *Eur. J. Pharmacol.* **306:** 257–264.
36. Hersi, A. I., J. W. Richard, P. Gaudreau, and R. Quirion. 1995. Local modulation of hippocampal acetylcholine release by dopamine D1 receptors: A common receptor autoradiography and in vivo dialysis study. *J. Neurosci.* **15:** 7150–7157.
37. Hersi, A. I., W. Rowe, P. Gaudreau, and R. Quirion. 1995. Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acteylcholine release in memory-impaired aged rats. *Neuroscience* **69:** 1067–1074.
38. Hicks, R. R., S. Numan, H. S. Dhillion, M. R. Prasad, and K. B. Seroogy. 1997. Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. *Brain Res. Mol. Brain Res.* **48:** 401–406.
39. Hovda, D. A., A. Yoshino, T. Kawamata, Y. Katayama, and D. P. Becker. 1991. Diffuse prolonged depression of cerebral oxidative metabolism following concussive brain injury in the rat: Cytochrome oxidase histochemistry study. *Brain Res.* **567:** 1–10.
40. Hovda, D. A., H. T. Chugani, J. R. Villablanca, B. Badie, and R. L. Sutton. 1992. Maturation of cerebral oxidative metabolism in the cat: A cytochrome oxidase histochemistry study. *J. Cereb. Blood Flow Metab.* **12:** 1039–1048.
41. Hsu, K. S. 1996. Characterization of dopamine receptors mediating inhibition of excitatory synaptic transmission in the rat hippocampal slice. *J. Neurosphysiol.* **76:** 1887–1895.
42. Kaelin, D. L., D. X. Cifu, and B. Mattheis. 1996. Methylphenidate effect on attention deficit in the acutely brain-injured adult. *Arch. Phys. Med. Rehab.* **77:** 6–9.
43. Keppel, G. 1991. *Design and Analysis: A Researcher's Handbook.* Prentice Hall, International, Englewood Cliffs, NJ.
44. Kieburtz, K., and I. Shoulson. 1996. Treatment of Parkinson's disease with deprenyl (selegiline) and other monoamine oxidase inhibitors. In *Neurodegeneration and Neuroprotection in Parkinson's Disease,* pp. 47–54. Academic Press, New York.
45. Kline, A. E., H. Q. Yan, J. Bao, D. W. Marion, and C. E. Dixon. 2000. Chronic methylphenidate treatment enhances water maze performance following traumatic brain injury in rats. *Neurosci. Lett.* **280:** 163–166.
46. Knoll, J. 1993. The pharmacological basis of the therapeutic effect of (−)-deprenyl in age-related neurological diseases. In *Inhibitors of Monoamine Oxidase B: Pharmacology and Clinical Use in Neurodegenerative Disorders* (I. Szelenyi, Ed.), pp. 145–168. Birkhauser Verlag, Basel.
47. Kraus, M. F., and P. M. Maki. 1997. Effect of amantadine hydrochloride on symptoms of frontal lobe dysfunction in brain injury: case studies and review. *J. Neuropsychol. Clin. Neurosci.* **9:** 22–230.

48. Lamensdorf, I., S. Porat, R. Simantov, and J. P. Finberg. 1999. Effect of low-dose treatment with selegiline on dopamine transporter (DAT) expression and amphetamine-induced dopamine release in vivo. *Br. J. Pharmacol.* **126:** 997–1002.

49. Leonard, J. R., M. S. Grady, M. E. Lee, J. C. Paz, and L. E. Westrum. 1997. Fluid percussion injury causes disruption of the septohippocampal pathway in the rat. *Exp. Neurol.* **143:** 177–187.

50. Leonard, J. R., D. O. Maris, and M. S. Grady. 1994. Fluid percussion injury causes loss of forebrain choline acetyltransferase and nerve growth factor receptor immunoreactive cells in the rat. *J. Neurotrauma* **11:** 379–392.

51. Levine, G. 1991. *A Guide to SPSS for Analysis of Variance.* Erlbaum, Hillsdale, NJ.

52. Lewen, A., and L. Hillered. 1998. Involvement of reactive oxygen species in membrane phospholipid breakdown and energy pertubation after traumatic brain injury in the rat. *J. Neurotrauma* **15:** 521–530.

53. McDowell, S., J. Whyte, and J. D'Esposito. 1998. Differential effect of a dopaminergic agonist on prefrontal function in traumatic brain injury patients. *Brain* **121:** 1155–1164.

54. McIntosh, T. K., T. Yu, and T. A. Gennarelli. 1994. Alterations in regional brain catecholamine concentrations after experimental brain injury in the rat. *J. Neurochem.* **63:** 1426–1433.

55. McIntosh, T. K., D. H. Smith, D. F. Meaney, M. J. Kotapka, T. A. Gennarelli, and D. I. Graham. 1996. Neuropathological sequelar of traumatic brain injury: Relationship to neurochemical and biomechanical mechanisms. *Lab. Invest.* **74:** 315–342.

56. Matsuoka Y., Y. Kitamura, I. Tooyama, H. Kimura, and T. Taniguchi. 1997. In vivo hypoxia-induced neuronal damage with an enhancement of neuronal nitric oxide synthase immunoreactivity in hippocampus. *Exp. Neurol.* **146:** 57–66.

57. Morris, R. G. M., P. Garrud, J. N. P. Rawlins, and J. O'Keef. 1982. Place navigation impaired in rats with hippocampal lesions. *Nature* **297:** 681–683.

58. Moser, M. B., M. Trommald, and P. Andersen. 1994. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. USA* **91:** 12673–12675.

59. Nickels, J. L., W. N. Schneider, M. L. Dombovy, and T. M. Wong. 1994. Clinical use of amantadine in brain injury rehabilitation. *Brain Inj.* **8:** 709–718.

60. Norusis, M. J. 1997. *SPSS for Windows Advanced Statistics Release 6.0.* SPSS, Chicago, IL.

61. Oyesiku, N. M., C. O. Evans, S. Houston, R. S. Darrell, J. S. Smith, Z. L. Fulop, C. E. Dixon, and D. G. Stein. 1999. Regional changes in the expression of neurotrophic factors and their receptors following acute traumatic brain injury in the adult rat brain. *Brain Res.* **833:** 161–172.

62. Patanow, C. M., J. R. Day, and M. L. Billingsley. 1997. Alterations in hippocampal expression of SNAP-25, GAP-43, stannin and glial fibrillary acidic protein following mechanical and trimethyltin-induced injury in the rat. *Neuroscience* **76:** 187–202.

63. Peterson, G. M. 1994. Sprouting of central noradrenergic fibers in the dentate gyrus following combined lesions of its entorhinal and septal afferents. *Hippocampus* **4:** 635–648.

64. Pettus, E. H., and J. T. Povlishock. 1996. Characterization of a distinct set of intra-axonal ultrastructural changes associated with traumatically induced alterations in axolemmal permeability. *Brain Res.* **722:** 1–11.

65. Phillips, L. L., B. G. Lyeth, R. J. Hamm, and J. T. Povlishock. 1994. Combined fluid percussion brain injury and entorhinal cortical lesion: a model for assessing the interaction between neuroexcitation and deafferentation. *J. Neurotrauma* **11:** 641–656.

66. Phillips, L. L., B. G. Lyeth, R. J. Hamm, J. Y. Jiang, J. T. Povlishock, and T. M. Reeves. 1997. Effect of prior receptor antagonism on behavioral morbidity produced by combined fluid percussion injury and entorhinal cortical lesion. *J. Neurosci. Res.* **49:** 197–206.

67. Phillips, L. L., B. G. Lyeth, R. J. Hamm, T. M. Reeves, and J. T. Povlishock. 1998. Glutamate antagonism during secondary deafferentation enhances cognition and axo-dendritic integrity after traumatic brain injury. *Hippocampus* **8:** 390–401.

68. Pike, B. R., and R. J. Hamm. 1997. Activating the posttraumatic cholinergic system for the treatment of cognitive impairment following traumatic brain injury. *Pharmacol. Biochem. Behav.* **57:** 785–791.

69. Pike, B. R., and R. J. Hamm. 1997. Chronic administration of a partial muscarinic M1 receptor agonist attenuates decreases in forbrain choline acetyltransferase immunoreactivity following experimental brain trauma. *Exp. Neurol.* **147:** 1–12.

70. Plenger, P. M., C. E. Dixon, R. M. Castillo, R. F. Frankowski, S. A. Yablon, and H. S. Levin. 1996. Subacute methylphenidate treatment for moderate to moderately severe traumatic brain injury: A preliminary double-blind placebo-controlled study. *Arch. Phys. Med. Rehab.* **77:** 536–540.

71. Povlishock, J. T., and H. A. Kontos. 1992. The role of oxygen radicals in the pathobiology of traumatic brain injury. *Hum. Cell* **5:** 345–353.

72. Povlishock,J. T., D. E. Erb, and J. Astruc. 1992. Axonal response to traumatic brain injury: Reactive axonal change, deafferentation, and neuroplasticity. *J. Neurotrauma* **9**(Suppl. 1): S189–S200.

73. Prasad, R. M., C. M. Tzigaret, D. Smith, H. Soares, and T. K. McIntosh. 1992. Decreased alpha 1-adrenergic receptors after experimental brain injury. *J. Neurotrauma* **9:** 269–279.

74. Prasad, R. M., C. Ramaiah, T. K. McIntosh, R. J. Dempsey, S. Hipkens, and D. Yurek. 1994. Regional levels of lactate and norepinephrine after experimental brain injury. *J. Neurochem.* **63:** 1086–1094.

75. Prasad, R. M., J. M. Dose, H. S. Dillon, T. Carbary, and P. J. Kraemer. 1995. Amphetamine affects the behavioral outcome of lateral fluid percussion brain injury in the rat. *Restor. Neurol. Neurosci.* **9:** 65–75.

76. Reeves, T. M., B. G. Lyeth, and J. T. Povlishock. 1995. Long-term potentiation deficits and excitability changes following traumatic brain injury. *Exp. Brain Res.* **106:** 248–256.

77. Reeves, T. M., J. Zhu, J. T. Povlishock, and L. L. Phillips. 1997. The effect of combined fluid percussion and entorhinal cortical lesions on long-term potentiation. *Neuroscience* **77:** 431–444.

78. Reeves, T. M., B. G. Lyeth, L. L. Phillips, R. J. Hamm, and J. T. Povlishock. 1997. The effects of traumatic brain injury on inhibition in the hippocampus and dentate gyrus. *Brain Res.* **757:** 119–132.

79. Ricci, A., M. Mancini, P. Strocchi, S. Bongrani, and E. Bronzetti. 1992. Deficits in cholinergic neurotransmission markers induced by ethylcholine mustard aziridinium (AF64A) in the rat hippocampus: Sensitivity to treatment with the monoamine oxidase-B inhibitor L-deprenyl. *Drugs Exp. Clin. Res.* **18:** 163–171.

80. Riva, M. A., R. Molteni, and G. Racagni. 1997. L-Deprenyl potentiates cAMP-induced elevation of FGF-2 mRNA levels in rat cortical astrocytes. *NeuroReport* **8:** 2165–2168.

81. Robertson, R. T., B. P. Yu, H. H. Liu, N. H. Liu, and G. H. Kageyama. 1991. Development of cholinesterase histochemi-

cal staining in cerebellar cortex: Transient expression of "non-specific" cholinesterase in Purkinje cells of the nodulus and uvula. *Exp. Neurol.* **114:** 330–342.

82. Seamans, J. K., S. B. Floresco, and A. G. Phillips. 1998. D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J. Neurosci.* **18:** 1613–1621.
83. Semkova, I., P. Wolz, M. Schilling, and J. Krieglstein. 1996. Selegiline enhances NGF synthesis and protects central nervous system neurons from excitotoxic and ischemic damage. *Eur. J. Pharmacol.* **315:** 19–30.
84. Seniuk, N. A., J. T. Henderson, W. G. Tatton, and J. C. Roder. 1994. Increased CNTF gene expression in process-bearing astrocytes following injury is augmented by R(−)-deprenyl. *J. Neurosci. Res.* **37:** 278–286.
85. Sharma, K. V., and J. W. Bigbee. 1998. Acetylcholinesterase antibody treatment results in neurite detachment and reduced outgrowth from cultured neurons: Further evidence for a cell adhesive role for neuronal acetylcholinesterase. *J. Neurosci. Res.* **53:** 454–464.
86. Sharp, F. R., J. Liu, J. Nickolenko, and B. Bontempi. 1995. NMDA and D1 receptors mediate induction of c-fos and junB genes in striatum following morphine administration: Implications for studies of memory. *Behav. Brain Res.* **66:** 225–230.
87. Sinson, G., B. R. Perri, J. Q. Trojanowski, E. S. Flamm, and T. K. McIntosh. 1997. Improvement of cognitive deficits and decreased cholinergic neuronal cell loss and apoptotic cell death following neurotrophin infusion after experimental traumatic brain injury. *J. Neurosurg.* **86:** 511–518.
88. Small, D. H. 1990. Non-cholinergic actions of acetylcholinesterases: Proteases regulating cell growth and development? *Trends Biochem. Sci.* **15:** 213–216.
89. Smith, D. O., D. Lowe, R. Temkin, P. Jensen, and H. Hatt. 1995. Dopamine enhances glutamate-activated currents in spinal motoneurons. *J. Neurosci.* **15:** 3905–3912.
90. Steward, O., and J. A. Messenheimer. 1978. Histochemical evidence for a post-lesion reorganization of cholinergic afferents in the hippocampal formation of the mature cat. *J. Comp. Neurol.* **178:** 697–709.
91. Steward, O., J. Loesche, and W. C. Horton. 1977. Behavioral correlates of denervation and reinnervation of the hippocampal formation of the rat: Open field activity and cue utilization following bilateral entorhinal cortical lesions. *Brain Res. Bull.* **2:** 41–48.
92. Stoll, S., U. Hafner, O. Pohl, and W. E. Muller. 1994. Age-related memory decline and longevity under treatment with selegiline. *Life Sci.* **55:** 2155–2163.
93. Tago, H., H. Kimura, and T. Maeda. 1986. Visualization of detailed acetylcholinesterase fiber and neuron staining in rat brain by a sensitive histochemical procedure. *J. Histochem. Cytochem.* **34:** 1431–1438.
94. Tang, Y. P., Y. Noda, and T. Nabeshima. 1997. Involvement of activation of dopaminergic neuronal system in learning and memory deficits associated with experimental mild traumatic brain injury. *Eur. J. Neurosci.* **9:** 1720–1727.
95. Truettner, J., R. Schmidt-Kastner, R. Busto, O. F. Alonso, J. Y. Loor, W. D. Dietrich, and M. D. Ginsberg. 1999. Expression of brain-derived neurotrophic factor, nerve growth factor, and heat shock HSP70 following fluid percussion brain injury in rats. *J. Neurotrauma* **16:** 471–486.
96. Verweij, B. H., J. P. Muizelaar, F. C. Vinas, P. L. Peterson, Y. Xiong, and C. P. Lee. 1997. Mitochondrial dysfunction after experimental and human brain injury and its possible reversal with a selective N-type calcium channel antagonist (SNX-111). *Neurol. Res.* **19:** 334–339.
97. Wadia, J. S., R. M. E. Chalmers-Redman, W. J. H. Ju, G. W. Carlile, J. L. Phillips, A. D. Fraser, and W. G. Tatton. 1998. Mitochondrial membrane potential and nuclear changes in apoptosis caused by serum and nerve growth factor withdrawal: Time course and modification by (−)-deprenyl. *J. Neurosci.* **18:** 932–947.
98. Wu, R. M., C. C. Chiueh, A. Pert, and D. L. Murphy. 1993. Apparent antioxidant effect of l-deprenyl on hydroxyl radical formation and nigral injury elicited by MPP+ in vivo. *Eur. J. Pharmacol.* **243:** 241–247.
99. Xiong, Y., Q. Gu, P. L. Peterson, J. P. Muizelaar, and C. P. Lee. 1997. Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. *J. Neurotrauma* **14:** 23–34.
100. Xiong, Y., P. L. Peterson, J. P. Muizelaar, and C. P. Lee. 1997. Amelioration of mitochondrial function by a novel antioxidant U-101033E following traumatic brain injury in rats. *J. Neurotrauma* **14:** 907–917.
101. Yang, K., J. R. Perez-Polo, X. S. Mu, H. Q. Yan, J. J. Xue, Y. Iwamoto, S. J. Liu, C. E. Dixon, and R. L. Hayes. 1996. Increased expression of brain-derived neurotrophic factor but not neurotrophin-3 mRNA in rat brain after cortical impact injury. *J. Neurosci. Res.* **44:** 157–164.
102. Zhang, X., D. M. Zuo, B. A. Davis, A. A. Boulton, and P. H. Yu. 1996. Immunohistochemical evidence of neuroprotection by R(-)-deprenyl and N-(2-hexyl)-N-methylpropargylamine on DSP-4-induced degeneration of rat brain noradrenergic axons and terminals. *J. Neurosci. Res.* **43:** 482–489.
103. Zoli, M., I. Zini, L. F. Agnati, D. Cuidolin, F. Ferraguti, and K. Fuxe. 1990. Aspects of neural plasticity in the central nervous system. I. Computer-assisted image analysis methods. *Neurochem. Int.* **16:** 383–418.