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Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats

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Abstract

Impairments of learning and memory are common neuropsychological sequelae of chronic alcohol abuse. Alcoholics often have impairments of anterograde memory, including spatial memory dysfunction, and a tendency toward response perseveration. This study was designed to assess the effects of binge ethanol exposure on neurodegeneration and cognitive function. Rats were given ethanol three times daily for 4 days. Silver staining revealed neurodegeneration in the olfactory bulb, piriform cortex, perirhinal cortex, entorhinal cortex, and dentate gyrus. After withdrawal, behavioral testing in the Morris water maze revealed significant differences in reversal learning between treatment groups. Ethanol-treated animals required more trials to learn the reversal task, entered the previously trained quadrant more often, and spent more time there than controls. [^{3}H]PK-11195 binding, an index of CNS damage, was elevated in the piriform cortex of ethanol-treated animals. Thus, binge ethanol exposure resulted in neurodegeneration of a corticolimbic circuit with common excitatory inputs from the olfactory bulb and was associated with perseverative responding on a spatial learning task. These studies suggest that a single binge drinking episode could cause neurodegeneration and cognitive dysfunction in humans. The perseverative nature of the behavioral deficit could be related to both cognitive dysfunction and the behavioral components of the addiction process.

Keywords: Alcoholism; Binge drinking; Cognitive dysfunction; Neurodegeneration; Reversal learning

1. Introduction

Chronic alcohol abuse is associated with cortical and limbic atrophy through white matter loss (Harding et al., 1997; Jensen and Pakkenberg, 1993; Kril et al., 1997; Pfefferbaum et al., 1997; Sullivan et al., 1995) and gray matter loss (Harper, 1998; Kril et al., 1997; Pfefferbaum et al., 1997; Sullivan et al., 1995). Alcoholics also often suffer from learning and memory impairments, including deficits in spatial memory (Beatty et al., 1996; Bowden and McCarter, 1993) and abnormal response perseveration (Oscar-Berman et al., 1992). Although human studies show neurodegeneration and cognitive dysfunction after long-term alcohol abuse, short-term animal models of binge alcohol exposure have found neurodegeneration in corticolimbic areas (Collins et al., 1996, 1998; Corso et al., 1998; Crews et al., 2000; Zou et al., 1996), which are involved in many aspects of learning and spatial memory (Haberly, 1998). The vulnerability of these regions to damage after a single binge ethanol episode suggests that long-term ethanol exposure is not required to produce neurotoxicity or cognitive impairments.

Animal models of binge drinking produce high blood alcohol levels (BALs), which are consistent with those reported clinically by emergency room physicians (Cartlidge and Redmond, 1990). In one such study, the average BAL of emergency room patients deemed ‘‘nonintoxicated’’ was 268 mg/dl, leading the authors to suggest that chronic alcohol abusers commonly have BALs approaching 300 mg/dl (Urso et al., 1981). Our study’s short-term alcohol binge

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in animals models binge drinking by heavy drinking human alcoholics.

The hippocampal formation has long been implicated in spatial learning and memory in the rat (Aggleton et al., 1986; Morris et al., 1982; Olton et al., 1979). More recently, research has focused on the contributions of extrahippocampal regions to spatial learning and memory. Lesions of the entorhinal cortex (Johnson and Kesner, 1994; Nagahara et al., 1995) and olfactory bulb (Amemori et al., 1989; Hall and Macrides, 1983; Van Rijzingen et al., 1995) lead to deficits in spatial working memory and reversal learning. It follows that rats exposed to a binge alcohol paradigm might show many of the same deficits in spatial working memory and reversal learning.

To investigate the effects of our binge alcohol model on spatial working memory and reversal learning, the Morris water maze was used to test animals subjected to a single 4-day binge. Amino cupric silver stain and [^{3}H]PK-11195 binding were used to assess areas of acute neurodegeneration and long-term CNS damage. Our findings indicate that binge alcohol exposed animals manifest enduring perseverative responses, which are accompanied by long-term CNS damage in corticolimbic areas.

2. Methods

2.1. Binge ethanol treatment

Male Sprague–Dawley rats (275–325 g) were anesthetized with pentobarbital (50 mg/kg) and polyethylene intragastric catheters were implanted in the stomach as previously described (Knapp and Crews, 1999). At 4–7 days after surgery, the ethanol-treated animals (ETOH) were given an initial dose of 5 g/kg ethanol in a solution of 25% (w/v) ethanol in diluted nutritionally complete diet (50% (v/v) Vanilla Ensure) (Collins et al., 1996). Additional ethanol was administered every 8 h for 4 consecutive days at 7 a.m., 3 p.m., and 11 p.m. Except for the initial 5 g/kg dose, doses were based on the animals' estimated blood ethanol level, determined using a six-point intoxication scale (Knapp and Crews, 1999; Majchrowicz, 1975). The average dose of ethanol per day was 7.6 g/kg, similar to the amount of ethanol that rats will self-administer in a chronic ethanol paradigm (Lukoyanov et al., 2000). This treatment model titrates the dose according to the animal's estimated blood ethanol level, thereby maintaining intoxicating levels of blood ethanol while minimizing mortality. Control animals (CON) received a diet of 50% (v/v) Vanilla Ensure made isocaloric with dextrose, equal to the average of all ethanol-treated animals. Two sets of animals were binge treated, one used to assess neurodegeneration by amino cupric silver staining and a second used in behavioral testing and long-term damage assessment by [^{3}H]PK-11195 binding. Tail blood samples were taken on the third and fourth days immediately before and 1.5 h after the 3 p.m. treatment. BELs were determined using a GM7 Anaylser (Analox, London, UK). BELs averaged 193±18 and 255±24 mg/dl before ethanol dosing and between 257±22 and 286±25 mg/dl after ethanol dosing. The mortality rate ranged 16% during the binge treatments and 20% during withdrawal. All animals had free access to water throughout the experiment, and after the completion of the binge, all animals were given free access to rat chow. All procedures were carried out in accordance with the University of North Carolina Institutional Animal Care and Use Committee.

2.2. Amino cupric silver staining

Animals were sacrificed after 48 h of treatment (2nd day) or immediately after the last dose of ethanol (4th day). There were six animals in each group. To sacrifice, animals were anesthetized with an overdose of pentobarbital and perfused transcardially with wash buffer (2 mM cacodylate, 0.9% NACl, 22 mM dextrose/sucrose, 2 mM $CaCl_2$, pH 7.4) and fixative (4% paraformaldehyde with 90 mM sodium cacodylate and 115 mM sucrose, pH 7.4). Brains were embedded in a gelatin matrix and sectioned at 40 μm. Silver staining was performed by the method of de Olmos et al. (1981) as modified by Switzer (2000) (Neuroscience Associates, Knoxville, TN). Representative brain sections were digitally imaged in Figs. 2 and 3 corresponding to the following brain levels (Paxinos and Watson, 1998): olfactory bulb 6.7 mm bregma; anterior piriform cortex (APC) 2.7 mm bregma; perirhinal cortex and temporal dentate gyrus −5.3 mm bregma; lateral entorhinal cortex −7.3 mm bregma.

2.3. Behavioral testing

There were eight animals in each treatment group that underwent behavioral testing. The animals lost approximately 15% of their body weight during the binges, though there was no significant difference in body weights between treatment groups across days. A timeline for behavioral testing is shown below (Fig. 1).

The ETOH rats were withdrawn from ethanol for 106 h (roughly 4.5 days). During this time, all animals were hyperactive with tail spasticity, and many animals showed tremors and splayed limbs, well-documented signs of ethanol withdrawal (Majchrowicz, 1975). After 106 h, all ETOH animals were free of any overt signs of withdrawal and were indistinguishable from CON animals. At this 106-h time point (24 h prior to the initiation of maze testing), locomotor activity was analyzed in an open field using an infrared beam activity box (Med Associates, St. Albans, VT). Each animal was placed in an activity box for a 5-min trial at 9 a.m. and 1 p.m.

2.4. Morris water maze

The apparatus consisted of a circular, galvanized steel pool 144 cm in diameter. The pool was placed in a room

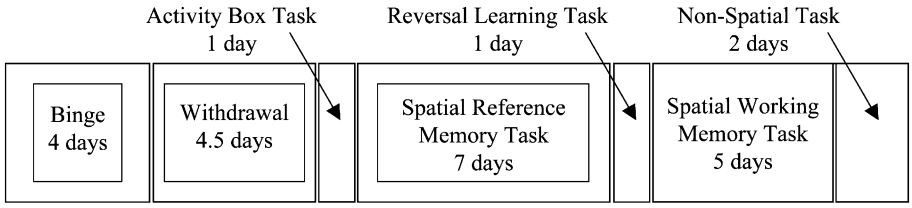

Fig. 1. Timeline of binge treatment and behavioral testing.

with different extramaze cues hung from each wall. The pool and a 10.25-cm circular platform were painted white. The pool was filled with water (20–21 °C) until it reached 2 cm above the platform surface and was opaqued with white tempura paint. Animals were marked on the top of the head with a nontoxic black marker. The animal's movement in the pool was tracked by video camera and analyzed using Chromotrack Version 4.02 software (San Diego Instrument, California). The water in the tank was stirred in between animal trials to disrupt odor trails.

2.5. Spatial reference memory task

Rats were trained to escape from the water onto the submerged platform. The platform was placed in the northeast quadrant and remained there through all 7 days of reference memory testing. Each animal was given four trials a day. Each trial had a 90-s ceiling, and there was a 60-s intertrial interval. The animal was introduced into the pool, facing the wall of the pool at each of the four quadrant edges, pseudorandomly chosen across trials. Once the animal reached the platform, it was allowed to remain on the platform for approximately 10 s. If the animal failed to reach the platform within the trial ceiling, the experimenter gently guided the animal through the water and placed it on the platform where it would remain for 10 s. The animal was then removed to its home cage, which was warmed with a heating pad, to await its next trial.

2.6. Reversal learning

Following the 7 days of place learning, the animals were tested in a reversal learning task. The submerged platform was placed in the quadrant opposite that in which it had been placed during the reference memory task (southwest quadrant). The animals were given four trials, each consisting of a 90-s ceiling and a 60-s intertrial interval. The animal was introduced into the pool, facing the wall of the pool, at each of the four quadrant edges, pseudorandomly chosen. Once the animal reached the platform, it was allowed to remain on the platform for approximately 10 s. If the animal failed to reach the platform within the trial ceiling, the experimenter gently guided the animal through the water and placed it on the platform where it would remain for 10 s. The animal was then removed to its home cage, which was warmed with a heating pad, to await the next trial.

2.7. Spatial working memory task

To test the working memory, the animals were tested with the submerged platform randomly placed in a different quadrant each day. Each animal was given four trials per day. Each trial had a 90-s ceiling, and there was a 60-s intertrial interval. The animal was introduced into the pool, facing the wall of the pool, at each of the four quadrant edges, pseudorandomly chosen. Once the animal reached the platform, it was allowed to remain on the platform for approximately 10 s. If the animal failed to reach the platform within the trial ceiling, the experimenter gently guided the animal through the water and placed it on the platform where it would remain for 10 s. The animal was then removed to its home cage, which was warmed with a heating pad, to await its next trial.

2.8. Nonspatial or visually cued task

Following completion of the spatial tasks, nonspatial learning was tested. The submerged platform was elevated until it was 2 cm above the water level. The edges of the platform were painted black to provide a visual cue within the maze. The animals were given four trials a day to locate the platform. The platform was pseudorandomly placed in a different quadrant for each trial, and the animal was introduced into the pool, facing the wall of the pool, at each of the four quadrant edges, pseudorandomly chosen. Once the animal reached the platform, it was allowed to remain on the platform for approximately 10 s. If the animal failed to reach the platform within the trial ceiling, the experimenter gently guided the animal through the water and placed it on the platform where it would remain for 10 s. The animal was then removed to its home cage, which was warmed with a heating pad, to await its next trial.

2.9. Autoradiography

Two days following the completion of the nonspatial task, animals were decapitated, and the brains were rapidly frozen in dry ice chilled isopentane. The brains were then sectioned on a cryostat at 10 μm and thaw mounted on slides. Sections were taken from 2.7 mm bregma, −5.3 mm bregma, and −7.3 mm bregma. The slides were equilibrated in 170 mM Tris buffer (pH 7.4) for 10 min. Binding was performed by incubating slides for 30 min at room temperature in Tris

buffer containing 2 nM [^{3}H]PK-11195 (specific activity 85 Ci/mmol, NEN, Boston, MA). Nonspecific binding was assayed by incubating adjacent sections in the radioligand with the addition of 3 μM PK-11195. Rinsing sections two times for 5 min in cold Tris buffer terminated the incubations. The sections were then dipped once in cold distilled water. The sections were dried and exposed to ^{3}H-Hyperfilm (Amersham, Arlington Heights, IL) for 4 weeks. Films were digitally imaged and analyzed using the MCID M4 software (Imaging Research, Ontario, Canada). [^{3}H]PK-11195 binding was determined by sampling the optical density for each specific brain region. The optical density measurement was converted to nCi/mg tissue using a standard curve generated from a tritium microscale (Amersham International, Amersham, UK). Both hemispheres were sampled for each brain region, and two sections from each brain level were quantified for binding. One section from each brain level was quantified for nonspecific binding. The measurement of nonspecific binding was subtracted from the average binding measurement to produce specific binding. Three animals lacked sections from all brain regions assess. Therefore, these animals were removed from the statistical analysis of [^{3}H]PK-11195 binding.

2.10. Statistical analyses

For all behavioral tests, a two-way mixed-design ANOVA was used. The within variable was either days or trials, and the between variable was treatment (CON or ETOH). When there was no repeated measure, an unpaired, two-tailed *t* test was used. To evaluate at which point an animal had successfully acquired the reversal task, a criterion level was determined. The level was set at two standard deviations greater than the distance traveled on the last day of the reference memory task.

For analysis of [^{3}H]PK-11195 binding, a two-way mixed design ANOVA was performed. The within variable was brain region, and the between variable was treatment. Post hoc analyses comparing damage in different brain regions were performed using unpaired, two-tailed *t* tests.

3. Results

3.1. Assessment of neurodegeneration using amino cupric silver staining

To visualize specific neuroanatomical structures vulnerable to binge alcohol exposure, neurodegeneration was assessed using the amino cupric silver stain, which makes dead neurons argyrophilic (i.e. neurons appear black). The entire brain of each animal was sectioned and surveyed for argyrophilia. Argyrophilia was found in the olfactory bulb, APC, and perirhinal cortex at the 4-day time point (Fig. 2), and the lateral entorhinal cortex and temporal dentate gyrus (Fig. 3). Note that CON show little or no argyrophilia. Extensive argyrophilia was apparent throughout the olfactory bulb at the 2-day time point. Argyrophilic processes and scattered argyrophilic cell bodies were also apparent in the cortical regions and dentate gyrus after 2 days of binge treatment (data not shown). After 4 days of binge treatment, argyrophilia remained prominent in the glomeruli of the olfactory bulb (Fig. 2), denoting degeneration of the axonal elements of the olfactory sensory neurons in ETOH animals. Degenerating granule cells were also found throughout the olfactory granule cell layer, apparent by the dendritic processes and lack of axon. The dendrites of these granule cells were also argyrophilic, showing granule cell innervation of the deep layers of the external plexiform layer. Neurodegeneration was apparent throughout the APC in ETOH animals (Fig. 2). Superficial pyramidal neurons (Layer II) and their processes (extending into Layers I and III) show argyrophilia in both the dorsal and ventral aspects of the piriform cortex. Neurons of the ventral bank of the perirhinal cortex (Fig. 2) also displayed argyrophilia in animals exposed to binge ethanol for 4 days. Pyramidal neurons in Layer III were argyrophilic with dendritic processes extending into Layer I. Extensive argyrophilia was found in the entorhinal cortex (Fig. 3) of ETOH animals. This neurodegeneration involved pyramidal and multipolar neurons of Layer III as described by Cajal (1911). The dendritic processes of these neurons extending into Layer I were argyrophilic as were the apical dendrites, which extend radially in Layer III. Argyrophilic neurons were clustered together, lending a columnar appearance to the damaged areas, suggesting that neurons from individual input fiber systems are vulnerable. Ethanol-induced neurodegeneration in the hippocampal formation was found in the temporal dentate gyrus (Fig. 3). Degenerating granule cells were apparent with argyrophilic dendrites extending into the stratum moleculare and argyrophilic axonal elements extending into the polymorphic layer (hilus). Damage was normally confined in the temporal dentate gyrus. Sparse argyrophilia was occasionally seen in the septal dentate gyrus but only concomitant with extensive argyrophilia in the temporal dentate gyrus. Neurodegeneration visualized by amino cupric silver staining was apparent in the olfactory bulb, piriform, perirhinal and lateral entorhinal cortex, and temporal dentate gyrus, consistent with previous studies (Collins et al., 1996, 1998; Corso et al., 1998; Crews et al., 2000; Penland et al., 2001; Zou et al., 1996). These cortical areas receive common excitatory inputs from the olfactory bulb and are interconnected to form a hippocampal circuit implicated in some forms of memory function (Haberly, 1998).

3.2. Behavioral testing

In order to assess the effects of binge ethanol exposure on nonspecific motor function and exploration, we assessed behavior in the open-field test. There was no significant difference between the groups as measured by the variables

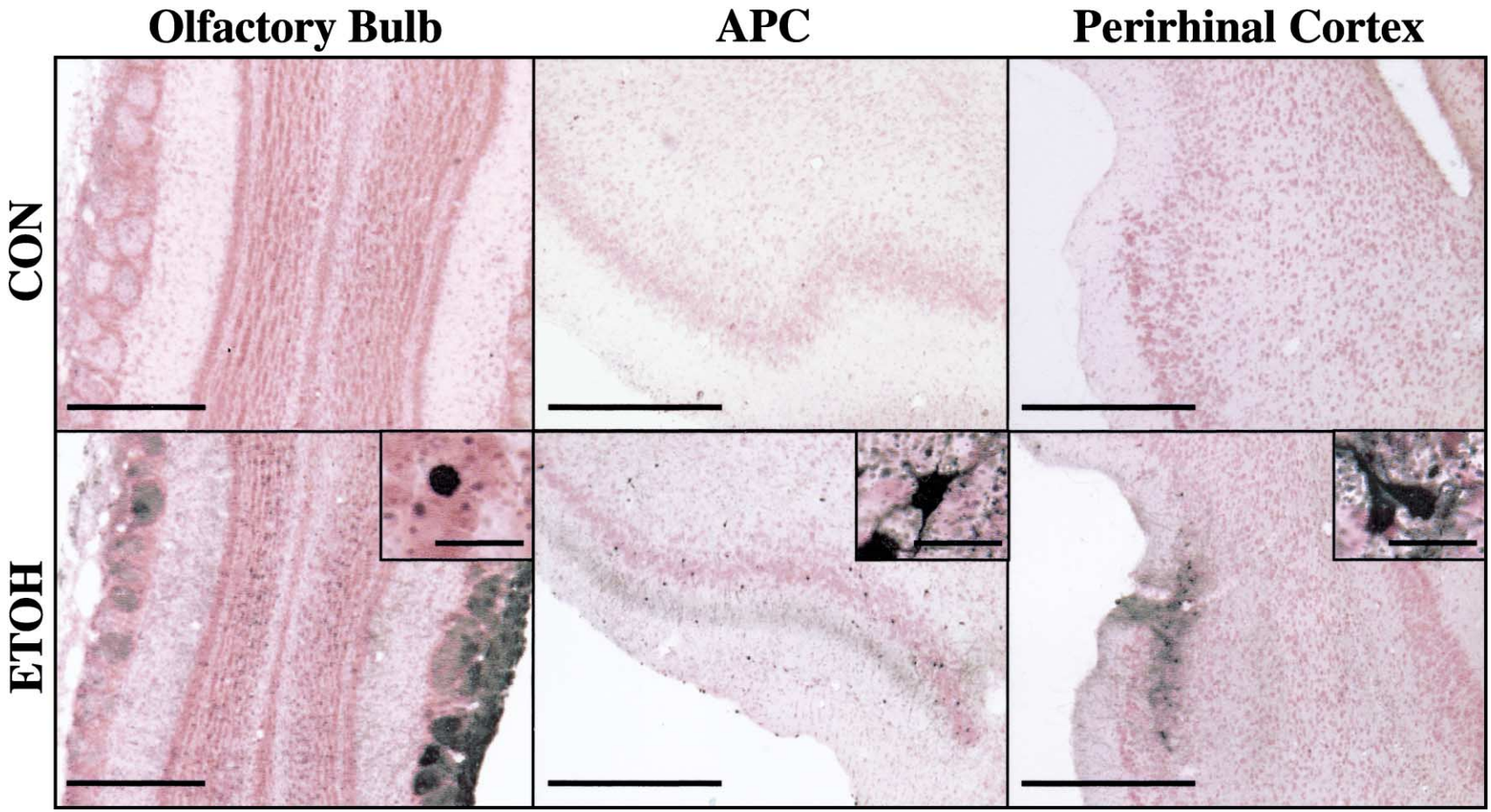

Fig. 2. Neurodegeneration caused by a 4-day ethanol binge. Sections (40 μm) were stained by amino cupric silver stain to visualize neurodegeneration. Dead neurons are argyrophilic and stain black. Shown in the top row are control animals that received isocaloric control diet, and in the bottom row are animals administered 25% ethanol diet for 4 days and immediately sacrificed. The brain regions shown are olfactory bulb, APC, and perirhinal cortex. The scale bar is 500 μm, inset bar is 20 μm.

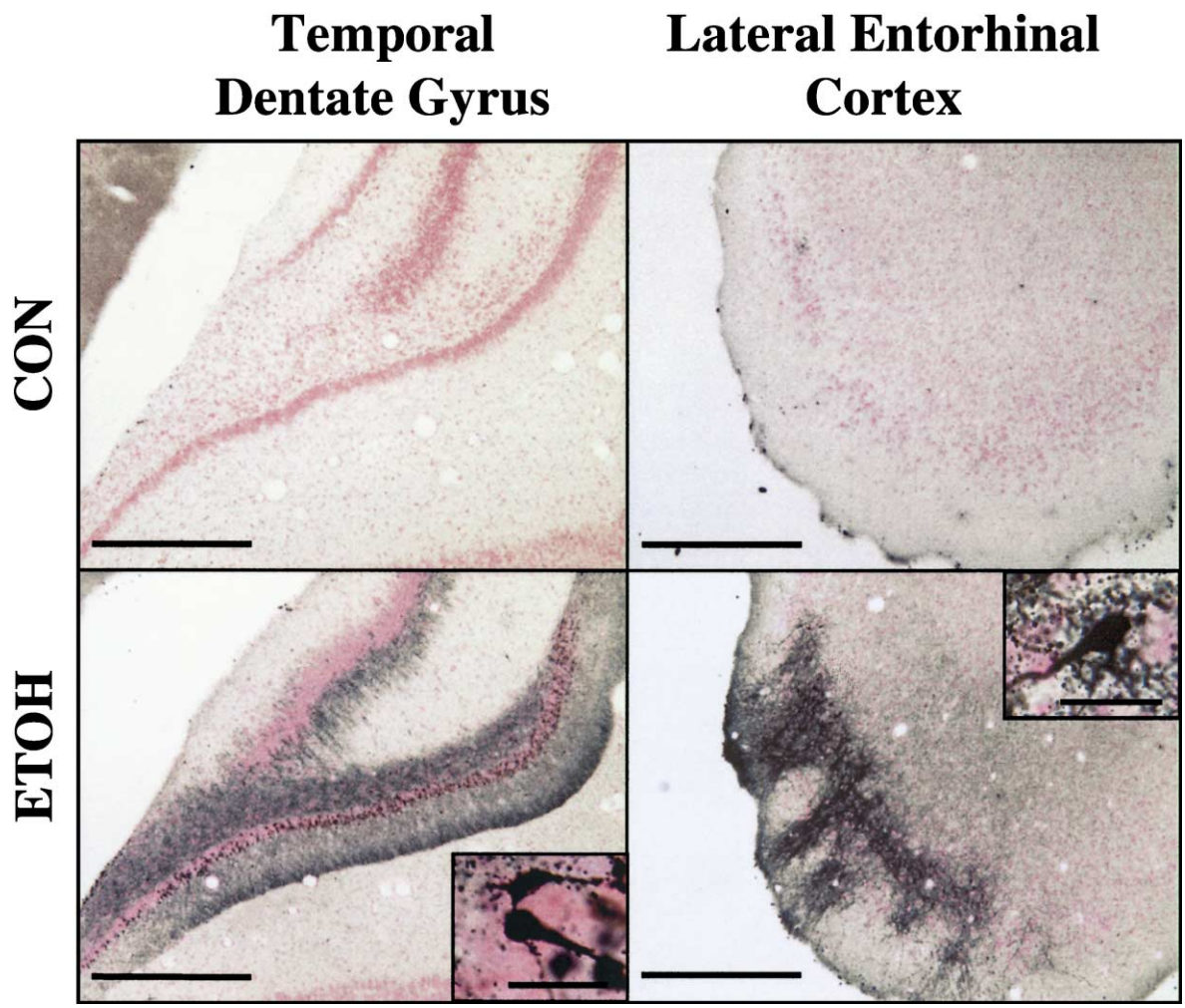

Fig. 3. Neurodegeneration caused by a 4-day ethanol binge. Sections (40 μm) were stained by amino cupric silver stain to visualize neurodegeneration. Dead neurons are argyrophilic and stain black. Shown in the top row are control animals that received isocaloric control diet, and in the bottom row are animals administered 25% ethanol diet for 4 days and immediately sacrificed. The brain regions shown are temporal dentate gyrus and lateral entorhinal cortex. Scale bars are 500 μm, inset bars are 20 μm.

of distance traveled [$F(1, 14)=2.824$; $P>.05$], time ambulatory [$F(1, 14)=2.575$; $P>.05$], and velocity [$F(1, 14)=0.015$; $P>.05$] (data not shown).

3.3. Spatial reference memory task: place learning

Binge ethanol exposure did not affect acquisition of the spatial reference memory task [$F(1, 14)=0.547$; $P>.05$] (Fig. 4A). Both treatment groups were able to learn the location of the submerged platform based on spatial (extramaze) cues, showing a decrease in distances swam with training [$F(6, 84)=28.101$; $P<.0001$]. There was no influence of ethanol exposure on the rate of acquisition [$F(6, 84)=0.344$; $P>.05$].

3.4. Spatial working memory task: four-trial place learning

Following the reversal learning task, animals were trained on the working memory task for 5 days (Fig. 4B). Each day, the submerged platform was moved to a new, randomly chosen quadrant. All animals acquired the task [$F(4, 56)=4.432$; $P<.005$], and there was no significant effect of binge ethanol treatment on this task [$F(1, 14)=0.031$; $P>.05$]. There was no influence of ethanol exposure on the rate of acquisition [$F(4, 56)=0.165$; $P>.05$].

To ensure that the ETOH animals did not have perceptual or motivational deficits, the animals were tested in a visually cued, nonspatial learning task (data not shown). All animals acquired the task [$F(1, 14)=19.923$; $P<.005$], and there was no significant effect of binge ethanol treatment on the nonspatial learning task [$F(1, 14)=0.876$; $P>.05$]. There was no influence of ethanol exposure on the rate of acquisition [$F(1, 14)=0.001$; $P>.05$].

3.5. Reversal learning task: new place learning

On the day following the completion of the 7-day reference memory task, animals were tested in a reversal

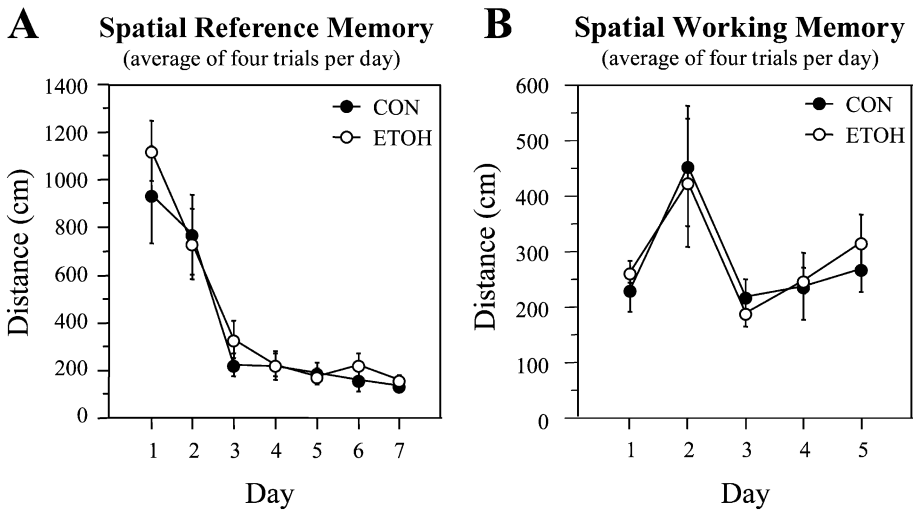

Fig. 4. Spatial reference and working memory tested in the Morris water maze. (A) Rats were trained to the location of a submerged platform by four trials a day over 7 days as a measure of place learning. Shown is the mean±S.E.M of the average of each animals four trials per day. There was no significant difference in acquisition of the task between treatment groups [$F(1, 14)=0.547$; $P>.05$]. (B) The submerged platform was moved to a new, pseudorandomly chosen quadrant each day for 5 days, and the animals were allowed four trials a day at the new position. Shown is the mean±S.E.M. of the average of each animals four trials per day. There was no significant difference in spatial working memory between treatment groups [$F(1, 14)=0.031$; $P>.05$].

learning task (Figs. 5 and 6). The submerged platform was placed in the southwest quadrant, opposite the platform location used in the reference memory task. Both treatment groups were able to learn the new location of the submerged platform [$F(3, 42)=37.805$; $P<.0001$] (data not shown). Analysis of raw distance data did not reveal an overall difference between groups [$F(1, 14)=3.98$; $P>.05$] or an influence of ethanol exposure on the rate of acquisition [$F(3, 42)=1.25$; $P>.05$]. However, there were significant differences between the treatment groups on several other learning related variables. To evaluate when an animal had successfully learned the reversal task, a criterion was established. The level was set at two standard deviations above the mean distance traveled by all animals on the last day of the reference memory task ($\bar{x}=147.3\pm46.9$ cm). The criterion (241.1 cm) includes the distances traveled by 95% of the population on this learned task. The number of trials required to learn the task (i.e. reach criterion) on the reversal learning task (Fig. 5A) was significantly different between treatment groups. Every CON animal reached this criterion level on the second trial of the reversal learning task, whereas four of eight ETOH animals required three or more

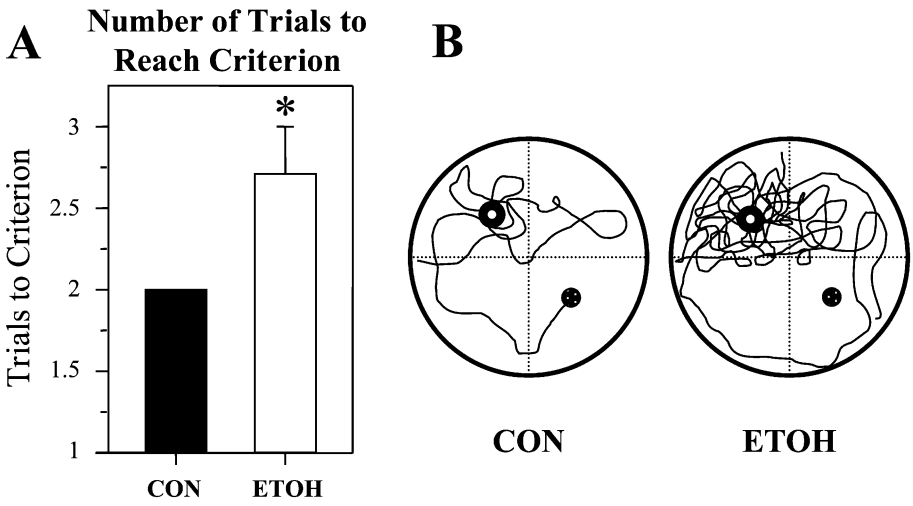

Fig. 5. Reversal learning task in the Morris water maze. After a 7-day acquisition of the platform location, the submerged platform was moved to the opposite quadrant and animals were given four trials (12 days post binge treatment). (A) Shown is the mean±S.E.M. of the number of trials to reach criterion. Criterion was set at two standard deviations from the distance to platform on the last day of the reference memory task. ETOH animals required a significantly greater number of trials to reach criterion than CON animals [$t(14)=-2.376$; $^*P<.05$]. (B) A vertical view of the track taken by a CON and an ETOH rat during the first trial of the reversal learning task. The open circle representation the location of the submerged platform the animals were trained to add the patterned circle represents the location of the platform during the reversal learning task. Note the perseverative behavior shown by the ETOH animal with numerous reentries into the original goal quadrant. The ETOH animal also failed to reach the new platform locations within the 90-s ceiling.

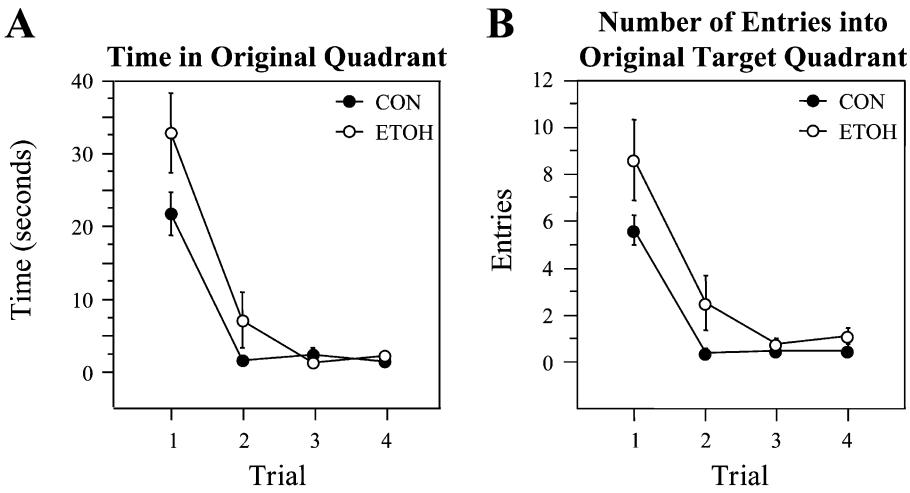

Fig. 6. Reversal learning task in the Morris water maze. After a 7-day acquisition of the platform location, the submerged platform was moved to the opposite quadrant and animals were given four trials (12 days post binge treatment). (A) Shown is the mean±S.E.M. of the time spent in the original goal quadrant. ETOH animals spent significantly more time in the original goal quadrant than CON animals [$F(1, 14)=4.64$; $P=.053$]. (B) Shown is the mean±S.E.M. of number of entries into the original goal quadrant. ETOH animals entered the original goal quadrant a significantly greater number of times than CON animals [$F(1, 14)=5.600$; $P<.05$].

trials to reach criterion level [$t(14)=-2.376$; $P<.05$]. ETOH animals entered the original goal quadrant more often [$F(1, 14)=5.600$; $P<.05$] and spent more time in the original goal quadrant [$F(1, 14)=4.64$; $P=.053$], indicating a failure to reverse the original learning as quickly as control animals. ETOH animals averaged 53% more entries into the original goal quadrant on the first trial compared to CON animals (Fig. 6B) and spent an average of 53% more time in the original goal quadrant on the first trial compared to CON animals (Fig. 6A). These differences can be noted in the tracings shown in Fig. 5B. Each tracing is the track taken by a CON or ETOH animal on the first trial of the reversal learning task. Binge-treated animals required more trials to learn the reversal task, spent more time in and had more entries into the original goal quadrant, indicating a deficit in reversal learning and a tendency to perseverate on the previously learned response.

3.6. [^{3}H]PK-11195 binding

To investigate binge ethanol-induced brain damage in rats that were tested behaviorally, [^{3}H]-PK binding was determined. Previous studies have indicated that silver stained neuronal damage is no longer apparent 1 week after binge treatment likely due to scavenging of dead neuronal elements by microglia and other cells (Crews et al., 2000; Switzer, 2000). Therefore, to assess brain damage in animals that underwent three weeks of behavioral testing, [^{3}H]PK-11195 binding to the peripheral benzodiazepine binding site was quantified as an index of microglial proliferation and CNS damage (Stephenson et al., 1995) (Fig. 7). Optical density measurements were sampled for the APC, perirhinal cortex, temporal dentate gyrus, and lateral entorhinal cortex, areas known to be damaged by binge treatment (Fig. 8). In general, [^{3}H]PK-11195 binding was increased by ethanol treatment [$F(1, 11)=5.119$; $P<.05$]. Further post hoc analysis revealed that [^{3}H]PK-11195 binding in the APC in ETOH was significantly different than CON ($P<.05$). [^{3}H]PK-

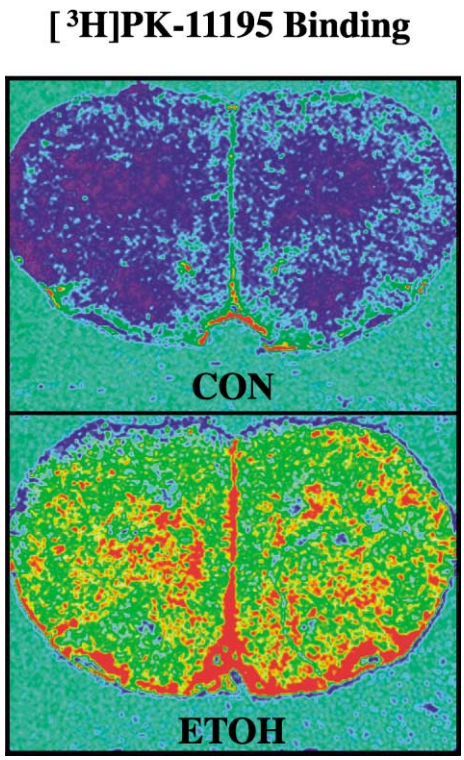

Fig. 7. [^{3}H]PK-11195 autoradiography. Shown is an artificially colored autoradiographic images of [^{3}H]PK-11195 binding at the level of the APC (2.7 mm bregma).

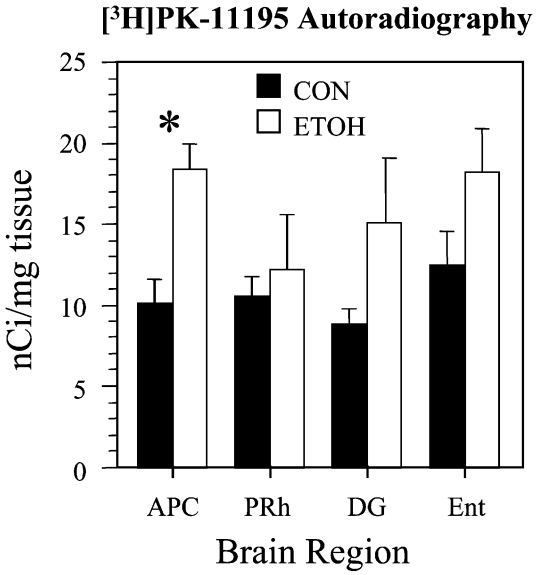

Fig. 8. Quantification of [^{3}H]PK-11195 autoradiography in animals subjected to behavioral testing. Two days after the end of behavioral testing, animals were sacrificed, and [^{3}H]PK-11195 autoradiography was performed on the sectioned brains. Shown is the mean±S.E.M. of [^{3}H]PK-11195 binding, in nCi/mg tissue. There was a significant effect of treatment [$F(1, 11)=5.119$; $P<.05$]. Post hoc analysis revealed that [^{3}H]PK-11195 binding in the APC in ETOH animals was significantly different than CON (*$P<.01$). APC—anterior piriform cortex; PRh—perirhinal cortex; DG—temporal dentate gyrus; Ent—lateral entorhinal cortex.

11195 binding increased 81% from 10.1±1.5 nCi/mg tissue to 18.3±1.9 nCi/mg tissue. Approximately 3 weeks after binge treatment, [^{3}H]PK-11195 binding was elevated, indicative of long-term changes in corticolimbic structures resulting from binge ethanol-induced neurodegeneration.

4. Discussion

Decades of chronic alcohol consumption in humans is known to cause neurodegeneration and cognitive dysfunction. Alcoholics suffer from impairment of spatial memory and abnormal response perseveration (Bowden and McCarter, 1993; Oscar-Berman et al., 1992). Alcoholics also show decreased neuropsychological performance compared to peer nonalcoholics on tests of learning, memory, abstract thinking, problem solving, visuospatial and perceptual motor functioning, and information processing (Parsons, 1993). These patients are not only less accurate but take considerably longer to complete tasks (Glenn and Parsons, 1990). Some abstinent alcoholics appear to have permanent cognitive impairments, particularly in memory and visual–spatial–motor skills (Di Sclafani et al., 1995). Alcoholics also suffer damage to the entorhinal cortex, hippocampal formation, and frontal cortex. This includes grey and white matter loss in the cortex (de la Monte, 1988; Jernigan et al., 1991), loss of hippocampal volume and shrinkage of hippocampal neurons (Harding et al., 1997; Laakso et al., 2000; Sullivan et al., 1995), and shrinkage in the entorhinal cortex (Ibanez et al., 1995). Thus, years of heavy alcohol consumption result in an array of cognitive deficiencies and damage to broad regions of the brain.

While researchers have made great strides in describing the neuroanatomical and cognitive changes that occur with chronic alcohol use, it is still very difficult to relate these two bodies of research. Though it is often hypothesized that the degree of deficit is dependent upon the duration and amount of alcohol consumed (National Institute on Alcohol Abuse and Alcoholism, 2000), researchers have been unable to consistently correlate total lifetime consumption of alcohol with cognitive deficits or structural changes. Several factors may cause this disparity, including nutrition (Caine et al., 1997), anemia (Pfefferbaum et al., 1992), and differences in BALs and/or alcohol metabolism (York and Welte, 1994).

One factor that has not been adequately explored is drinking pattern. One study, in particular, illustrates the differences between a binging alcoholic and a chronic alcoholic. Researchers found that binging and chronic alcoholics consumed equal amounts of alcohol per month, but binging alcoholics had an average of 83 days between drinking episodes compared to 0.7 days in chronic alcoholics (McMahon et al., 1991). Binging alcoholics consume huge amounts of alcohol in very short periods of time. It is possible that these episodes of sustained, high BALs are responsible for the cognitive and structural changes seen in long-term alcoholics, explaining the lack of correlation between lifetime consumption and degree of dysfunction. High BALs and binge drinking have already been documented to cause neuronal death in fetal alcohol syndrome, where a small daily dose of alcohol is more damaging than a larger daily dose, if it is consumed in a binge-like pattern that produces higher BALs (Goodlett et al., 1997; West et al., 1990). Thus, the cognitive deficits and structural changes seen in long-term alcoholics may be due to episodes of binge drinking and not chronic, daily consumption of alcohol.

In this study, we found that our 4-day binge ethanol paradigm resulted in both learning impairments and damage to the corticolimbic system (olfactory bulb, piriform cortex, perirhinal cortex, entorhinal cortex, and the hippocampal dentate gyrus). To our knowledge, this is the first evidence that short-term binge ethanol exposure can lead to long-term changes in cognition. Although our binge exposure paradigm represents only one binge, whereas alcoholics have likely had multiple binges, there appear to be many similarities in the pattern of brain damage and dysfunction.

The corticolimbic system is a circuit comprised of portions of the olfactory cortex and hippocampus. The olfactory cortex is comprised of regions receiving input from the olfactory bulb, such as the olfactory tubercle, piriform, entorhinal, agranular insular, perirhinal, and amygdaloid cortices (Haberly, 1998; Liu and Bilkey, 1997). The circuit then continues from the entorhinal cortex into the hippocampal formation, considered to be critical for learn-

ing and memory formation (Jarrard, 1993). The cognitive functions associated with these brain regions were discovered through lesion studies. Aspiration lesions of the entorhinal–perirhinal cortices cause modest spatial learning deficits (Nagahara et al., 1995), while ibotenate- and NMDA-induced entorhinal cortex lesions cause deficits in reversal learning in a spatial task (Hagan et al., 1992; Pouzet et al., 1999). Aspiration lesions of the hippocampus cause spatial reference and working memory deficits (Aggleton et al., 1986; Jarrard, 1993). This research suggests that the extensive damage to the entorhinal cortex is responsible for the reversal learning deficits found following a single episode of binge ethanol treatment.

In this study, we did not detect deficits in the initial acquisition of spatial learning and memory or motor function weeks after a single, 4-day binge. Nor did we find changes in visually cued behavior, indicating that there was no change in perception or motivation due to the ethanol exposure. However, the single binge exposure did impair reversal learning. This impairment could be interpreted to mean that the binge-treated animals learned the original reference memory task better than control animals, and thus spent more time searching in the original goal quadrant during the reversal learning task. However, this is unlikely since there was no difference between treatment groups in either the rate of initial learning or the performance on the final day of reference memory training before the reversal task. Another possible interpretation of the reversal learning impairment is that binge-treated animals were more likely to perseverate in behavior (i.e. returning to the original goal quadrant), which was no longer consistent with successful completion of the task. While the reversal learning task in the Morris water maze is not a classical test of perseveration, the greater number of entries and increased amount of time spent in the original goal quadrant by the ethanol-treated animals do indicate perseverative responses toward the original goal quadrant. This could indicate a deficit in the ability to shift sets within a learning paradigm and suggests a cognitive substrate for the kinds of deficits in executive functioning that are often manifested by alcoholics. In addition, this type of deficit in response inhibition could be a component of alcohol addiction, related to the difficulty that alcoholics have changing their behavior even when their alcohol use is associated with negative consequences (Bowden et al., 2001).

Some of the CNS deficits alcoholics suffer appear to improve over a 4- to 5-year period of abstinence (Brandt et al., 1983). This is consistent with the recovery of brain mass in alcoholics over several months of abstinence (Pfefferbaum et al., 1995). Our previous studies with silver stain indicated that dying neurons were scavenged and the silver stain no longer apparent one week after binge treatment (Crews et al., 2000). Studies of long-term ethanol treatment have indicated increased [^{3}H]PK-11195 binding in brain (Syapin and Alkana, 1988), an index of CNS damage that quantifies gliosis following brain damage (Stephenson et al., 1995). We found a significant increase in [^{3}H]PK-11195 binding in our ethanol-treated animals that showed deficits in reversal learning, consistent with long-term CNS damage following our binge treatment.

It is interesting that after a relatively short binge treatment, we found significant neuronal degeneration and learning deficits. Individuals who abuse alcohol often binge drink for years before reaching criteria for alcohol dependence, however, it is possible that brain damage and perseverative dysfunction, which contribute to the progression to addiction, may precede the emergence of frank physical dependence among people who drink in a binge pattern. Recent studies have indicated that two risk factors for developing alcohol dependence, adolescent drinking and genetics, are also risk factors for excessive binge ethanol-induced brain damage (Bowden et al., 2001). Epidemiological studies have indicated that 40% of college students are heavy drinkers, and during the 1990s, there was a dramatic increase in frequent heavy drinking among college students (Wechsler et al., 2000). Although our binge drinking paradigm and behavioral studies model extreme binge drinking, we see brain damage after only 2 days of this treatment. Greater cumulative damage should result following multiple binges, which more closely models human binge drinking patterns. In any case, our finding that a single binge episode causes long-term CNS and behavioral changes highlights the importance of understanding the effects of binge drinking on CNS function and addiction.

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