4-Hydroxynonenal, a Product of Lipid Peroxidation, Damages Cholinergic Neurons and Impairs Visuospatial Memory in Rats

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Abstract. The mechanisms that underlie cholinergic neuronal degeneration in Alzheimer disease (AD) are unclear, but recent data suggest that oxidative stress plays a role. We report that 4-hydroxynonenal (HNE), an aldehyde product of lipid peroxidation, damages and kills basal forebrain cholinergic neurons when administered intraparenchymally. Examination of Nissl-stained brain sections following unilateral HNE infusion revealed widespread neuronal loss in basal forebrain ipsilaterally to the injection, but not on the contralateral side. Levels of choline acetyltransferase activity and immunoreactivity in the ipsilateral basal forebrain and hippocampus were significantly reduced by 60–80% seven days following HNE administration. Performance in Morris water maze tasks of visuospatial memory was severely impaired in a dose-dependent manner seven days following bilateral administration of HNE. Bilateral infusion of FeCl₃ (an inducer of membrane lipid peroxidation) into the basal forebrain caused neuron loss and decreased choline acetyltransferase immunoreactivity and deficits in visuospatial memory. Additionally, FeCl₃ infusion increased HNE immunoreactivity, implicating HNE in iron-induced oxidative damage. Because recent studies have demonstrated HNE adds to degenerating neurons in AD brain, the present findings suggest a role for HNE in damage to cholinergic neurons in AD.

Key Words: Alzheimer disease; Choline acetyltransferase; Hippocampus; Iron; Morris water maze.

INTRODUCTION

Although the cause of neuronal death in Alzheimer disease (AD) has not been established, increasing data support a prominent role for oxidative stress (1, 3, 4). Increased levels of protein (5), lipid (6), and DNA (7) oxidation have been documented in postmortem tissue taken from vulnerable regions of AD brain. Moreover, the major proteins comprising neurofibrillary tangles (tau) and senile plaques (amyloid β peptide-Aβ) are glycolated (8–10), suggesting high levels of oxidative stress in the microenvironment of degenerating neurons. Although the mechanisms leading to oxidative stress in AD have not been established, considerable data suggest a role for Aβ, including Aβ is associated with degenerating neurons in AD brain (4); mutations in βAPP are causally linked to some inherited forms of AD (11–13); transgenic mice expressing a human βAPP mutation exhibit age-dependent Aβ deposition and some neuropathological and behavioral features similar to AD (14, 15); and Aβ is toxic to cultured neurons with features of cell death similar to those seen in neurofibrillary tangles of AD (16, 17). The mechanism of Aβ toxicity appears to involve induction of membrane lipid peroxidation (18, 19) and disruption of ion homeostasis (16, 20). Aβ can impair, apparently via peroxidative mechanisms, a variety of regulatory systems in the plasma membrane, including ion-motive ATPases (20), glutamate transport (21), glucose transport (22), and the coupling of muscarinic acetylcholine receptors to the GTP-binding protein Gq11 (23).

When membrane lipids are peroxidized, the aldehyde 4-hydroxynonenal (HNE) is released. Studies of nonneural cells showed that HNE is cytotoxic at concentrations achieved in cells exposed to oxidative insults (24). Recent studies have also documented increased levels of HNE adducts in neurofibrillary tangles in AD (25) and increased levels of HNE in brain and cerebrospinal fluid of AD patients (26, 27). Cell culture studies have demonstrated that HNE can crosslink cytoskeletal proteins, and lead to glutathione depletion and inactivation of glutathione peroxidase (28, 29). HNE in vitro can also impair ion-motive ATPase activities, increase intracellular calcium levels, and promote excitotoxic cell death and apoptosis in cultured rat hippocampal neurons (30, 31), thus suggesting that HNE is an important mediator of Aβ-induced disruption of neuronal ion homeostasis and cell death. HNE also disrupts glucose transport in cultured hippocampal neurons (22) and glutamate transport in cortical synaptosomes (21). However, it is not known whether HNE is neurotoxic in vivo.

Selective degeneration of cholinergic neurons in the basal forebrain is a prominent feature of AD that is strongly correlated with deficits in cognitive function (32, 33). Basal forebrain cholinergic neurons innervate brain regions critically involved in learning and memory, notably the hippocampus and cerebral cortex, and animal

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studies have shown that damage to the cholinergic neurons can impair learning and memory (34). Cholinergic neuronal loss induced by axotomy or excitotoxins has been correlated with impaired visuospatial memory (35–38). In the present study we administered HNE and FeCl₂ (an inducer of lipid peroxidation) into the basal forebrain of adult rats, and assessed their effects on performance in Morris water maze learning and memory tasks and degeneration of cholinergic neurons. We found that FeCl₂ and HNE damage basal forebrain cholinergic neurons and impair visuospatial memory in rats, suggesting that lipid peroxidation and HNE production could contribute to the cognitive decline and neuronal damage observed in AD.

MATERIALS AND METHODS

Stereotoxic Administration of HNE and FeCl₂, and Histological Analyses

Adult male Sprague-Dawley rats (200–250 g) were anesthetized with ketamine/rompun, placed in a stereotoxic head holder, and the skull exposed along the midline. HNE (0.64 or 3.2 μmol in a volume of 0.5 μl), FeCl₂ (0.2 μmol in a volume of 1 μl), or vehicle were injected unilaterally or bilaterally into the basal forebrain using the following stereotoxic coordinates (39): D/V = −5.5, M/L = +2.5, A/P = −1.4 from bregma. HNE (Cayman Chemicals) was dissolved in ethanol and FeCl₂ (Sigma) was dissolved in sterile water. At the indicated times following HNE, FeCl₂ or vehicle administration rats were anesthetized with sodium pentobarbital (75 mg/ml; i.p.), and perfused transcardially with saline followed by cold phosphate-buffered 4% paraformaldehyde. Coronal brain sections (30 μM) were cut on a freezing microtome. Sections were stained with cresyl violet, or were immunostained with ChAT or HNE antibodies using methods described previously (40–42). Briefly, following an overnight incubation at 4°C in the presence of blocking solution (5% horse serum in PBS containing 0.2% Triton X-100), sections were incubated for 24 hours (h) in the presence of primary antibody (4°C). Sections were then incubated for 4 h in the presence of biotinylated secondary antibody at room temperature with continued incubation overnight at 4°C. The sections were then treated with ABC reagent (Vector Laboratories) for 1 h, followed by a 20 minute incubation in a solution containing 0.1% hydrogen peroxide, 0.05% diaminobenzidine tetrahydrochloride, 0.025% cobalt chloride, and 0.02% nickel ammonium sulfate. Sections were then mounted on slides, dehydrated, and mounted. To control for nonspecific staining, additional sections were subjected to the immunostaining procedure without primary antibody. Additionally, nonspecific staining was determined by competition for HNE antibody with a solution of HNE-conjugated bovine serum albumin, which has been demonstrated to compete effectively for HNE antiserum (43). Briefly, 1 mM HNE was incubated overnight at room temperature with a 5-fold excess of bovine serum albumin, after which this solution was added to a concentrated solution of HNE antiserum, and applied to tissue sections. Either of such control procedures completely abolished HNE immunoreactivity in both FeCl₂ and HNE injected animals (data not shown). Stained sections were visualized and photographed under bright-field optics.

ChAT Activity Assay

ChAT activity was quantified using a modification of the method of Shea and Aprison (44). Briefly, tissue was homogenized in 50 mM sodium phosphate buffer (pH 7.4) and protein concentration was determined (Lowry method). Samples (250 μl) were mixed with 10 μl of a solution containing 20 mM NaHPO₄, 0.3 mM eserine sulfate, and 2 mM choline. The reaction was initiated by addition of 10 μl 2.5 mM [³H]-acetyl coenzyme A, and the reaction was allowed to proceed for 1 h at 37°C. The reaction was stopped by the addition of 230 μl of a solution containing 1 mM acetylcholine chloride and 0.26% formic acid. The radiolabeled acetylcholine was extracted by addition of 600 μl of 30 mg/ml sodium tetraphenylboride in butanitrite, and vortexed for 30 sec. The samples were centrifuged at 800 × g for 5 min, and 350 μl of the organic layer was added to 5 ml scintillation cocktail, and radioactivity was quantified using a Packard 2500 TR automated counter. Values were calculated as pmol acetylcholine produced/mg protein/min.

Behavioral Testing

The apparatus for performing Morris water maze tasks of visuospatial memory has been described in detail previously (45). The testing procedure was essentially identical to that described in our previous studies (46, 47). Each rat received 3 four-trial blocks of training, with each block separated by 1 h; within each block trials were separated by 5 min. On each trial, the rat was released at one of four compass locations (North, South, East, or West) with its head facing the side of the pool. It was allowed to swim until it climbed onto the hidden platform or until 60 seconds had elapsed. The goal latency measure consisted of the time to locate the platform, with a maximum score set at 60 seconds. Rats that failed to find the platform within 60 seconds were placed there so that all subjects spent 10 seconds on the platform at the end of each trial. Each rat was given a probe trial immediately after the last training trial. The hidden platform was removed and the subject was allowed to swim for 90 s after being released from the north compass point. Each probe trial was videotaped by an overhead camera, and the videotapes were analyzed by a video motion analyzer (Videomex-V, Columbus Instruments). The dependent variable for probe trials was the number of target crossings.

TBARS Assay

Malondialdehyde concentration was determined by using the thiobarbituric acid reactive substances assay (TBARS), as described by Kovatchich and Mishra (48). Briefly, dissected basal forebrain and hippocampus were homogenized in ice cold PBS, precipitated in 5% trichloroacetic acid, and an aliquot of the trichloroacetic acid precipitate was incubated with 0.335% thiobarbituric acid in 50% glacial acetic acid for 1 hour at 90–95°C. Lipids were then extracted in butanol and fluorescence detected using a Millipore Cytosensor 2350 fluorescence plate reader with excitation and emission wavelengths set at 500 and 525 nm.

Statistical Analyses

All data were analyzed using one-way ANOVA, followed by Scheffe’s post-hoc analyses to determine statistical significance. p values <0.05 were considered statistically significant.
RESULTS
HNE Damages Basal Forebrain Cholinergic Neurons

Rats were divided into 3 groups, with one group receiving a vehicle (ethanol) injection into the basal forebrain, one group receiving unilateral 0.64 μmol HNE, and one group receiving unilateral 3.2 μmol HNE. Half of the rats in each group were killed 24 h later, and those remaining in each group were killed 7 days later. Examination of Nissl-stained sections from rats killed at the 24 hour time point revealed a large decrease in cresyl violet-positive cells in the ipsilateral basal forebrain of rats administered HNE compared with sections from rats administered the vehicle, with greater damage occurring in those receiving the higher dose of HNE (Fig. 1). At the 7 day time point, damage to basal forebrain was extensive in the HNE-injected rats, whereas no evidence of neuron loss was seen in the vehicle-injected basal forebrain (data not shown). No damage to neurons in the contralateral basal forebrain was observed in either vehicle- or HNE-injected rats (Fig. 1). HNE-induced cytotoxicity was not limited to neurons, and at high doses appears to damage all cells in the vicinity of the injection (Fig. 1). In vehicle-injected basal forebrain, numerous intact ChAT-immunoreactive neurons were present; the pattern of ChAT immunoreactivity was essentially identical to that of the contralateral uninjected basal forebrain (Fig. 2). In contrast, a marked decrease in the number of ChAT-immunoreactive neurons was observed in basal forebrain administered HNE, with the loss being more pronounced in rats receiving the high dose of HNE (Fig. 2). Moreover, loss of ChAT immunoreactivity was considerably more pronounced at the 7 day time point compared with the 24 hour time point. HNE did not affect the pattern of ChAT immunoreactivity in the contralateral basal forebrain (Fig. 2).

HNE Causes a Decrease in ChAT Activity Levels in Basal Forebrain and Hippocampus

Twenty-four h following administration of 0.64 and 3.2 μmol HNE, there was a small (10–15%) decrease in ChAT activity in basal forebrain on the injected side, but the decrease did not reach statistical significance (Fig. 3). However, at the 7 day time point, there were large and significant decreases in ChAT activity in the ipsilateral basal forebrain of rats receiving 0.64 μmol HNE (40% decrease) and 3.2 μmol HNE (70% decrease). ChAT activity was also significantly reduced in ipsilateral hippocampus at the 7 day time point in rats receiving 3.2 μmol HNE (Fig. 3). No differences in ChAT activity among treatment groups were seen in the contralateral basal forebrain and hippocampus (data not shown).

HNE Impairs Visuospatial Learning and Memory

Damage to cholinergic basal forebrain neurons is correlated with impairments in learning and memory in humans with AD (32), and with impaired performance on visuospatial memory tasks in rats (34–37). We therefore examined the effects of bilateral HNE injection into the basal forebrain on performance in goal latency and probe trials in the Morris water maze. Rats were tested 7 d following stereotaxic injection of vehicle or HNE. Whereas goal latencies significantly decreased across trial blocks in vehicle-injected rats (indicating that animals were learning the location of the platform), there was only a small decrease in goal latency in animals treated with 3.2 μmol HNE (Fig. 4A). Goal latencies for the group receiving 0.64 μmol HNE were intermediate to those of the groups receiving vehicle or 3.2 μmol HNE. ANOVA revealed a highly significant difference between the vehicle group and the 3.2 μmol HNE group (p < 0.001), and a significant difference between the vehicle group and the 0.64 μmol HNE group (p < 0.05). In the probe trial, there were significant differences between the vehicle group and the 0.64 μmol HNE group (p < 0.05), and between the vehicle group and the 3.2 μmol HNE group (p < 0.001) in the number of target crossings (Fig. 4B). Swim times for rats among the various groups were not significantly different (data not shown), indicating that the impaired performance of the rats administered HNE was not due to motor deficits. Unilateral injections of HNE into the basal forebrain did not lead to impaired performance in goal latency or probe trials (data not shown).

Iron Administration Damages Basal Forebrain Neurons, Induces Lipid Peroxidation and HNE Production, and Impairs Visuospatial Memory

Our previous data from studies of cultured neural cells indicated that neuronal death induced by oxidative insults that induce membrane lipid peroxidation is mediated, at least in part, by HNE (22, 30, 31). Previous studies in adult rats had shown that injection of FeCl₃ into the cortex or hippocampus results in extensive lipid peroxidation (49, 50). We therefore examined the effects of FeCl₃ injection into the basal forebrain on cholinergic neurons and spatial memory. FeCl₃ (0.2 μmole) or vehicle was injected bilaterally into the basal forebrain, and 7 days later, rats were tested in the Morris water maze, euthanized, and the brains processed for histological analyses. Examination of Nissl-stained sections revealed a large decrease in cresyl violet-positive cells in the basal forebrain of rats administered FeCl₃ compared with rats administered vehicle (Fig. 5). There was also a marked loss of ChAT-immunoreactive neurons in the basal forebrain of animals that had received FeCl₃ (Fig. 5). To determine if iron administration induced HNE formation, brain sections from FeCl₃- and vehicle-injected animals were processed for HNE immunoreactivity as described previously (41, 43). Seven days following vehicle infusion, HNE immunoreactivity was limited to the cells in the immediate vicinity of the needle track (Fig. 5). However, in
**FeCl₂**-injected rats, HNE immunoreactivity was increased in enlarged, swollen cell bodies in the outer vicinity of the lesion (Fig. 5). The level of thiobarbituric acid-reactive substances (TBARS), an index of malondialdehyde formation consequent to lipid peroxidation, was also determined in rats 24 h following either HNE or FeCl₂ administration. FeCl₂, but not HNE, induced statistically significant increases in TBARS in the basal forebrain, but not in the hippocampus (Table 1). These data, combined with increased HNE immunoreactivity, indicates that FeCl₂ produces significant increases in membrane lipid peroxidation throughout the basal forebrain.

FeCl₂ induced profound deficits in both the goal latency and probe trials in the Morris water maze analyses (Fig. 6). Rats receiving bilateral FeCl₂ injections into the basal forebrain showed no decrease in goal latency over
the 3 trial blocks. The probe trial revealed highly significant differences between the FeCl$_2$ group and the vehicle group in the number of target crossings (Fig. 6). Taken together with the data for rats injected with HNE, these data suggest that membrane lipid peroxidation and HNE accumulation result in degeneration of basal forebrain cholinergic neurons and impaired visuospatial learning and retention.

**DISCUSSION**

Ferrous iron is a potent inducer of membrane lipid peroxidation that acts by catalyzing the conversion of hydrogen peroxide to the highly reactive hydroxyl radical (51, 52). Previous studies have shown that Fe$^{2+}$ can induce membrane lipid peroxidation in cultured hippocampal and cortical neurons (53). At the dose employed (0.2
Fig. 3. HNE administration into the basal forebrain induces a time-dependent decrease in ChAT activity levels in basal forebrain (BF) and hippocampus (Hipp). HNE at the indicated doses, or vehicle, was infused unilaterally into the basal forebrain; 24 h or 7 days later the rats were euthanized and levels of ChAT activity in basal forebrain and hippocampus were quantified. Values are the mean and SEM (n = 4–6 rats). *p < 0.05, **p < 0.001 compared with corresponding control value (ANOVA with Scheffe’s post-hoc tests).

μmole), FeCl₃ was previously shown to induce lipid peroxidation, as measured by malondialdehyde production, when injected into the hippocampus (49) or cerebral cortex (50) of adult rats. We found that introduction of Fe²⁺ into the basal forebrain caused both increased malondialdehyde levels and accumulation of HNE-protein conjugates as detected by immunoreactivity with anti-HNE antibody, suggesting the occurrence of lipid peroxidation in basal forebrain neurons. HNE immunoreactivity was broadly distributed throughout the regions of basal forebrain in which neuron damage and loss of ChAT immunoreactivity occurred. As with Fe²⁺, administration of HNE into the basal forebrain induced damage and death of cholinergic neurons as indicated by loss of ChAT-immunoreactive cells. Although it is not known whether Fe²⁺ plays a major role in the increased membrane lipid peroxidation that occurs in AD brain (6), recent studies have shown that levels of iron are increased in vulnerable brain regions of AD patients (54–56). Our data demonstrate that Fe²⁺ can induce the degeneration of basal cholinergic neurons, a neuronal population lost in AD (32, 33).

In addition to Fe²⁺, increasing evidence suggests that Aβ plays a prominent role in inducing membrane lipid peroxidation in neurons in AD. Recent histological analyses of AD brain tissue have shown that levels of HNE adducts (25, 27) and the lipid peroxidation–inducing agent peroxynitrite (57) are increased in degenerating neurons in Aβ-laden regions of AD brain, but not in brains from age-matched controls. Cell culture studies and analyses of synaptosomes have shown that Aβ induces membrane lipid peroxidation and accumulation of HNE-protein conjugates in hippocampal and cortical neurons (21, 22, 30), and that HNE can covalently bind to the microtubule-associated protein tau and prevent its dephosphorylation (58), suggesting a role for HNE in the
Fig. 5. Administration of FeCl₃ into the basal forebrain damages cholinergic neurons and induces accumulation of HNE. Vehicle or 0.4 μmol FeCl₃ was injected into the basal forebrain, and 7 days later the rats were killed, perfused, and coronal brain sections stained with cresyl violet, or processed for ChAT or HNE immunohistochemistry. Bright-field micrographs of the basal forebrain region reveals extensive loss of Nissl-positive cells in the FeCl₃-injected rats (upper right), compared with no cell loss in the vehicle-injected control rats (upper left). ChAT-positive neurons were markedly decreased in the FeCl₃-injected rats compared with vehicle-injected rats (arrows in middle panels). HNE immunoreactivity was induced in vehicle animals only in the immediate vicinity of the needle track (arrow in lower left panel), but was also increased in both the needle track (arrow) and in enlarged cells throughout the basal forebrain (arrow) in FeCl₃-treated rats. Similar results were obtained in 5 vehicle-injected and 5 FeCl₃-injected rats.
TABLE 1
Effects of FeCl₂ and HNE Administration on Thiobarbituric Acid Reactivity in Hippocampus and Striatum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Basal forebrain</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5.37 ± 0.38</td>
<td>4.03 ± 0.14</td>
</tr>
<tr>
<td>FeCl₂</td>
<td>9.21 ± 0.99**</td>
<td>4.84 ± 0.32</td>
</tr>
<tr>
<td>HNE</td>
<td>7.25 ± 0.61</td>
<td>3.93 ± 0.35</td>
</tr>
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Vehicle, 0.4 μmol FeCl₂, or HNE (3.2 μmole) was injected into the basal forebrain; 24 h later the rats were killed, and the basal forebrain and hippocampal areas dissected out and processed for TBARS assay as described in the Materials and Methods section. Values are the mean and SEM (n = 4–6 rats), **p < 0.005 compared with corresponding control value (ANOVA with Scheffe’s post-hoc tests).

Pathogenesis of neurofibrillary tangle formation. Our data directly demonstrate that HNE can also induce degeneration of cholinergic neurons in vivo. Although the concentrations of HNE that accumulated in cholinergic neurons exposed to FeCl₂ and HNE in the present study are unknown, previous studies have shown that levels of HNE in the range of 1–50 μM are produced in cultured neurons exposed to neurotoxic concentrations of Aβ and Fe²⁺ (30, 31). In light of the severe damage to cholinergic neurons induced by Fe²⁺ in the present study, it seems likely that neurotoxic levels of HNE were attained. While data in this manuscript demonstrates that HNE administration to the basal forebrain destroys cholinergic neurons and abolishes performance in the water maze task of spatial memory, when put into the context of available data demonstrating increased HNE in brains of Alzheimer patients (25–27), and HNE formation upon Aβ application in culture (30), it would suggest that the HNE could participate in the cell loss and cognitive decline in AD.

HNE may play a central role in neuronal injury and death resulting from exposure to agents that induce membrane lipid peroxidation (30, 31). While the mechanism whereby HNE damaged cholinergic neurons in vivo was not established, prior cell culture data suggest that it acts by conjugating to, and impairing the function of, membrane ion-motive ATPases and glutamate and glucose transporters (21, 22, 30). Impairment of the transport systems promotes excitotoxic cascades involving membrane depolarization and massive calcium influx (16, 30, 52). This scenario for damage to cholinergic neurons induced by Fe²⁺ and HNE is consistent with data showing that excitatory amino acids induce degeneration of basal forebrain cholinergic neurons in adult rats (38, 59–61). Although this manuscript does not address the role of excitotoxicity in HNE damage, further experiments using this model could evaluate potential anti-excitotoxic therapeutic agents, such as MK801, in HNE neurotoxicity and behavioral deficits. Previous studies have shown that oxidative insults relevant to the pathogenesis of AD can damage and kill cholinergic neurons in culture. AF64A, a toxin that selectively damages basal forebrain cholinergic neurons and induces memory impairment in adult rats, induced lipid peroxidation in rat brain (62), and vitamin E administration prevented the memory impairment and cholinergic neuron damage caused by AF64A (63). Aβ decreased levels of ChAT and acetylcholine synthesis in a neuroblastoma/basal forebrain neuron cell line (64). In addition, Aβ and Fe²⁺ impaired coupling of muscarinic receptors to the GTP-binding protein Gₘ₁ in cultured cortical neurons by a mechanism involving membrane lipid peroxidation and HNE production (23, 65). The ability of Fe²⁺ and HNE to damage cholinergic neurons in vivo and to impair performance on learning and memory tasks suggests an important role for lipid peroxidation cholinergic deficits in AD.

Fig. 6. Bilateral FeCl₂ administration into the basal forebrain impairs performance in visuospatial memory tasks. Vehicle or 0.4 μmol FeCl₂ was injected bilaterally into the basal forebrain, and 7 d later the rats were tested in the Morris water maze. (A) Vehicle treated animals showed significant decreases in goal latency over the trial blocks (p < 0.001), whereas animals that had received iron showed no decreases in latency to reach the platform. Values are the mean and SEM (n = 5 rats/group). (B) Mean target crossings in the probe trial were determined. Values are the mean and SEM (n = 5 rats/group). *p < 0.001 compared with vehicle value (ANOVA with Scheffe’s post-hoc tests).
Bilateral injection of FeCl₃ and HNE into the basal forebrain resulted in marked deficits in performance of both the goal latency and probe trial tasks in the Morris water maze 7 days later. The detrimental effect of HNE on these spatial navigation tasks was more severe in rats administered the higher dose of HNE. Our findings are in accord with previous studies showing that a variety of traumatic and toxic insults that damage basal forebrain cholinergic neurons bilaterally impair performance in spatial learning tasks (66, 67). The cholinergic-hippocampal circuitry is believed to be critical for Morris water maze tasks of visuospatial memory in rats (68, 69), and interventions that replace cholinergic deficits ameliorate memory deficits (70, 71). Our data demonstrate that an agent that induces membrane lipid peroxidation (FeCl₃), and the lipid peroxidation product HNE, can induce deficits in visuospatial learning and memory consistent with disruption of cholinergic input to the hippocampus. The cognitive impairments in AD patients are strongly correlated with loss of basal forebrain cholinergic neurons and decreased levels of ChAT. In the present study, HNE induced significant decreases in ChAT levels in basal forebrain and hippocampus, consistent with loss of ChAT-immunoreactive neurons in basal forebrain. Further studies of basal forebrain neurons in AD patients and animal models of AD (14, 15) will be required to clarify the role of HNE in the pathogenesis of the dysfunction and death of cholinergic neurons in AD.

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