Research report

Age-related working memory impairment is correlated with increases in the L-type calcium channel protein \( \alpha_{1D} \) (\( \text{Ca}_{1.3} \)) in area CA1 of the hippocampus and both are ameliorated by chronic nimodipine treatment

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Abstract

The hippocampus is critical for spatial memory formation in rodents. Calcium currents through L-type voltage-sensitive calcium channels (L-VSCCs) are increased in CA1 neurons of the hippocampus of aged rats. We have recently shown that expression of the calcium conducting L-VSCC subunit \( \alpha_{1D} \) (\( \text{Ca}_{1.3} \)) is selectively increased in area CA1 of aged rats. We and others have speculated that excessive Ca\(^{2+} \) influx through L-VSCCs may be detrimental to memory formation. Therefore, we investigated the relationship between age-related working memory decline and \( \alpha_{1D} \) expression in the hippocampus. In addition, we studied the effects of chronic treatment with the L-VSCC antagonist nimodipine (NIM) on age-related working memory deficits and \( \alpha_{1D} \) expression in the hippocampus. Here we report that age-related increases in \( \alpha_{1D} \) expression in area CA1 correlate with working memory impairment in Fischer 344 rats. Furthermore, we demonstrate that chronic NIM treatment ameliorates age-related working memory deficits and reduces expression of \( \alpha_{1D} \) protein in the hippocampus. The present results suggest that L-VSCCs participate in processes underlying memory formation and that increases in L-VSCC protein and currents observed with aging may play a role in age-related memory decline. Furthermore, the amelioration in age-related memory decline produced by NIM treatment may be mediated, at least in part, by reductions in the abnormally high levels of \( \alpha_{1D} \) protein in the aged hippocampus. These findings may have implications for patients with Alzheimer’s disease, who show increased L-VSCC protein expression in the hippocampus, and for patients receiving chronic treatment with L-VSCC antagonists.

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1. Introduction

L-VSCCs are voltage sensitive channels that mediate long-lasting Ca\(^{2+} \) currents in response to depolarization in excitable cells. Brain L-VSCCs consist of five subunits: \( \alpha_{1}, \alpha_{2}, \beta, \gamma \) and \( \delta \) \([24,42,50]\). The \( \alpha_{1} \) subunits form the ion-conducting pore of the channel and contain the binding sites for the dihydropyridine class of L-VSCC antagonists \([7]\). Two different brain L-VSCC \( \alpha_{1} \) subunits have been identified: \( \alpha_{1C} \) (\( \text{Ca}_{1.2} \)) and \( \alpha_{1D} \) (\( \text{Ca}_{1.3} \)) \([25,48]\). Increasing evidence suggests that L-VSCC currents are elevated in CA1 neurons of the hippocampus in aged rats and rabbits \([6,37,47,51,52]\), perhaps due to increases in the density of L-VSCCs in neuronal cell membranes \([6,52]\). Indeed, \( \alpha_{1D} \) mRNA is increased in area CA1 of aged rats \([20]\) and L-VSCC currents correlate with levels of \( \alpha_{1D} \) mRNA in
single neurons [10]. We have recently shown that $\alpha_{1D}$ protein is selectively increased in area CA1 of the aged rat hippocampus while expression of the alternative pore forming subunit $\alpha_{1C}$ remains unchanged [54].

It has been proposed that age-related deficits in cognitive processing are due to dysfunction of one or more of the components involved in hippocampal synaptic plasticity. Long-term potentiation (LTP) is a synapse-specific, activity-dependent enhancement in synaptic efficacy [5] that can be induced at hippocampal synapses. LTP is a favored candidate for a cellular mechanism of memory [4] and, like memory, some forms of LTP decline with advancing age [34]. LTP induction requires Ca$^{2+}$ influx through NMDAR [4], or L-VSCC [36] but the mechanism for induction of LTP by L-VSCC appears to be distinct from LTP resulting from NMDAR activation [8,9,19].

Interestingly, while NMDAR-dependent LTP is decreased in area CA1 of aged rats [11,34], L-VSCC-dependent LTP is increased [47]. However, the direct relationship between L-VSCC current increases and memory formation remains unclear, although some data suggest that excessive Ca$^{2+}$ influx through L-VSCCs may in fact be detrimental to memory formation. For instance, Landfield et al. reported that increases in L-VSCC currents in area CA1 of aged hippocampus correlated with degree of learning impairment on a hippocampal-dependent task [52]. Furthermore, the L-type calcium channel antagonist nimodipine (NIM) has been shown to enhance memory in both young and aged animals or animals with experimentally-induced ischemia or hypoxia [2,13,14,32,41,55]. However, the molecular basis for these effects remains elusive and questions regarding the long-term neurobiological effects of chronic NIM treatment remain unanswered.

The aim of the current study was twofold: (1) to investigate the role of $\alpha_{1D}$ expression in age-related working memory decline, and (2) to investigate whether chronic treatment with the L-VSCC antagonist nimodipine (NIM) affects levels of the L-type VSCC $\alpha_{1}$ subunits or other proteins known to be involved in hippocampal-dependent memory, i.e. the NMDA-R subunits NR2A and NR2B, and CaMKII, in brains of aged rats.

2. Methods

2.1. Animals

All young (4 months old) and aged (24 months old) Fischer 344 rats were obtained from the National Institutes of Aging colony at Harlan Laboratories under a pilot study award (LMV and MBD). In accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, every effort was made to minimize the quantity and suffering of the rats employed in this study. Accordingly, rats were housed in a climate controlled environment with a 12-h light–dark cycle (lights on at 07:00 h) in microinsulated clear polycarbonate cages in groups of 2–3 with water and rat chow available ad libitum. All rats were allowed to acclimate for 7 days before experiments began.

2.2. Radial arm water maze

The radial arm water maze (RAWM) combines the physical features of a radial arm maze [40], with a water escape task. The water version of the radial arm maze has previously been used to demonstrate working memory deficits in rodents [1,3,18,35]. The RAWM has 12 arms (15 cm wide, 43 cm long) that radiate from a circular choice area (60 cm diameter). The maze was set up in a room with many extra-maze visual cues, including visually distinct posters on two walls, a checker board pattern on another wall, and a wall featuring a table with rat cages. The experimenter always stood in the same place, was another wall, and a wall featuring a table with rat cages.

2.3. Experiment 1

2.3.1. Behavioral testing

Six young and 10 aged Fischer 344 rats were tested on a time-delay, win-stay RAWM task to assess differences in working memory performance. Rats underwent 7 days of pretraining with four trials/day (180 s maximum/trial) to learn to locate a submerged platform in one of the 12 arms of the RAWM. If the rat did not find the hidden platform in 180 s it was gently guided to its location and allowed to climb onto the platform. After landing on the platform the rat was removed to its home cage where it stayed for the 30-s inter-trial interval. The location of the escape platform changed daily and the rats thus had to learn the new location on each day. Start location was at the end of one arm and varied for each trial. The number of arms entered prior to entering the arm containing the escape platform was recorded as working memory errors. Following pretraining, rats were tested for their ability to find the hidden platform on 8 consecutive days. The first 4 days of testing the rats were given four consecutive trials with a 30-s inter-trial interval. Then for the next 4 days, a 3-h delay was introduced between the third and fourth trial. During the delay, the rats were placed in their home cages.

2.3.2. Semi-quantitative Western blotting analysis

Following behavioral testing the rats were sacrificed, both hippocampi dissected free, and 400-µm-thick transverse hippocampal slices were generated by sectioning through the hippocampus using a McIlwan tissue chopper.
Hippocampal slices were bathed in oxygenated aCSF, held on ice while being micro-dissected to isolate area CA1, and tissue homogenates prepared for Western blotting analysis as previously described [54]. Briefly, 10–12 CA1 mini-slices per rat were sonicated in solubilization buffer (1% Triton-X 100, 20 mM EDTA, 10 mM EGTA, 10 mM Tris–HCl, pH 7.4, 5 µl protease inhibitor cocktail II/ml buffer (Sigma-Aldrich, St. Louis, MO, USA), 8 µg/ml Calpain inhibitor I and II (ICN Biochemicals, Aurora, OH, USA)), and the protein content of each sample was determined by the BCA assay using bovine serum albumin (BSA) as a standard (Pierce, Rockford, IL, USA). Aliquots of each sample were then prepared for Western blots and the protein concentration normalized to 4 mg/ml by suspension in 4X sample buffer (270 mM Tris-base, 9.2% SDS, 40% glycerol, 20% beta-mercaptoethanol and a trace of bromophenol blue) and dH2O. Duplicate lanes containing 20 µg of protein were separated by SDS–PAGE and transferred to PolyScreen PVDF membranes, which were probed with antibodies against α1D (1:500; Alomone Laboratories, Jerusalem, Israel) at 4°C overnight. On the following day, blots were washed, incubated with a horseradish-peroxidase labeled goat-anti-rabbit secondary antibody (1:5000; Amersham, Piscataway, NJ, USA), washed again, and antibody reactive bands were visualized by chemiluminescence using the SuperSignal Femto chemiluminescence kit (Pierce). Data were captured using the ChemiImager 4400 and Alpha Ease software (Alpha Innotech, San Leandro, CA, USA) and the optical densities of bands representing the 180 kD isoform of Calpain inhibitor I and II (ICN Biochemicals, Aurora, OH, USA), and a kaleidoscope molecular weight marker (BioRad, Hercules, CA, USA) loaded onto each gel. Samples were only evaluated if they fell within the linear range of the hippocampal standard for each antibody. In cases where samples did not fall within the linear range of the hippocampal standard the analysis was repeated with a different standard dilution. All results were confirmed by repeating the analysis at least one time.

2.4. Experiment 2

2.4.1. Nimodipine treatment

Aged Fischer 344 rats were implanted with pellets containing vehicle matrix (vehicle; n=9) or 40 mg NIM (n=9, Innovative Research America, Sarasota, FL, USA) by an incision at the nape of the neck under isoflurane anesthesia. Pellets were designed to supply a steady release of drug for 35 days as previously reported [31].

2.4.2. Behavioral testing

Twenty-one days following implantation of NIM or vehicle pellets, a subset of rats (six from each treatment group) were trained in a win-shift version of the RAWM [3]. This version of the RAWM is highly analogous to a land radial arm maze, in that rats are required to sequentially locate eight hidden platforms over eight daily trials, with working memory load increasing with successive trials. Due to failure to comply with the physical demands of the task (unwillingness to swim for the entire 180 s of several consecutive trials), two rats in the vehicle group were eliminated during the course of testing. Rats received eight trials/day for 12 consecutive days between the hours of 08:00 and 17:00. Platform location was randomized such that one animal in each group was assigned the same pattern of platform locations. The platform locations remained the same across all days for each rat. The object of this task is to learn the location of all eight escape platforms and to not enter arms that never contain a platform. On each training day, the rats were placed in the water facing the center of the maze at the end of the start arm. The start arm never contained a platform and was constant across days and groups. After being released into the water, the rat was allowed 180 s to locate an escape platform and climb onto it. The rat then remained on the platform for 15 s, was removed from the platform, dried with a towel, and returned to a heated cage for 30 s until the next trial. During the 30-s inter-trial interval, the platform that the rat had escaped onto was removed from the maze. This sequence was repeated until all platforms had been found for a total of eight trials/day. During testing, two types of errors were recorded: working memory (WM) errors occurred when a rat entered an arm that earlier in that session had contained an escape platform but no longer did, or made repeat entries into an arm that never contained an escape platform (i.e. entries into arms that had previously been entered on that day). Reference memory (RM) errors occurred the first time during a session that a rat entered an arm that never contained an escape platform.

2.4.3. Semi-quantitative Western blotting analysis

One day following behavioral testing rats were sacrificed, both hippocampi dissected free, the tissue sonicated in solubilization buffer, and Western blotting performed as described above and previously [54]. Primary antibodies were used at the following dilutions: NR2B (1:2000; Browning), NR2A (1:1000; Browning), Ca2+-calmodulin dependent kinase II (CamKII; 1:500, Browning), α1D or α1C (both 1:500; Alomone Laboratories, Jerusalem, Israel).

2.5. Statistical analyses

In experiment 1, behavioral testing was grouped into two conditions: no-delay or delay (see Section 2.3.1). The average number of errors that each rat made on trials 1–4 over the 4 days of no-delay and 4 days of delay testing was computed and group differences were evaluated by repeated measures analysis of variance (ANOVA). Posthoc
3. Results

3.1. Experiment 1

Young and aged rats were trained to locate a hidden platform in one of 12 arms of the RAWM. The platform location changed daily and thus the rats were required to learn the platform’s location on each testing day. Following 7 days of pretraining, rats underwent testing for 4 days in a no-delay condition where the rats were re-released into the maze after a 30-s inter-trial interval for four successive trials. Fig. 1A shows the average number of errors (arms entered) the rats made in locating the hidden platform during the 4 days of testing in the no-delay condition. Young and aged rats were both proficient in learning the platform location and both groups reduced the number of errors over successive trials (ANOVA: $F=40.305$, $P<$...
0.001; Fig. 1A). Next, the rats were tested in the same manner but a 3-h delay was introduced between the third and fourth trial (delay condition) in order to test memory for the platform location. Fig. 1B shows the average number of errors the rats made in locating the hidden platform during the 4 days of testing in the delay condition. Both groups were equally proficient in locating the hidden platform on trials 1–3 of the delay testing and reduced the number of errors over trials ($F=34.107, P<0.001$, Fig. 1B). However, statistical analysis revealed a significant interaction ($F=5.795, P<0.01$), and post hoc analysis showed that aged rats made a greater number of errors on the fourth trial after the 3-h delay than did young rats ($F=17.7, P<0.001$; Fig. 1B). Fig. 1C shows a scatter plot of the number of errors made by the young and aged rats on the fourth trial of testing in the delay condition averaged over 4 days. Note that some rats made equal numbers of errors; thus some data points overlap. Like many other studies of normal aging, not all aged rats in our study exhibited the same degree of working memory impairment and some aged rats performed similarly to the young rats (Fig. 1C).

One to 7 days following behavioral testing, rats were sacrificed and 400-μm-thick hippocampal slices were generated, microdissected to yield area CA1 strata pyramidale and radiatum, homogenized and subjected to semi-quantitative Western blotting analysis in order to determine the expression of the 180 kD isoform of $\alpha_{1D}$ protein in this area of the hippocampus. Fig. 3A shows representative bands from Western blots of $\alpha_{1D}$ protein from area CA1 of young and aged rats. The bar graph in Fig. 3B shows the quantitative analysis of 180 kD $\alpha_{1D}$ expression in area CA1 of young and aged rats. Similar to our previous report [54], we found that expression of 180 kD $\alpha_{1D}$ protein is significantly elevated in the aged CA1 ($F=6.726, P<0.05$, Fig. 2A and B). Several investigators have speculated that excessive Ca$^{2+}$ influx through L-VSCCs may be detrimental to memory formation [16,17,39,52,53] and have reported correlations between L-VSCC currents and age-related memory decline. Therefore, we have tested the hypothesis that increases in 180 kD $\alpha_{1D}$ protein in the hippocampus of aged rats correlate with age-related memory decline. Fig. 2C shows a scatterplot of errors on the fourth trial of the delay-testing and $\alpha_{1D}$ protein expression in area CA1 of the hippocampus. Our correlation analysis showed that working memory error and $\alpha_{1D}$ protein expression in area CA1 are positively correlated ($r=0.664, P<0.05$), suggesting that age-related increases in $\alpha_{1D}$ protein may be detrimental to processes underlying working memory.

### 3.2. Experiment 2

We next examined the ability of the L-VSCC antagonist nimodipine to ameliorate the age-related behavioral and biochemical effects detected in experiment 1. Aged rats were implanted with NIM or vehicle pellets and 21 days following implantation, a subset of rats were trained in a different version of the RAWM for 12 consecutive days. Both groups were able to learn the reference memory component of the task and showed a reduction in the
The number of reference memory errors made across days of testing (ANOVA, $F=3.27, P<0.001$; Fig. 3A). Aged NIM- and vehicle-treated rats did not differ in the number of reference memory errors made on the RAWM during either phase of testing (Fig. 3A). On the working memory component of the task, the two groups performed similarly during the acquisition phase (days 1–6). However, the NIM-treated rats showed significant improvement compared to the vehicle-treated rats during the testing phase (days 7–12) as NIM-treated rats made significantly fewer working memory errors than vehicle-treated rats (ANOVA, $F=6.19, P<0.05$; Fig. 3B). Indeed, during the last 6 days of testing about half of the aged NIM-treated rats performed as well as young rats that we had trained in this task previously. However, the other aged NIM-treated rats performed similarly to vehicle controls, making variability in working memory errors in the NIM-treated group slightly higher than in the vehicle group. No overlap in working memory performance was observed between the aged vehicle group and the young rats previously examined.

Expression of the L-VSCC subunits $\alpha_{1C}$ and $\alpha_{1D}$ as well as the NMDA-R subunits NR2A and NR2B is influenced by $\text{Ca}^{2+}$ influx through L-VSCC [21], presumably due to $\text{Ca}^{2+}$-dependent transcriptional regulation of the genes encoding these proteins [12,15]. Therefore, we assessed

A

![Graph A](image)

B

![Graph B](image)

Fig. 3. Chronic NIM treatment improves spatial working memory in aged rats. Vehicle- (n=4) and NIM- (n=6) treated aged rats were trained to sequentially locate eight hidden escape platforms in 12 arms of the RAWM during eight daily trials over 12 days. (A) NIM- and vehicle-treated aged rats were equally proficient in learning the reference memory component of the task, which does not depend on the hippocampus. Both groups reduced the number of reference memory errors over the 12 days of testing ($F=3.27, P<0.001$; Fig. 3A). (B) However, hippocampal-dependent working memory performance was improved in aged NIM-treated rats compared to vehicle. NIM-treated rats made significantly fewer working memory errors during the last 6 days of testing than did aged vehicle-treated rats ($F=6.19, P<0.05$), suggesting that NIM selectively affects working memory.
the effects of chronic NIM treatment on expression of the 190 kD isoform of α_{1C}, the 180 kD isoform of α_{1D}, NR2A, NR2B, as well as the Ca^{2+}-dependent kinase CaMKII in the hippocampus using semi-quantitative Western blotting analysis. Fig. 4A shows representative bands from Western blots of these proteins detected in hippocampal homogenates from NIM- and vehicle-treated aged rats. The bar graph in Fig. 4B shows the quantitative analysis of α_{1C}, α_{1D}, NR2A, NR2B, and CaMKII expression in the hippocampus of NIM- and vehicle-treated rats. NIM treatment reduced levels of α_{1D} in the hippocampus of aged rats by ~35% compared to vehicle treatment, as revealed by a one-way ANOVA (F = 4.091, P < 0.05; Fig. 4A and B). To further examine the relationship between α_{1D} protein in the hippocampus and working memory deficits in aged rats we performed a correlation analysis between cumulative working memory error on the last 6 days of testing and α_{1D} protein expression in whole hippocampal homogenates. Although we observed a positive relationship between these two variables in the aged vehicle- and NIM-treated rats, the effect did not reach statistical significance (r = 0.48, P = 0.09). We detected no significant effects of NIM treatment on hippocampal levels of α_{1C}, NR2A, NR2B, or CaMKII (Fig. 4B).

4. Discussion

We have used the RAWM to demonstrate spatial memory impairments in aged rats. In the first study, aged rats performed as well as young rats when inter-trial intervals were short and working memory load was low. However, after a 3-h delay aged rats demonstrated significant impairments in memory for the location of the hidden platform. In these aged rats, expression of α_{1D} protein was greatly increased in area CA1 of the hippocampus, an area thought to be critical for spatial memory encoding [22,28,43]. We found that α_{1D} expression was positively correlated with working memory errors in the RAWM task used in experiment 1, suggesting that age-related increases in α_{1D} protein in the hippocampus may contribute to spatial memory impairments in aged rats.

**Fig. 4.** NIM treatment significantly and selectively reduces levels of the L-type calcium channel subunit α_{1D} in the hippocampus of aged rats. Western blotting analysis was performed on hippocampal homogenates from aged NIM- (n = 9) and vehicle- (n = 9) treated rats to determine protein expression of the L-VSCC subunits α_{1C} and α_{1D}, the NMDA-R subunits NR2A and NR2B, as well as CaMKII. (A) Representative bands from Western blots show expression levels of NR2A, NR2B, CaMKII, the 190 kD isoform of α_{1C} and the 180 kD isoform of α_{1D} in the hippocampus of NIM- and placebo-treated aged rats. (B) Protein expression from Western blots was quantified by comparison to a hippocampal standard. Bar graph shows group differences in expression of NR2A, NR2B, CaMKII, 190 kD α_{1C}, and 180 kD α_{1D} in the hippocampus of NIM- and vehicle-treated aged rats. Expression of 180 kD α_{1D} was reduced by ~35% in the hippocampus of NIM-treated compared with vehicle-treated aged rats (F = 4.09, P < 0.05). No significant differences in expression of NR2A, NR2B, CaMKII or α_{1C} were detected.
expression in this area of the hippocampus. This may be detrimental to processes underlying spatial memory encoding. In the second study, aged rats treated with NIM showed improved working memory performance in the RAWM and $\alpha_{1D}$ expression was reduced in hippocampus as compared to vehicle controls. These results suggest that NIM has direct effects in the brain and that the beneficial effects of chronic NIM on hippocampal dependent memory may be due, at least in part, to a reduction in expression of $\alpha_{1D}$ protein. Although we also found a trend towards a positive correlation between working memory error and $\alpha_{1D}$ expression in hippocampus in experiment 2, this effect did not reach statistical significance. There are several possible explanations for this result. First, in experiment 1 we examined $\alpha_{1D}$ expression in area CA1 of the hippocampus, while in experiment 2 we examined expression of this protein in homogenates from whole hippocampus. Thus, it is possible that age-related changes in $\alpha_{1D}$ expression in area CA1 of the hippocampus have a greater impact on spatial memory than expression of this protein in other areas of the hippocampus. Secondly, the two studies described here used two different versions of the RAWM to study spatial memory in aged rats. In experiment 1, we examined memory for a spatial location following a 3-h delay. Performance on the delayed version of this task relies heavily on consolidation of spatial memory, a process that may be specifically impacted in aged animals since the aged rats we examined performed the same task without impairment when inter-trial intervals were short. In experiment 2, however, we used an RAWM task that instead places great demands on spatial working memory, which is a type of memory that, by definition, has a very limited time span [40]. Therefore, it is possible that the two different tasks, which may address different roles for the hippocampus in spatial memory, are not equally sensitive to changes in hippocampal L-VSCC protein expression induced by aging or NIM treatment.

The question of how L-VSCC-dependent Ca$^{2+}$ influx may affect hippocampal information processing arises. Ca$^{2+}$ influx through L-VSCC is known to activate Ca$^{2+}$-dependent K$^+$ channels, causing the afterhyperpolarization (AHP) phase following action potentials in hippocampal neurons [45]. This particular feature of L-VSCC-dependent Ca$^{2+}$ influx is thought to play a key role in NMDAR-dependent hippocampal excitability and plasticity processes that are especially vulnerable to changes in aging. The fact that aged hippocampal neurons show increased L-VSCC currents [6], as well as increased expression of $\alpha_{1D}$ [54], which activate at significantly more hyperpolarized potentials than channels containing the $\alpha_{1C}$ subunit [26,56], may explain both the robust L-VSCC-dependent potentiation [47] as well as the larger AHP observed [27,38] in the aged hippocampus. It has been suggested that increases in the AHP may contribute to impairments in synaptic plasticity in aged animals [16,17,39] because the larger AHP impedes summation of incoming excitatory potentials in the aged neuron. Thus, the larger AHP makes it more difficult for the aged neuron to fire a series of action potentials, a condition necessary for relieving the Mg$^{2+}$ block of the NMDAR and allowing this receptor to participate in LTP. We hypothesize that changes in L-VSCC expression in the aged hippocampus may contribute to changes in synaptic plasticity that underlie cognitive processes, and thereby contribute to changes in hippocampal-dependent spatial memory processes.

It is noteworthy that NIM enhances hippocampal excitability in a dose- and age-dependent manner, due to its ability to normalize the increased, L-VSCC-dependent AHP in aged neurons [37,38,53]. As discussed above, normalizing the AHP may facilitate synaptic plasticity, and thus memory, in the aged hippocampus. In support of this idea, Disterhoft et al. have shown that NIM enhances hippocampal-dependent learning in aged rabbits at a dose that also increases excitability of aged CA1 neurons [49]. Indeed, several investigators have reported that NIM enhances hippocampal-dependent memory in a variety of animals and tasks [13,14,29,31,44,46,53]. In the present study we found no effect on reference memory but a significant enhancement of working memory in rats treated with chronic NIM. Our finding agrees well with another study of NIM effects on performance in the Morris water maze [31]. These investigators found no effect of NIM on latency to find the hidden platform, which is mainly a reference memory task after several days of training. However, when the platform location was changed and the rats had to rely on working memory to find the platform in its new location, NIM enhanced search behavior for the new location. Together, these results suggest that NIM has greater effects on working memory than reference memory.

Interestingly, there appears to be both rapid and secondary effects of NIM treatment. For instance, in studies using a within-subjects design, both aged rats and monkeys treated acutely with NIM show improved working memory performance on NIM trials but not on trials were they did not receive drug [29,46], suggesting that NIM has rapid but reversible effects on working memory. Furthermore, Quartermain et al. [44] reported that several dihydro- pyridine antagonists enhance memory in adult mice when administered either peripherally or centrally. Together, these results suggest that NIM acts directly in the CNS and that its rapid effects are likely due to direct antagonism of neuronal L-VSCC. Levy et al. reported that chronic NIM treatment, with implants as in this study, improved working memory and increased acetylcholine levels in the hippocampus of young rats [30], while others report memory improvements accompanied by lasting changes in the arborization of cortical dendrites [33]. Our present finding, that NIM reduces levels of $\alpha_{1D}$ in the hippocampus, supports the hypothesis that chronic treatment with NIM may cause long-lasting molecular changes in the CNS, in addition to the more immediate effect of L-VSCC.
antagonism. The NIM-induced downregulation of α1D is presumably due to Ca\(^{2+}\)-dependent transcriptional regulation of the mRNA encoding this protein, as L-VSCC-dependent Ca\(^{2+}\) influx has been shown to regulate transcription of the α1C subunit in cardiac myocytes [12, 15]. However, to our knowledge, transcriptional regulation of α1D has not been studied so the proposed mechanism for α1D downregulation in our study remains purely speculative. Regardless of mechanism, our data suggest that long-term treatment with L-VSCC antagonists may be effective in normalizing α1D expression in the aged hippocampus and ameliorating the excessive Ca\(^{2+}\) influx that is associated with impaired synaptic plasticity and memory in aging. However, further studies are warranted to elucidate the effects of chronic L-VSCC antagonism on synaptic plasticity in aged animals.

In summary, we have demonstrated that age-related spatial memory impairments are associated with increased expression of α1D protein in the aged hippocampus. Furthermore, chronic treatment with the L-VSCC antagonist NIM can ameliorate age-related working memory impairments as well as normalize levels of α1D in the hippocampus of aged rats.

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