Carbachol-Induced Long-Term Synaptic Depression Is Enhanced During Senescence at Hippocampal CA3–CA1 Synapses

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Kumar A. Carbachol-induced long-term synaptic depression is enhanced during senescence at hippocampal CA3–CA1 synapses. J Neurophysiol 104: 607–616, 2010. First published May 26, 2010; doi:10.1152/jn.00278.2010. Dysregulation of the cholinergic transmitter system is a hallmark of Alzheimer’s disease and contributes to an age-associated decline in memory performance. The current study examined the influence of carbachol, a cholinergic receptor agonist, on synaptic transmission over the course of aging. Extracellular excitatory postsynaptic field potentials were recorded from CA3–CA1 synapses in acute hippocampal slices obtained from young adult (5–8 mo) and aged (22–24 mo) male Fischer 344 rats. Bath application of carbachol elicited a transient depression of synaptic transmission, which was followed by a long-lasting depression (CCh-LTD) observed 90 min after carbachol cessation in both age groups. However, the magnitude of CCh-LTD was significantly larger in senescent animals and was attenuated by N-methyl-D-aspartate receptor blockade in aged animals. Blockade of L-type Ca2+ channels inhibited CCh-LTD to a greater extent in aged animals compared to young adults. Finally, the expression of CCh-LTD was dependent on protein synthesis. The results indicate that altered Ca2+ homeostasis or muscarinic activation of Ca2+ signaling contribute to the enhanced CCh-LTD during senescence.

INTRODUCTION

Pronounced dysregulation of cholinergic neurotransmission is a characteristic of Alzheimer’s disease, and it is believed to be partially responsible for the cognitive impairment associated with the disease (Collerton 1986; Kasa et al. 1997; McKinney and Jacksonville 2005; Schliebs 2005). Additionally, the decline in memory performance during “normal” aging is associated with deficits in the cholinergic system, which includes a loss of cholinergic neurons, a marked decline in acetylcholine synthesis, a decreased expression of cholinergic muscarinic receptors, and an increase in the enzyme acetylcholine esterase, which is responsible for the hydrolysis and breakdown of acetylcholine (Amenta et al. 1995; Bartus et al. 1982; Blaker et al. 1988; Decker 1987; Frotscher and Leranth 1985; Galagher and Colombo 1995; Mesulam 2004; Strong et al. 1980; Tayebati et al. 2002).

The hippocampus, a brain region that plays an important role in learning and memory, receives cholinergic projections from the septal medial nucleus and the diagonal band of Broca (Amaral and Kurz 1985; Frotscher and Leranth 1985; Gaykema et al. 1990; Lewis et al. 1967). Previous studies suggest that intact cholinergic innervation is necessary for certain forms of hippocampal-dependent memory processing (Bartus et al. 1982; Callahan et al. 1993; Dekker et al. 1991; Drachman and Leavitt 1974; Fibiger 1991; Hasselmo 1999). Acetylcholine mediates its effects both through the nicotinic acetylcholine receptors and the G-protein-coupled muscarinic acetylcholine receptors (mAChRs). A family of five muscarinic receptor genes encoding five functionally distinct mAChRs (M1–M5) is expressed throughout the CNS, including the hippocampus (Bonner et al. 1987; Buckley et al. 1988; Caulfield 1993; Vilaro et al. 1993), which are divided into two classes based on signal transduction; subtype one mAChRs include M1, M3, and M5, which are coupled with Gq/11 proteins to activate phospholipase C and mobilize the release of Ca2+ from intracellular Ca2+ stores. M2 and M4 receptors, which comprise subtype two, predominantly signal through Gi/o proteins to inhibit adenylate cyclase and reduce the intracellular concentration of cAMP. Pharmacological activation of mAChR in the hippocampus is associated with a reduced presynaptic release of glutamate (Irving and Collingridge 1998; Kimura 2000; Nicoll et al. 1990; Valentino and Dingledine 1981) and increased hippocampal cell excitability (Disterhoft and Matthew Oh 2003; Jiang and Khanna 2006; Muller and Mispéld 1986; Oh et al. 1999; Pittler and Alger 1992; Rouse et al. 1999). Furthermore, mAChR activation facilitates long-term potentiation (LTP) and produces lasting potentiation of synaptic transmission (Auerbach and Segal 1996, 1994; Blitzer et al. 1990; Brocher et al. 1992; Huerta and Lisman 1993; Ito et al. 1988; Koijima and Ondera 1998; Li et al. 2007; Patil et al. 1998; Shimoshige et al. 1997; Shinoe et al. 2005; Williams and Johnston 1988). Interestingly, activation of mAChR also facilitates the induction of long-term depression (LTD) in various brain regions, including the hippocampus of younger animals (Kirkwood et al. 1999; Kremien et al. 2006; Massey et al. 2001; McCoy and McMahon 2007; McCutchen et al. 2006; Scheiderer et al. 2006, 2008; Volk et al. 2007). Although all subtypes of mAChRs (M1 and M4) play an important role in synaptic plasticity (Kamslser et al. 2010; Sanchez et al. 2009; Shirley et al. 2008), the M1 subtype is especially important for the regulation of hippocampal synaptic plasticity and cognitive function (Anagnostaras et al. 2003; Andrews et al. 1994; Aura et al. 1997; Fornari et al. 2000; Hagan et al. 1987; Hunter and Roberts 1988; Kamslser et al. 2010; Messer et al. 1990; Sala et al. 1991). Thus, activation of mAChRs could influence memory by regulating synaptic plasticity, which is altered with advanced age and hypothesized to contribute to the age-associated impairments in memory function (Burke and Barnes 2006, 2010;...
Foster 1999, 2007; Giocomo and Hasselmo 2007; Rosenzweig and Barnes 2003).

METHODS

Subjects

Procedures involving animal subjects have been reviewed and approved by the university’s institutional animal care and use committee and were in accordance with guidelines established by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Male Fischer 344 rats, young adult (5–8 mo) and aged (22–24 mo), were group housed (2 per cage), maintained on a 12:12 h light schedule and provided ad lib access to food and water.

Hippocampal slice preparation

The methods for hippocampal slice preparation have been published previously (Bodhinathan et al. 2010; Kumar and Foster 2004, 2007; Kumar et al. 2007). Briefly, rats were anesthetized with isoflurane (Halocarbon Laboratories, River Edge, NJ) and swiftly decapitated. The brains were rapidly removed, and the hippocampi were dissected. Hippocampal slices (~400 μm) were cut parallel to the alvear fibers using a tissue chopper. The slices were incubated in a holding chamber (room temperature) containing standard artificial cerebrospinal fluid (ACSF) at a flow rate of 2 ml/min. The pH and temperature were maintained at 7.4 and 30 ± 0.5°C, respectively. Humidified air (95% O₂-5% CO₂) was continuously blown over the slices.

Electrophysiological recordings

Extracellular synaptic field potentials from CA3–CA1 synaptic contacts were recorded with glass micropipettes (4–6 MΩ) filled with recording medium (ACSF). Two concentric bipolar stimulating electrodes (outer pole: stainless steel, 200 μm diam; inner pole: Platinum/Iridium, 25 μm diam, FHC, Bowdoinham, ME) were positioned ~1 mm from either side of the recording electrode localized in the middle of the stratum radiatum. A single diphasic stimulus pulse of 100 μs was passed via stimulators (SD9 Stimulator, Grass Instrument, West Warwick, RI) to the Schaffer collateral commissural pathway to evoke field potentials at 0.033 Hz. A response baseline of the excitatory postsynaptic potential (EPSP, ~1.0 mV) was collected for ~10 min prior to experimental manipulations (drug application) and for ~90 min following drug washout.

The signals were amplified, filtered between 1 Hz and 1 kHz and stored for off-line analysis. Two cursors were placed around the initial descending phase of the waveform, and the maximum slope (mV/ms) of the EPSP was determined by a computer algorithm that found the maximum change across all sets of 20 consecutively recorded points (20 kHz sampling rate) between the two cursors. Changes in transmission properties induced by application of drug were calculated as the percent change from the average baseline responses collected. For paired pulse, a 50 ms interpulse interval was used. The paired-pulses ratio was calculated by dividing the slope of the second synaptic response by the slope of the first response. To determine the effects of carbachol on the paired-pulse ratio, the paired-pulse ratio for each response was normalized by the average ratio calculated for the baseline recording.

Drugs

All drugs were bath applied by the addition of ACSF. 2-Amino-5-phosphonomopentanoic acid (AP-5) was obtained from Tocris (Tocris Bioscience, Ellisville, MO). Carbamylcholine chloride (carbachol), atropine, pirenzepine, nifedipine, and anisomycin were obtained from Sigma-Aldrich. Carbachol (50 μM), atropine (1 μM), pirenzepine (1 μM), and AP-5 (100 μM) were dissolved directly in ACSF. Nifedipine (10 μM) was initially dissolved in a small amount of dimethyl sulfoxide (DMSO) and diluted further by ACSF to a final DMSO concentration of 0.01%. Anisomycin (20 μM) was initially dissolved in a small amount of ethyl alcohol and diluted further by the ACSF.
All antagonists were bath applied for 20–40 min before application of carbachol and had no noticeable effect on baseline synaptic transmission (data not shown).

**Statistical analysis**

All statistical analyses were performed using StatView 5.0 (SAS Institute). Student’s t-test, with a significance set at \( P < 0.05 \), was employed to determine any changes in synaptic response induced by carbachol, as well as any possible differences between baseline and PPF ratio. ANOVA was used to examine the interaction of age and drug treatment, and follow-up ANOVAs were employed to localize age differences. Post hoc comparisons of the effects of pharmacological treatment relative to the control condition on the level of carbachol (CCh)-LTD were performed using Fisher’s PLSD with a significance set at \( P < 0.05 \).

**RESULTS**

**Synaptic depression induced by muscarinic \( M_1 \) activity is enhanced during senescence**

The application of the nonselective cholinergic receptor agonist, CCh (50 \( \mu \)M for 10 min), induces LTD in visual and perirhinal cortices, as well as the hippocampus of young animals, (Kirkwood et al. 1999; Massey et al. 2001; McCoy and McMahon 2007; McCoy et al. 2008; McCutchen et al. 2006; Scheiderer et al. 2006, 2008; Volk et al. 2007). Here, in hippocampal slices obtained from young adult (5–8 mo) and aged (22–24 mo) animals, I show that bath application of CCh (50 \( \mu \)M for 10 min) rapidly decreased synaptic responses to a similar magnitude in young adult (22.02 ± 1.68% of baseline, \( n = 23 \)) and aged (23.83 ± 1.59, \( n = 29 \)) animals. However, age differences began to emerge ~30 min following the start of washout such that the synaptic responses were significantly decreased for aged animals, relative to young adults, at 30 min \( F(1,15) = 14.18, P < 0.0004 \), 60 min \( F(1,49) = 22.82, P < 0.0001 \), and 90 min \( F(1,23) = 6.02, P < 0.02 \) following cessation of CCh application (Fig. 1).

Preincubation (30–40 min) of the slices with atropine (1 \( \mu \)M) completely blocked the CCh-induced synaptic depression in young adult (\( n = 5 \)) \( F(1,16) = 143.99, P < 0.0001 \) and aged (\( n = 6 \)) \( F(1,16) = 197.27, P < 0.0001 \) animals compared to the respective control groups (young \( n = 12 \), aged \( n = 14 \); Fig. 2, A and B). Furthermore, preincubation of slices with the \( M_1 \) muscarinic receptor selective antagonist, pirenzepine (1 \( \mu \)M, 20–30 min) reduced the transient depression, such that the response recovered to baseline within 30 min of CCh cessation in both age groups (Fig. 2, C and D). A higher dose of pirenzepine (10 \( \mu \)M) completely blocked the transient phase of CCh-induced synaptic depression (data not shown).

**Mechanism for enhanced synaptic depression in aged animals**

To determine whether presynaptic mechanisms mediate the age-related difference in CCh-induced synaptic depression, paired-pulse facilitation was examined. In general, a change in the paired-pulse ratio suggests a presynaptic locus of expression, whereas no change indicates involvement of postsynaptic mechanisms (Dobrunz and Stevens 1997). For young adults, CCh significantly increased the paired-pulse ratio relative to baseline during agonist application (\( P < 0.005, n = 15 \)) and at 15 min (\( P < 0.0002, n = 15 \)), 30 min (\( P < 0.0002, n = 15 \)), and 60 min (\( P < 0.008, n = 14 \)) following drug washout. No change in the paired-pulse ratio was observed 90 min after CCh.

**FIG. 2.** Synaptic depression induced by CCh is dependent on activation of muscarinic receptors. Cholinergic receptor selective antagonist, atropine (1 \( \mu \)M) completely blocked CCh-induced long-term depression (LTD) in young adult (\( n = 5 \)) and aged (\( n = 6 \)) rats. Time course of synaptic responses showing the 10 min baseline before CCh application, during application (—), and following 30 min CCh washout in presence (○) and absence of atropine (--) for young adult (A) and aged (B) rats. CCh applied in presence of pirenzepine (1 \( \mu \)M) in slices obtained from young adult (C) and aged (D) rats reduced the carbachol mediated synaptic depression in young adult (\( n = 5 \)) and aged (\( n = 5 \)) rats. Representative traces of EPSP responses for the time points indicated in C and D for control (left) and pirenzepine (right) slices obtained from young adult and aged rats.
cessation. Similarly, in the case of aged rats, the paired-pulse ratio was significantly increased above baseline during agonist application ($P < 0.007, n = 17$) and at 15 min ($P < 0.0002, n = 17$) following cessation of CCh. Furthermore, there was no difference in the paired-pulse ratio between baseline and at 90 min after CCh washout (Fig. 3, B and C). Finally, young adult rats exhibited a larger increase in the paired-pulse ratio than senescent animals at 15 min [$F(1,30) = 12.01, P < 0.002$] and 30 min [$F(1,30) = 6.64, P < 0.02$], following CCh cessation (Fig. 3C). The results suggest that reduced transient presynaptic transmitter release contributes to the early phase of CCh-induced synaptic depression in young adult and aged animals; this is consistent with previous results reported in cortex (Kimura and Baughman 1997; McCoy and McMahon 2007; Mrzljak et al. 1993). However, the absence of a shift in

**FIG. 3.** Effect of CCh on the paired-pulse ratio in young adult and aged rats. Time course of CCh effects on the 1st (●) and 2nd (○) synaptic response and paired-pulse ratio (△) in slices obtained from young adult (A) and aged (B) rats. Each individual response was computed as a percent of the mean baseline response (-----) collected during the 10 min just prior to CCh application. For the purpose of clarity, each data point represents the mean of 4 consecutive responses. C: mean paired-pulse ratio during CCh application 15, 30, 60, and 90 min following CCh washout in aged (■) and young adult (□) rats. Error bars equal SE. *, a significance difference relative to baseline; #, a significant difference between the aged and young adult groups. The number above each bar indicates number of slices recorded for each group at each time point.

**FIG. 4.** Expression of CCh-induced synaptic depression is dependent on protein synthesis. Time course of the effects of preincubation of slices with protein synthesis inhibitor anisomycin on CCh-induced synaptic depression. Preincubation of slices with anisomycin (20 μM) obtained from young adult (A, $n = 7$) or aged (B, $n = 10$) animals significantly attenuated the CCh-LTD. Bar diagrams showing the mean of EPSP responses 90 min following wash out of CCh recorded in slices obtained from young adult and aged rats in presence (■) and absence of anisomycin (○). Relative to control (Cont), CCh-LTD was significantly reduced by preincubation with anisomycin (Aniso) in both groups. *, a significant depression from the control group. Representative traces of EPSP responses for the time points indicated in A and B of carbachol-induced LTD with and without (control) anisomycin from both age groups.
paired-pulse ratio at ∼60–90 min, following the start of drug washout, suggests that a shift in transmitter release does not mediate the age-difference in long-term synaptic depression induced by CCh.

Activation of metabotropic glutamate or muscarinic receptors induces a form of long-term depression that requires protein synthesis (Huber et al. 2000; Kumar and Foster 2007; Massey et al. 2001; McCoy and McMahon 2007; Nosyreva and Huber 2005; Volk et al. 2007). To determine the role of protein synthesis in CCh-LTD, slices were preincubated (30–40 min) in the protein synthesis inhibitor, anisomycin (20 μM). Anisomycin significantly attenuated the long-lasting synaptic depression in young adults \( F(1,15) = 4.60, P < 0.04 \) compared to the respective interleaved vehicle control (\( n = 10, \) Fig. 4A).

Preincubation of slices obtained from aged rats in anisomycin significantly reduced the CCh-LTD \( F(1,23) = 5.54, P < 0.02 \) compared to the vehicle control (\( n = 10, \) Fig. 4B). These results suggest that the expression of synaptic depression induced by muscarinic agonist is dependent on protein synthesis in young adult and aged animals; however, the dependence of CCh-induced synaptic depression on protein synthesis probably wanes with aging.

Another form of synaptic depression requires Ca\(^{2+}\) influx from N-methyl-D-aspartate (NMDA) receptors and/or L-type voltage-gated Ca\(^{2+}\) channels and is protein synthesis independent (Christie et al. 1997; Norris et al. 1998; Oliet et al. 1997; Thiels et al. 1994). To determine whether CCh-LTD requires Ca\(^{2+}\) from these sources, I preapplied (30–40 min) the NMDA receptor blocker, AP-5, or the L-type Ca\(^{2+}\) channel antagonist, nifedipine, to the bath solution of slices obtained from young adult and aged animals. Preincubation of slices with AP-5 (100 μM) failed to block the CCh-LTD in slices obtained from young adults (\( n = 5 \)) but significantly reduced CCh-LTD in aged rats (\( P < 0.008, n = 5 \)) compared to the respective interleaved control groups (young, \( n = 12, \) aged, \( n = 14; \) Fig. 5, A and B). Prior application of nifedipine (10 μM) reduced the long-lasting synaptic depression in young adult animals (\( P < 0.007, n = 5 \)) compared to control (\( n = 12, \)) and blocked the CCh-LTD in aged animals (\( P < 0.0001, n = 5 \)) compared to control (\( n = 14; \) Fig. 5, C and D). However, nifedipine failed to attenuate the transient phase of CCh-LTD in either group.

Synaptic depression induced by CCh does not require synaptic activation

The reduction of CCh-LTD by the NMDA receptor antagonist, AP-5 suggests a role for synaptic activity in induction
processes. To elucidate the role of synaptic activation in mediating the enhanced synaptic depression induced by CCh during senescence, I performed experiments wherein CCh was applied without concurrent synaptic stimulation. The baseline synaptic activation was turned off to the second path during CCh application and 10 min following CCh cessation. No difference was observed in the level of synaptic depression induced by CCh with and without synaptic stimulation in young adult (Fig. 6) and aged (Fig. 7, A and C) animals. To control for the possibility that CCh was inducing activity in CA3 cells (Bianchi and Wong 1994), we examined the effects of afferent stimulation during CCh application in slices wherein the CA3 region was surgically removed. Compared with intact slices, there was no difference in the magnitude of CCh-LTD in slices in which the CA3 region was removed, regardless of whether stimulation occurred during CCh application (Fig. 7, B and D). The results are consistent with earlier studies, demonstrating that CCh-LTD is independent of synaptic activation in younger animals (Massey et al. 2001; Volk et al. 2007).

**DISCUSSION**

The principal finding of the present study is that the CCh-induced synaptic depression at CA3-CA1 hippocampal synapses is enhanced in aged animals relative to young adults. At least two major forms of LTD can be observed in the CA1 hippocampal region. One form can be observed following activation of metabotropic glutamate receptors, something that is dependent on protein synthesis (Huber et al. 2000; Kumar and Foster 2007; Massey et al. 2001; McCoy and McMahon 2007; Nosyreva and Huber 2005; Volk et al. 2007). The other form is protein-synthesis independent and requires membrane depolarization, as well as a rise in postsynaptic Ca$^{2+}$ influx through NMDA receptors and L-channels (Bolskakov and Siegelbaum 1994; Christie et al. 1997; Dudek and Bear 1992; Mulkey and Malenka 1992; Norris et al. 1998; Oliet et al. 1997; Thiels et al. 1994). It is now apparent that the susceptibility and expression of these forms of LTD change across the life span (Dudek and Bear 1993; Kemp et al. 2000; Kumar and Foster 2007; Norris et al. 1996; Nosyreva and Huber 2005). The results of the current study reveal that CCh-LTD, like stimulation-induced LTD (Foster 2002; Foster and Kumar 2002; Norris et al. 1996, 1998) and metabotropic receptor activation induced LTD (Kumar and Foster 2007), is enhanced during senescence. In each case, there is an essential role for L-channels in mediating age differences, supporting the idea that altered Ca$^{2+}$ homeostasis contributes to age differences in synaptic depression (Foster 2007). However, it should be noted that the metabotropic receptor induced LTD and CCh-LTD during senescence also involve a significant contribution of NMDA receptors.

NMDA receptor and L-channel activity depend on the membrane potential, and cholinergic activity can increase excitation of hippocampal neurons (Benson et al. 1988; Blitzer et al. 1991; Fraser and MacVicar 1996; Haas 1982; Halliwell and Adams 1982; Muller and Missfeld 1986). Previous researchers have suggested that an increase in Ca$^{2+}$ signaling following M$_1$ muscarinic receptor activation is likely due to the inhibition of the afterhyperpolarization and enhancement of a slow onset depolarization (Egorov et al. 1999; Wu et al. 2004). Moreover, muscarinic activity can have direct effects that increase NMDA receptor currents (Markram and Segal 1990) and enhance the Ca$^{2+}$ signaling associated with backpropagating action potentials (Cho et al. 2008), which augment NMDA receptor activity and increase Ca$^{2+}$ influx due to increased cell discharge activity. A reduction in synaptic depression by NMDA receptor blockade suggests that CCh-LTD depends on synaptic activity. However, we observed that CCh induces significant synaptic depression both in stimulated and nonstimulated pathways in the same slice, suggesting another source of glutamate. Recent work indicates that CCh can act on muscarinic receptors on astrocytes, increasing intracellular Ca$^{2+}$ and triggering the release of glutamate (Araque et al. 1998a,b; Kang et al. 1998; Parpura et al. 1994; Shelton and McCarthy 2000). The results are consistent with earlier studies, demonstrating that CCh-LTD is independent of synaptic activation in younger animals.
(Massey et al. 2001; Volk et al. 2007). In contrast, other studies have shown that CCh-LTD in the visual cortex (Kirkwood et al. 1999) and in the hippocampus (Scheiderer et al. 2006) of juvenile animals depends on synaptic activation, suggesting the possibility of a developmental shift in the induction mechanism as observed during metabotropic glutamate receptor activation-induced synaptic depression (Dumas and Foster 1997; Kumar and Foster 2007; Luscher and Huber 2010; Nosyreva and Huber 2005).

Previous studies have demonstrated that M₁ muscarinic cholinergic receptors contribute to the regulation of synaptic plasticity in young rats (Kirkwood et al. 1999; Scheiderer et al. 2006). Furthermore, a recent study indicates that M₁ receptors are required for another form of long-term synaptic depression induced by metabotropic glutamate receptor agonist (s)-3,5-dihydroxyphenylglycine (Kamsler et al. 2010). Results of the current study that pirenzepine, a selective M₁ cholinergic receptor antagonist, significantly attenuated the CCh-LTD in both young adult and aged rats, extend previous findings to aged animals, demonstrating that M₁ cholinergic receptors play a role in modulating synaptic plasticity in the aging hippocampus. Expression of CCh-LTD likely involves presynaptic mechanisms and is protein-synthesis dependent that is consistent with earlier findings, which show that the expression of various forms of LTD requires protein synthesis (Huber et al. 2000; Kumar and Foster 2007; Massey et al. 2001; McCoy and McMahon 2007; Nosyreva and Huber 2005; Sajikumar and Frey 2003; Volk et al. 2007).

The CCh-LTD imitates endogenous- and cholinergic-dependent synaptic depression of glutamate transmission, and studies have established that cholinergic innervations to the hippocampus are pivotal to normal learning and memory functions (Bartus et al. 1982; Callahan et al. 1993; Dekker et al. 1991; Drachman and Leavitt 1974; Fibiger 1991; Hasselmo 1999). Alterations in cholinergic transmission due to aging or pathological conditions could contribute to a decline in cognitive functions. The results indicate that CCh-LTD is one of the few forms of synaptic plasticity that can change during senescence (Foster 2002) and may contribute to an age-related reduction in synaptic transmission in the CA1 region (Barnes et al. 1992; Jouveenceau et al. 1998; Landfield et al. 1986), thus causing a memory decline in aged animals (Barnes et al. 2000; Deupree et al. 1993).

Aging is associated with a marked decrease in the cholinergic system, including a decline in acetylcholine synthesis, release, and presence in the synapse. Furthermore, the cholinergic hypothesis of aging states that serious loss of function of the cholinergic neurons arising from the basal forebrain and terminating in the cortex and hippocampus contributes to impairment in cognitive function associated with advanced age.
and neurodegenerative diseases (Bartus 2000; Bartus et al. 1982). Despite years of research, enhancing cholinergic neurotransmission is the primary pharmacological intervention for treating and forestalling the cognitive deficit associated with neurodegenerative diseases. The results of the current study demonstrate that even in the face of less cholinergic tone in the hippocampus with advanced age, the senescent CA3–CA1 synapses are capable of cholinergic modulation. In addition, the higher concentration of muscarinic agonist used in this study, as well as several previous studies (Kirkwood et al. 1999; Massey et al. 2001; McCoy and McMahon 2007; McCoy et al. 2008; McCutchen et al. 2006; Scheideler et al. 2006, 2008; Volk et al. 2007), may not represent the in vivo condition but emphasizes the functional significance of modulating cholinergic transmission exogenously. Future studies using a lower concentration of muscarinic agonist could possibly represent such a condition in vivo and may provide an explanation about both the waning cholinergic function with advanced age and neurodegenerative diseases, as well as the intrinsic mechanisms that contribute to enhanced synaptic depression.

In conclusion, the present study demonstrates that CCh-LTD is enhanced in aged animals when compared to young adults. The data provide evidence for a role of L-channels in the induction of CCh-LTD during aging as reported previously in the visual cortex (McCoy and McMahon 2007). The finding of age-related differences in CCh-induced synaptic depression, including a role of NMDA receptors and L-channels, provides an avenue for investigating the role of modulating cholinergic transmission in learning and memory during aging. Future studies will better elucidate the signaling cascades involved in the enhanced CCh-induced synaptic depression at CA3–CA1 hippocampal synapses, as well as the influence of cholinergic modulation on other forms of synaptic plasticity and cognitive function during senescence.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES
Auerbach JM, Segal M. Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. J Physiol 492(Pt 2):479–493, 1996.
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