Reduced Synaptic Plasticity in the Lateral Perforant Path Input to the Dentate Gyrus of Aged C57BL/6 Mice

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INTRODUCTION

It has been shown that aged rats can learn and retain information in the short term, but long-term learning in older animals is often slower than in younger rats and decays more rapidly (Barnes 1979; Dunnett et al. 1988, 1990; Mabry et al. 1996; Martinez and Rigter 1983; Oler and Markus 1998; Winocur 1988; Zornetzer et al. 1982). The most pronounced memory deficit associated with age is typically the poor retention of newly acquired information over time. This suggests that aged animals are, to some extent, lacking the mechanisms required for the storage and maintenance of new memories, but are not necessarily deficient in the mechanisms necessary for the acquisition and initial formation of memories. A number of studies have also shown impaired synaptic function in the hippocampus of aged animals, possibly indicating that these changes may underlay the poor learning performance in older animals (Barnes 1994; Geinisman et al. 1995; Landfield 1988; Thibault et al. 2001).

Long-term potentiation (LTP) of synaptic efficacy serves as the main biological model for learning and memory processes in the CNS (for review, see Bliss and Collingridge 1993). The majority of studies are performed in young rats (<3 mo of age), presumably because they provide a well-established animal model and possess a large hippocampus, amenable to study both in vivo and in vitro (e.g., see Blank et al. 2002; Foster and Dumas 2001; Ross and Soltész 2001; Rush et al. 2000). LTP in older animals has mainly been described in the mediodentral perforant path inputs to the dentate gyrus or the Schaffer collateral inputs of the CA1 region of the hippocampus proper. In these studies, it is primarily the longevity of LTP that is compromised, not the initial posttetanic magnitude (Barnes 1979, 1985; Deupree et al. 1991, 1993; Landfield and Lynch 1977; Landfield et al. 1978).

METHODS

C57BL/6 mice were used in the present study (UBC Animal Care Protocol AO1-0089). Following decapitation, brains were rapidly removed while submerged in chilled artificial cerebrospinal fluid (ACSF; pH 7.2) containing (in mM) 125.0 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25.0 NaHCO3, 2 CaCl2, 1.3 MgCl2, and 10.0 dextrose, and continuously bubbled with 95% O2-5% CO2. While submerged in

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chilled ACSF, transverse slices containing the hippocampal formation were sectioned at 400 μm using a vibratome. For recovery, each slice was sequentially placed in a beaker containing warm ACSF (30°C) continuously bubbled with 95% O₂-5% CO₂ for ≥30 min. Individual slices were transferred to the recording chamber as required. Responses were obtained using a 1–3 MΩ recording electrode filled with ACSF and a Dagan BVC-700 amplifier. Responses were evoked with a sharpened tungsten electrode (A-M systems) using biphasic current pulses (120 μs, 10–400 μA) and a digital stimulus isolation unit (Getting Instruments). All data acquisition and analysis was performed using software provided by Getting Instruments (Lee Campbell).

An Olympus BX50wi microscope (10× objective) was used to visually position both the recording and stimulating electrodes for each experiment. Electrodes were positioned in the outer third of the molecular layer, adjacent to the hippocampal fissure, and distal from the dentate granule cell layer. Stimulation intensity was adjusted to yield response amplitudes approximately 30% of the maximum. All evoked responses were initially tested with paired-pulse stimuli (50-ms interpulse interval). During experiments, individual synaptic responses were continuously elicited at 15-s intervals, except during the application of the conditioning stimulation. After a minimum of 15 min of stable baseline responses were obtained, LTP was induced by applying four bursts of 50 pulses at 100 Hz (30 s between bursts). Single pulse stimulation was again initiated immediately following the last tetanus and continued for a minimum of 30 min. All data were acquired at 5–10 kHz, and the initial slope of the negative going waveform was used to assess changes in synaptic efficacy (Christie et al. 1999). Amplitude measurements were taken as the voltage difference between the initial 20 ms of data (acquired before presenting a stimulus), and the most negative component of the resulting stimulus induced waveform. Paired-pulse (PP) responses were presented as the normalized difference between the slopes of the two responses and are presented as a percentage change. LTP is quantified as the percentage change in individual responses collected following the application of conditioning stimuli compared with the average of 60 responses acquired prior to tetanic stimulation. In all figures, data are presented as the mean ± SE for that data set. For analysis purposes, data for unpaired t-tests were grouped according to whether the animal was older or younger than 12 mo of age.

RESULTS

Lateral perforant path responses exhibit normal morphology, but reduced PP facilitation and amplitude, in aged animals

To determine whether there are age-related changes in the response profile for lateral perforant path (LPP)-evoked responses, we evaluated the morphology of evoked responses in mice 3–25 mo of age in vitro. Using a constant intensity stimulus, we found that, overall, the size of the evoked response elicited with LPP stimulation declined as a function of the age of the animal \[ y = -0.1382x + 19.627, R^2 = 0.046, F(1,121) = 23.8, P < 0.05; \text{Fig. 1A, top} \]. The difference in response size was not, however, due to changes in any specific

![Image](https://example.com/image1.png)

**FIG. 1.** Paired-pulse facilitation of field excitatory postsynaptic potentials (EPSPs) recorded at LPP synapses is diminished in slices obtained from aged (24 mo) but not young (3 mo) mice. A: scatterplots showing a decrease in EPSP peak amplitude (mV) and paired-pulse facilitation with age. Paired-pulse facilitation data are plotted as ratio of initial slope of the 2nd EPSP/1st EPSP (S2/S1). B: representative samples of EPSPs recorded in the outer molecular layer. Responses from 3- (gray line) and 24- (black line) mo-old mice are presented individually and superimposed to illustrate the similarity in response onset and decay measures (see superimposition). C and D: averaged paired-pulse responses taken from 3 separate 3- and 24-mo-old animals. Slices from 3-mo-old mice showed significant paired-pulse facilitation (C) of the 2nd EPSP evoked with paired-pulse stimuli (50 ms), while paired-pulse facilitation was not observed when these same stimuli were presented to slices taken from older animals (D).
components of the response. Field EPSPs were similar to those observed in young animals in previous in vitro studies (Colino and Malenka 1993; Hanse and Gustafsson 1992) in all ages of animals examined here. As shown in Fig. 1B, stimulation of the LPP in both old and young animals elicited negative field EPSPs that were identical in both latency and duration.

Stimulation of the LPP has reliably been shown to result in PP facilitation of responses recorded in the DG in young animals both in vivo and in vitro (Christie and Abraham 1992a,b; Colino and Malenka 1993; Hanse and Gustafsson 1992; McNaughton and Barnes 1977). Presumably this reflects a lower release probability in lateral compared with medial perforant path synapses, further differentiating the two inputs (McNaughton and Barnes 1977; Zucker 1989). In animals up to 1 yr in age, application of the PP stimuli to the LPP produced a significant PP facilitation in the DG (24.65 ± 2.4%, n = 47; t(46) = 10.05, P < 0.05; Fig. 1C). Conversely, in animals over 1 yr of age, the application of these same stimuli failed to reliably produce a significant PP facilitation (6.74 ± 5.6, n = 23; t(22) = 1.20, P > 0.05). All recordings were visually confirmed as being in the outer molecular layer for both groups. We next repeated these experiments with inhibition intact. Similarly, synapses from animals less than 1 yr in age reliably exhibited PP facilitation (12.89 ± 2.4, n = 42; t(41) = 5.38, P < 0.05), while slices from animals older than 1 yr failed to exhibit significant PP facilitation (3.20 ± 5.3, n = 8; t(22) = 0.60, P > 0.05; Fig. 2A). Taken together, these data indicate that there may be differences in neurotransmitter release that accompany the aging process, and that lateral path synapses in older animals may have a higher probability of release. Figure 1, C and D, shows examples of PP responses recorded from both old and young animals. Regression analysis of PP responses elicited in young and old animals revealed that slices obtained from older animals exhibited significantly less PP facilitation than did slices obtained from younger animals (y = 0.0326x + 13.668, R² = 0.0033, F(1,118) = 10.7, P < 0.05; Fig. 1A, bottom). To determine if the size of the initial evoked response may have played a role in determining the degree of PP facilitation, regression analysis was performed using the initial amplitude of the evoked response across animals as the independent variable and the degree of PP facilitation as the dependent variable. This analysis revealed that initial response amplitude did not predict the degree of PP facilitation exhibited (P > 0.05), confirming our previous observations in vivo (Christie and Abraham 1992a,b).

Ability of the lateral perforant path to exhibit LTP declines with age

There is some controversy in the literature regarding the susceptibility of the medial perforant path input to age-related declines in its ability to exhibit LTP. A number of studies have failed to find any age-related decrease in the ability of the medial perforant path input to exhibit LTP, while others have shown an age-related reduction in the ability of the medial perforant pathway to sustain LTP (Barnes 1979; De Toledo-Morrell et al. 1988; Landfield and Lynch 1977; Maroun and Richter-Levin 2002). In normal ACSF, the application of high-frequency stimuli (HFS) at 100 Hz for 0.5 s (repeated 4 times) to LPP fibers reliably elicited robust short-term potentiation (STP; 15.0 ± 1.9%, n = 42; t(41) = 7.98; P < 0.05) and LTP (13 ± 4%, n = 42; t(41) = 3.24; P < 0.05) in animals <12 mo in age (see Fig. 2B). These findings are in agreement with previous work examining the ability of lateral perforant path evoked responses to exhibit LTP in younger animals (<3 mo; Colino and Malenka 1992; Hanse and Gustafsson 1992). In contrast, when we applied these same high-frequency stimuli to hippocampal slices obtained from animals >12 mo in age, we did not observe significant LTP. Although significant STP

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was observed in these slices (17.3 ± 3.3%, n = 42, \( t_{41} = 5.3 \); \( P < 0.05 \)), this short-term effect did not translate into robust long-lasting LTP, and moreover, a slight depression was observed (9 ± 6%, \( n = 8 \), \( P > 0.05 \)). Thus although there was no significant difference between the amount of STP observed between slices obtained from animals 1–12 mo of age (15.0 ± 1.9%) or 12–25 mo of age (17.3 ± 3.3%), slices obtained from older animals displayed significantly less LTP than slices obtained from animals in the younger group (\( t_{48} = 2.28 \); \( n = 49 \), \( P < 0.05 \)). Figure 3 illustrates these findings and shows that for all animals tested, although STP is equivalent across age (\( y = 0.031x + 20.755 \); \( R^2 = 0.0032 \)), LTP declines significantly as a function of age (\( y = -0.1382x + 19.627 \); \( R^2 = 0.046 \)).

Blocking \( \text{GABA}_A \)-mediated inhibition facilitates synaptic plasticity in young, but not old, animals

As the level of synaptic inhibition changes with age (Distefano and Teyler 1994), we tested the effects of blocking \( \text{GABA}_A \) inhibition in slices obtained from old and young animals by adding 1.0 \( \mu \)M bicuculline to the perfusion medium. This procedure is routinely used to aid in the induction of LTP of medial path evoked responses in vitro, presumably by aiding in the spread of depolarization into the dendrites being stimulated (Colino and Malenka 1992; Sloviter and Brisman 1995; Tomasulo et al. 1993; Wang and Wojtowicz 1997; White et al. 1990). There was a small, but nonsignificant, increase in the amount of STP observed in slices taken from older animals (>12 mo) compared with that observed in normal ACSF (Bicuc: 28.3 ± 5.0%; Normal: 17.3 ± 3.2%). Surprisingly, this effect was greater in slices obtained from young animals, where STP increased significantly from 15.0 ± 1.8% to 27.7 ± 3.2% (\( t_{87} = 3.30 \); \( n = 88 \), \( P < 0.05 \), Fig. 4). As was observed in normal ACSF, slices from young animals (<12 mo) exhibited significant LTP (\( t_{68} = 2.24 \); \( n = 69 \), \( P < 0.05 \); 16.0 ± 3.0%). Slices taken from older animals also showed significantly more STP in the presence of bicuculline

**FIG. 3.** STP and LTP as a function of age. Induction of STP (A) was equivalent in all animals independent of age, while in contrast, LTP (B) declined significantly as a function of age.
layer have a higher probability of release in older animals. It appears that synapses in the outer portion of the molecular LPP as a function of age. Because a similar decrease was still some disagreement in the literature as to whether, or to these pre- and postsynaptic changes.

We next used PP facilitation to examine whether changes occurred in presynaptic release properties. Although there is still some disagreement in the literature as to whether, or to what extent, hippocampal synapses change during aging, our examinations did reveal that PP facilitation can change in the LPP as a function of age. Because a similar decrease was observed whether or not bicuculline was added to the ACSF, it appears that synapses in the outer portion of the molecular layer have a higher probability of release in older animals. It is interesting to note that this finding suggests that the decrease in response size we observed in aged animals is not due to a reduction in presynaptic release properties. However, further testing is required to discern whether the observed reduction in PPF is due to some structural/functional change that occurs in existing lateral path inputs in this region, or whether there might be some age-related infiltration of medial path synapses into this region in older animals.

Another major finding of this study was that LTP of synaptic efficacy was significantly decreased in lateral path synapses in older animals. STP was reliably produced in both young and old animals, suggesting that the synaptic mechanisms activated by the conditioning stimuli were intact, regardless of age. However, STP was only reliably translated into LTP in slices obtained from animals <1 yr in age, indicating that synapses in older animals are not as plastic as those of their younger counterparts. This deficiency was not the result of differences in the initial size of the evoked response or due to alterations in levels of inhibition in these animals. As part of the LTP induction protocol, all animals were tested using stimulus intensities that elicited responses approximately 30% of maximal amplitude. At this intensity, there was no significant difference in the initial response size between young and old animals. Similarly, addition of bicuculline to the bathing medium did not alter response sizes in either young or old animals. Slices taken from young animals showed a similar degree of LTP whether bicuculline was present (16 ± 3.0%) or absent (13 ± 4.0%). In the case of older animals, no significant change in the initial slope of the EPSP was observed whether bicuculline was present or absent. Taken together, these data support the conclusion that the ability of the lateral perforant path to exhibit changes in synaptic efficacy declines with age, independent of changes in GABA_A-mediated inhibition. In studies involving responses elicited by stimulation of the medial perforant path, there is some evidence that putative “mature” cells may require GABA_A-mediated inhibition to be blocked for them to exhibit LTP (Wang et al. 2000). In this study, “mature” neurons were differentiated from “young” neurons based on a number of morphological criteria, and it was proposed that the “young” neurons resulted from the process of neurogenesis and had different physiological properties compared with more mature neurons. Wang et al. (2000) further showed that cells that were putatively identified as “young” neurons were unaffected by the addition of bicuculline to the bathing medium. It may be that lateral perforant path stimulation preferentially activates these “young” neurons, but that under normal circumstances their numbers are insufficient in older animals to sustain LTP of field responses normally.

Mechanisms of age-related LTP deficits

Another difference between this and other studies examining age-related deficits in LTP is the fact that these differences are observed following HFS-induced LTP. Studies in CA1 have shown that age-related deficits in the expression of LTP are evident when LTP is induced with physiologically patterned stimulation, but that they are obscured when HFS is used (Barnes et al. 1996; Chang et al. 1991; Deupree et al. 1993; Landfield et al. 1978; Moore et al. 1993; Norris et al. 1996). Moreover, it has been suggested that patterns of stimulation that are more physiologically relevant than conventional HFS paradigms are required to accurately assess the ability of the hippocampus for encoding information (Moore et al. 1993). This does not seem to be the case with lateral perforant path synapses given the present results. In addition, there is widening support that the HFS stimulation protocol is also physiologically relevant. First, increases in postsynaptic intracellular Ca^{2+} concentrations have been associated with high-frequency patterns of stimulation (Jager et al. 2002). Second, gamma oscillations (30–100 Hz) are known to be evoked by bursts of LTP inducing bursts of HFS (100 Hz) (Poschel et al. 2003) and by sensory stimulation (Poschel et al. 2002). Finally, frequencies in this range are also thought to play a role in binding sensory information (Gray et al. 1989), the formation of short-term memories (Lisman and Idiart 1995), and to be important for synchronizing the activity of local and anatomically distributed populations of neurons (Buzsaki and Chrobak 1995). Although our study did not investigate whether changes in receptor populations were responsible for the decrease in LTP observed, Clayton et al. (2002) have reported age-related (6 vs. 16–24 mo) decreases in hippocampal NR1 and NR2B subunits of the NMDA receptor. They have also shown that decreased NR2B levels are correlated with reduced LTP and spatial ability in the Morris water-maze. In contrast, mice overexpressing the NR2B subunit show enhanced LTP effects, improved object recognition, and heightened contextual fear conditioning (Tang et al. 1999). In addition, Magnusson et al. (2002) have recently shown that NR2B mRNA levels decline as a function of age in C57BL/6 mice like those used here, supporting the hypothesis that an age-related decrease in NR2B expression may underlie the reduction in LTP expressed in aged animals. Clayton et al. (2002) have suggested that increased concentrations of calcium associated with age may be the cause of the

D I S C U S S I O N

Effects of age on synaptic plasticity

The present results demonstrate that the lateral perforant path input to the DG exhibits numerous changes that manifest themselves in electrophysiological indices of synaptic plasticity. First, although responses evoked with LPP stimulation did not differ morphologically, they did tend to be of smaller amplitude in older animals. This may reflect either a change in the number of postsynaptic spines present on granule cell dendrites; the number or density of postsynaptic receptors, and/or their respective subtypes; some alteration in the release properties of presynaptic fibers; or a combination of any of these pre- and postsynaptic changes.

Effects of age on synaptic plasticity of LTP whether bicuculline was present or absent. Taken together, these data support the conclusion that the ability of the lateral perforant path to exhibit changes in synaptic efficacy declines with age, independent of changes in GABA_A-mediated inhibition. In
selective down-regulation of NR2B levels. Age-related increases in cytosolic Ca\(^{2+}\) levels can result from altered calcium homeostasis (Thibault et al. 1998) and increased activity at voltage-gated calcium channels (Thibault and Landfield 1996). Thus the age-associated reduction of LTP may reflect an increase in cytosolic Ca\(^{2+}\) and shift the kinase-phosphatase balance to facilitate long-term depression (LTD) and impair LTP (Foster 1999). In partial support of this theory, overexpression of CaN in young rats has been shown to produce similar impairments of synaptic plasticity (Mansuy et al. 1998; Mayford and Kandel 1999; Winder et al. 1998; Zhuo et al. 1999).

According to this hypothesis, LTD should be easier to induce in older animals owing to the fact that protein dephosphorylation is a critical component of long-term decrements in synaptic efficacy. In hippocampal CA1 in vitro, both LTD and depotentiation, the reversal of previously established LTP, were, in fact, shown to be greater in magnitude in aged compared with adult Fischer 344 rats, but this difference was attributed to an age-dependent alteration in Ca\(^{2+}\) regulation (Norris et al. 1996). Furthermore, this facilitation of LTD induction and impairment of LTP induction in aged rats could be reversed by L-type Ca\(^{2+}\) channel blockade (Norris et al. 1998). It is interesting that in our preparation, high-frequency stimulation actually induced a modest depression in aged animals, rather than LTP, giving some credence to this hypothesis.

While altered synaptic function in hippocampal circuits has been associated with age-related learning and memory deficits (Barnes 1994; Foster and Norris 1997), little is known about age-related alterations in LTP in the dentate gyrus or the behavioral implications of such changes. Lesions of the lateral entorhinal cortex have been shown to prolong the duration of recognition memory on olfactory tasks (Ferry et al. 1996; Wirth et al. 1998). Thus the lateral path input to the dentate gyrus is an integral part of a normal functioning hippocampal circuit, and our research indicates that it may play a key role in age-related changes in synaptic plasticity and learning and memory. This research represents an important step in elucidating the mechanisms underlying age-related declines in learning and memory functions by indicating that there may be structural or mechanistic changes in the integrity of the mammalian dentate gyrus across the life span. Furthermore, this research suggests that such age-related deficits may be apparent first in the more distal processes of DG cells.

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