Altered dendritic integration in hippocampal granule cells of spatial learning-impaired, aged rats

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Abstract

Glutamatergic transmission at central synapses undergoes activity-dependent and developmental changes. In the hippocampal dentate gyrus, the non-N-methyl D-aspartate (NMDA) receptor component of field excitatory postsynaptic potentials (fEPSPs) increases with age in Fischer-344 rats. This effect may not depend on the animal’s activity or experience, but could be part of the developmental process. Age-dependent differences in synaptic transmission at the perforant path-granule cell synapse may be caused by changes in non-NMDA and NMDA receptor-mediated currents. To test this hypothesis we compared whole-cell excitatory postsynaptic currents (EPSCs) in dentate granule cells evoked by perforant path stimulation in young (3-4 months) and aged (22-27 months) Fischer-344 rats using a Cs⁺-based intracellular solution. Aged animals as a group showed spatial learning and memory deficits in the Morris water maze. With these recording methods, slope conductances of both non-NMDA and NMDA EPSCs at holding potentials -10 to +50 mV were significantly reduced in aged animals and the non-NMDA/NMDA ratio in aged animals was found to be significantly smaller than in young animals. In contrast, we detected no differences in basic electrophysiological parameters, or absolute amplitudes of non-NMDA and NMDA EPSCs. Extracellular Cs⁺ increased the fEPSP in young slices to a greater degree than was found in the aged slices, while it increased population spikes to a greater degree in the aged rats. Our results not only provide evidence for reduced glutamatergic synaptic responses in Fischer 344 rats, but also point to differential changes in Cs⁺-sensitive dendritic conductances, such as Iₜ, or inwardly rectifying potassium currents, during aging.
Introduction

A large body of literature indicates that aged rats show spatial learning and memory deficits compared to young animals, as assessed by hippocampal-dependent behavioral tasks (for review, see Burke and Barnes 2006; Foster 2007; Penner and Barnes 2007; Wilson et al. 2006). Synaptic transmission at the perforant path-granule cell synapse undergoes a number of changes during aging. These changes include a reduction of the number of synaptic contacts in the termination zone of the perforant path during aging (for review, see Geinisman et al. 1995), and a larger EPSP in aged rats (after adjusting for presynaptic fiber potential amplitude), which decreases as a function of age (Barnes and McNaughton 1980). Furthermore, aged rats show a larger granule cell spike discharge for a given EPSP amplitude (Barnes 1979) together with a larger unitary EPSP (McNaughton et al. 1981) and larger quantal content (Foster et al. 1991) of perforant path synapses. In the dentate gyrus the non-NMDA-mediated fEPSP increases during aging, while the NMDA receptor-mediated fEPSP decreases (Rao et al. 1994). Lastly, the threshold to induce long-term potentiation (LTP) in the dentate gyrus of aged animals is higher compared to young animals (Barnes et al. 2000), and LTP decays more rapidly over the course of days in this part of the hippocampus in aged rats (Barnes 1979). Some of these electrophysiological changes observed in old animals might be interpreted as compensatory responses to loss of synaptic contacts, although the faster rate of LTP decay at granule cell synapses is correlated with age-related memory deficits (Rosenzweig and Barnes 2003).

While field potential recordings in the molecular layer of dentate gyrus have yielded larger non-NMDA/NMDA fEPSP ratio in aged rats compared to young (Rao et al. 1994), a confirmation of these findings at the level of evoked ionotropic glutamatergic currents is still missing. Thus, the present study was designed to determine whether and how individual components of the excitatory ion currents at the perforant path-granule cell synapse change during aging. To do this, we recorded excitatory postsynaptic currents (EPSCs) from granule cells using the standard Cs+-based internal solution in the patch pipettes. The responses were evoked by stimulation of the perforant path that arises from layer II entorhinal cortical cells (Steward and Scoville 1976) in slices obtained from young and aged Fischer-344 rats.

In recent years evidence has accumulated for the expression and function of voltage-sensitive ion channels expressed in dendrites of central neurons (Johnston et al. 1996; Magee 2000; Stuart et al. 1997). To test whether active dendritic conductances such as the cationic current $I_h$ (Bender et al. 2007; Chen 2004; Poolos et al. 2002) or inwardly rectifying potassium
currents (Karschin et al. 1996; Koyrakh et al. 2005; Ponce et al. 1996), which are blocked in whole-cell recordings by intracellular Cs⁺, alter synaptic potentials in an age-dependent manner (Bender et al. 2001; Magee 1999), we also recorded fEPSPs and population spikes in the granule cell layer of young and old rats in the absence and presence of extracellular Cs⁺. For both sets of experiments spatial learning and memory deficits were confirmed in aged animals by testing the young and old rats in the Morris water maze prior to the electrophysiological recordings. Portions of these results have been published previously in abstract form (Houston et al. 2004; Krause et al. 2002).
Material and Methods

Two experiments were conducted, referred to here as Experiment 1 and Experiment 2. Both experiments consisted of an initial behavioral screening test of spatial and visually-cued learning and memory in the Morris water maze task, followed by electrophysiological analysis in acute hippocampal slices from these animals.

Animals

For Experiment 1 we used 11 young (mean age 3.0 months at the time of behavioral testing) and 11 aged (mean age 22.2 months at the time of behavioral testing) male Fischer-344 rats. For Experiment 2 we used 6 young (mean age 4.0 months at the time of behavioral testing) and 6 aged (mean age 26.0 months at the time of behavioral testing) male Fischer-344 rats. All animals were obtained from the National Institute of Aging colony at Harlan (Indianapolis, IN). The time between the behavioral and electrophysiological testing was approximately 2 weeks. None of the included rats had cataracts or other visual or motor problems that prevented them from good performance on cued versions of the swim task. Rats were housed in pairs, and had access to food and water ad libitum. All experiments were conducted during the dark phase of their 12-h light/12-h dark cycle. After behavioral testing in Experiment 1 animals were shipped to the Max-Planck-Institute for Experimental Medicine in Göttingen, Germany, for the whole-cell patch-clamp experiment for electrophysiological testing described below. For Experiment 2 rats were shipped, following behavioral testing, to Tensor Biosciences in Irvine, CA, for the field potential recording experiment examining the effects of extracellular Cs⁺ described below. In each experiment, rats were allowed to accommodate to their new environment for at least a week after arrival.

Morris Water Maze

To assess the rats’ spatial learning ability, we tested all animals in a 185-cm-diameter pool essentially as previously described (Shen and Barnes 1996). Procedures used for water maze training in Experiment 1 and 2 were identical except that in Experiment 2 the rats’ movements were recorded and analyzed with a camera mounted over the pool and computer software (HVS Image, UK). These data were used to obtain the corrected integrated path length (CIPL) in addition to the latency to find the platform. Because aged animals swim more slowly than do young animals, CIPL is a preferred measure to test the animals’ spatial memory.
since it is independent of the animals’ swim speed; however, for Experiment 1, the tracking data were lost due to a technical error, and thus only latency measures are available.

During the spatial (hidden-platform) version of the swim task, a circular escape platform was present in a constant location, submerged about 1 cm below the water surface. Each rat performed three training blocks per day (two training trials per block) for 4 days (24 trials total), with the release location varied randomly from trial to trial. Rats were allowed a 60-s inter-trial interval within a given training block, and 30-60 min of recovery time between training blocks, where rats were in a warm chamber so that body temperature was maintained.

Animals were also given a probe trial, which immediately followed the last spatial trial on day 4 of the spatial task. For the probe trial, the platform was removed from the tank, and the rats were allowed to swim and search for the platform for a total of 60 s (data for this procedure are only available for Experiment 2).

To control for possible age-related deficits in visual acuity and swimming ability, the same rats were also trained on a second version of this task, in which a visible platform was moved randomly to one of four locations in the tank after each trial (cued version). A total of 12 visible-platform trials were performed (6 trials daily for two days). In all trials, we recorded the time to reach the platform.

**In vitro hippocampal slice preparations**

**Patch-clamp recordings:** Animals were deeply anesthetized with halothane (Hoechst, Germany) and subsequently decapitated. The brain was quickly removed and submerged in ice-cold oxygenated ACSF containing (in mM): NaCl 125; KCl 1.25; KH2PO4 1.25; MgCl2 1.5; NaHCO3 25; CaCl2 1, and D-glucose 10 (pH 7.4). Transverse hippocampal slices were prepared with a vibratome (Leica, Germany), (400-µm-thick). The slices were held in CSF and a mixture of 95% O2 and 5% CO2 for up to 6 h at room temperature. After ≥ 1 h incubation, individual slices were transferred to a submerged chamber and kept in place by a light grid made out of nylon stocking for electrophysiological recording.

**Field potential recordings:** Animals were deeply anesthetized with halothane (Sigma, MO) and subsequently decapitated. Transverse slices (400 µm thick) were obtained from the dorsal hippocampus with a tissue chopper. Slices were incubated and recorded from at 32°C in an interface-style chamber in ACSF solution containing (in mM): NaCl 124, KCl 3, NaH2PO4 1.25, MgSO4 3.75, NaHCO3 25, CaCl2 2, glucose 10, gassed with 95% O2 and 5% CO2 (pH 7.4). A warm, humidified mixture of 95% O2/5% CO2 was added to the chamber throughout recovery
and recording periods. Slices were allowed to recover for a period of at least 1 h before recordings began.

**Electrophysiological recordings, stimulation and data acquisition**

**Patch-clamp recordings:** During recordings from hippocampal slices the [Ca\(^{2+}\)] was raised to 2.5 mM and 20 µM (-)-bicuculline-methochloride (Research Biochemicals International) was added to block GABA\(_A\)-receptor-mediated currents (flow rate: 2 ml/min). In some experiments we added CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) or d-AP5 (Tocris) to the bath solution to block AMPA/kainate- and NMDA-mediated currents, respectively. Patch-pipettes (3-5 MΩ) were pulled (Narishige PP-830, Japan) from borosilicate glass (ID 0.9 mm, OD 1.5 mm, Hilgenberg, Germany) and were filled with a solution containing (in mM): CsCl 115; tetraetylammonium chloride (TEA) 20; NaCl 5; Mg-ATP 4; HEPES 10; EGTA 5; QX-314 5; GTP 0.4 (titrated to pH 7.3 with CsOH; osmolarity 300 mOsm). In this solution, potassium currents and \(I_h\) were blocked by Cs\(^+\) and TEA and voltage-sensitive sodium channels were blocked by QX-314 (Calbiochem). The liquid junction potential was -4 mV, and was not subtracted from the command voltages. Dentate granule cells were identified by their location in the slice, patched using the “blind method” (Blanton et al. 1989), and recorded in the whole-cell configuration. Granule cells were further identified by the presence of synaptically-activated glutamatergic currents upon afferent stimulation of the perforant path. Synaptic currents (~60% of maximal amplitude) were evoked once every 10 s by orthodromic stimulation of the perforant path (100 µs duration) with 70-300 µA stimulation intensity (Isoflex, AMPI, Israel), using bipolar stainless steel electrodes (distance between tips ~200 µm). The distance between the stimulation electrode and the recorded cell was 200-250 µm. The majority of the recordings were made in the dorsal (or attached) blade of the dentate gyrus. Synaptic currents were low-pass filtered at 1 kHz, amplified with a patch-clamp amplifier (EPC-9, Heka, Germany) and digitized at 4 kHz using the software package Pulse (Heka, Germany). In all recordings, series resistance was regularly monitored by –5-mV test pulses of 100 ms duration at -70 mV holding potential (sampled at 40 kHz). Series resistance was estimated from the current difference between the transient peak at the beginning and the steady state at the end of that pulse. Average series resistance was 12.4 ± 0.7 MΩ in the young animals and 16.4 ± 1.0 MΩ in the aged animals, respectively. Only recordings with series resistances below 25 MΩ and changes no greater than 15% were accepted. The input resistance was estimated from the 100-ms pulse by measuring the current difference prior to the pulse and at steady state near the end of the test pulses. Between 1 and 5 neurons were recorded from each animal, but data were averaged for
a given animal to use for the age comparisons, in order to prevent introducing a sampling bias. All recordings were made at room temperature (20-22°C).

**Field potential recordings:** One or 2 slices were recorded from each animal. Input-output relationships were obtained by stimulating the perforant path (80 µs pulse width, 10-50 µA stimulation intensity) with a bipolar stimulation electrode. Excitatory postsynaptic field potentials (fEPSPs) were recorded with a glass pipette filled with 2 M NaCl (2-4 MΩ), which was placed near the granule cell layer/hilar border. Maximal population spike (PS) amplitudes were initially ascertained. Five other intensities were determined, the lowest being set at 1/5 the intensity of the maximal, and the other four set at equal intervals between this maximum and minimum. Three sample responses were recorded at each intensity (sampled at 20 kHz), with an overall stimulation rate of once per 30 sec. Each slice was first tested in ACSF, followed by the same protocol in 2 mM CsCl, which was added to the perfusion medium (~1 ml/min flow rate). If not otherwise specified, all chemicals used were purchased from Sigma.

**Data analysis**

**Water maze:** To analyze latency and corrected integrated path length in the hidden platform and visible platform task, a two-way repeated-measures ANOVA was used with age (young vs. aged) as the between-subjects factor and training (24 trials) as the repeated within-subjects factor. Post-hoc tests were used if appropriate. Rat’s dwell times in the 4 quadrants of the pool during the probe task were analyzed using one-way ANOVA. Fisher’s protected least-significant difference post-hoc tests were used to determine whether rats spent significantly more time in the target quadrant over other quadrants.

**Whole-cell patch-clamp recordings:** To establish current-voltage relationships (I-V relationship) the maximal amplitudes were measured (between 6-14 ms after stimulation onset, representing non-NMDA-mediated currents) and the amplitudes 70-80 ms after stimulation onset (representing NMDA-mediated currents) were obtained at every holding potential of the averaged waveforms (Fig. 3C). Current waveforms shown are averages of 5-10 single samples. The time constants of current onsets or decays were calculated using a single exponential fit. To estimate the positive slope conductance of non-NMDA- and NMDA-mediated currents, a line (least square method) was fit to the averaged current amplitudes at holding potentials -10, 10, 30, and 50 mV. Regression coefficients (Pearson) were ≥ 0.93, and correlations of all line fits were significant (p<0.05). Student’s t-tests were used for comparisons of two means, and alpha was set at the 0.05 level. For data analysis, the programs PulseFit (Heka, Germany) and Igor (Wavemetrics, OR) were used.
**Field potential recordings:** Custom-written software was used to estimate fEPSP slope and area of the population spike (Matlab, Mathworks, MA).

## Results

### Experiment 1

#### Behavioral testing in the Morris water maze

In the spatial learning portion of the water maze task a significant main effect of age was found \(F(1,20)=23.27, p<0.0001\), indicating that young animals reached the platform faster than did aged animals. A significant main effect of training was also found \(F(23,460)=15.47, p<0.0001\), indicating that the time to reach the platform was reduced with training. There was also a significant interaction of age and training \(F(23,460)=2.19, p<0.001\), suggesting that young animals benefited significantly more from the training than did the aged animals (Fig. 1A). A comparison of latencies of young and aged rats on each day of training shows that the young animals were significantly faster on any given day (day 1: \(t(112)=3.35, p<0.0001\); day 2: \(t(112)=5.19, p<0.0001\); day 3: \(t(112)=9.62, p<0.0001\); day 4: \(t(112)=6.77, p<0.0001\)).

----INSERT FIGURE 1 HERE----

In the cued-training version of the water maze task a significant main effect of age was found \(F(1,20)=6.13, p<0.0001\), indicating again that young rats navigated significantly faster to the platform. A main effect of training \(F(11,220)=3.68, p<0.0001\) was also found, but there was no statistically significant interaction \(F(11,220)=0.66, p=0.778\) of training and age. These results indicate that both age groups benefited equally from the training sessions, and that the rats became significantly faster at finding the platform over trials (Fig. 1B). The comparison of averaged latencies on each training day shows that the young rats found the platform significantly faster than did the old rats (day 1 (trials 1-6): \(t(65)=3.73, p<0.0005\); day 2 (trials 7-12): \(t(65)=3.11, p<0.005\)).

### Dentate gyrus granule cell EPSCs and non-NMDA/NMDA ratios in young and aged rats
To study the influence of aging on synaptic transmission in hippocampal granule cells, excitatory postsynaptic currents (EPSCs) were recorded in the whole-cell configuration. Currents were evoked by orthodromic stimulation of the perforant path from young (27 neurons) and aged (24 neurons) rats. No differences were detected in several basic electrophysiological measures such as input resistances, current rise and decay time constants, reversal potentials, and absolute current amplitudes (Table 1, p>0.05 in all cases). EPSCs evoked at different holding potentials were overall similar in both age groups, and comparable to those recorded in very young (20-30 days) animals (see examples in Fig. 2; (Keller et al. 1991).

----INSERT FIGURE 2 AND TABLE 1 HERE----

As expected, when the AMPA and kainate receptor antagonist CNQX (10 µM) was applied in combination with the NMDA-receptor antagonist d-AP5 (50 µM) the evoked EPSC was completely abolished (Fig. 3A). CNQX (10 µM) applied alone at -70 mV (N=6 animals in each group) reduced evoked EPSCs by 90% in both age groups (young vs. aged t(10)=2.2, p>0.9, Fig. 3B). At holding potentials positive to -10 mV EPSCs were composed of NMDA and non-NMDA currents (Fig. 3C). To analyze individual contributions of the NMDA and non-NMDA receptor-mediated currents to the total, the currents were separated on the basis of decay times. Figure 3C shows that the NMDA component is not contaminated by non-NMDA currents 70-80 ms after stimulus onset, allowing separate measurements of NMDA and non-NMDA components to be made from the same response. This method was favored over a pharmacological approach, which has also been used to separate currents (e.g., d-AP5), because we experienced a change in series resistances after wash-in of the blocker on several occasions.

----INSERT FIGURE 3 here----

The relationship between non-NMDA and NMDA-mediated currents in young and aged animals were determined by constructing current-voltage plots (I-Vs, Fig. 4). The currents were separated on the basis of their kinetics, as described earlier. I-V relationships were linear (correlation coefficient r>0.93; p<0.05) for both age groups from holding potentials positive of –10 mV showed a positive slope conductance (Fig. 4A). The age effects observed were: (i) the absolute values of slope conductances for non-NMDA currents were 3.29 ± 0.58 nS for young and 1.55 ± 0.20 nS for aged animals (t(20)= 2.75, p<0.05), and the absolute values of slope
conductances for NMDA currents were $2.10 \pm 0.35$ nS for young and $1.14 \pm 0.14$ nS for aged animals ($t(20)=2.53$, $p<0.05$) (Fig. 4B); (ii) the ratio of non-NMDA/NMDA slope conductance was $1.54 \pm 0.05$ for young and $1.34 \pm 0.04$ for aged animals, and was statistically different ($t(20) = 2.90$, $p<0.01$) (Fig. 4C).

In summary, although many electrophysiological parameters in dentate gyrus granule cells of young and aged animals are similar, differences were found in the absolute slope conductances of non-NMDA and NMDA-mediated currents, as well as a significant decrease in the non-NMDA/NMDA ratio in aged animals.

Experiment 2

The results from whole-cell patch-clamp recordings in dentate gyrus granule cells in Experiment 1 indicate a significant decrease in the non-NMDA/NMDA current ratio in aged animals. On the other hand, field potential recording experiments have suggested that there is an increase in this ratio with increasing age (c.f. Yang et al. 2008). One potential difference between the two recording methods is the fact that the patch-clamp recordings were conducted with Cs$^+$-containing patch pipettes to aid in isolating glutamatergic currents, while no Cs$^+$ was present in the conditions used for field potential recording. To test the hypothesis that the Cs$^+$ could play a role in the differences observed between studies, field potentials were recorded in young and old memory-characterized rats in the presence and absence of extracellular 2 mM Cs$^+$. Intracellular Cs$^+$ blocks $I_h$ and several potassium currents (Coetzee et al. 1999). When applied extracellularly, Cs$^+$ blocks $I_h$ (Chen 2004; Maccaferri et al. 1993) and inwardly rectifying currents (Lesage et al. 1995), such as potassium channels of the GIRK and IRK families (Halliwell and Adams 1982). The HCN channels (Ludwig et al. 1998; Santoro et al. 1998) underlying the $I_h$, are expressed in hippocampal granule cell dendrites (Bender et al. 2007), and can shape dendritic EPSPs (Magee 1998, 1999, 2000; Poolos et al. 2002; Williams and Stuart 2000). The field potential recordings allowed assessment of the effects of Cs$^+$ on both the synaptic and population spike components of the evoked responses. The rationale for Experiment 2 was thus to determine whether the effect of the presence or absence of $I_h$ or inwardly rectifying currents would produce effects on the fEPSP and PS that could explain the difference between the results obtained in the present experiment using patch-clamp methods versus those obtained using extracellular field potential recording methods.
Behavioral testing in the water maze

Hidden Platform Test: There was a significant main effect of age in the hidden platform, spatial version of the swim task. Young rats learned to take a more direct route to the hidden platform over trials than did the old rats (F(1,10)=35.95; p<0.0001). In addition, there was a significant effect of training (F(23,230)=5.01; p<0.0001), and an interaction effect (F(23,230)=1.84; p<0.013), indicating that the two groups did not benefit equivalently from the training sessions (Fig. 5A).

Probe Test: To test the animal’s ability to recall accurately the location of the hidden platform, it was removed for the spatial probe test that immediately followed the last session of the spatial training on day 4. Young rats spent significantly more time swimming in the target quadrant (quadrant 1), compared to any of the other 3 quadrants (one–way ANOVA: F(3, 23)=20.9, p<0.0001; followed by Tukey-Kramer’s post-hoc comparison). In contrast, the amount of time aged animals spent in the target quadrant was not significantly different from time spent in any of the other quadrants (one–way ANOVA: F(3, 23)=1.46, p=0.25, Fig. 5B). This indicates that the aged animals as a group did not remember the location of the escape platform as well as did the young animals.

Visible Platform Test: In order to test the possibility that the age difference in performance on the hidden platform test was a result of visual impairments, a visible platform test was conducted. There was no significant main effect of age between groups in this test (F(1,10)=7.55; p=0.21). There was, however, a significant effect of training, (F(11,110)=2.59; p<0.006), with no significant interaction (F(11,110)=1.07; p=0.39), indicating that there was no difference detected between groups in the improvement of their navigational accuracy towards the platform over trials (Fig. 5C).

Differential effects of extracellular Cs⁺ on dentate fEPSPs in young and aged rats

At least one week following behavioral testing, the slice electrophysiology experiments were initiated. fEPSPs and PSs in the dentate granule cell layer were measured before and after extracellular bath application of Cs⁺ (2 mM). Seven slices from young and 8 slices from aged animals were investigated (6 animals in each group). Figure 6A shows typical examples of responses obtained from young and aged animals during recordings of field potential responses at several different intensities. To ensure that the stimulation and recording
procedure by itself did not alter the response characteristics, the experiments were begun by obtaining responses at a low intensity up to the highest intensity, and then again back down to the lowest intensity. For each age group, the fEPSP slope and PS area obtained under the increasing or decreasing intensity protocols were not different. Thus, this stimulation paradigm per se did not change response magnitudes.

----INSERT FIGURE 6 here----

The effect of Cs⁺ was assessed by subtracting the responses with Cs from those in ACSF. Overall, the effect of Cs⁺ on fEPSP slopes and PS area is of opposite direction (Fig. 6B and C). While fEPSP slopes of young animals were greater affected by Cs⁺ (Fig. 6B, left panel; Fig. 6C), it affected PS area more in aged animals (Fig. 6B, right panel; Fig. 6C). Using a two-way ANOVA, with age and stimulus intensity as factors, we found significant main effects of age (F(1,59)=8.68, p<0.005) and intensity (F(4,59)=2.72, p<0.05) for fEPSP slopes. Using two-way ANOVA for the PS area we found significant main effect of intensity (F(4,59)=5.22, p<0.005), but not for age (F(1,59)=2.10, p>0.15).

These data suggest that extracellular Cs⁺, which blocks mainly H-currents in dentate gyrus granule cells (Chen 2004), affects field potentials differently in young and aged rats, possibly indicating different expression levels of the channel protein underlying this current, and/or a different age-dependent distribution of Cs⁺-sensitive channels in soma, proximal and distal dendrites.

Discussion

Whole-cell recordings and extracellular field recordings were used to describe, characterize and compare glutamatergic currents in dentate gyrus granule cells of young and aged, memory-impaired Fischer-344 rats. The primary finding from the standard whole cell patch method was that old rats, compared to young animals, show a decrease in overall slope conductance in the current-voltage relationship of glutamatergic currents, as well as a decrease in the ratio of non-NMDA/NMDA currents in dentate granule cells. The whole-cell patch-clamp recordings were performed with a Cs⁺-based internal solution for optimal voltage control. The second principal finding obtained from the field potential recording experiments, in which Cs⁺ concentration was directly manipulated, indicate an age-dependent change in the expression or sensitivity of Cs⁺-sensitive ion channels.
Glutamate receptor-mediated synaptic transmission in dentate granule cells of young and old rats

The data obtained in this study show that glutamatergic currents in dentate granule cells from adult and aged rats are very similar to those in 3-4 week-old rats. Reported rise time constants of glutamatergic EPSCs for these very young rats are 0.5-1.9 ms for non-NMDA EPSCs and 4-9 ms for NMDA EPSCs (Keller et al. 1991) for hippocampal granule cells, and 0.5-4 ms non-NMDA EPSCs and 4-8 ms for NMDA EPSCs in CA1 pyramidal cells (Hestrin et al. 1990; Otmakhova et al. 2002). These values do not substantially deviate from the values obtained from the mature and old hippocampal granule cells reported here. The reduced slope conductance in the I-V relationship of aged animals reported here is in agreement with smaller fEPSP amplitudes in aged animals (c.f. Yang et al. 2008), and is expected given the loss of perforant path synaptic contacts during aging (Geinisman et al. 1995). This synaptic loss is accompanied by a reduction in ionotropic glutamate receptor mRNA and protein expression in the hippocampus of aging rodents (Barnes and McNaughton 1980, 1983; Clark et al. 1992; Geinisman et al. 1995; Lippa et al. 1981; Magnusson 2000; Magnusson and Cotman 1993; Nicolle et al. 1996; Pagliusi et al. 1994; Tamaru et al. 1991; Wenk and Barnes 2000).

Patch-clamp recording experiments are typically conducted on animals at substantially younger ages than those in the present experiments. Because of the greater challenge of applying these methods for use in mature tissue, it was important to establish appropriate voltage control in these hippocampal granule cells. Thus, the series resistance in the present study was carefully monitored to assess electrical access to the inside of the cell. In addition, intracellular Cs⁺ and the sodium blocker QX-314 was used to increase the input resistance by blocking several ion channels. The median input resistance was 520 MΩ for young and 600 MΩ for aged animals. These values are, in fact, similar to values obtained by other investigators when using Cs⁺-containing patch pipettes in young animals (Stabel et al. 1992). Given that the highest accepted series resistance was 25 MΩ in the present study, the maximal voltage error near the soma was 7%, but was less than 5% for the majority of the recordings. A larger voltage error in the distal dendrites, however, cannot be excluded due to limited space-clamp conditions. Nonetheless, current-clamp recordings using sharp electrodes, where access resistance plays no role, does reveal very similar results to those obtained in the present study. In agreement with the present study, no differences in resting membrane potential, input resistance or action potential threshold in granule cells of young and old Fischer-344 rats were detected (Barnes and McNaughton 1980).
Reduction of glutamatergic currents at the perforant path - granule cell synapse during aging

Pharmacological characterization indicates that non-NMDA (AMPA and kainate) and NMDA-receptors mediated currents are the two main components of the EPSCs evoked by perforant path stimulation. Ratios of these currents have been studied in several brain regions such as neocortex, hippocampus, and ventral tegmental area, and are generally considered to be a dynamic measure of synaptic modification (Heynen et al. 1996, 2000; Otmakhova et al. 2002; Sjostrom et al. 2007; Sprengel et al. 1998; Ungless et al. 2001). Several methods are available to establish measures of a non-NMDA/NMDA response ratio in single neurons: (i) a pharmacological method, where a neuron is held at +40 mV and first recorded in normal ACSF and subsequently in the presence of APV (an NMDA receptor blocker). This method allows separation of the dual-component EPSC (Ungless et al. 2001), (ii) an alternative pharmacological method, where the charge at -20 mV holding potential in normal ACSF after application of APV is measured (Otmakhova et al. 2002); and (iii) a biophysical method, where an I-V relationship is recorded and the slope conductance for each receptor subtype is measured on the basis of the kinetics of the individual component (Sprengel et al. 1998). The latter biophysical method was preferred in the present study as a potential control for the possibility of changing series resistances before and after administration of glutamatergic blockers. Although several groups have reported results of non-NMDA/NMDA ratios, different recording conditions and analyses along with different species do not allow direct and meaningful comparisons of these ratios, and thus will not be attempted here. We can confidently conclude, however, that under the whole-cell recording conditions of the present study, non-NMDA and NMDA EPSCs decline with age in hippocampal granule cells, and this decline occurs in aged rats with hippocampal-dependent learning and memory deficits.

Role of Cs⁺-sensitive currents in synaptic transmission in dentate gyrus

Results from the present study illustrate a decline in glutamatergic EPSCs (Fig. 4B), and smaller dentate granule cell fEPSP slopes and PS areas (Fig. 6B and C) in aged, learning- and memory-impaired rats consistent with previous studies (Barnes 1979, Barnes and McNaughton 1980, Yang et al. 2008). The increase in the non-NMDA-mediated portion of the fEPSP in aged animals in the Yang et al. study is not congruent with the smaller non-NMDA/NMDA ratio observed in the whole-cell recording data collected in Experiment 1 of the present study. There are a number of technical differences between these preparations that may explain this
apparent discrepancy. Among these is the fact that the patch pipette contained blockers for sodium currents, $I_h$, and several potassium currents. None of these currents were blocked in the fEPSP recordings reported in the Yang et al. (2008) study. Internal Cs$^+$ blocks $I_h$ and inwardly rectifying potassium currents (e.g., GIRKs) in granule cells (Chen 2004; Mellor et al. 2002). Both HCN channels, which underlie $I_h$, and GIRKs are expressed in the dentate gyrus (Karschin et al. 1996; Monteggia et al. 2000; Moosmang et al. 1999; Ponce et al. 1996; Santoro et al. 2000). Functionally, these currents stabilize the neuron’s resting membrane potential, and could play a role in the size of fEPSPs and overall excitability. Particularly in dendrites, $I_h$ (Berger et al. 2001; Magee 1998, 1999; Poolos et al. 2002) and GIRKs (Takigawa and Alzheimer 1999, 2002, 2003) attenuate excitatory input. The activation time of these currents are on a similar time scale to that of dendritic fEPSPs (Magee 1998). Thus, amplitude and/or time course of the fEPSPs recorded in the molecular layer of the dentate gyrus may be differentially affected by an age-dependent expression of $I_h$ and GIRKs. The data obtained in the present report, is at least consist with this hypothesis. Our results indicate a stronger attenuation of fEPSPs in young rats by Cs$^+$-sensitive currents. This could be due to higher expression of these channels, and/or a shift of their activation curve to more depolarized potentials in young animals (e.g., in the case of $I_h$, the results might be explained by higher cAMP levels in younger animals; (Beaumont and Zucker 2000; Pedarzani and Storm 1995; Tombaugh et al. 2005).

Although our data implicate changes in Cs$^+$-sensitive potentials as an explanation for the differences observed between the present study and the Yang et al. (2008) results, we cannot entirely exclude other possible contributing factors. Specifically, the age-related changes observed in the non-NMDA/NMDA receptor ratios appear to be dependent on whether field potential recording methods were used to establish the ratio (see Yang et al., 2008), or whether whole-cell patch-clamp recordings were used, as in the present study. Besides the presence of cesium in the patch pipette, other possible technical explanations for the differences in results between these studies include the fact that the whole-cell recordings were performed in a submerged chamber while the field potential recordings used an interface chamber, recording temperature was different (22ºC in field potential recordings), there were minor differences in ACSF composition (CA$^{2+}$ was slightly higher in whole-cell recording, and K$^+$ and Mg$^{2+}$ was slightly higher in field potential recordings), and during whole-cell recordings there was the possibility of wash out of intracellular constituents. While these differences may have contributed to the differences between the data observed here and the Yang et al. (2008) data, it is likely that Cs$^+$-sensitive currents remain a major explanatory factor.
In summary, the present data suggest that aging not only changes expression and function of ionotropic glutamate receptor-mediated currents, but also results in changes of the dendritic integration of excitatory inputs.
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Disclosures

There are no disclosures.
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Figure Legends

Figure 1: Aged rats show a spatial learning deficit
Eleven young (mean: 3.0 months) and 11 aged (mean: 22.2 months) male Fischer-344 rats were tested in the Morris water maze.  

A: Spatial version of the water maze with the escape platform hidden. Rats were given 3 blocks of 2 trials of training every day for 4 days. Plotted are averaged latencies for each training day to find a hidden platform. Aged rats learned to find the hidden platform significantly more slowly than did young rats and benefited significantly less from the training.  

B: Cued version of the water maze with the escape platform visible. Here the platform was visible and its location in the pool and the rat’s starting location was randomly changed in each trial. Plotted are latencies of young and aged animals (same legend as in A). Rats were given 6 trials per day for two days. Both age groups learned to find the platform, but young animals reached the platform significantly faster than did old animals, possibly due to slower swim speed of aged animals. In this task, both age groups benefited equally well from the training.

Figure 2: Dentate granule cell EPSCs in young and aged F-344 rats show similar waveform kinetics
Hippocampal dentate granule cells from young or aged rats were voltage-clamped in the whole-cell configuration. EPSCs were evoked by orthodromic stimulation of the perforant path once every 10 s. Shown are averages of 5-10 sweeps recorded at the indicated holding potentials. Note that synaptic currents of both age groups show similar time course of current decays. Stimulation artifacts are blanked out for clarity.

Figure 3: Granule cell EPSCs are mediated by NMDA and non-NMDA receptors in both age groups
A: Evoked EPSCs are composite currents of non-NMDA and NMDA currents. EPSCs recorded in ACSF at -70 and +50 mV (gray waveforms) are completely blocked in the presence of 50 µM D-APV and 10 µM CNQX (black waveforms) in young and aged animals. Scale bars: 200 pA (young), 100 pA (aged), 10 ms. B: Evoked EPSC of young and aged animals at -70 mV, in the absence (gray waveforms) and presence (black waveforms) of 10 µM CNQX. On average, CNQX blocks ~90% of the peak current at -70 mV in both age groups (N=6 animals in each group). Note the similar time course of EPSCs in both age groups. Scale bar: 50 pA (young), 40 pA (aged), 20 ms. C: EPSCs recorded at -10 mV in ACSF (gray waveforms) and in 10 µM CNQX (black waveforms). To isolate the non-NMDA current (smallest waveform in each example), the current in CNQX was subtracted from the one in ACSF. The gray bar shows the window in which NMDA current amplitudes were measured (70-80 ms after the stimulus artifact). Note that non-NMDA current amplitudes in that window are close to pre-stimulus levels in both age groups, and therefore do not contaminate the measurement of the NMDA component. Scale bar: 50 pA, 50 ms.

Figure 4: Aged rats show a smaller non-NMDA/NMDA ratio in dentate granule cell EPSCs
A: Current-voltage relationships of the currents shown in Fig. 2 of evoked EPSCs of a young and an aged rat. Amplitudes were measured as described in Fig. 3. Amplitudes at holding potentials -10 to +50 mV were fitted by linear regression (Young: r(non-NMDA)=1.0, r(NMDA) = 0.99, p<0.05 in both groups; Aged: r(non-NMDA)=1.0, r(NMDA) = 0.98, p<0.05 in both groups). The slope conductance was 10.4 nS for non-NMDA currents and 7.2 nS for NMDA currents in young animals. In aged animals, the slope conductance was 0.6 nS for non-NMDA currents and 0.5 nS for NMDA currents. B: Summary data for 11 young and 11 aged animals showing the slope conductances for non-NMDA and NMDA currents. In A and B, gray depicts the fits of the non-NMDA currents, and black depicts the fits of the NMDA currents. (* p<.05) C: Summary
data for 11 young and 11 aged animals showing ratio of non-NMDA/NMDA slope conductances.

(*** p<.01)

Figure 5: Behavioral analysis of rats for the Cs⁺ experiment
Prior to the electrophysiological analysis, rats were tested in the spatial and cued versions of the water maze task.  
A: Corrected integrated path length (shortened here as “path length”) is plotted over days of training for young and aged rats navigating in the water maze.  Young rats found the platform significantly faster than did aged rats from the first day on (indicated by the asterisks).  
B: To test the animals’ recall of the platform location, the platform was removed in a single trial following the last hidden platform trial (A).  Young rats spent significantly more time in the target quadrant (1) than in any of the other quadrants (indicated by the asterisk).  In contrast, aged rats as a group did not spend more time in one quadrant over other quadrants.  
The dashed line indicates chance level.  
C: Corrected integrated path length plotted over trials during the visible platform task.  There was no difference between the performance of young and aged rats in this task.  This result confirms that differences seen in A are due to better spatial learning of young animals.

Figure 6: Cs⁺ differently affects fEPSP slopes and PSs in young vs. aged rats
A: Once responses to weak stimuli were stable, input-output (I-O) curves were recorded at 5 different intensities, starting from a low intensity to the highest intensity, and then back down to the lowest intensity (not shown).  A concentration of Cs⁺ (2 mM) was washed into the slice chamber over a period of about 30 min.  
By 30 min, responses to weak stimuli were stable, and I-O curves were then recorded using the same paradigm as for the control condition.  Example sweeps of I-O curves for 5 different stimulation intensities are shown.  Scale bars: 2 ms, 1 mV.  
B: Summary plots of the effects of Cs⁺ on fEPSP slope (left panel) and PS area (right panel) in young and aged animals.  Shown are the responses obtained in Cs⁺ subtracted from those in
ACSF. The effect of Cs⁺ on fEPSP slopes is larger in young rats compared to aged rats. C: Summary plots of PS area vs. fEPSP slope in young and aged animals, illustrating the differential effects of Cs⁺ on the two age groups. Every data point represents one stimulation intensity (same legend as in B).
Table 1: Basic Electrophysiological Parameters of Granule Cell EPSCs in Young and Aged F-344 rats (Mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rise time constant non-NMDA-mediated currents(^1)</td>
<td>5.3±0.4 ms (4)</td>
<td>4.2±0.9 ms (5)</td>
</tr>
<tr>
<td>Rise time constant NMDA-mediated currents(^2)</td>
<td>10.4±1.0 ms (6)</td>
<td>12.0±1.4 ms (7)</td>
</tr>
<tr>
<td>Decay time constant non-NMDA-mediated currents(^1)</td>
<td>16.6±1.6 ms (4)</td>
<td>22.4±3.3 ms (5)</td>
</tr>
<tr>
<td>Decay time constant NMDA-mediated currents(^2)</td>
<td>162±21 ms (6)</td>
<td>183±30 ms (7)</td>
</tr>
<tr>
<td>Input resistance (range)</td>
<td>568±27 MΩ (353-947 MΩ) (11)</td>
<td>639±68 MΩ (365-1072 MΩ) (11)</td>
</tr>
<tr>
<td>Reversal potential(^1)</td>
<td>13±2 mV (11)</td>
<td>16±2 mV (11)</td>
</tr>
<tr>
<td>Amplitude non-NMDA(^2)</td>
<td>-134±53 pA (4)</td>
<td>-118±24 pA (5)</td>
</tr>
<tr>
<td>Amplitude NMDA(^3)</td>
<td>77±33 pA (6)</td>
<td>38±10 pA (7)</td>
</tr>
</tbody>
</table>

\(^1\) liquid junction potential (4 mV) subtracted

\(^2\) recorded at -70 mV in the presence of 50 µM D-AP5

\(^3\) recorded at +50 mV in the presence of 10 µM CNQX

Numbers in parenthesis indicated numbers of recorded animals. All time constants were estimated by mono-exponential fits.
Figure 1

A

B
Figure 2

Young
Aged

+50 mV
+30 mV
+10 mV
-10 mV
-30 mV
-50 mV
-70 mV

50 pA

100 ms

100 pA

100 ms
Figure 3

A. **CNQX + APV**

- Young
- Aged

B. **CNQX**

- Young
- Aged

C. **Current separation (-10 mV)**

- Young
- Aged

residual current (in %)

![Graph showing residual current comparison between young and aged groups for CNQX and CNQX + APV treatments.](image-url)
Figure 4

A

Young

Aged

B

C

non-NMDA
NMDA
fit non-NMDA
fit NMDA

slope conductance (nS)

non-NMDA/NMDA ratio

Young Aged

**
Figure 5

A

B

C

days of training

quadrant

trial (6 per day)
Figure 6

A

ACSF

+ Cs⁺

Young

Aged

B

Δ fEPSP slope

Δ PS area

stimulation intensity

C

Δ PS area

Δ fEPSP slope