Synaptic Commitment: Developmentally Regulated Reciprocal Changes in Hippocampal Granule Cell NMDA and AMPA Receptors Over the Lifespan

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Yang Z, Krause M, Rao G, McNaughton BL, Barnes CA. Synaptic commitment: developmentally regulated reciprocal changes in hippocampal granule cell NMDA and AMPA receptors over the lifespan. J Neurophysiol 99: 2760–2768, 2008. First published April 16, 2008; doi:10.1152/jn.01276.2007. Synaptic transmission in hippocampal field CA1 is largely N-methyl-D-aspartate receptor (NMDAR) dependent during the early postnatal period. It becomes increasingly mediated by α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors until an adult ratio of AMPA to NMDA receptors is achieved. It is shown here that increases in the AMPA receptor (AMPA<sub>R</sub>)-mediated field potential response continue over the life span of the F-344 rat at the perforant path–granule cell synapse in the dentate gyrus. In contrast, the NMDAR<sub>R</sub>-dependent component of the response decreases with age between 1 and 27 mo, leading to an increase of AMPA<sub>R</sub>/NMDA<sub>R</sub> ratio with age. One possible explanation of this age difference is that the AMPA<sub>R</sub>/NMDA<sub>R</sub> ratio can be modified by experience. To test the idea that the changed ratio is caused by the old rats' longer lives, an intensive 10-mo period of enrichment treatment was given to a group of animals, beginning at 3 mo of age. Compared with animals housed in standard cages, the enrichment treatment did not alter the glutamatergic response ratio measured with field potential recording methods. These data provide support for the conclusion that the observed change with age is developmentally regulated rather than experience dependent. Given the role of the NMDA<sub>R</sub> in synaptic plasticity, these changes suggest a progressive commitment of perforant path synapses to particular weights over the life span. One possible implication of this effect includes preservation of selected memories, ultimately at the expense of a reduced capacity to store new information.

INTRODUCTION

Whole cell recording studies (Durand et al. 1996; Isaac et al. 1995; Liao et al. 1995) indicate that, in the early stages of synaptogenesis, many synapses in the Schaffer collateral input to the hippocampal CA1 region express a functional complement of N-methyl-D-aspartate receptors (NMDAR) but do not express the α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPA<sub>R</sub>) that come to mediate fast glutamatergic synaptic transmission in the adult (Blake et al. 1988; Davies and Collingridge 1989). At this early stage, functional AMPA receptors can be expressed by induction of long-term potentiation (LTP) (Bliss and Collingridge 1993), which depends on calcium entry through NMDA<sub>R</sub> channels (Durand et al. 1996; Isaac et al. 1995; Liao et al. 1995). In the Schaffer collateral pathway, the adult strengths of the NMDA<sub>R</sub>- and AMPA<sub>R</sub>-mediated components of the compound excitatory postsynaptic potential (EPSP) are achieved by ~1 mo of age, remain at similar levels in adulthood (9 mo), but show a decline at 26 mo of age. Nonetheless, the ratio of AMPA<sub>R</sub>-to NMDA<sub>R</sub>-mediated EPSPs remains virtually constant across the young, adult, and aged groups (Barnes et al. 1997). Thus a progressive increase of the AMPA<sub>R</sub>-mediated component of transmission at individual synapses during experience cannot be a mechanism of long-term information storage in the CA1 network, unless a compensatory reduction at other synapses occurs. This suggests that if AMPA<sub>R</sub> currents are increased as a consequence of LTP, as some studies suggest (Bliss and Collingridge 1993; Durand et al. 1996; Foster and McNaughton 1991; Isaac et al. 1995; Kauer et al. 1988; Liao et al. 1995; Lynch et al. 1982; Malenka and Nicoll 1999), this increase must be accompanied by an equal net reduction at other synapses, possibly through a long-term depression (LTD) mechanism. In fact, there is a decrease in the number of AMPA<sub>R</sub> clustered at synapses following the induction of LTD (Carroll et al. 1999; Shepherd et al. 2006). The induction of LTD, similar to LTP, is usually regulated by NMDA<sub>R</sub> currents (Christie et al. 1994; Dudek and Bear 1992; Mulkey and Malenka 1992), even though these two types of synaptic plasticity may depend on different subtypes of NMDA<sub>R</sub> (Liu et al. 2004).

The associative LTP/LTD learning principle is useful for a variety of information storage applications in neural networks (Bear and Malenka 1994; Coussens and Teyster 1996; Lisman and Idiart 1995), but suffers the problem that new information gradually overwrites the old. One way in which a network might function as a relatively long-term repository of information would be to restrict further changes in AMPA<sub>R</sub>-mediated transmission by reducing NMDA<sub>R</sub>-mediated currents once a synapse was committed to the storage of specific items. Such a network, however, would require large numbers of synapses and would have to use extremely sparse coding principles (Marr 1971), if its capacity is not to be used up too quickly. The region of the hippocampus that contains by far the largest total number of modifiable synapses (Amaral et al. 1990) and which uses the sparsest coding scheme (Jung and McNaughton 1993; Leutgeb et al. 2005) is the dentate gyrus granule cells. Thus the dentate gyrus would be a candidate system for durable information storage.

Whereas the number of axospinous synapses in the CA1 stratum radiatum remains the same in young and old rats (Geinisman et al. 2004), perforant path synapses from the entorhinal cortex to the dentate gyrus do undergo a net loss in
numbers over the life span (Geinisman et al. 1992, 1995). Interestingly, the perforant path to granule cell synapses that remain in the dentate gyrus of old rats have previously been found to be functionally more powerful (Foster et al. 1991). Motivated by the foregoing theoretical considerations, the questions addressed in this experiment were whether this net increase in synaptic efficacy in the dentate gyrus reflects an increase of AMPAR-mediated currents in aged rats and whether such changes are accompanied by a net increase or decrease in NMDAR currents. In addition, to address the question of whether changes in NMDAR- and AMPAR-mediated responses over the life span result from a fixed developmental process or are shaped by accumulated experience through life, the effect of experience on glutamatergic responses in hippocampal granule cells was examined in young adult rats.

**METHODS**

**Animals and treatment procedures**

In total, 54 male F-344 rats at ages 1, 9, and 27 mo (n = 18 per group) were used to study the effect of age on glutamatergic response ratios. The rats used in the aging experiment were housed singly in standard cages. In the experiment designed to study the effects of experience on glutamatergic response ratios, F-344 rats were raised under three different treatment conditions for 10 mo, beginning at 3 mo of age. In the standard control group (n = 9), rats were housed singly in standard cages, with no specific handling other than the routine weekly weighing and cage cleaning. In the wheel running/social enrichment group (n = 9), on each of 3 consecutive wk, the rat spent 1 wk in a cage in which a running wheel could be accessed freely and the other 2 wk in a cage with another rat from the same treatment group. In the enriched environment group (n = 10), all the rats were housed together in a big cage (~100 × 100 × 80 cm), scattered with frequently changed toys and tunnels. A running wheel was added into the cage every other week. In addition, the rats were taken to a bigger environment for free exploration for 1 h twice per week. All procedures were in accordance with the University of Arizona’s Institutional Animal Care and Use Committee.

**Morris swim task**

The protocol used for the Morris swim task is described in detail elsewhere (Barnes et al. 1997). In brief, the rats were first given spatial trials for 4 consecutive days, in which a 14-cm platform was hidden in a constant location inside of a 180-cm-diam tank. There were three training blocks each day (two 60-s trials per block, 60-s intertrial interval, 30- to 60-min interblock interval), with the releasing locations changed from trial to trial. One probe trial was given immediately following the last spatial trial on day 4, in which the platform was removed, and the rats were allowed to swim freely for 60 s. The rats were then given six “cued trials,” in which the platform became visible but its location was randomly changed across trials. Another six cued trials were given on day 5. Rats’ performance on the swim task was analyzed off-line with in-house software (WMAZE, M. Williams). In the spatial and cued trials, the latency and the path length to escape the water were measured. In the probe task, the search time of the rat in each quadrant and the number of target crossings, that is, how many times the rat swims across the location where the hidden platform used to be, were recorded to measure the rat’s memory for the location of the platform.

**Brain slices**

To study the effect of age on the glutamatergic response ratio, hippocampal slices from different age groups were prepared as described previously (Barnes et al. 1997). Briefly, rats were first anesthetized with methoxyfluorane (Metofane), and the hippocampi were rapidly dissected. Slices (450 μm) were cut parallel to the alvear fibers using a tissue chopper and were transferred to an interface style brain slice chamber perfused (2.0 ml/min) with artificial cerebrospinal fluid (ACSF) at 32 ± 1°C, oxygenated with a 95% O2-5% CO2. For the initial hour, the ACSF consisted of the following (in mM): 124 NaCl, 2 KCl, 1.25 KH2PO4, 2 MgSO4, 26 NaHCO3, 10 dextrose, and 2 CaCl2. The Mg2+ was then reduced to 200 μM to facilitate recording of the NMDAR component of the EPSP. After another 1 h of incubation, a recording pipette and a bipolar stimulating electrode were placed ~300 μm apart in the stratum molecular of the dentate gyrus (Fig. 1A). Rats from the three enrichment treatment groups were killed at least 1 wk after being tested on the Morris swim task.

**Field potential recording**

Field EPSPs (fEPSPs) and presynaptic fiber potentials were recorded from the lower (detached) blade of the dentate gyrus from rats in the different age groups, and the amplitude was maximized by adjusting the depths of the electrodes into the tissue. Data were acquired and analyzed using Workbench software (DataWave). The responses were characterized by recording stimulus input–response output functions at 12 stimulus intensities using 100-μs biphasic, constant-current stimulus pulses. The synaptic components of these responses are referred to as the AMPAR fEPSPs because they were measured as the voltage difference between the prestimulus baseline and the response at 1.5 ms after stimulus onset. At this latency, there is a negligible contribution from the slower NMDAR-mediated fEPSP, although it is likely that there is some contribution to this response from receptors responsive to kainate.

To compensate for the reduced numbers of synapses with aging, synaptic responses at a given stimulus intensity were expressed as a ratio of the corresponding amplitude of the presynaptic fiber potential. This procedure also minimizes variation caused by electrode location. For each stimulus intensity, the ratio of the synaptic response to the presynaptic fiber potential was computed. Following the measurement of the AMPAR-mediated fEPSP, the NMDAR antagonist 2,3-dioxo-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (CNXQ), a selective non-NMDA receptor blocker, was applied with a pipette (20 μm tip diam) containing 2 mM CNXQ. Diffusion of the CNXQ was facilitated by applying via the pipette to the surface of lens paper placed over the outer molecular layer (shown in Fig. 1A). The stimulus input–response output functions were again recorded at 12 stimulus intensities, and the synaptic responses measured 2.5 ms after stimulus onset (referred to as the NMDAR-mediated component) were computed against their respective presynaptic fiber potentials.

The application of CNXQ resulted in a large reduction in fEPSP magnitude (Fig. 1B) leaving a residual component that was slower in time course. After measuring the residual fEPSP component, its NMDAR-mediated nature was confirmed by largely abolishing it with a subsequent application of the competitive NMDAR antagonist, D-amino-phosphono-valerate (APV), which was applied by pipette (1 mM) in the same manner as for CNXQ application (Fig. 1C). At least one slice in each rat was tested in this manner. At the end of the session, the NMDAR-mediated nature of the residual fEPSP in the last slice recorded from was also confirmed by abolishing it with 2 mM Mg2+ added to the perfusion medium (Fig. 1D). The APV and Mg2+ treatments reduced the residual EPSPs equivalently in both age groups by ~95 and ~87%, respectively.

The field potentials of slices from different treatment groups were recorded similarly from the upper (attached) blade of the dentate gyrus. The amplitude of the fEPSPs, however, was measured differently. The amplitude of the AMPAR and NMDAR fEPSPs was measured as the slope of the EPSP before and after the
application of CNQX, respectively. The cursors for the slope measurement were set to the initial half of the EPSP so that the measurement performed before the application of CNQX was not contaminated by the NMDA component.

To calculate the presynaptic fiber potential, AMPA and NMDAR-mediated EPSPs, and the AMPAR-to-NMDAR response ratio across different groups, the values collected at the middle three to five stimulus intensities were averaged for each response type in each slice. To avoid introducing sampling bias, data from different slices of one animal (which varied from 1 to 3) were averaged before the final ratios were calculated, and statistics were performed with ANOVA (i.e., $n =$ number of rats).

**Statistical analyses**

Statview software was used for statistical analysis. The path length or corrected integrated pathlength (CIPL) in the spatial trials of the Morris swim test was analyzed with repeated-measures ANOVA, with age or environmental treatment as the between-subjects factor and path length across days as the repeated within-subject factor. The cued trials were analyzed similarly. In the probe trial, rats’ dwell times in the target quadrant or the number of times they crossed the previous target location were analyzed using one-way ANOVA. Fischer’s protected least significant difference analysis was used when a post hoc test was needed.
The presynaptic fiber volley and postsynaptic field potential responses, as well as the ratio of AMPA$_{K^+}$ to NMDA$_{K^+}$-mediated iEPSPs, were analyzed with one-way ANOVA in both experiments. The Fischer’s PLSD test was used for post hoc analysis, and $\alpha$ was set at the 0.05 level.

**RESULTS**

Changes in NMDA$_{K^+}$ and AMPA$_{K^+}$-mediated neurotransmission in hippocampal granule cells in young, adult, and old memory-impaired rats

A significant age effect was detected for the spatial trials of the Morris swim task $[F(2,51) = 51.36; P < 0.0001]$. The young (1 mo) and adult (9 mo) rats performed very similar to each other, and they both did significantly better than did the old (27 mo) rats ($P < 0.0001$) except on the first day (Fig. 2A). A significant age effect was also detected in the probe trials $[F(2,51) = 27.95; P < 0.0001]$. Young rats spent significantly more time in the target quadrant than did the adult rats ($P < 0.001$), and the adult rats spent significantly more time in the target quadrant than did the old rats ($P < 0.001$; Fig. 2B). Although old rats took significantly longer paths to find the visible platform in the first cued trial compared with the young and adult rats $[F(2,51) = 8.66; P < 0.001]$, there was no significant difference on the performance of the three age groups by the last cued trial $[F(2,51) = 1.31; P = 0.28]$; Fig. 2C), suggesting that there was no impairment in visual discrimination ability in old rats.

Hippocampal slices were prepared from young, adult, and old rats to record synaptic field potentials and presynaptic fiber potentials in the stratum moleculare region of dentate gyrus (Fig. 1A). Measurement of AMPA$_{K^+}$-mediated EPSPs and NMDA$_{K^+}$-mediated EPSPs were obtained systematically from young, adult, and old rats. In the old rats, the number of perforant path synapses declines (Geinisman et al. 1995). This reduction is accompanied by a corresponding reduction in the magnitude of the presynaptic fiber potential (Foster et al. 1991), presumably reflecting pruning of the perforant path collateral axons arising from the entorhinal cortex. For this reason, and also to reduce variance caused by electrode positioning, the synaptic component of each response was normalized by the magnitude of the corresponding presynaptic fiber potential for analysis (Barnes et al. 1997). In addition, for each slice, the ratio of the AMPA$_{K^+}$ to NMDA$_{K^+}$-mediated components was computed. These ratios are independent of any normalization factor. Typically, data were collected from several slices in each rat. These “within-rat” data were averaged so that the final $n$ is number of animals.

The changes in response characteristics over the life span are summarized in Fig. 3. As found in several previous studies (Barnes et al. 1992), the presynaptic fiber potential was significantly reduced in 27-mo-old animals compared with the adult group ($P < 0.05$). There was no statistically significant difference between the fiber potential of young and adult rats (Fig. 3A). The AMPA$_{K^+}$-mediated EPSP, however, was increased for a given presynaptic fiber potential amplitude ($P < 0.05$), while the NMDA$_{K^+}$ EPSP declined in the old rats ($P < 0.05$). These changes were reflected in an approximately twofold increase in the mean ratio of AMPA$_{K^+}$ to NMDA$_{K^+}$ components between 1 and 27 mo of age ($P < 0.0001$; Fig. 3D).

**Effects of experience on spatial memory performance**

Three treatment conditions were administered to different groups of animals for a 10-mo period, including a standard control group, a wheel running/social enrichment group, and an...
Effects of experience on NMDA<sub>R</sub>- and AMPAR-mediated neurotransmission in hippocampal granule cells

Following the 10 mo of controlled housing and testing in the Morris swim task, the rats were killed, and their hippocampi were used for field potential recording. The amplitude of the presynaptic fiber potential was not significantly affected by treatment conditions (Fig. 5, A and B). For a given presynaptic input amplitude, neither the amplitude of the AMPAR<sub>R</sub>-mediated fEPSPs nor the amplitude of the NMDA<sub>R</sub>-mediated fEPSPs was significantly different among slices from the enriched environment, the standard control, and the wheel running/social enrichment groups (Fig. 5, C and D). Furthermore, the ratio of AMPAR<sub>R</sub>-to-NMDA<sub>R</sub>-mediated fEPSPs was also not significantly different among the three treatment groups (Fig. 6). These results suggest that substantial enrichment experience does not alter NMDA<sub>R</sub>- and AMPAR<sub>R</sub>-mediated neurotransmission in the dentate gyrus.

**DISCUSSION**

These data contribute to a growing body of evidence indicating that the process of aging involves a complex, regionally specific pattern of neurobiological changes, not all of which are necessarily deteriorative (Burke and Barnes 2006; Rosenzweig and Barnes 2003; Wilson et al. 2006). There are two main findings in this study. 1) The AMPAR<sub>R</sub>-mediated fEPSP response increased with age in hippocampal granule cells, whereas the NMDA<sub>R</sub>-mediated fEPSP response decreased. This resulted in an increase of the AMPAR<sub>R</sub>-to-NMDA<sub>R</sub> response ratio with age. 2) The AMPAR<sub>R</sub>-to-NMDA<sub>R</sub> response ratio is not altered by extensive experience, suggesting that the observed change in this ratio with aging is a consequence of a fixed developmental process.

Over the past decade, it has become clear that multiple activity-dependent mechanisms cooperate to maintain stability in neural circuit function in response to dynamic changes in synaptic drive (Turrigiano 2007; Turrigiano et al. 1998). The first descriptions of this homeostatic plasticity mechanism came from observations of the phenomenon of denervation supersensitivity in muscle (see Davis 2006 for review). Similar
principles, however, also are in play in the brain, where average neural activity levels in networks are continuously adjusted to prevent either excessive excitation or quiescence. Because synapses can change dramatically in response to experience, this mechanism serves to keep cellular excitability levels within a range that effectively “normalizes” total synaptic weights. These mechanisms can be pre- or postsynaptic and can work either at a global network level, a cellular level, or even selectively at individual synapses. Postsynaptic forms of homeostatic plasticity include mechanisms of synaptic “scaling” that adjust synaptic weights up or down to normalize firing rates (O’Brian et al. 1998; Turrigiano et al. 1998). Proportional adjustments of synaptic strengths, for example, can provide a means of preserving relative differences between synapses that have been modified by LTP or LTD, because the weights are modified in proportion to their initial strengths (Liu et al. 2004; Moga et al. 2006; Myme et al. 2003).

In fact, in neocortical areas such as visual or prefrontal cortex, it seems that a constant AMPA<sub>R</sub>-to-NMDA<sub>R</sub> response ratio is maintained, even in the face of ongoing changes in synaptic plasticity (Myme et al. 2003; Umemyia et al. 1999; Watt et al. 2000, 2004). In hippocampus, however, there are considerable data that suggest that LTP can result in increased AMPA<sub>R</sub>-mediated currents (for review, see Malinow et al. 2000) and an altered AMPA<sub>R</sub>-to-NMDA<sub>R</sub> ratio (Heynen et al. 2000; Liao et al. 1995; Lu et al. 2001). Although the data on NMDA-LTP are less consistent, the possibility that there is a “lag” in the expression of NMDA-LTP may explain the discrepancies in observations made in the hippocampus. In neocortex, however, it is clear that this receptor ratio is proportionally regulated (Watt et al. 2004), at least in young animals. This study is consistent with the idea that some form of receptor normalization occurs during the course of information processing, because the enriched environment treatment that dramatically increased the “amount of experience” rats received over a 10-mo period did not affect AMPA<sub>R</sub>-to-NMDA<sub>R</sub> ratios. Studies designed to examine the magnitude of LTP induction in the hippocampus following enrichment treatment have generated inconsistent results, with one laboratory reporting more LTP induced in animals following the treatment (Duffy et al. 2001) and another suggesting that LTP induction was inhibited following enrichment (Foster et al. 1996). Overall, it seems that there are mechanisms at play that act to dynamically adjust synaptic strengths in circuits, regulating AMPA and NMDA current alterations so that new information can be stored effectively.

Interestingly, the age-related change in the AMPA<sub>R</sub>-to-NMDA<sub>R</sub> response ratio is region specific and correlates with differential effects of aging on LTP induction thresholds in different hippocampal subregions. In old rats, there is no effect of age on the maximal expression of LTP at either the Schaffer collateral–pyramidal cell or perforant path–granule cell synapse (Barnes 1979; Diana et al. 1994a,b; Landfield and Lynch 1977; Landfield et al. 1978; Tombaugh et al. 2002) when the stimulation parameters used are well above the LTP induction threshold. When mild stimulation of afferent fibers is paired with intracellularly applied depolarization, however, the threshold for LTP induction is increased with age in the dentate gyrus (Barnes et al. 2000). This is consistent with our observation of an increase in the AMPA<sub>R</sub>-to-NMDA<sub>R</sub> response ratio in old rats. Additionally, fewer granule cells express the immediate early gene Arc in old animals compared with young following behavioral exploration (Small et al. 2004), and the expression of Arc is necessary for the maintenance of LTP and the consolidation of spatial memory (Guzowski et al. 2000). In CA1, on the other hand, where the AMPA<sub>R</sub>-to-NMDA<sub>R</sub> response ratio does not increase over the life span (Barnes et al.
there is no age-related change in LTP threshold following intracellular peri-threshold stimulation pairings (Barnes et al. 1996) or in the numbers of cells that express Arc (Small et al. 2004). Importantly, these results are unlikely to reflect alterations in the function of the aged cells involving resting membrane potential, input resistance or membrane time constants, because these variables do not change across age (for review, see Barnes 1994).

One possible interpretation of an increment in AMPA$_R$-mediated responses at granule cells synapses of aged rats is that it reflects a mechanism to preserve previously stored information. Granule cells are ideal candidates for information storage sites because of their large numbers and the sparse coding scheme in the dentate gyrus. Additionally, the decreased NMDA$_R$-mediated response in granule cells of aged rats may concurrently prevent stored information from being overwritten by restricting the induction of further plasticity. The observation that administration of the NMDA$_R$ antagonist CPP following spatial memory training enhances subsequent memory retention (Villarreal et al. 2002) provides strong support for this hypothesis. Although most memory consolidation theories suggest that the hippocampus is only a temporary repository of information (Squire and Alvarez 1995), an alternative view is that the storage and retrieval of spatial memory may always depend on the hippocampus (Moscovitch et al. 2006). If the hippocampus is involved in such long-term memory maintenance, the dentate gyrus could be a subregion involved in this durable synaptic commitment.

In summary, the AMPA$_R$-to-NMDA$_R$ response ratio increases in the dentate gyrus from 1 to 27 mo of age, and this increment seems to be developmentally regulated rather than experience dependent. These results suggest that the dynamics of information storage mechanism in hippocampal granule cells may differ in an important way from other hippocampal subfields. Furthermore, aged rats may preserve the storage of old information by sacrificing the learning of new information (Burke and Mackay 1997; Wilson et al. 2006).

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