Altered Plasticity in Hippocampal CA1, But Not Dentate Gyrus, Following Long-Term Environmental Enrichment

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Eckert MJ, Bilkey DK, Abraham WC. Altered plasticity in hippocampal CA1, but not dentate gyrus, following long-term environmental enrichment. J Neurophysiol 103: 3320–3329, 2010. First published April 14, 2010; doi:10.1152/jn.01037.2009. Exposure to an enriched environment can improve cognitive functioning in normal animals as well as in animal models of neurological disease and impairment. However, the physiological processes that mediate these changes are poorly understood. Previously we and others have found changes in hippocampal synaptic transmission and plasticity after 2–4 wk of enrichment although others have not observed effects. To determine whether long-term enrichment produces more robust changes, we housed rats continuously in an enriched environment for a minimum of 3 mo and then tested for effects on hippocampal physiology in vitro and in vivo. Enriched housing improved spatial learning compared with social and isolated housing, but surprisingly this was not accompanied by changes in basal synaptic transmission in either CA1 or the dentate gyrus as measured either in vitro or in vivo. This lack of change may reflect the operation of homeostatic mechanisms that keep global synaptic weights within a narrow range. In tests of synaptic plasticity, the induction of long-term potentiation was not changed in either CA1 or the dentate gyrus. However, in CA1 of enriched rats, there was less long-term depression in stratum radiatum, less depotentiation in stratum oriens, and altered paired-pulse inhibition of population spikes evoked in stratum oriens. These effects suggest that there are altered synaptic and network dynamics in hippocampal CA1 that contribute to the enrichment-related cognitive improvement.

INTRODUCTION

The complexity of stimuli in an environment can have profound effects on brain structure and function. Increasing environmental complexity beyond that typically provided by standard laboratory housing can improve performance on a range of learning and memory tasks. Hebb (1949) first noted that rats reared as pets in his house performed better on a maze task compared with rats reared in standard laboratory cages. Numerous subsequent experiments have demonstrated a beneficial effect of enriched environment exposure on hippocampus-dependent memory tasks among others (Duffy et al. 2001; Irvine and Abraham 2005; Kempermann et al. 1997; Schrijver et al. 2004; Teather et al. 2002). Besides these beneficial effects in normal animals, environmental enrichment also facilitates recovery from neural damage or disease (Jankowsky et al. 2005; Laviola et al. 2008; Lazarov et al. 2005; Nithianantharajah et al. 2008; Ohlsson and Johansson 1995; Young et al. 1999). While these results suggest a profound effect of enrichment on neural function, the physiological mechanisms that underpin the cognitive enhancements are not clearly understood.

One hypothesis is that enriched environment exposure causes a learning-induced change in synaptic physiology that, in the hippocampus for example, might be measured as a change in basal synaptic transmission or a change in the induction or persistence of long-term potentiation (LTP) and long-term depression (LTD). Some previous studies using in vitro hippocampal slices have reported increases in basal synaptic transmission after 3–5 wk of enrichment (Foster and Dumas 2001; Foster et al. 2000; Green and Greenough 1986), whereas others have reported no difference (Duffy et al. 2001; Feng et al. 2001; Parsley et al. 2007). Using in vivo recordings made before and after repeated overnight exposure to an enriched environment (EE), we have observed a small increase in synaptic strength and a substantial increase in cellular excitability in the dentate gyrus (Irvine et al. 2006). In CA1, the effect of enrichment was more complicated, and input/output curves showed an increase in field excitatory postsynaptic potentials (fEPSPs) at high stimulus intensities but a decrease in fEPSPs at low stimulus intensities (Irvine and Abraham 2005).

Even in the absence of any observable changes in basal synaptic transmission, there is the possibility that enrichment alters the ability to induce synaptic plasticity. In CA1, an increased degree of LTP has been reported (Artola et al. 2006; Duffy et al. 2001), but there is also evidence of no change in LTP (Foster and Dumas 2001). In the dentate gyrus, a reduction in LTP induction was observed and attributed to an LTP-like process occurring during enrichment which occluded subsequent LTP induction (Foster et al. 1996, 2000). However, there is also evidence for no effect on dentate gyrus LTP (Feng et al. 2001). In contrast, when given days after LTP or LTD induction, enrichment leads to their rapid reversal (Abraham et al. 2002, 2006).

One possible explanation for some of the preceding variability in the results is the duration of exposure to the complex environments. Some studies used continuous living in the environment, whereas others used brief daily exposures to the environment. Furthermore, the environmental exposure has been typically ~1 month in duration prior to the experiments. A few reports have shown that longer periods of enrichment (~2 yr) reliably improve cognitive performance, including performance on hippocampus-dependent tasks such as the Morris water maze (Kempermann et al. 2002; Kobayashi et al. 2002; Pham et al. 1999). In the present study, we hypothesized that maximizing the exposure to an enriched environment would amplify any effects on hippocampal synaptic function and thereby help clarify the nature of the physiological effects generated by EE exposure. We found, surprisingly, that this treatment produced no observable effects on basal synaptic transmis-
sion in either the dentate gyrus or CA1 but did lead to subtle changes in CA1 synaptic plasticity, particularly in the stratum oriens.

METHODS

Environmental enrichment

Male Sprague-Dawley rats (aged postnatal days 28–30) were randomly assigned to three living conditions: EE, social control (SC), and isolated control (IC). For the enriched environment, four rats were housed continuously in a large fiberglass box (80 × 80 × 80 cm) that contained a three-dimensional (3-D) arrangement of objects such as tunnels, ladders, boxes, and plastic toys (Fig. 1A). The objects were changed twice every week to ensure continued novelty and complexity. Social control animals lived in groups of four in a standard plastic group cage (35 × 25 × 25 cm) with only bedding. Isolated controls lived individually in standard cages (35 × 25 × 25 cm) with only bedding. All animals had ad libitum access to food and water and were maintained on a normal 12 h light/dark cycle. Following a minimum of 3 mo of differential housing (range: 3–5 mo), the behavioral and electrophysiological experiments began. All procedures were approved by the University of Otago Animal Ethics Committee.

Water maze testing

Reference spatial memory was tested using a standard water maze with visual cues on the surrounding room walls (Liu and Bilkey 2001). Briefly, training consisted of four trials per day for 6 days. On each trial, the rat had 60 s to find a submerged platform in a fixed location for each animal. If it failed, it was guided to the platform and allowed to rest on it for 10 s before being gently dried and moved to a holding cage for the 2 min inter-trial interval. During training, the rat was videotaped from a fixed overhead camera, and custom software was used to score performance. The three measures scored were escape latency, path length, and swim speed. Probe trials (platform removed from maze) were conducted on day 5 after completing training on that day, and a final probe trial was conducted on day 7, 24 h after the final training session. The rat was released from a fixed starting position and then swam freely for 60 s before being removed. Performance on a probe trial was measured by percent time in the correct quadrant and number of platform crossings.

In vitro electrophysiology

For in vitro electrophysiology, the animal was taken directly from its experimental housing and deeply anesthetized with ketamine (100 mg/kg ip) before decapitation and slice preparation. Transverse hippocampal slices (400 μm) were cut and placed in a submerged recording chamber that was maintained at 32.5°C and perfused continuously with artificial cerebrospinal fluid (composition in mM: 124 NaCl, 3.2 KCl, 1.25 NAH2PO4, 26 NaHCO3, 2.5 CaCl2, 1.3 MgCl2, and 10 d-glucose). Slices were allowed to equilibrate for ≥1.5 h before testing began. Throughout the slice experiments, the experimenter was blind to treatment condition until the completion of the experiments.

fEPSPs were elicited by diphasic constant-current pulses (100 μs half-wave pulse duration) delivered through a Teflon-insulated tungsten wire. Evoked responses were recorded through glass pipettes filled with 2 M NaCl (1–2 MΩ), then filtered (0.1–3 kHz) and digitized for off-line analysis. Input-output curves were performed using an increasing series of stimulation intensities (range: 10–250 μA). In both CA1 and the dentate gyrus, recordings were made simultaneously in the dendritic and cell-body layers. Measurements were made of the initial slope of the fEPSP and the height of the population spike. In CA1, recordings were made following separate stimulation of the stratum radiatum and the stratum oriens. In the dentate gyrus, recordings were made following separate stimulation of the lateral and medial perforant paths.

Paired-pulse facilitation (PPF) of the fEPSP and paired-pulse inhibition of the population spike (PPI) were assessed with a range of inter-pulse intervals (IPI). For PPF in both CA1 and DG, the stimulation intensity was adjusted below the level that evoked a population spike to avoid activation of recurrent inhibition. The ratio of fEPSP slopes was then calculated. For PPI in CA1, the first pulse was...
delivered via a stimulating electrode positioned in the alveus at an intensity set to elicit a 75% maximal antidromic population spike. This activated recurrent inhibition while largely avoiding any synaptic activation and facilitation. The second pulse was then delivered to the afferent fibers of either the radiatum or oriens at an intensity set to elicit a 50% maximal spike. The population spike amplitude of this second response was then compared with the population spike evoked by a single pulse to the afferent fibers.

To induce LTP in the stratum radiatum and stratum oriens, the baseline stimulation intensity was first adjusted to elicit a 1 mV fEPSP. Following a 40 min baseline recording period (0.017 Hz), a high-frequency stimulus (HFS) protocol was delivered (2 \( \times \) 100 Hz, 1 s trains, 20 s apart). The pulse intensity during the tetanus was increased to elicit a 2 mV fEPSP that required currents in the range 100–250 \( \mu \)A. This protocol was chosen to give a submaximal and decrementing LTP in control animals, capable of being either facilitated or impaired by the EE treatment. Two hours following LTP induction, depotentiation was attempted by delivering 1,200 pulses at 3 Hz. Naive slices were used to test LTD induction. The baseline intensity was adjusted to elicit a 0.5 mV fEPSP. Following a 30 min baseline, LFS was delivered (1,200 pulses at 3 Hz, 1 mV amplitude initial response) and then responses monitored for a further hour (Mockett et al. 2002). This protocol was chosen to elicit a moderate LTD that could reveal either facilitation or inhibition by EE treatment. LTP in the dentate gyrus was performed in 10 \( \mu \)M picrotoxin to reduce but not completely block GABAergic inhibition as LTP is otherwise difficult to induce in this region. Besides this difference, the same stimulation parameters were used as for CA1.

**In vivo electrophysiology**

After \( \sim \)2.5 mo of environmental treatment, animals were anesthetized with a mixture of ketamine (75 mg/kg) and domitor (0.5 mg/kg), and stainless steel wire electrodes (75 \( \mu \)m diameter) were positioned in the hilus of the dentate gyrus (3.8 mm posterior and 2.5 mm lateral to bregma) and the medial perforant path (4.5 mm lateral to lambda). Jewelers screws inserted in the frontal and occipital bones served as reference and earth electrodes, respectively. Additional jewelers screws positioned over the skull surface served as anchors for dental acrylic. Gold Amphenol connectors soldered on the ends of the electrode wires were inserted into a plastic headcap, and the assembly was fixed to the skull with dental acrylic. The animals were allowed to recover for 10 days in individual cages before being returned to the experimental housing for the duration of the experiment. Individual housing for 4–5 wk after EE treatment has been reported not to modify the effects of EE on hippocampal synaptic transmission (Green and Greenough 1986).

During the recording sessions, each animal was taken to a small room that was different from either the enriched or control housing rooms. Baseline recording consisted of single diphasic pulses (150 \( \mu \)s pulse width, 0.07 Hz pulse rate) delivered for 30 min. From this recording, the slope measurements of the fEPSP for the final 15 min were averaged to yield a single slope measurement for that day. There were three recording sessions per week, and they continued until the response was stable for \( \geq 1 \) wk. Once the response was stable, an input-output series was recorded using a range of stimulus currents to the (10–600 \( \mu \)A). LTP was first induced with a weak HFS protocol that typically results in a small, decremental LTP (2 sets of 5 trains delivered at 1 Hz where each train consisted of 10 pulses delivered at 400 Hz and with 10 min between sets). Baseline recordings continued until the responses had decayed to baseline levels and were stable for \( \geq 1 \) wk. A second, stronger HFS was then applied ([the 4*ST protocol from Abraham et al. (2002): similar to the first tetanus except that the set of trains was delivered 4 times instead of twice]. LTP persistence was monitored for 17 days post-HFS.

**Statistical analysis**

Significant differences on all measures were determined by repeated-measures ANOVA. The data for in vitro LTP/LTD were averaged into 5 min bins before being analyzed. Depotentiation measures were expressed as a percentage of the average potentiated value 5 min prior to LFS before being binned into 5 min averages and analyzed. In all figures, data are presented as means \( \pm \) SE.

**RESULTS**

In these experiments, we never observed any statistically significant differences between the IC and SC groups (see for example Supplementary Fig. S1\(^1\)), so we combined the data from these groups to improve the statistical power in finding an effect of EE treatment.

**Enrichment improves water maze performance**

Before assessing synaptic transmission and plasticity, we wanted to confirm that the long-term continuous enrichment protocol improved cognitive functioning as we have previously demonstrated for shorter periods of overnight EE treatment (Irvine and Abraham 2005). Enriched \((n = 12)\) and control \((n = 23)\) rats were tested in the Morris water maze over a period of 6 days. Compared with control rats, enriched rats had significantly shorter escape latencies, resulting in a significant main effect of group \([F(1,33) = 31.7, P < 0.001; \text{Fig. 1B}]\). The shorter escape latency of the enriched rats was evident even on the first day of training, raising the possibility that their improved performance might be due in part to a general enhancement in motor skill instead of being purely due to improved spatial memory. A comparison of the swim speed of the two groups did not reveal any significant effects suggesting that this was not the case \([F(1,33) = 1.8, P = 0.18; \text{Fig. 1C}]\). However, there was a tendency for the enriched rats to have faster swimming speeds on the first day, so we next analyzed the water maze performance using path length, a measure that is independent of swim speed. Enriched rats swam significantly shorter path lengths than control rats, indicating that the extended enrichment protocol had indeed improved the rats’ spatial memory \([\text{ANOVA } F(1,33) = 15.3, P < 0.001; \text{Fig. 1D}]\). Post hoc \(t\)-test revealed that enriched rats’ path lengths were significantly shorter on all days except days 2 and 6. Although the enriched rats performed better when the platform was present, they were not significantly different from controls during probe trial tests conducted on days 5 and 7 (as measured by the number of platform crossings and time spent in the target quadrant; Suppl. Fig. S2). This is likely because sufficient training had occurred to overcome the initial learning deficit. Indeed post hoc comparisons on the path length data when the platform was present \([\text{Fig. 1D}]\) revealed that by day 6 the control rats performed as well as enriched rats.

**CA1 electrophysiology in vitro**

Having confirmed that the enrichment protocol improved functioning on a hippocampus-dependent task, we began to test for electrophysiological changes that might account for the improvement. We recorded field potentials from hippocampal slices and looked for changes in basal synaptic transmission or

\(^{1}\) The online version of this article contains supplemental data.
plasticity. We first tested whether enrichment caused a lasting increase (or decrease) in basal synaptic strength by recording input-output (IO) series prior to any plasticity protocols. In CA1, there was no significant difference between groups in the fEPSP IO curves for both stratum radiatum and stratum oriens recordings (2-way repeated-measures ANOVA, NS.; Fig. 2, A and B). The IO curves for the population spike amplitude were also completely overlapping between the groups, indicating that there was no difference in cell excitability in either pathway (Fig. 2, C and D).

To test for differences in short-term presynaptic plasticity, PPF of the fEPSP was assessed in both the radiatum and oriens pathways. There was no difference between groups in the paired-pulse ratio for either pathway (Fig. 3, A and B), suggesting that presynaptic short-term plasticity mechanisms were unaltered by enrichment. To assess network inhibitory dynamics, we measured paired-pulse inhibition of the population spike. In this paradigm, the first spike of a pair was generated by antidromic stimulation of the alveus to isolate the effects of recurrent inhibition from any confounding fEPSP PPF that would arise from stimulating the same pathway twice above spike threshold. In stratum oriens, enriched rats exhibited more spike inhibition at short inter-pulse intervals and less inhibition at longer intervals, yielding a significant interaction \([F(12,120) = 4.7, P < 0.001;\) Fig. 3D]. A qualitatively similar effect appeared to occur for the radiatum-generated population spike, but the interaction was not statistically significant \([F(12,120) = 1.5, P = 0.12;\) Fig. 3C].

Because of the improved memory performance of enriched rats, we predicted that there would be differences in long-term plasticity in these rats. We were somewhat surprised to find that most measures of LTP were unaffected by enrichment. For example, there was no difference between groups in either the amount of LTP induced following HFS (2 \(\times\) 100 Hz) or its persistence during the 2-h period following tetanization. This was true for both the radiatum (Fig. 4A) and oriens pathways (B). Although the LTP in the oriens pathway appeared to be somewhat more stably induced in the EE group, there was no statistically significant group main effect \([F(1,18) = 1.1, P = 0.31]\) or group by time interaction \([F(23,414) = 1.2, P = 0.27]\). Interestingly, however, the oriens LTP in the EE group was more resistant to reversal by an LFS protocol (1,200 pulses at 3 Hz) that was capable of depotentiating the control LTP. Both groups had a similar initial level of response suppression, but the LTP in enriched rats recovered during the 40-min follow-up period, whereas the control LTP remained depotentiated during this time \([F(7,126) = 4.6, P < 0.001]\). There was no difference between groups in the amount of depotentiation induced in stratum radiatum.

We next tested whether there was a group difference in de novo LTD using naïve slices given the same LFS protocol and found a different pattern of results to that seen for depotentiation. In stratum radiatum, there was a significant reduction in LTD induction. In control slices, LFS induced an initial depression of 41 \(\pm\) 7% that recovered to a stable depression of 14 \(\pm\) 5% 1 h later, whereas in enriched slices, there was less initial depression, and it recovered completely back to baseline levels 1 h following LFS \([2 \pm 4\%; F(1,20) = 5, P < 0.05]\) compared with EE slices; Fig. 4C). In stratum oriens, in contrast, there was no difference in LTD between groups (Fig. 4D).

![Fig. 2. Basal synaptic transmission and excitability in CA1 is similar for enriched and control rats. Input-output curves measured in vitro for field excitatory postsynaptic potential (fEPSP) and population spike are similar in enriched and controls in both stratum radiatum (A and C) and stratum oriens (B and D).](http://jn.physiology.org/)

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Dentate gyrus electrophysiology in vitro and in vivo

When we performed a similar set of measurements in the dentate gyrus in vitro, we did not observe any effects of environmental enrichment. Basal synaptic transmission was unaltered for both the lateral and medial perforant pathways (Fig. 5). Paired-pulse plasticity was similarly unaltered in both pathways (Supplementary Fig. S3). When testing LTP and depotentiation, a moderate concentration of picrotoxin (10 μM) was added to the bath to reduce GABAergic inhibition, as LTP was difficult to produce in the dentate gyrus in normal artificial cerebrospinal fluid (ACSF). The HFS and LFS protocols were the same as those used in CA1. In the lateral path, there was no difference in the amount of LTP or depotentiation observed (Supplementary Fig. S4). In the medial path, enriched slices tended to have more potentiation shortly after induction, but this decayed to control levels over the 2-h post-HFS period. The ANOVA group by time interaction did not quite reach statistical significance \( F(23,345) = 1.5, P = 0.07; \) Supplementary Fig. S4]. There was no group difference in the amount of depotentiation in the medial path.

The lack of observable effects in the dentate gyrus in vitro made us consider the possibility that an in vivo preparation might be more sensitive in detecting effects on plasticity because recordings can be made over several weeks. We also considered the possibility that the slice preparation procedure introduces synaptic changes (Kirov et al. 2004) that could mask effects caused by EE treatment. To address these possibilities, we implanted enriched and control rats that had experienced ~2.5–3 month of differential housing with electrodes in the medial perforant path and the dentate gyrus. Except for the recording sessions, the rats continued to live in either the enriched or control housing for the duration of the experiment. As for the in vitro slices, basal synaptic transmission was not significantly different between groups. There was a trend for the fEPSP to be increased in enriched animals across all stimulation intensities, but this did not yield a significant group main effect \( F(1,22) = 1.5, P = 0.24; \) Fig. 6A]. The population spike appeared to be larger in enriched animals at higher stimulation intensities, but the interaction was also not significant \( F(14,308) = 1.3, P = 0.2; \) Fig. 6B]. When testing LTP, we first tried a mild tetanus protocol that might highlight any increased plasticity in the enriched rats (2 sets of 5 trains, each consisting of 10 pulses at 400 Hz). In both groups, this yielded a modest initial potentiation (EE: 17.3 ± 2.6%; control: 18.8 ± 3.7%) that decayed to baseline levels within 2 wk (data not shown). After waiting ≥3 wk after the first tetanus, and ensuring that recordings were stable for at least 1 wk, a second, stronger tetanus was applied (4 sets of 5 400-Hz trains). The two groups showed a similar amount of initial LTP, but there was a trend for a faster decay of fEPSP LTP in enriched rats during the

*FIG. 3. Enrichment effects on paired-pulse plasticity. Enrichment did not alter paired-pulse facilitation of fEPSPs in radiatum or oriens (A and B). Paired-pulse inhibition was not altered in radiatum (C). Enrichment did significantly alter paired-pulse inhibition of population spikes in oriens (D). Here an antidromic population spike triggered by stimulation of the alveus was used to inhibit the orthodromic population spike of slices.*
first 2 wk following the tetanus. This effect was not statistically significant [group main effect, $F(1,16) = 2.7$, $P = 0.12$; Fig. 6C]. Population spike potentiation was also similar for both groups [group main effect, $F(1,16) = 0.04$, $P = 0.84$; Fig. 6D].

**DISCUSSION**

Exposure to an enriched environment can improve cognitive functioning, including memory tasks that involve the hippocampus. This is true for normal animals as well as disease models, such as Alzheimer’s models, that affect memory performance (Irvine and Abraham 2005; Jankowsky et al. 2005; Kempermann et al. 1997; Laviola et al. 2008). As memory storage is believed to occur as a result of changes in synaptic strength, it is considered likely that enrichment alters synaptic transmission or plasticity in some way, and elucidating the mechanism by which this happens could be important for understanding enriched environment effects. Interestingly, previous studies examining this issue have yielded mixed results, a problem that might be due to the variability of enrichment protocols. There are several variables that affect the degree of enrichment experienced by the animal, including the species used, the dimensions of the housing container, number and type of objects in the container, the presence of running wheels, the presence of conspecifics, and the duration of exposure to the environment. It is also worth noting that the standard isolated housing typically experienced by laboratory rodents is a state of sensorimotor deprivation, while the “enriched” environment only approximates what the animal might experience in the wild.

In this study, we aimed to understand the synaptic effects of prolonged environmental enrichment by exposing rats to ≥3 month of continuous enrichment and predicted this treatment would produce larger and more reliable effects on hippocampal synaptic physiology than commonly reported in previous studies employing shorter enrichment procedures. We initially used two control groups, one of which was isolated housing and the other was social housing in groups of four. While others have noted a difference between social and isolated housing conditions on hippocampal function (e.g., Artola et al. 2006), we did not observe any differences between these groups, and we combined them to focus our study more directly on the spatial complexity and novelty of the environment. In the present experiment, enrichment for 3 month improved watermaze performance, consistent with other studies showing that prolonged enrichment of adult rodents for ≥10 mo significantly increases hippocampal neurogenesis and performance on hippocampus-dependent memory tasks (Kempermann et al. 2002; Kobayashi et al. 2002; Pham et al. 1999). It was surprising, then, that contrary to prediction we failed to observe any significant effects of prolonged EE treatment on basal synaptic transmission in either CA1 or the dentate gyrus. Furthermore LTP induction was unchanged, although there were significant reductions in LTD in CA1 stratum radiatum and in depotentiation in CA1 stratum oriens.
Lack of change in basal synaptic strength

In this study we did not observe any enrichment-related changes in basal synaptic strength either in slices or in recordings from chronically implanted rats. Given that effects on basal transmission have been observed in some studies, including our own, using shorter periods of enrichment that was available for only a limited number of hours each day (Foster and Dumas 2001; Foster et al. 1996; Irvine and Abraham 2005; Irvine et al. 2006), the present findings suggest that some process occurs during the prolonged enrichment that reverses the effects occurring earlier during enrichment. Indeed we have shown that EE treatment can reverse previously induced LTP and LTD (Abraham et al. 2002, 2006) and that LTP induced during a period of EE exposure is less persistently maintained over days (Irvine et al. 2006). Furthermore, EE-induced increases in cell excitability peaked after ~1 wk of treatment and then substantially decayed after a further week (Irvine et al. 2006). Similarly, Parsley et al. (2007) have suggested that plasticity induced early during EE exposure is reversed later on. We propose therefore that any synaptic changes induced early during EE exposure are overwritten by potentially one of two processes. First, the synaptic changes might be actively reversed in response to further novelty exposure perhaps because of facilitated depotentiation and dedepression (Abraham et al. 2006; Artola et al. 2006). Alternatively, any potentially prolonged change in the net synaptic weight on any given neuron might be intrinsically adjusted through a homeostatic scaling process to bring the net weight back to a basal level. For example, neurons have been shown to scale their synaptic weights and somatodendritic channel distributions up or down to maintain a certain firing rate in response to prolonged periods of relative (in)activity (Turrigiano 1999, 2008). If a net increase in fEPSP size occurred early in the enrichment paradigm, a homeostatic scaling down of synaptic strength may have renormalized the net synaptic input, making the population response indistinguishable from controls.

Two other explanations may account for the stability of basal synaptic transmission. First, it may be that with sufficient EE exposure to novelty and learning opportunities there is eventually a balance of LTP and LTD induction at different synapses, leading to no net change in fEPSPs. This is consistent with the suggestions that both LTP and LTD are important for learning (Kemp and Manahan-Vaughan 2004; Manahan-Vaughan and Braunewell 1999; Pastalkova et al. 2006; Whitlock et al. 2006). Another potential explanation is that the procedure of making slices for in vitro recordings erased any evidence of synaptic plasticity due to enrichment. Although others have reported changes in basal synaptic strength in slices taken from enriched animals (Foster and Dumas 2001; Foster et al. 1996; Green and Greenough 1986), this has not been the case consistently. There is evidence that dendritic spines undergo a marked retraction and regrowth during the chilling and subsequent rewarming that occurs during typical slice preparation (Kirov et al. 2004). Under these conditions, it is possible that subtle changes in synaptic strength generated in the intact animal (Irvine and Abraham 2005; Irvine et al. 2006) would not survive the structural reorganization and would be reduced or absent in the slice. Counter to this argument, however, is the fact that we also failed to observe any differences in basal synaptic transmission in awake freely moving animals at least for perforant path synapses in the dentate gyrus.

EE-induced reduction in CA1 LTD and depotentiation

In the present experiments, two effects of enrichment on long-term plasticity were observed in area CA1: a reduced
amount of depotentiation in stratum oriens and a reduced induction of LTD in stratum radiatum. We did not observe a difference in LTP in this study, but others have reported increased LTP in enriched animals (Artola et al. 2006; Duffy et al. 2001). It is possible that our choice of HFS protocol was too strong (2 × 100 Hz) to detect a difference as the previous studies used only a single tetanus. To us this does not seem likely, however, as the LTP induced in our study was decremental in both groups, suggesting that LTP had not been saturated. Furthermore, although a single tetanus was used by Artola et al. (2006) to examine LTP persistence, they also observed that two and three tetani resulted in an even greater difference in LTP between enriched and control animals. Thus it seems likely that our HFS protocol should have revealed any difference in LTP in enriched animals had any existed, but we cannot discount the possibility that a weaker protocol would have revealed a more robust potentiation in enriched animals.

Although we did not observe a difference in the amount of LTP induced in either the radiatum or oriens, the combined effect of reduced depotentiation and reduced LTD would bias CA1 synapses toward increased synaptic strength during learning. This result is in agreement with the previous reports showing increased LTP in enriched animals because both these reports found no difference in the initial amount of LTP but an increased persistence (Artola et al. 2006; Duffy et al. 2001). Interestingly, Duffy et al. (2001) observed this effect in mice following 8 wk of EE exposure, in the absence of any observable effect on basal synaptic transmission, a result that we also observed in the present experiments. However, Artola et al. (2006) found an increased LTD in enriched animals in addition to an increased LTP, suggesting a larger range of synaptic modification in enriched animals instead of a bias toward increases in synaptic strength. It is possible that this difference is due to the shorter enrichment protocol they used (5 wk) or some other difference in the nature of the enrichment protocol. Other studies using shorter periods of EE have reported different patterns of effects of enrichment on plasticity and synaptic transmission in CA1. Foster and Dumas (2001) found an increase in basal synaptic transmission, but no change in the amount of LTP in rats, whereas Feng et al. (2001) did not observe any changes in either basal transmission or LTP in mice.

Conclusions

Two principal outcomes of this study were the lack of EE effects on a large range of measures of basal synaptic transmission, excitability, and plasticity in both CA1 and the dentate gyrus and the subtle but significant effects of EE treatment on depotentiation, LTD and PPI of the population spike in CA1. The first outcome was unexpected as we had predicted that a long period of enrichment would amplify the many previously reported effects of EE treatment. It is notable as well that the impoverished environment treatment (IC) was not significantly worse on any measure when compared with the SC group. This contrasts with other studies that have shown significant differ-
ences between IC and SC groups across a range of behavioral measures (Gresack et al. 2010; Ibi et al. 2008; Koike et al. 2009; Weiss et al. 2004). This latter finding could reflect an insufficient differentiation of the IC and SC conditions as the social groups involved only four animals, no objects, and standard caging for group-housed animals. On the other hand, the general lack of EE effects despite a behavioral difference suggests that synaptic transmission and plasticity mechanisms homeostatically adjust in response to different levels of activity to keep overall function within a narrow range. This hypothesis will require further testing.

Despite the general lack of EE-related change, we did observe a number of plasticity differences in CA1. What is the significance of these changes for spatial information processing and memory? In a recent preliminary study using a combination of place cell recording and immunohistochemical analysis of the expression of the immediate early gene Arc (activity-regulated cytoskeleton-associated protein), we found that the hippocampus of EE rats uses a sparser neural representation for encoding a novel environment compared with control animals (Eckert et al. 2007). Sparse coding gives a network a greater storage capacity and better pattern discrimination, both properties that could explain some of the cognitive benefits of enrichment. The altered plasticity in CA1 might then reflect a necessary adjustment of the network’s learning rule as it adopts a sparser coding strategy (Finelli et al. 2008).

At a behavioral level, it has been proposed that CA3 stores the patterns of activity generated by the dentate gyrus and that CA1 uses this information to form associations with neocortical activity, either through the back-projections to the neocortex or from the incoming activity of the direct perforant path-CA1 pathway (McClelland et al. 1995; Rolls and Kesner 2006). CA3 projects to both stratum radiatum and stratum oriens, but whether these two pathways play different roles in information processing is poorly understood. Thus while there are somewhat different effects of prolonged EE on radiatum and oriens plasticity, the combined effect of these changes appears to be a relative strengthening of the output from CA3 to CA1. This should enhance the recall of stored spatial relationships and facilitate detection of spatial novelty when new or altered environments are encountered.

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DISCLOSURES
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