

Effects of Adult Neurogenesis on Synaptic Plasticity in the Rat Dentate Gyrus

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Snyder, J. S., N. Kee, and J. M. Wojtowicz. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. *J Neurophysiol* 85: 2423–2431, 2001. Ongoing neurogenesis in the adult hippocampal dentate gyrus (DG) generates a substantial population of young neurons. This phenomenon is present in all species examined thus far, including humans. Although the regulation of adult neurogenesis by various physiologically relevant factors such as learning and stress has been documented, the functional contributions of the newly born neurons to hippocampal functions are not known. We investigated possible contributions of the newly born granule neurons to synaptic plasticity in the hippocampal DG. In the standard hippocampal slice preparation perfused with artificial cerebrospinal fluid (ACSF), a small (10%) long-term potentiation (LTP) of the evoked field potentials is seen after tetanic stimulation of the afferent medial perforant pathway (MPP). The induction of this ACSF-LTP is resistant to a *N*-methyl-D-aspartate (NMDA) receptor blocker, D,L-2-amino-5-phosphonovaleric acid (APV), but is completely prevented by ifenprodil, a blocker of NR2B subtype of NMDA receptors. In contrast, slices perfused with picrotoxin (PICRO), a GABA-receptor blocker, revealed a larger (40–50%), APV-sensitive but ifenprodil-insensitive LTP. The ACSF-LTP required lower frequency of stimulation and fewer stimuli for its induction than the PICRO-LTP. All these characteristics of ACSF-LTP are in agreement with the properties of the putative individual new granule neurons examined previously with the use of the whole cell recording technique in a similar preparation. A causal relationship between neurogenesis and ACSF-LTP was confirmed in experiments using low dose of gamma radiation applied to the brain 3 wk prior to the electrophysiological experiments. In these experiments, the new cell proliferation was drastically reduced and ACSF-LTP was selectively blocked. We conclude that the young, adult-generated granule neurons play a significant role in synaptic plasticity in the DG. Since DG is the major source of the afferent inputs into the hippocampus, the production and the plasticity of new neurons may have an important role in the hippocampal functions such as learning and memory.

INTRODUCTION

It is now well established that the mammalian dentate gyrus (DG) produces a large number of neurons in adult individuals of all species examined, including rodents (Altman and Das 1967; Kaplan and Hinds 1977) and primates (Gould et al. 1999b). This adult neurogenesis is regulated by age (Kuhn et al. 1996; Seki and Arai 1995), stress (Gould et al. 1997), exercise (van Praag et al. 1999), learning (Gould et al. 1999a), and seizures (Parent et al. 1997). One outstanding issue is the

functionality of the adult-generated young neurons and their contribution to hippocampal plasticity. A previous publication showed that the putative young neurons, located near the proliferative zone in the DG, produce long-term potentiation (LTP) more readily than the mature ones (Wang et al. 2000). One reason for the difference was the lack of GABAergic inhibition in the young neurons, but likely other reasons were not determined. Due to a limited sampling power of intracellular recordings, the overall contribution of the young neurons within a much larger population of adult granule neurons was difficult to assess. In the present study, we used the field potential recordings that can sample from a larger number of neurons.

Specifically, we examined LTP of field excitatory postsynaptic potentials (EPSPs) in the terminal region of the medial perforant pathway (MPP) in response to tetanic stimulation of the afferent axons. We predicted that LTP induced in slices perfused with a standard artificial cerebrospinal fluid (ACSF-LTP) ought to be produced by primarily the young, uninhibited neurons since the mature neurons are strongly inhibited in these conditions. In contrast, in the presence of picrotoxin (PICRO), a blocker of GABAergic synapses, we expected to obtain LTP contributed by both the mature and immature neurons (PICRO-LTP).

The specific objectives of this study should be seen in light of the extensive body of evidence describing LTP within the DG of mature and developing animals. It has been established that the granule neuron excitation is strongly modulated by GABAergic synapses both in vitro (Wigstrom and Gustafsson 1983) and in vivo (Buckmaster and Schwartzkroin 1995; Moser 1996). Induction of LTP in DG in vitro hippocampal slices is often difficult (Hanse and Gustafsson 1992; Nguyen and Kandel 1996; Wigstrom and Gustafsson 1983) presumably due to the strong GABAergic inhibition. However, in other studies, using similar preparations, consistent induction of LTP was achieved (Bramham et al. 1997; Trommer et al. 1995). Results showing LTP in DG perforant path synapses in vivo are more consistent (Bramham et al. 1991a; Morris et al. 1986; Nosten-Bertran et al. 1996). It is also generally accepted that LTP induction in MPP is *N*-methyl-D-aspartate (NMDA)-receptor dependent in vivo (Morris et al. 1986) and in vitro (Trommer et al. 1995).

Possible differences between the mechanisms of LTP induc-

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tion between the lateral (LPP) and medial divisions of the perforant pathway have been described with some studies (Bramham et al. 1991b) claiming NMDA independence and others disputing this evidence (Hanse and Gustafsson 1992; Xie and Lewis 1991). The results from different studies may be reconciled if the differences in the synaptic interactions between the *in vitro* and *in vivo* preparations are taken into account (Bramham and Sarvey 1996).

We report here that in hippocampal slices there are two pharmacologically and physiologically distinct types of LTP in MPP, consistent with the presence of young and mature populations of neurons. Preliminary account of these results has been published and presented in an abstract form (Snyder and Wojtowicz 2000).

METHODS

Subjects

Experiments were performed on 60- to 70-day-old male Wistar rats (320–360 g) obtained from Charles River. In several experiments, we also used Long-Evans rats of similar size also from Charles River to

confirm that the effects of neurogenesis on synaptic plasticity are not limited to the Wistar strain. Animals were individually housed on 12 h light/dark schedule and used at 11:00–12:00 a.m. during the light phase. All experiments were conducted according to the protocols approved by the University of Toronto Animal Care Committee. Slices were prepared according to the procedures used previously in our laboratory (Wang and Wojtowicz 1997; Wang et al. 2000). Briefly, the rats were anesthetized with halothane and decapitated. The brains were removed and the hippocampi were dissected. Four-hundred-micrometer-thick hippocampal slices were prepared on a tissue chopper and incubated at least 1.5 h in a holding chamber at room temperature prior to experiments. For electrophysiological recordings, the slices were transferred into a perfusion chamber and viewed with a dissecting microscope. All experiments were done on slices maintained *in vitro* for 2–8 h. Generally, only one slice per rat was used for each type of experiment except for several experiments like those illustrated in Fig. 1, where LTP was measured twice in the same slices. These experiments were done to demonstrate as many features of LTP as possible, in a most comprehensive manner.

Electrophysiological recordings and LTP induction

The field potential recordings were obtained in the medial molecular layer of the dentate gyrus on stimulation of the medial perforant

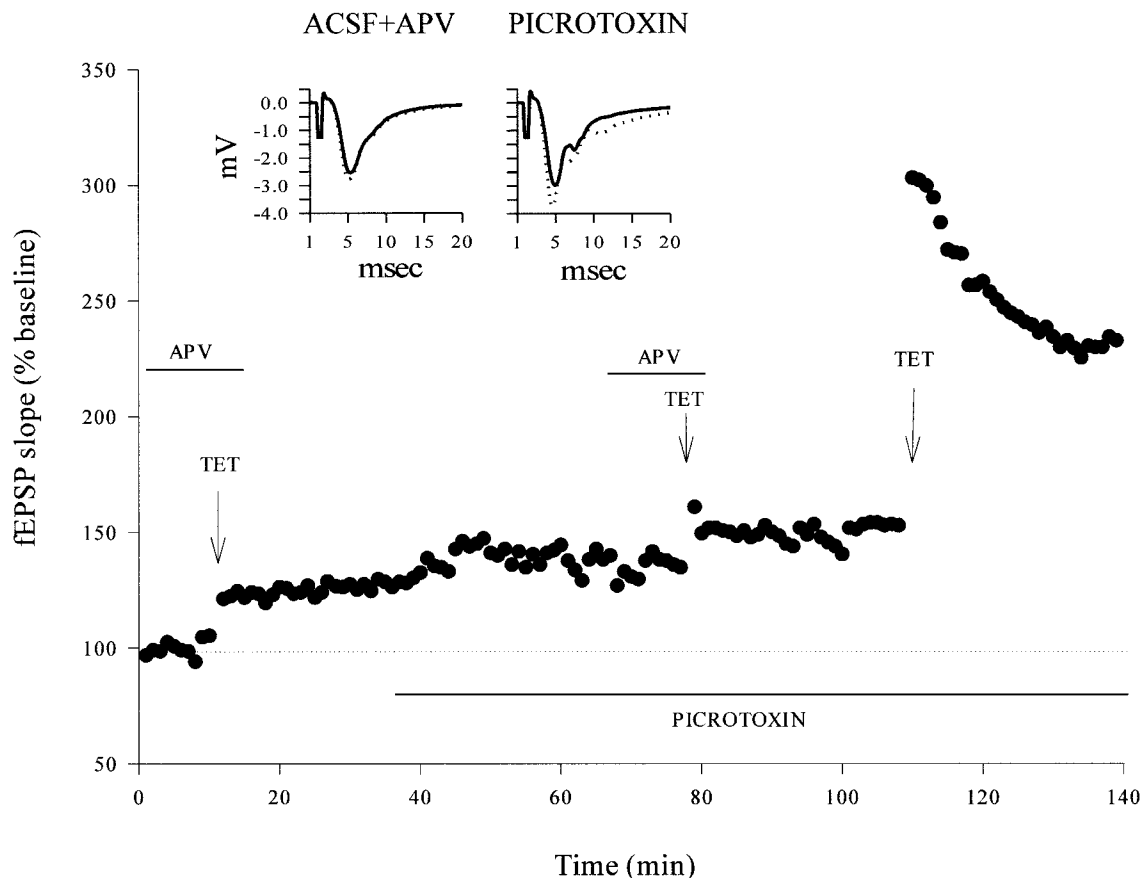


FIG. 1. Time course of an experiment illustrating 2 types of long-term potentiation (LTP) in a single slice. Sample field excitatory postsynaptic potential (fEPSP) traces before (—) and after tetanization (···) are shown in the *inset*. Each point in the main graph shows the average slope of the fEPSP in the middle molecular layer in response to slow (1/20 s) stimulation. APV (50 μ M) was perfused for 10 min prior to the tetanic stimulation with 4 100-Hz trains (TET) and switched off 1–2 min afterward. The 20% enhancement [artificial cerebrospinal fluid (ACSF)-LTP] of fEPSPs is seen for at least 30 min without any decline before application of 50 μ M picrotoxin. Sample traces before and 30 min after tetanization are shown on the left side of the *inset*. Picrotoxin produced a slight increase of the fEPSP slope as seen on the graph and in comparing ··· on the left and — on the right in the *inset*. An enhanced population spike was also clear in the presence of picrotoxin. The 2nd tetanus, applied at 80 min in presence of picrotoxin and APV, did not have a significant effect. The 3rd tetanus applied without APV produced a large posttetanic potentiation that declined within 5–10 min to a more stable “plateau” (PICRO-LTP).

pathway. Baseline recordings were obtained by electrical stimulation in the middle region of the molecular layer. Stimulation was performed with insulated bipolar tungsten electrodes with tip diameters of approximately 40 μm . Input-output curves were obtained after 20–30 min of stable recordings. The stimulation intensity that produced a half-maximal response (300–400 μA , 10–20 μs) was chosen for test pulse and tetanic stimulation. Only those slices which produced the field excitatory postsynaptic potentials (EPSPs) of 1 mV or higher in amplitude were accepted for experiments. The baseline frequency of stimulation was 1 per 20 s. During the experiments, the slices were continuously perfused with the artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2 -5% CO_2 at the rate of approximately 3 ml/minute. The temperature in the perfusion chamber was kept at 30–32°C. LTP was induced with a protocol developed previously (4 trains, 500 ms each, 100 Hz within the train, repeated every 20 s). The stimulation intensity was kept at the baseline intensity at all times (Wang and Wojtowicz 1997). Two other modified induction protocols were used to measure the LTP induction threshold. These included two trains instead of four and 50 Hz instead of 100 Hz. In all experiments, the number of stimuli within a train remained constant at 50.

Application of pharmacological blockers

Picrotoxin (50 μM) was applied to the perfusate 15–20 min prior to the induction of LTP. Ifenprodil was obtained from RBI in a water soluble-tartrate-form and from Tocris in a water insoluble form. In the latter case, ifenprodil was dissolved in DMSO and later diluted into ACSF with the final concentration of DMSO of 0.1%. At this level, DMSO had no noticeable effects on either baseline responses or LTP. In either case, ifenprodil was applied at 3 μM for 15 min prior to LTP induction and produced the same results. Several attempts to wash ifenprodil from the slices for 60 min were apparently unsuccessful. There was no reversal of the LTP blockade. Nimodipine and nifedipine (Calbiochem) were dissolved in DMSO prior to experiments and diluted with ACSF to yield the final concentrations of 10 and 30 μM respectively. D,L-2-amino-5-phosphonovaleric acid (APV) and D-(–)-2-amino-5-phosphonovaleric acid (Sigma) were dissolved directly in ACSF. In most experiments, D,L-APV at 50 μM was used, but the identical results were obtained with 50 μM D-APV, so the results from with both compounds are pooled.

Irradiation experiments

Rats (42 days old) were anesthetized with sodium pentobarbital (6.5 mg/100 g body wt), wrapped in a lead shield that left the head exposed, and placed in the gamma irradiating chamber (MDS, Nordion, Kanata, Ontario). The procedure was repeated on two consecutive days at the dose of 5 Gy for a total dose of 10 Gy following the previously described studies of (Parent et al. 1999; Peissner et al. 1999). Subsequently, the irradiated animals and sham controls were placed back in their cages and kept in the animal facility for 3 wk. On the day of the experiment, two hippocampi were dissected as described in the preceding text, with one side used for preparation of hippocampal slices for electrophysiological experiments and the other for immunolabeling of mitotic cells. For immunohistochemistry, the tissue was fixed in 4% paraformaldehyde by immersion and further processed for 5-bromo-2'-deoxyuridine (BrdU) or CRMP-4 immunohistochemistry as described previously (Kee et al. 2001; Scott et al. 1998). Three days prior to the dissection the animals were injected intraperitoneally with a mitotic indicator, BrdU (Sigma), three times, every 4 h for a total dose of 300 mg/kg. BrdU immunoreactivity was detected in 40- μm hippocampal sections using a procedure described previously (Scott et al. 1998) and viewed with a fluorescent or confocal microscope. The tissue from three irradiated and three sham animals was processed for immunohistochemistry to measure the total number of the mitotic cells using BrdU. The number of BrdU-positive

cells was measured stereologically by examining every 18th section from the complete left hippocampus in each animal using an optical dissector method (Coggeshall 1992). The contralateral right hippocampus was used for electrophysiology.

The collapsin response mediator protein (CRMP-4; formerly known as TOAD-64) immunoreactivity is a histological marker for young neurons (Kee et al. 2001; Quinn et al. 1999). The cell bodies of the CRMP-4⁺ neurons are located in the well-defined innermost layer of the granule cell layer and in the subgranular zone bordering the hilus. For quantification, we included the neuronal counts within the granule cell layer and in the subgranular zone of approximately 20 μm in width. We quantified the density of CRMP-4⁺ cells in the following manner. Beginning at one-third into the hippocampus from the dorsal end, every 15th section was analyzed to a total of 4 sections. The number of the CRMP-4⁺ cells in the selected sections was measured using an optical dissector method. The volume of the granule cell layer in the sampled sections was measured, and the density of CRMP-4⁺ cells was calculated as the ratio of the total number of the cells to the volume sampled.

RESULTS

Two types of LTP in a single slice

In a representative experiment, the slice was perfused with ACSF and the MPP was stimulated in the middle third of the molecular layer of the dentate gyrus with a brief electrical pulse required to evoke a field potential (fEPSP) in the granule neuron dendrites. A low rate of stimulation (1 stimulus/20 s) was used to avoid the frequency depression characteristic for this pathway (McNaughton 1980). In a standard protocol, LTP was induced by four trains of 100-Hz pulses, lasting 500 ms each, applied every 20 s. This tetanic stimulation produced a small (approximately 10%) but consistent LTP lasting at least 30 min (Fig. 1). Subsequent addition of 50 μM picrotoxin into the perfusate produced effective disinhibition of the slice and allowed the induction of additional, larger LTP (40–60%) with the identical tetanic stimulation. Also shown in Fig. 1 and fully documented in subsequent graphs are the different sensitivities of the two LTPs to the NMDA receptor blocker, APV. The ACSF-LTP was resistant to the application of 50 μM APV in the perfusate, whereas the PICRO-LTP was fully blocked with the same dose. Several ($n = 4$) experiments were done with three tetanic stimulations applied to the same slice in sequence in different conditions as shown in Fig. 1. However, in the majority of experiments shown in the following text we applied only one tetanus per slice. Results were the same in both protocols suggesting that ACSF-LTP had no effect on subsequent PICRO-LTP.

Pharmacological properties of two LTPs

Ifenprodil (3 μM), a blocker of NR2B/NMDA receptor subtype (Chenard and Menniti 1999; Kirson and Yaari 1996; Tovar and Westbrook 1999) completely blocked the ACSF-LTP but not PICRO-LTP (Figs. 2 and 3).

The results of the pharmacological experiments are summarized in Fig. 3. These experiments showed that although ACSF-LTP was relatively resistant to APV, it was blocked by 3 μM ifenprodil, a blocker of the NR2B/NMDA receptor subtype. In contrast, PICRO-LTP was fully blocked by APV but not by ifenprodil. Because, at higher concentrations, ifenprodil was reported to block the L-type calcium channels

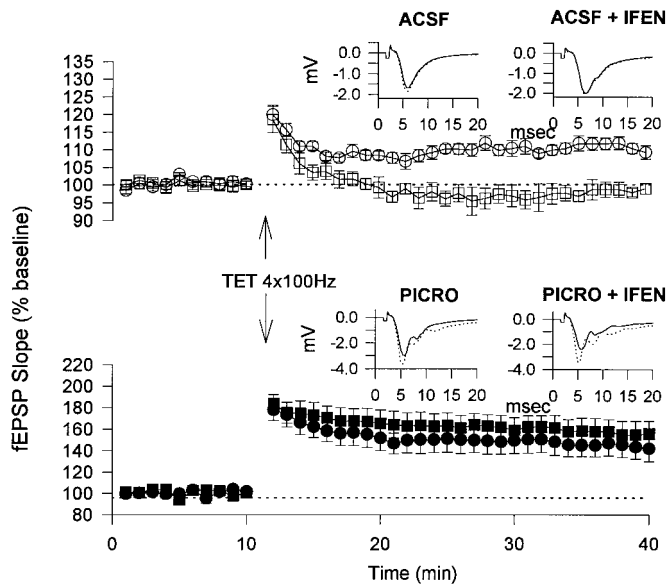


FIG. 2. Effect of ifenprodil (IFEN), NR2B receptor blocker, on LTP. *Top*: ACSF-LTP induced in control solution (○) and in the presence of 3 μ M ifenprodil (□). *Bottom*: PICRO-LTP in absence (●) and presence of 3 μ M ifenprodil (■). The mean time course of 6 experiments in each group is shown. Standard errors are indicated. The sample traces were taken from representative experiments in each group to show the baseline (—) and the potentiated (···) fEPSP responses.

(Church et al. 1994) and some forms of LTP are dependent on the L-type channels for induction (see DISCUSSION), we also tested whether ACSF-LTP is blocked by the selective L-type channel blockers nifedipine (30 μ M) and nimodipine (10 μ M). However, in a series of several such experiments, we have seen no effect by either blocker. The mean ACSF-LTP induced in the presence of L-channel blockers was $108.3 \pm$ (SE) 2 ($n = 7$). This is not different from the control values presented in Fig. 3. Thus it can be assumed that ifenprodil acted by a selective blockade of the NR2B/NMDA receptors.

Frequency requirements and time course of two LTPs

We also tested some of the stimulus parameters required for induction of the two types of LTP. These experiments showed

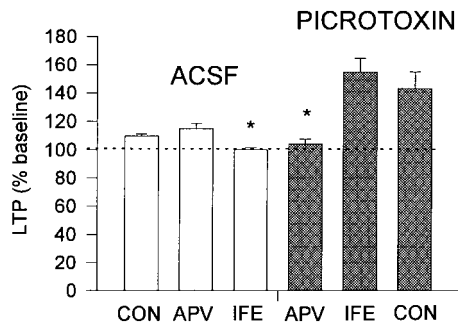


FIG. 3. Two LTPs can be distinguished pharmacologically. Summary of experiments with each bar representing the magnitude of LTP measured 25–30 min after tetanization under different conditions. □ (with SE), experiments done in normal ACSF; ■, those done in presence of 50 μ M picrotoxin. Data shows that the ACSF-LTP ($109.7 \pm 1.5\%$, $n = 6$) was blocked by ifenprodil ($99.9 \pm 1.6\%$, $n = 7$) but not by 50 μ M APV ($115 \pm 3.5\%$, $n = 6$). In contrast, PICRO-LTP ($143 \pm 11.7\%$, $n = 5$) was blocked by APV ($104 \pm 3.5\%$, $n = 5$) but not by ifenprodil ($154.5 \pm 10.0\%$, $n = 6$). Two-way ANOVA followed by a Bonferroni's test for comparison among groups confirmed that these effects were statistically significant ($P < 0.05$, *).

that fewer high-frequency trains are needed for the induction of ACSF-LTP than for the induction of PICRO-LTP (Fig. 4). Similar series of experiments established that ACSF-LTP can be fully induced by 50-Hz trains in comparison to 100-Hz trains required for PICRO-LTP (confirmed statistically with ANOVA test followed by a pairwise comparison among groups, $P < 0.05$, $n = 6$ in each group). Together, these experiments show that ACSF-LTP is relatively easier to induce. The two LTPs had different time courses of expression, with the ACSF-LTP maintaining its magnitude for the duration of the recording and PICRO-LTP showing a decline (Fig. 4). The contrast between the two was particularly clear when the ACSF-LTP was isolated pharmacologically by the addition of APV, and PICRO-LTP by the addition of ifenprodil (Fig. 5).

Inhibition of ACSF-LTP by cranial irradiation

It has been shown previously that neurogenesis in the DG can be prevented by low doses of cranial ionizing radiation (Parent et al. 1999). We have used this method to determine if a causal link between neurogenesis and ACSF-LTP can be

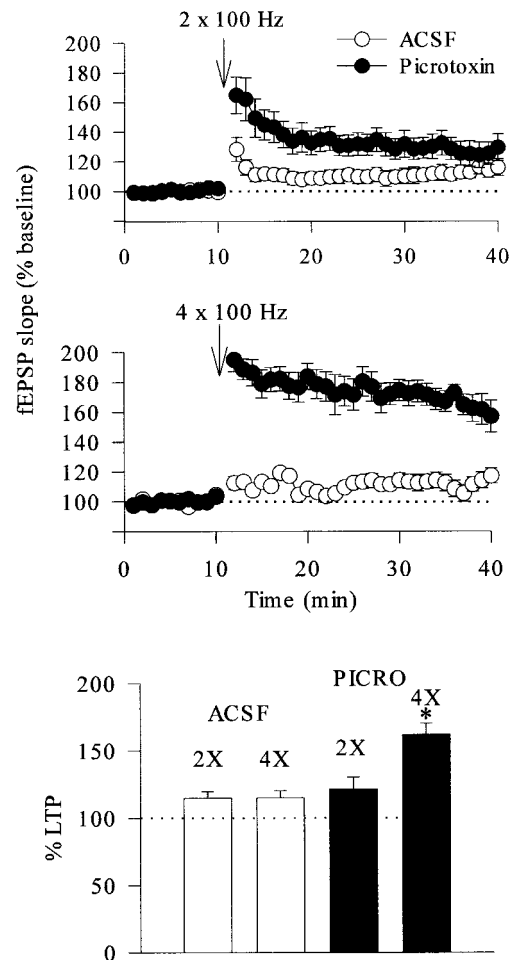


FIG. 4. ACSF-LTP requires fewer trains for induction. The graphs show the time course and magnitudes of the 2 LTPs induced by 2 or 4 trains at 100 Hz. The graph below summarizes the data. The magnitude of LTP at 25–30 min posttetanus was taken in each case. Only the PICRO-LTP induced by 4 trains was significantly different from the other treatments (ANOVA followed by pairwise comparison among groups, $P < 0.05$). Results show that PICRO-LTP requires at least 4 trains for induction whereas ACSF-LTP requires only 2. Each group included 5 experiments (5 slices from 5 animals).

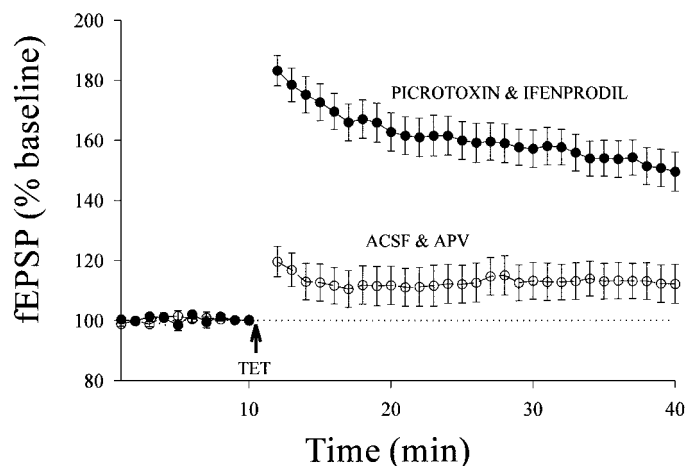


FIG. 5. PICRO-LTP and ACSF-LTP differ in time course of expression. In all cases, LTP was induced by a standard tetanic protocol (TET). ACSF-LTP was induced in presence of 50 μ M APV and showed a steady magnitude from the time of induction. PICRO-LTP was induced in the presence of 3 μ M ifenprodil. It showed a strong posttetanic potentiation and a gradually declining LTP. Each group included 7 experiments (7 slices from 7 animals).

established. By preventing new cell proliferation and conducting electrophysiological experiments after a dwell time of 3 wk, we eliminated new cells that would have been born during that time. In three control and three irradiated animals, we confirmed stereologically that there were few mitotically active cells present using the BrdU as a mitotic indicator (Fig. 6A). The total number of BrdU⁺ cells was reduced from $2,315 \pm 863$ (mean \pm SD, $n = 3$) in controls to 737 ± 132 ($n = 3$) in the irradiated animals. This change was statistically significant ($P < 0.05$). We also measured the density of the immature cells using CRMP-4 as a marker. In normal, control animals, CRMP-4 labels the immature neurons, which span ages from a few days up to 5 wk of age (Kee et al. 1999). Their density/mm³ of the granule cell layer was reduced from $48,243 \pm 7351$ (mean \pm SD, $n = 3$) in controls to $25,301 \pm 1153$ ($n = 3$) in the irradiated animals (Fig. 6A). The remaining CRMP-4-positive cells are likely to be more than 3 wk old since the irradiation was done 3 wk prior to the labeling, but the additional young neurons born after the irradiation were also present since the reduction of the mitotic activity was not complete. In all other irradiated rats, the effects of irradiation on the mitotic activity appeared similar but were not quantified stereologically. The electrophysiological measurements of LTP on six irradiated rats showed unequivocal reduction in ACSF-LTP (Fig. 6B). The potentiation of fEPSPs immediately after the tetanic stimulation was not changed nor was the baseline amplitude of the response or their paired pulse depression at the 40-ms interval (Fig. 6B, inset). The mean of PICRO-LTP in the controls was $135 \pm 9.6\%$ ($n = 6$). This was reduced to $130 \pm 5.6\%$ ($n = 7$) after irradiation, but the change was not statistically significant ($P > 0.05$).

To examine the possibility that the absence of ACSF-LTP was due to an unspecific effect such as an enhanced inhibition within the DG, we measured the paired-pulse depression of field EPSPs in the two groups of rats. The second EPSP within the pair was typically reduced in control animals (Fig. 6B) by $7.8 \pm 2.8\%$. The magnitude of the depression was similar ($6.7 \pm 3.2\%$) in the irradiated animals, ruling out the possibility of enhanced inhibition. Furthermore the stimulus intensities

required to evoke half-maximal fEPSPs in DG of irradiated animals fell in the range of the control values (see METHODS). Finally, we compared the magnitude of LTP in the CA1 field of the hippocampus induced by stimulation of the Schaffer collateral/associational pathway (same stimulation protocol as for DG) and found no difference between the control and irradiated animals (127 ± 2 vs. $122.5 \pm 4.9\%$, $n = 3$, $P > 0.05$). In summary, we found that the functional profile of the putative young neurons established previously, i.e., low threshold for LTP induction, stable course of LTP expression, and insensitivity to GABAergic inhibition (Wang et al. 2000), also applies to ACSF-LTP in DG. The effects of irradiation and of the pharmacological blockers further strengthen the hypothesis that this new type of LTP originates from the young neurons.

DISCUSSION

Two groups of neurons or two sets of synapses?

The strong GABAergic inhibition in the DG of the hippocampus has been described both in slices (Wigstrom and Gustafsson 1983) and in vivo (Moser 1996). We have described a new form of LTP in the DG that can apparently overcome this inhibition. We further propose that this new ACSF-LTP originates from a separate population of neurons classified as young, adult-generated granule neurons. An alternative hypothesis, that the two LTPs are induced at different sets of synapses in the same population of neurons, is unlikely since in such case the GABA_Aergic inhibition, acting predominantly on postsynaptic receptors, would be expected to affect both equally. Instead, picrotoxin, a GABA_A receptor blocker, is required to unmask only one form of LTP. It should also be noted that in all experiments, the same populations of synapses, located in the medial region of the granule cell dendrites, were stimulated, so a differential degree of GABAergic inhibition on different dendritic regions influencing the two sets of synapses is unlikely. Finally, the presence of two distinct populations of neurons, one with strong and the other with weak GABAergic inhibition, has been demonstrated with single-cell recordings (Wang et al. 2000). These putative young neurons occupy only the innermost (next to hilus) of the six-seven layers of granule neurons (approximately 15% of the total population). Nevertheless, if all these individual neurons produce a uniform, stable LTP of about 60%, approximately 9% enhancement of the total fEPSP sampled from young and old neurons together, would be expected in slices. This is reasonably close to the average of 10% LTP seen in our experiments.

Comparison with previous studies

Previous studies on slices have produced mixed results regarding the magnitude and induction requirements of MPP LTP. In a majority of studies (but see Bramham et al. 1997), it is found that only a small amount of LTP can be obtained in a standard ACSF bath (Hanse and Gustafsson 1992; Nguyen and Kandel 1996; Wigstrom and Gustafsson 1983), leading many experimenters to turn to pharmacological disinhibition as a routine procedure necessary to study DG LTP (Beck et al. 2000; Colino and Malenka 1993; Motro et al. 1996). This disinhibition may block LTP in very young neurons, which are thought to possess depolarizing inhibitory postsynaptic potentials (Ben-Ari et al. 1997), making their contribution to the

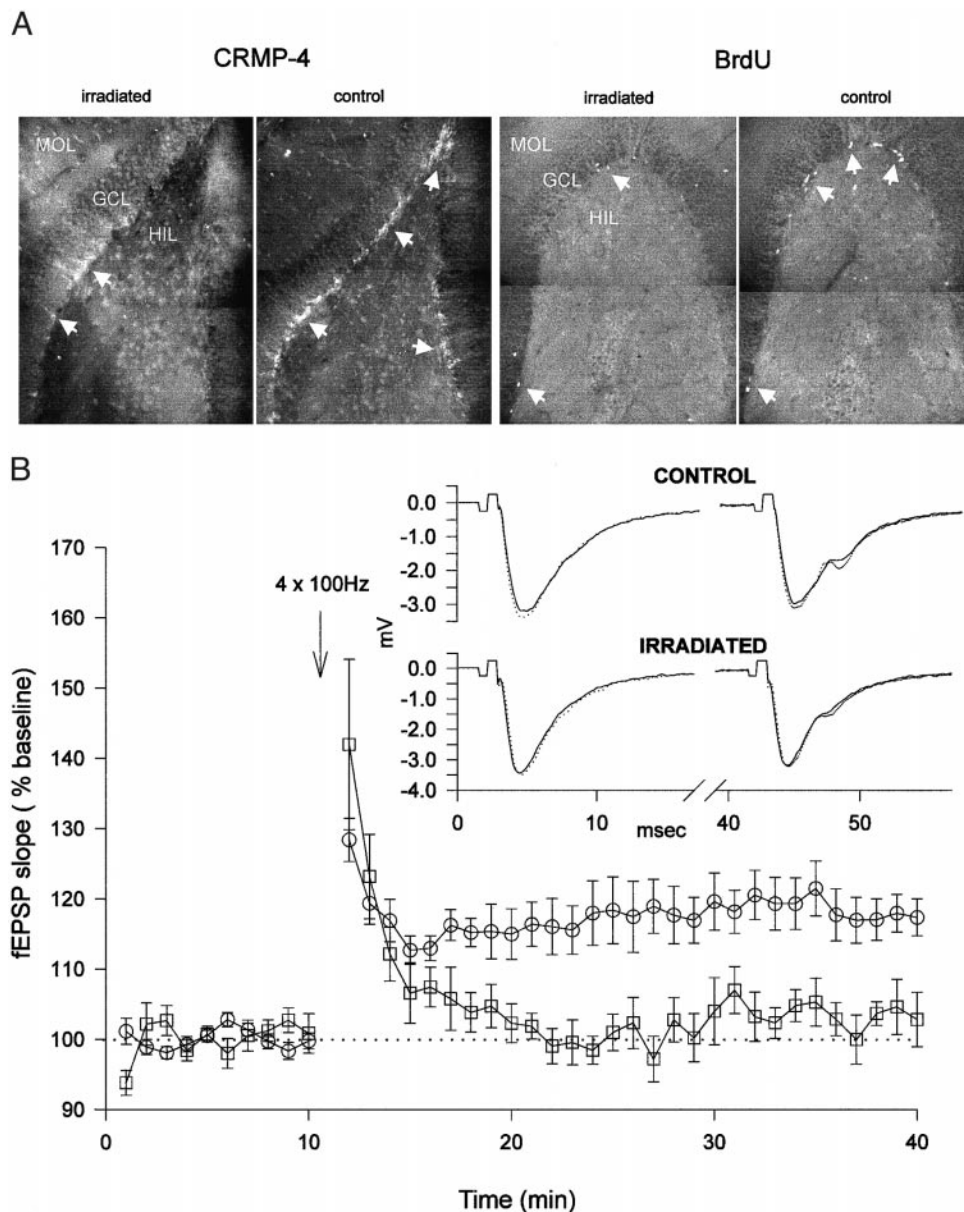


FIG. 6. Cranial irradiation reduces neurogenesis and ACSF-LTP. **A:** the densities of CRMP-4⁺ young cells and BrdU⁺ mitotic cells (→) lining the border between Hilus (HIL) and granule cell layer (GCL) were clearly reduced in the animals examined 3 wk after irradiation. **B:** time course of ACSF-LTP in control (○) and irradiated (□) animals ($n = 6$). Note that a short-lasting posttetanic potentiation was clearly present in the irradiated animals, suggesting a healthy state of afferent axons, their terminals, and synapses. However, ACSF-LTP at 30 min after the tetanus was almost completely blocked. The sample traces shown in the inset were taken from representative experiments to show similar amplitudes of the evoked fEPSPs in both groups and similar paired pulse depression at 40 ms, suggesting that the strength of synaptic inhibition was unchanged by the irradiation. —, baseline responses; ···, potentiated responses.

overall fEPSP LTP undetectable. Such effect could explain our results showing no reduction of PICRO-LTP by ifenprodil and no residual LTP on addition of APV in presence of picrotoxin (Figs. 2 and 3).

Complicating the issue and contrary to our results, greater DG LTP has been shown in the inhibited slice preparation (Bramham et al. 1997; Trommer et al. 1995). Furthermore the magnitude and reliability of DG LTP shown by Trommer et al. (1995) depended significantly on the type of recording chamber used, demonstrating the influence of experimental conditions. Apart from these technical differences, it is plausible that the variability in ACSF-LTP may be due to differences in animal species and strains used since it is clear that mouse strain has a significant effect on the magnitude of DG (Bampton et al. 1999) and CA1 LTP (Nguyen et al. 2000).

In vivo studies reliably produce LTP, albeit usually of a greater magnitude (20–30%) than ACSF-LTP seen in our slices (Bramham et al. 1991a; Errington et al. 1987; Morris et al. 1986; Nosten-Bertran et al. 1996). Numerous differences in

experimental conditions, as mentioned in the preceding text, can account for this discrepancy. It is important to note that *in vivo* LTP, which may be more closely related to a natural learning environment, doesn't require disinhibition. This may signify the young neuron's greater ability to contribute to learning processes as compared with more mature neurons.

Induction of ACSF-LTP requires presence of young neurons

The block of ACSF-LTP after irradiation suggests strongly that this new type of LTP is produced by the young, adult-generated granule neurons (Fig. 6). Since the time interval between the irradiation and the electrophysiological experiments was 3 wk, it can be concluded that neurons that normally produce ACSF-LTP are no more than 3 wk old. The intact PICRO-LTP in the DG, as well as, the lack of changes in the synaptic inhibition as demonstrated by unchanged posttetanic potentiation and PPD confirm that the irradiation had a desired effect of acting selectively on the proliferating neurons. It

should be noted that not all CRMP-4⁺ neurons were eliminated by the irradiation. This is not surprising since the survival period of 3 wk allows for the presence of adult-generated young neurons that were born before the irradiation. The number of mitotic, BrdU⁺ cells 3 wk after irradiation was approximately 30% of the control. The functional status of these new cells born after irradiation is not known.

Induction of ACSF-LTP requires a young type of NMDA receptors

ACSF-LTP is characterized by a relatively low threshold for its induction as determined by fewer trains and lower frequencies of stimulation within the trains. How can this property be explained? The induction of ACSF-LTP apparently requires activation of NR2B/NMDA receptor subtype that is known to be present in the developing brain but is largely replaced by the NR2A/NMDA subtypes during maturation (Monyer et al. 1994; Williams et al. 1993). Although some NR2B immunolabeling persists in the adult brain of mice, its highest levels are present at 15–21 days postnatal (Okabe et al. 1998), approximately the age of the neurons that we think are responsible for ACSF-LTP (see the preceding text). We are not aware of any reports of ifenprodil blocking LTP in any other brain region, and preliminary data with another blocker of NR2B, CP101,606 showed no effect in CA1 region of the hippocampus (Weber et al. 1999). One characteristic of the NR2B/NMDA receptor-channel complexes is their prolonged opening and the resulting large calcium influx into the cytoplasm. This characteristic could account for the lower threshold of ACSF-LTP induction as compared with PICRO-LTP, presumably requiring NR2A/NMDA receptors. The differential effects of APV and ifenprodil on the two LTPs support this explanation. The approximately twofold higher affinity of APV toward the recombinant NR2A/NR1a, as compared with NR2B/NR1a receptors, has been reported (Priestley et al. 1995). Our results suggest that this selectivity is greater for endogenous, presumably heteromeric, receptors in the DG since the APV at the concentrations sufficient to completely block PICRO-LTP had no effect on ACSF-LTP. This selectivity cannot be regarded as absolute and it is possible that higher (>50 μ M) APV concentrations will block ACSF-LTP.

The lack of sensitivity of ACSF-LTP to dihydropyridines, nifedipine and nimodipine, rules out the contribution of the high-voltage activated L-type calcium channels in this phenomenon although some other forms of LTP in other brain regions have been reported to rely on L-type channels (Cavus and Teyler 1996; Weisskopf et al. 1999). In the hippocampus, the presence of L-type channels increases with age (Thibault and Landfield 1996), making their significant contribution to calcium influx in old neurons, not young ones, more likely. Thus the pharmacological characteristics of ACSF-LTP suggest that it originates primarily from young, adult generated granule neurons, while the PICRO-LTP may be mostly due to the mature neurons. This result is in agreement with the recent data reported by Christie et al. (2000), who observed lower threshold for LTP induction in animals with enhanced rate of adult neurogenesis induced by running.

ACSF-LTP may be longer lasting

Further evidence for a different mechanism of the two LTPs is presented by the data shown in Fig. 4, showing different decay times for the two phenomena. The steady time course of ACSF-LTP is reminiscent of the LTPs in the hippocampi of juvenile rats measured in the hippocampal CA1 area in vivo (Grover and Teyler 1994) and of LTP in transgenic mice artificially endowed with NR2B receptors (Tang et al. 1999). It should also be pointed out that the characteristics of the ACSF-LTP, i.e., low threshold and stable time course, closely resemble those of the LTP in the putative young neurons described by Wang et al. (2000). These physiological characteristics were corroborated by the morphological appearance of the dendritic tree and the relative paucity of dendritic spines on the young neurons, again the common characteristic of the young neurons seen in the developing brain.

Consequences for hippocampal-dependent learning

Functional contributions of the young neurons may be particularly significant in vivo where the GABAergic inhibition of the granule neurons within the DG is strong and paradoxically enhanced during exploration by the animals (Moser 1996). In view of the evidence for a crucial role of the intact DG, and of the MPP in particular (Ferbinteanu et al. 1999), in spatial learning, one may wonder if the relatively uninhibited and plastic young neurons play a major role in the hippocampal-dependent learning. The artificially induced LTP is not an accurate model of learning (Atwood and Wojtowicz 1999), but a long-lasting synaptic change of this type, occurring in vivo in a selective group of neurons, may be (Trommald et al. 1996). The discovery of adult neurogenesis and its regulation by physiological stimuli suggest a possible role of new neurons in learning (Gould et al. 1999a). Consequently, the exceptional ability of the young neurons to produce LTP under physiological conditions in slices may be related to the proposed role of the dentate gyrus in learning.

Although LTP is currently the best cellular model for hippocampal-dependent, spatial learning, the exact contribution of this phenomenon to learning is under debate. The NMDA receptor blockers appear to prevent only the initial, pretraining phase of the water maze task, frequently used to test for spatial learning (Bannerman et al. 1995; Saucier and Cain 1995). Recent results suggest that this effect is not likely due to some nonspecific effects of the blockers because the saturation of LTP by prior electrical stimulation of the perforant pathway disrupted learning in naive but not in pretrained animals (Ot-naess et al. 1999). In future studies, the possible contributions of the newly generated neurons within the DG to learning and memory could be examined given the data on their plasticity presented in this report.

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REFERENCES

- ALTMAN J AND DAS GD. Postnatal neurogenesis in the guinea pig. *Nature* 214: 1089–1101, 1967.
- ATWOOD HL AND WOJTOWICZ JM. Silent synapse in neural plasticity: current evidence. *Learn Mem* 6: 542–571, 1999.

- BAMPTON ETW, GRAY RA, AND LARGE CHH. Electrophysiological characterization of the dentate gyrus in five inbred strains of mouse. *Brain Res* 841: 123–134, 1999.
- BANNERMAN DM, GOOD MA, BUTCHER SP, RAMSEY M, AND MORRIS RGM. Distinct components of spatial learning revealed by prior training and NMDA blockade. *Nature* 378: 182–186, 1995.
- BECK H, GOUSSAKOV IV, LIE A, HELMSTAEDTER CH, AND ELGER CHE. Synaptic plasticity in the human dentate gyrus. *J Neurosci* 20: 7080–7086, 2000.
- BEN-ARI Y, KHAZIPOV R, LEINEKUGEL X, CAILLARD O, AND GAJARASA J-L. GABA_A, NMDA and AMPA receptors: a developmentally regulated “ménage à trois.” *Trends Neurosci* 20: 523–529, 1997.
- BRAMHAM CR, BACHER-SVENDSEN K, AND SARVEY JM. LTP in the lateral perforant path is beta-adrenergic receptor-dependent. *Neuroreport* 8: 719–724, 1997.
- BRAMHAM CR, MILGRAM NW, AND SREBRO B. Activation of AP5-sensitive NMDA receptors is not required to induce LTP of synaptic transmission in the lateral perforant path. *Eur J Neurosci* 3: 1300–1308, 1991a.
- BRAMHAM CR, MILGRAM NW, AND SREBRO B. Opioid receptor activation is required to induce LTP of synaptic transmission in the lateral perforant path in vivo. *Brain Res* 567: 42–50, 1991b.
- BRAMHAM CR AND SARVEY JM. Endogenous activation of μ and δ -1 opioid receptors is required for long-term potentiation induction in the lateral perforant path: dependence on GABAergic inhibition. *J Neurosci* 16: 8123–8131, 1996.
- BUCKMASTER PS AND SCHWARTZKROIN PA. Interneurons and inhibition in the dentate gyrus of the rat in vivo. *J Neurosci* 15: 774–789, 1995.
- CAVUS I AND TEYLER T. Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. *J Neurophysiol* 76: 3038–3047, 1996.
- CHENARD BL AND MENNITI FS. Antagonists selective for NMDA receptors containing the NR2B subunit. *Curr Pharm Design* 5: 381–404, 1999.
- CHRISTIE BR, VAN PRAAG H, AND GAUGE FH. Effects of voluntary exercise on long-term potentiation and neurogenesis in the dentate gyrus in vivo. *Soc Neurosci Abstr* 26: 888, 2000.
- CHURCH J, FLETCHER EJ, BAXTER K, AND MACDONALD F. Blockade by ifenprodil of high-voltage-activated Ca channels in rat and mouse cultured hippocampal pyramidal neurons: comparison with NMDA receptor antagonist actions. *Br J Pharmacol* 113: 499–507, 1994.
- COGGESHALL RE. A consideration of neural counting methods. *Trends Neurosci* 15: 9–13, 1992.
- COLINO A AND MALENKA RC. Mechanisms underlying induction of long-term potentiation in rat medial and lateral perforant paths in vitro. *J Neurophysiol* 69: 1150–1159, 1993.
- ERRINGTON MI, LYNCH MA, AND BLISS TVP. Long-term potentiation in the dentate gyrus: induction and increased glutamate release are blocked by D-aminophosphonovalerate. *Neuroscience* 20: 279–284, 1987.
- FERBINTEANU J, HOLSINGER RMD, AND McDONALD RJ. Lesions of the medial and lateral perforant path have different effects on hippocampal contributions to place learning and on fear conditioning to context. *Behav Brain Res* 101: 65–84, 1999.
- GOULD E, BEYLIN A, TANAPAT P, REEVES A, AND SHORS TJ. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci* 2: 260–265, 1999a.
- GOULD E, McEWEN BS, TANAPAT P, GALEA LAM, AND FUCHS E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J Neurosci* 17: 2492–2498, 1997.
- GOULD E, REEVES AJ, FALLAH M, TANAPAT P, GROSS CG, AND FUCHS E. Hippocampal neurogenesis in adult Old World primates. *Proc Natl Acad Sci USA* 96: 5263–5267, 1999b.
- GROVER LM AND TEYLER TJ. Activation of NMDA receptors in hippocampal area CA1 by low and high-frequency orthodromic stimulation and their contribution to induction of long-term potentiation. *Synapse* 16: 66–75, 1994.
- HANSE E AND GUSTAFSSON B. Long-term potentiation and field EPSPs in the lateral and medial perforant paths in the dentate gyrus in vitro: a comparison. *Eur J Neurosci* 4: 1191–1201, 1992.
- KAPLAN MS AND HINDS JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* 197: 1092–1094, 1977.
- KEE N, PRESTON E, AND WOJTOWICZ JM. Enhanced neurogenesis after transient ischemia in the dentate gyrus of the rat. *Exp Brain Res* 136: 313–320, 2001.
- KEE N, SMITH ML, AND WOJTOWICZ JM. Differential expression of neuronal markers in adult-generated granule neurons. *Soc Neurosci Abstr* 25: 522, 1999.
- KIRSON ED AND YAARI Y. Synaptic NMDA receptors in developing mouse hippocampal neurons: functional properties and sensitivity to ifenprodil. *J Physiol (Lond)* 497: 437–455, 1996.
- KUHN HG, DICKINSON-ANSON H, AND GAUGE FG. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 16: 2027–2033, 1996.
- MCNAUGHTON BL. Evidence for two physiologically distinct perforant pathways to the fascia dentata. *Brain Res* 199: 1–19, 1980.
- MONYER H, BURNASHEV N, LAURIE DJ, SAKMANN B, AND SEEBURG PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12: 529–540, 1994.
- MORRIS RGM, ANDERSON E, LYNCH GS, AND BAUDRY M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319: 774–776, 1986.
- MOSER EI. Altered inhibition of dentate granule cells during spatial learning in an exploration task. *J Neurosci* 16: 1247–1259, 1996.
- MOTRO B, WOJTOWICZ JM, BERNSTEIN A, AND VAN DER KOOY D. “Steel” mutant mice are deficient in hippocampal learning but not long-term potentiation. *Proc Natl Acad Sci USA* 93: 1808–1813, 1996.
- NGUYEN PV, DUFFY SN, AND YOUNG JZ. Differential maintenance and frequency-dependent tuning of LTP at hippocampal synapses of specific strains of inbred mice. *J Neurophysiol* 84: 2484–2493, 2000.
- NGUYEN PV AND KANDEL ER. A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *J Neurosci* 16: 3189–3198, 1996.
- NOSTEN-BERTRAN M, ERRINGTON MI, MURPHY KPSJ, TOKUGAWA Y, BARBONI E, KOZLOVA E, MICHALOVICH D, MORRIS RGM, SILVER J, STEWART CL, BLISS TVP, AND MORRIS RJ. Spatial learning is unaffected by a selective impairment of LTP in vivo in Thy-1 mutant mice. *Nature* 29: 826–829, 1996.
- OKABE S, COLLIN C, AUERBACH JM, MEIRI N, BENGZON J, KENNEDY MB, SEGAL M, AND MCKAY RD. Hippocampal synaptic plasticity in mice overexpressing an embryonic subunit of the NMDA receptor. *J Neurosci* 18: 4177–4188, 1998.
- OTNAESS MK, BRUN VH, MOSER MB, AND MOSER EI. Pretraining prevents spatial learning impairment after saturation of hippocampal long-term potentiation. *J Neurosci* 19: RC49, 1999.
- PARENT JM, TADA E, FIKE JR, AND LOWENSTEIN DH. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. *J Neurosci* 19: 4508–4519, 1999.
- PARENT JM, YU TW, LEIBOWITZ RT, GESCHWIND DH, SLOVITER RS, AND LOWENSTEIN DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* 17: 3727–3738, 1997.
- PEISSNER W, KOCHER M, TREURER H, AND GILLARDON F. Ionizing radiation-induced apoptosis of proliferating stem cells in the dentate gyrus of the adult rat hippocampus. *Mol Brain Res* 71: 61–68, 1999.
- PRIESTLEY T, LAUGHTON P, MYERS J, LE BOURDELLES B, KERBY J, AND WHITING PJ. Pharmacological properties of recombinant human NMDA receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Mol Pharmacol* 48: 841–848, 1995.
- QUINN CC, GRAY GE, AND HOCKFIELD S. A family of proteins implicated in axon guidance and outgrowth. *J Neurobiol* 41: 158–164, 1999.
- SAUCIER D AND CAIN DP. Spatial learning without NMDA receptor-dependent long-term potentiation. *Nature* 378: 186–189, 1995.
- SCOTT BW, WANG S, BURNHAM WM, DE BONI U, AND WOJTOWICZ JM. Kindling-induced neurogenesis in the dentate gyrus of the rat. *Neurosci Lett* 248: 73–76, 1998.
- SEKI T AND ARAI Y. Age-related production of new granule cells in the adult dentate gyrus. *Neuroreport* 6: 2479–2482, 1995.
- SNYDER JS AND WOJTOWICZ JM. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. *Soc Neurosci Abstr* 26: 889, 2000.
- TANG YP, SHIMIZU E, DUBE GR, RAMPON C, KERCHNER GA, ZHUO M, LIU G, AND TSJEN JZ. Genetic enhancement of learning and memory in mice. *Nature* 401: 63–69, 1999.
- THIBAUT O AND LANDFIELD PW. Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* 272: 1017–1020, 1996.
- TOVAR KR AND WESTBROOK GL. The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J Neurosci* 19: 4180–4188, 1999.

- TROMMALD M, HULLEBERG G, AND ANDERSEN P. LTP is associated with new excitatory spine synapses on rat dentate granule cells. *Learn Mem* 3: 218–228, 1996.
- TROMMER BL, KENNELLY JJ, COLLEY PA, OVERSTREET LS, SLATER NT, AND PASTERNAK JF. AP5 blocks LTP in developing dentate gyrus and unmasks LTD. *Exp Neurol* 131: 83–92, 1995.
- VAN PRAAG H, KEMPERMANN G, AND GAUGE FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2: 266–270, 1999.
- WANG S, SCOTT BW, AND WOJCIWICZ JM. Heterogenous properties of dentate gyrus granule neurons in the adult rat. *J Neurobiol* 42: 248–257, 2000.
- WANG S AND WOJCIWICZ JM. Control of associative synaptic interactions by GABA_B receptors in dentate gyrus of the rat. *Neuroscience* 79: 117–127, 1997.
- WEBER ML, TAYLOR CP, AND DONEVAN SD. CP101,606 an NR2B selective NMDA receptor antagonist, does not block hippocampal LTP. *Soc Neurosci Abstr* 25: 1485, 1999.
- WEISSKOPF MG, BAUER EP, AND LEDOUX JE. L-type voltage-gated calcium channels mediate NMDA-independent associative LTP at thalamic input synapses to the amygdala. *J Neurosci* 19: 10512–10519, 1999.
- WIGSTROM H AND GUSTAFSSON B. Large long-lasting potentiation in the dentate gyrus in vitro during blockade of inhibition. *Brain Res* 275: 153–158, 1983.
- WILLIAMS K, RUSSELL SL, SHEN YM, AND MOLINOFF PB. Developmental switch in the expression of NMDA receptors occurs in vivo and in vitro. *Neuron* 10: 267–278, 1993.
- XIE C-W AND LEWIS DV. Opioid-mediated facilitation of long-term potentiation at the lateral perforant path-dentate granule cell synapse. *J Pharmacol Exp Therap* 256: 289–296, 1991.