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AP5 Blocks LTP in Developing Rat Dentate Gyrus and Unmasks LTD

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The hippocampal dentate gyrus undergoes active neuronogenesis as well as growth and regression of neuronal elements and connections during the early postnatal period. In some brain regions, most notably in the visual system, both activity-dependent synaptic plasticity and NMDA receptor activation are candidate mechanisms by which neuronal architecture may be refined during brain maturation. To investigate whether similar mechanisms might obtain in developing dentate, we studied the effects of tetanic stimulation before and after NMDA receptor blockade in hippocampal slices from rats at 7–33 days. Field potentials were recorded in the suprapyramidal granule cell layer in response to stimulation of the medial perforant path. Robust long-term potentiation (LTP) of population spike amplitude (~200% of baseline) was produced by a single tetanus (100 Hz, 2 s, 200 μs) at all ages studied. Application of 10 μ*M* AP5 depressed population spike amplitude only in the younger slices (~81% of baseline at 8–15 days; ~86% of baseline at 16–24 days), suggesting that the NMDA receptor-mediated component of normal synaptic transmission is higher in early development and decreases with maturation. AP5 prevented or significantly diminished LTP at all ages, establishing the NMDA dependence of LTP induction in the medial perforant path throughout development. AP5 also unmasked tetanus-induced homosynaptic long-term depression (62–75% of baseline) in the younger slices (8–24 days). Thus, prominent NMDA receptor-mediated activity and the capacity for bidirectional synaptic plasticity are characteristic of immature dentate. These processes may influence dentate morphogenesis by contributing to the growth, regression, and stabilization of neuronal elements.

INTRODUCTION

Long-term potentiation (LTP) was initially described in the perforant path–granule cell synapse of rabbit hippocampal dentate gyrus *in vivo* (6, 7). Extensive study in adult animals and hippocampal slices has established the NMDA dependence of LTP induction in this pathway (8, 10, 37) and provided evidence for its role as a model of learning and memory (5, 32). Another line of reasoning, largely based on studies from visual systems (2, 15, 26, 28, 31), suggests that both NMDA receptor activation and LTP also play a prominent role in developmental synaptic plasticity—that is in the activity-dependent "sculpting" of the mature nervous system in which neurons and synapses that remain in adult brain are presumed to be survivors of a rigorous competition for stabilization during development (14). In this regard, the immature rat dentate gyrus is a fertile region for electrophysiologic investigation because the majority of its neuronogenesis occurs postnatally (16) and its developmental anatomy is well-studied (1, 17, 24, 25, 29, 36, 38, 39). We investigated whether tetanic stimulation would produce LTP in the medial perforant path–granule cell synapse as early as the end of the first postnatal week (the peak time of cell birth in this region) and whether LTP induction in this path is NMDA receptor dependent during development. Some of this data has been published in abstract form (43).

MATERIALS AND METHODS

Hippocampal slices from Sprague–Dawley rats of both genders (7–33 days) were prepared as follows. Animals were anesthetized with ketamine (50 mg/kg ip, age ≥ 10 days only), and decapitated, and the brains were removed and placed in chilled buffer gassed with 95% O_2/5% CO_2 and containing (in m*M*): NaCl 124, KCl 3, $MgSO_4$ 1.3, $NaHPO_4$ 1.25, $CaCl_2$ 2.4, $NaHCO_3$ 24, and glucose 10 (pH 7.4). Slices (350–400 μm thick) were cut manually with a vertical tissue chopper (Stoelting). Slices from ketamine-treated animals were rinsed with fresh oxygenated buffer at room temperature for ≥ 2 h after preparation and then transferred individually to a recording chamber; others were incubated in a beaker at room temperature for at least 1 h before being transferred as needed.

Recording electrodes were positioned in the suprapyramidal limb of the granule cell layer and stimulating

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electrodes in the middle third of the molecular layer to activate the medial perforant path. In most slices electrophysiologic verification of relatively pure medial perforant path stimulation was obtained by demonstrating a current sink when the recording electrode was transiently placed at the same level as the stimulating electrode in the middle third of the molecular layer, and a current source when recording from the outer third of that layer leaving the stimulating electrode position unchanged (see Fig. 1) (13, 19). Stimulating electrodes consisted of twisted bipolar platinum iridium wire (25 μm). Recording electrodes were pulled from glass capillaries (o.d. 1.00 mm, i.d. 0.50 mm, Dagan), filled with 2 *M* NaCl, and had tip impedances of 2–6 MΩ. Constant current stimuli were delivered through stimulus isolation units (A 360, World Precision Instruments) driven by Grass S88 or S8800 stimulators, driven in turn by custom written computer software also used for data acquisition and analysis. Stimulus intensities (75–500 μA) were set such that population spike (PS) threshold occurred at pulse widths of 30–50 μs and were not varied within an experiment; stimulus–response curves were constructed in response to test pulses of 30–200 μs delivered at 0.1–0.033 Hz. Responses were allowed to equilibrate for 30–60 min before the start of data collection. In most slices, the response to paired stimuli (interpulse interval 10–20 ms, pulse width 150 μs) was recorded prior to the start of the experiment.

The first set of experiments was designed to determine if LTP could be induced at all developmental stages. Sixty-one slices from 48 rats (7–30 days) were studied in a submersion chamber (Medical Systems Corp) with bath temperature 30.4 ± 0.2°C. Thirty-five additional slices from the same population of rats (n = 26) were studied in an interface chamber (Fine Science Tools) at 34 ± 0.2°C. Stimulus–response curves were obtained at baseline and 15 and 30 min following the delivery of a single tetanus (100 Hz, 2 s, 200 μs).

A second set of experiments was designed to determine the NMDA receptor dependence of LTP throughout development. In 54 slices from a second population of rats (8–33 days, submersion chamber only) 10 μ*M* D-AP5 (Tocris Neuramin) was dissolved in buffer and bath-applied for 20 min. In 39 of these slices (n = 17, 12, and 10 at ages 8–15, 16–24, and 25–33 days, respectively) a single tetanic stimulation (100 Hz, 2 s, 200 μs) was delivered in the presence of AP5 (Tetanus 1) and again approximately 40 min after washout of the drug (Tetanus 2). Stimulus–response curves were obtained at the following times: (1) Prior to AP5 application (baseline); (2) during the last 5 min of AP5 application; (3) 15 and 30 min after Tetanus 1 (i.e., after return to drug-free buffer); (4) 15 and 30 min after Tetanus 2. The remaining 15 slices (n = 4, 6, and 5 at ages 8–15, 16–24, and 25–33 days, respectively) were used to document the effects of exposure to AP5 in the absence of a tetanus. Stimulus–response curves for these slices were obtained (1) at baseline; (2) during the last 5 min of AP5 administration; and (3) 15 and 30 min after washout of AP5.

PS amplitude (mV) was defined as the vertical distance between the tangent connecting the two peak positivities of the EPSP and the peak of the spike; EPSP slope was defined as the maximum slope of the evoked

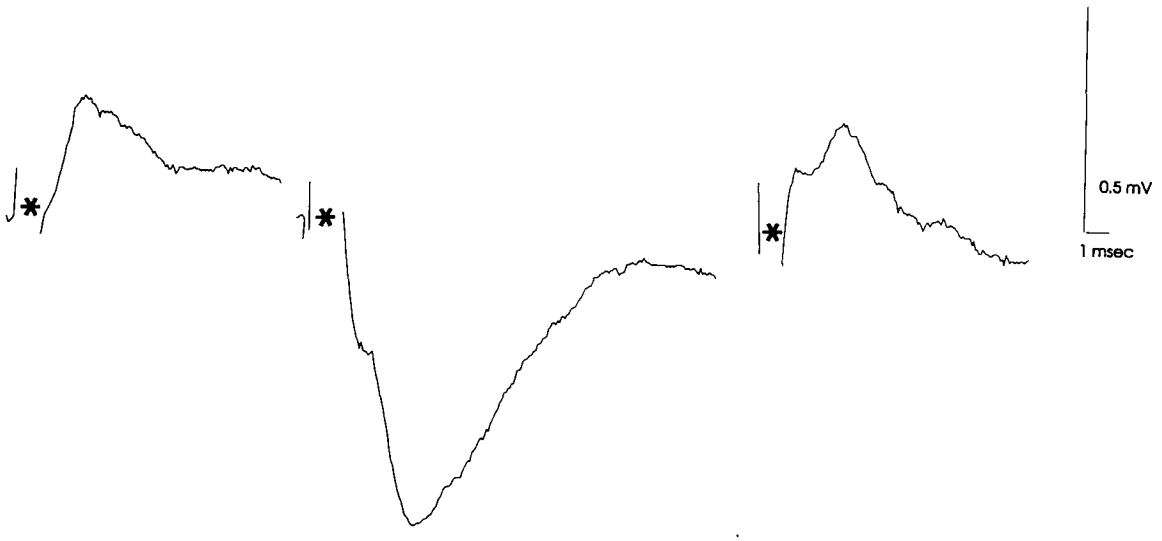

FIG. 1. Separation of lateral and medial perforant path activation in a 9-day slice recorded in the submersion chamber. In the center sweep, stimulating and recording electrodes are placed at the same level within the middle third of the molecular layer (medial perforant path) and a typical current sink (maximal negative EPSP) is demonstrated. A reversal in polarity of the evoked response is shown either when the recording electrode is moved to the outer third of the molecular layer (lateral perforant path), leaving the stimulating electrode unchanged (left sweep), or when the stimulating electrode is moved to the lateral perforant path, leaving the position of the recording electrode unchanged (right sweep). Asterisks (*) indicate that stimulus artifacts have been blanked for clarity.

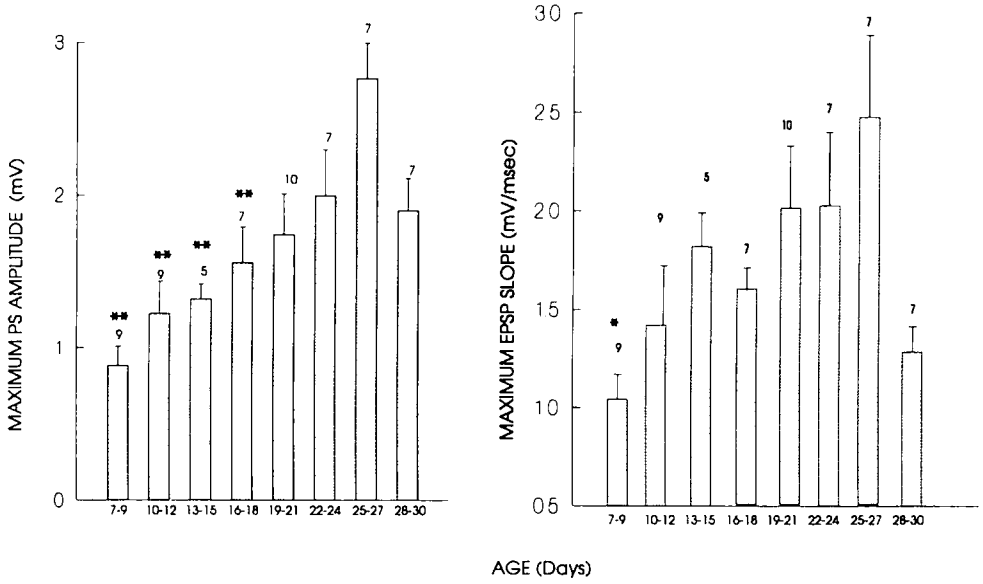

FIG. 2. Developmental increases in maximum PS amplitude and EPSP slope. (Left) PS amplitude and (right) EPSP slope recorded in the granule cell layer of the dentate gyrus at the ages indicated. Numbers above each bar represent the number of slices in each 3-day epoch. Values are means ± SE. **Indicates mean maximum PS amplitude was significantly smaller than at 25–27 days ($F = 5.456$, $df = 7.53$, $P < 0.0001$, ANOVA and $P < 0.04$ each comparison, Tukey post hoc HSD). *Indicates mean maximum EPSP slope was smaller than at 25–27 days ($F = 2.802$, $df = 7.53$, $P < 0.02$, ANOVA, and $P < 0.02$ Tukey post hoc HSD).

response between its onset and the first peak positivity. Data were analyzed at three representative points along the stimulus–response curves (corresponding to 25–30, 60–70, and 100% of the maximum baseline PS amplitude or EPSP slope). Since results did not differ according to stimulus intensity, they are reported for the 60–70% maximum range (linear portion of the curve). Three responses were averaged for each data point, normalized for each animal, and grouped by age as indicated in the analyses below. In the first set of experiments, responses were normalized by conversion to a percentage of the baseline value. In the second set, changes produced by Tetanus 1 were also expressed as a percentage of the baseline since drug washout was begun within 5 min of the tetanus. Changes in response to Tetanus 2 were expressed as a percentage of those obtained 30 min after Tetanus 1. When bidirectional changes were detected in response to tetanic stimulation, responses greater than 110% or less than 90% of baseline were considered to show LTP or long-term depression (LTD), respectively, and responses 90–110% of baseline were considered to show no plastic change. All values are expressed as means ± SD unless otherwise stated.

RESULTS

Properties of Dentate Gyrus Field Potentials during Development

A consistent developmental increase was seen in the absolute amplitude of evoked responses from 7 to 25 days. Mean maximum PS amplitudes and EPSP slopes were significantly larger at 25–27 days than in the younger age groups as is illustrated for responses recorded in the submersion chamber (Fig. 2). Developmental increases in maximum PS amplitude and EPSP slope were also seen in the interface chamber. Baseline responses were significantly larger in the interface than the submersion chamber (Table 1).

Paired stimuli were delivered to 74 slices from both chambers. Paired pulse depression (ratio of second to first population spike < 1) was detected in 32/33 slices at 7–15 days, and 29/41 slices at 16–30 days. Although the mean ratio of second to first PS was greater in the submersion (0.51 ± 0.28, $n = 40$) than the interface

TABLE 1

A Comparison of Baseline Mean Maximum Evoked Responses by Chamber Type

Chamber	Age (days)	*N*	PS max (mV)	EPSP max (mV/ms)
Submersion	7–15	23	1.11 ± 0.4	1.36 ± 0.7
	16–30	38	1.98 ± 0.8	1.98 ± 0.9
Interface	7–15	16	1.99 ± 0.9	2.70 ± 1.1
	16–30	19	3.95 ± 2.4	5.50 ± 2.8

Note. Baseline mean maximum PS amplitude was larger in the interface than in the submersion chamber at 7–15 days ($t = 3.824$, $P < 0.0001$) and 16–30 days ($y = 4.592$, $P < 0.0001$). Baseline mean maximum EPSP slope was also larger in the interface chamber in both age groups ($t = 4.716$, $P < 0.0001$ and $t = 7.204$, $P < 0.0001$ for 7–15 and 16–30 days, respectively).

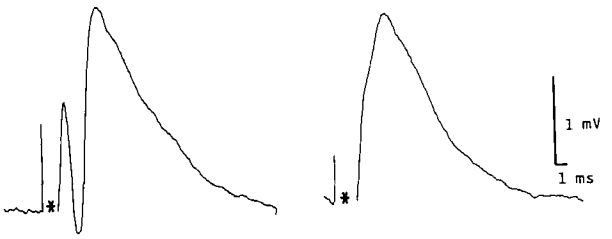

FIG. 3. Paired pulse depression is robust in immature dentate. Illustrated is a single sweep showing the cell body response to paired stimuli delivered to the medial perforant path (interpulse interval 20 ms) recorded in the interface chamber. In this slice the ratio of amplitudes of second to first PS was 0. Asterisks (*) indicate that stimulus artifacts have been blanked for clarity.

chamber (0.44 ± 0.36, $n = 21$) these differences were not significant ($t = 0.838$, $P = 0.41$, ages combined). In contrast, the mean paired pulse ratio was smaller (i.e., paired pulse depression was greater) in 7- to 15-day (0.41 ± 0.30, $n = 32$) than in 16- to 30-day rats (0.57 ± 0.3, $n = 29$), $t = 2.035$, $P < 0.04$, chambers combined). The greatest paired pulse depression (ratio 0.32 ± 0.27) was seen at 7–9 days ($n = 14$). Figure 3 illustrates an example recorded in the interface chamber in an 8-day slice in which the paired pulse ratio is 0.

LTP of Population Spike

In the submersion chamber, LTP of PS was demonstrated at all ages (range 123–423% baseline, mean 187 ± 52%) and was greater at 7–15 days (206 ± 65%; $n = 23$) than at 16–30 days (176 ± 40%; $n = 38$) $t = 2.247$, $P < 0.03$ (Fig. 4). LTP of PS was also demonstrated at all ages in the interface chamber and was greater in magnitude than in the submersion chamber (224 ± 94% vs 187 ± 52%, ages combined; $t = 2.977$, $P < 0.005$). Figure 5 illustrates responses before and after tetanus obtained from a 7-day slice in the submersion chamber and an 8-day slice in the interface chamber.

Plasticity of EPSP

As has previously been shown in dentate gyrus somatic recordings *in vivo* (6, 44), LTP of the EPSP was not routinely detected in the submersion chamber in slices that showed robust LTP of PS. LTP of EPSP was seen in 11/23 (48%) of slices 7–15 days and 26/38 (68%) of slices 16–30 days. The remainder showed no plastic change [7/23 (30%) at 7–15 days, 8/38 (21%) at 16–30 days] or a decrease in slope [5/23 (22%) at 7–15 days, 4/38 (11%) at 16–30 days]. In those slices showing LTP, the magnitude of potentiation (130 ± 18% at 7–33 days, 140 ± 28% at 16–33 days) did not differ between age groups ($t = 1.019$, $P = 0.315$). The magnitude of the decrease in slope in those slices showing a decrease (79 ± 6% of baseline at 7–15 days, 77 ± 8% at 16–30 days) also did not vary with age ($t = 0.333$, $P = 0.749$). These decreases in EPSP slope were not considered to represent LTD because of the possibility that EPSP slope measurements were reduced secondary to "contamination" by the markedly enhanced PS.

In contrast to the variable and limited plastic changes in the submersion chamber, EPSP slopes recorded in the interface chamber showed changes as prominent as those obtained for PS in both chambers. LTP of the EPSP slope was seen in 34/35 (97%) slices (range 112–463%, mean 199 ± 69%), and no plastic change was seen in one 10-day slice (108%). The mean magnitude of LTP (218 ± 92% at 7–15 days, 183 ± 38% at 16–33 days) did not differ with age ($t = 1.514$, $P = 0.14$). However, in those slices showing LTP of EPSP, the mean magnitude of enhancement was significantly greater in the interface chamber (199 ± 69%, $n = 34$) than in the submersion chamber (137 ± 26%, $n = 37$) (ages combined, $t = 5.066$, $P < 0.0001$).

NMDA Receptor Blockade with AP5

Acute effects of AP5 application. In the presence of AP5, mean PS amplitude was depressed at 8–15 days (81 ± 25%, $n = 22$) and 16–24 days (86 ± 14%, $n = 18$) and enhanced slightly at 25–33 days (105 ± 24%, $n = 25$) (Fig. 6). These effects of AP5 on PS amplitude differed according to age ($F = 5.584$; $df = 2,51$; $P < 0.007$, ANOVA). Post hoc testing revealed that the 8- to 15-day group differed significantly from the 25- to 33-day group ($P < 0.006$, Tukey HSD). Differences between the 16- to

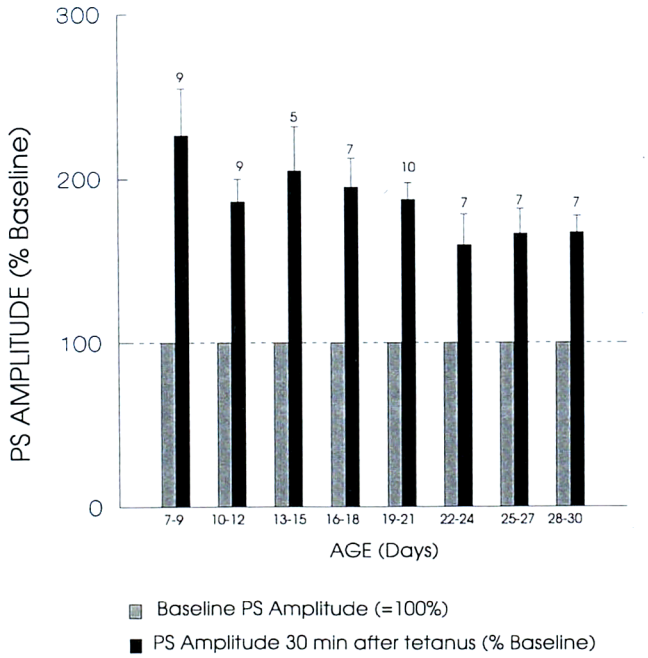

FIG. 4. LTP of PS is seen throughout development. PS amplitudes recorded 30 min following tetanic stimulation of the medial perforant path are expressed as a percentage of the baseline value for each animal and grouped by age. LTP was greater at 7–15 days than at 16–30 days ($P < 0.03$). Numbers above bars represent the number of slices in each age group. Values are means ± SE.

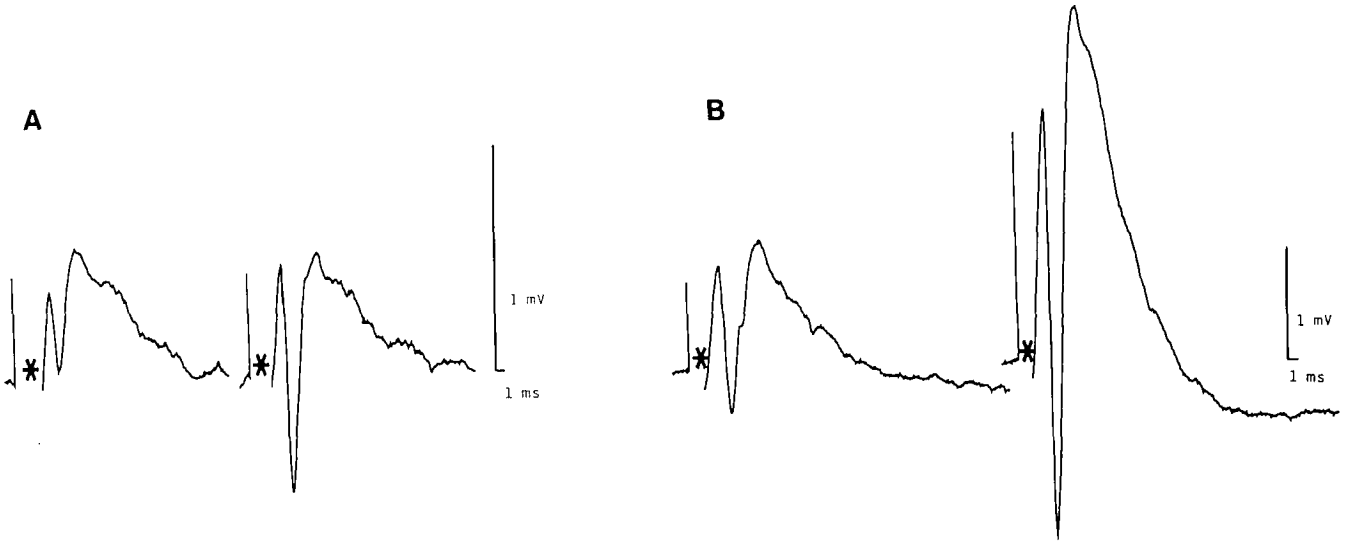

FIG. 5. Comparison of chamber type with respect to detection of LTP. Shown are single sweeps recorded from a 7-day rat in the submersion chamber (left) and an 8-day rat in the interface chamber (right) at baseline and 30 min following tetanic stimulation. Baseline responses and LTP of PS were greater in magnitude in the interface than the submersion chamber. LTP of EPSP was demonstrated with greater consistency in the interface chamber (34/35 = 97% of slices) than in the submersion chamber (37/61 = 61% of slices) and was significantly greater in magnitude as well (see Table 1 and text). Asterisks (*) indicate that stimulus artifacts have been blanked for clarity.

24-day and 25- to 33-day age groups approached, but did not reach, statistical significance ($P = 0.064$). AP5 application produced only slight changes in mean baseline EPSP slope (92 ± 19% at 8–15 days, 97 ± 21% at 16–24 days, 105 ± 11% at 25–33 days) and these age-related differences were not significant ($F = 2.123$; $df = 2,51$; $P = 0.130$). The lack of a prominent effect of AP5 on the initial EPSP slope is consistent with the slower onset of the NMDA-mediated synaptic current; the smaller PS amplitude in AP5 suggests that in the absence of the contribution from NMDA-mediated currents, fewer granule cells reached action potential threshold.

Effects of tetanic stimulation in the presence of AP5. AP5 caused complete or relative blockade of LTP at all ages ($n = 17$, 12, and 10 at ages 8–15, 16–24, and 25–33 days, respectively). In particular, although a total of eight slices in the three age groups exhibited LTP after tetanus in AP5 by our criteria, the mean magnitude of potentiation evoked by this tetanus (125 ± 14%) was significantly less than the potentiation produced by a second tetanus in the same slices after washout (214 ± 76%, $t = 3.231$, $P < 0.007$). These findings confirm that the induction of LTP in the dentate gyrus is NMDA receptor dependent throughout development. Figure 7 illustrates blockade of LTP by AP5 in a 10-day slice.

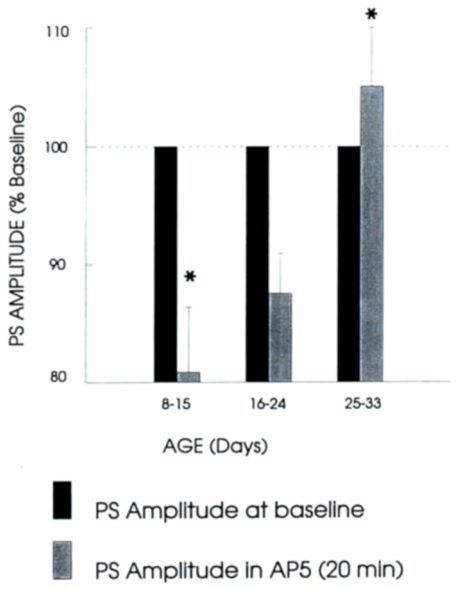

FIG. 6. Effects of NMDA blockade on synaptic transmission. PS amplitudes were measured at baseline and during the last 5 min of a 20-min bath application of 10 μ*M* AP5. Numbers above the bars represent the *N* for each age group. The effect of AP5 on PS amplitude differed according to age ($P < 0.007$, ANOVA) with significant differences seen between the 8- and 15-day and the 25- and 33-day age groups ($P < 0.006$, post hoc Tukey HSD) as indicated by the asterisks (*).

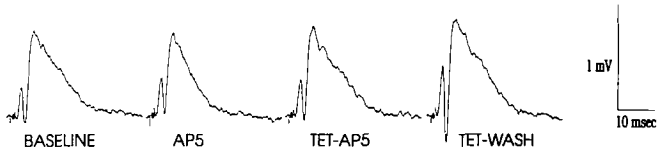

FIG. 7. AP5 blocks LTP throughout development. Responses recorded in a 10-day slice in the submersion chamber. In this slice, AP5 had no effect on baseline PS amplitude. Thirty minutes after Tetanus 1 in AP5 (TET-AP5) the response was also unchanged. Robust LTP is demonstrated 30 min following Tetanus 2 delivered after AP5 washout (TET-WASH). Asterisks indicate that stimulus artifacts have been blanked for clarity.

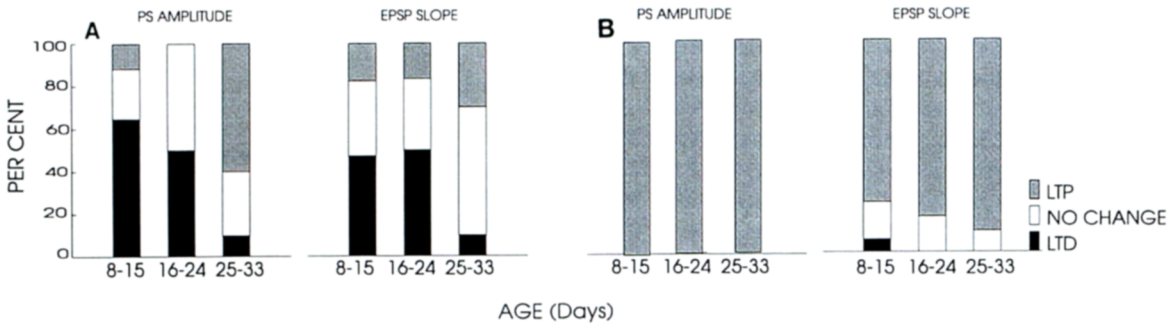

FIG. 8. Tetanic stimulation during NMDA blockade unmasks LTD. (A) Percentage of slices showing LTD, no plastic change, or LTP of PS amplitude (left) and EPSP slope (right) recorded in the cell body layer 30 min after tetanic stimulation delivered to the medial perforant path in the presence of 10 μM AP5 (Tetanus 1). N = 17, 12, and 10 for ages 8–15, 16–24, and 25–33 days, respectively. The percentage of slices exhibiting LTD at 8–15 and 16–24 days was significantly greater than at 25–33 days ($P < 0.01$, χ^2). (B) In the same slices after washout, tetanic stimulation (Tetanus 2) induced LTP of PS in 100% of slices and LTP of EPSP in the majority. Fifteen of the 16 slices that showed LTD of the EPSP after Tetanus 1 showed LTP of EPSP after Tetanus 2.

A surprisingly high percentage of slices exhibited LTD of PS in response to tetanus during NMDA blockade. Figure 8A illustrates the percentage of animals showing LTD, no plastic change, or LTP of PS 30 min after tetanus in AP5. The direction of plastic change (LTD vs no change or LTP) was found to differ as a function of age ($\chi^2 = 8.623, df = 2, P < 0.02$). Post hoc partitioning revealed that the percentage of slices exhibiting LTD of PS was similar at 8–15 days (11/17 = 65%) and at 16–24 days (6/12 = 50%) ($\chi^2 = 0.612, df = 1, P > 0.25$), but that together these age groups differed from the 25- to 33-day group in which LTD occurred in only 1/10 (10%) of slices ($\chi^2 = 7.073$, $df = 1$, $P < 0.01$). Furthermore, the two younger age groups were comparable with respect to mean magnitude of LTD (62 ± 19% of baseline at 8–15 days; 75 ± 15% at 16–24 days; $P > 0.1$, Fig. 9), whereas the single 30-day animal that met our criteria for LTD had a PS amplitude 86% of baseline. Figure 8B shows that 100% of these slices exhibited subsequent LTP of PS following Tetanus 2, enabling us to confidently distinguish LTD from deterioration in slice health.

Identical analyses were conducted for EPSP slope. In these analyses, decreases in EPSP slope were considered to represent LTD for two reasons: (1) they were not likely to be artifactually lowered because LTP of PS was minimal or absent and (2) 15 of the 16 slices that showed sustained decreases in EPSP slope after Tetanus 1 showed subsequent LTP after Tetanus 2. As is illustrated in Figs. 8 and 9, results paralleled the findings for PS amplitude but the age-related differences did not reach statistical significance. The mean magnitude of LTD of EPSP was comparable at 8–15 days (72 ± 10% of baseline) and 16–24 days (76 ± 23%; $P > 0.6$).

Effects of control AP5 exposure without tetanus. The effects of 20-min AP5 exposure without tetanus measured 30 min after washout were as follows: Mean PS amplitude was 100 ± 8, 102 ± 11, and 99 ± 5% of baseline at ages 8–15, 16–24, and 25–33 days, respectively. Mean values for EPSP slope at these ages were 102 ± 10, 104 ± 4, and 100 ± 18%. No age-dependent differences were detected in either measure ($P > 0.7$, ANOVA). Inspection of these data suggests that AP5 application alone did not induce long-term plastic changes in the evoked potentials. Since the most robust effect of tetanic stimulation in AP5 was the high incidence of LTD in the 8- to 15-day and 16- to 24-day slices, it was particularly important to establish that AP5 application without tetanus did not account for this effect. As these age groups did not differ with respect to incidence or magnitude of LTD they were combined for comparison with control animals from the same age groups. This comparison revealed that at 8–24 days, significantly more experimental than control animals showed LTD of both PS amplitude (17/29 = 59% vs 1/10 = 10%; $\chi^2 = 7.073$; $df = 1$; $P < 0.01$) and EPSP slope (14/29 = 48% vs 1/10 = 10%; $\chi^2 = 4.602$; $df = 1$; $P < 0.05$). Furthermore, the single PS amplitude and EPSP slope values that met our criteria for LTD in the control group did so with values of 88 and 89% of baseline, respectively. These data confirm that when LTD was detected in the experimental group it was attributable to tetanic stimulation in the presence of AP5 rather than AP5 exposure alone.

DISCUSSION

The main findings of this study are that robust LTP can be produced by tetanic stimulation of the medial perforant path–dentate gyrus synapse as early as Postnatal Day 7; that LTP is NMDA receptor dependent throughout development; and that between 8 and 24 days NMDA receptor blockade unmasks tetanus-induced homosynaptic LTD. In addition, we have demonstrated that developing dentate is characterized by prominent functional inhibitory circuitry, an orderly ontogenetic progression (7–25 days) in the magnitude of evoked responses to excitatory stimulation, and the

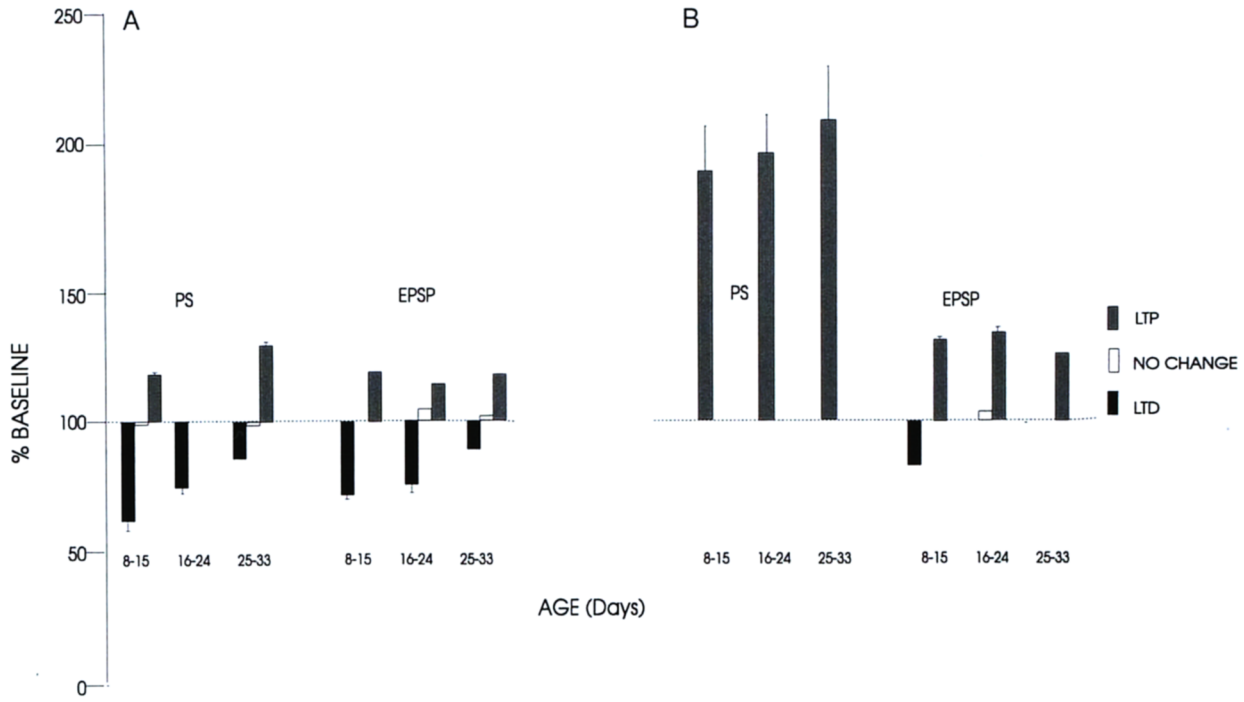

FIG. 9. Direction and magnitude of plastic change following tetanus in AP5 and after washout. (A) The mean magnitude of PS LTD in response to tetanus in AP5 was comparable at 8–15 days (62 ± 19% of baseline, $n = 11$) and 16–24 days (75 ± 15%, $n = 6$, $P > 0.1$); the single 30-day animal that met our criteria for LTD had PS amplitude 86% of baseline. The mean magnitude of EPSP LTD was similarly comparable in the two younger age groups (72 ± 10% at 8–15 days, $n = 8$; 76 ± 23% at 16–24 days, $n = 6$; $P > 0.6$) and 89% in the single 30-day animal that showed LTD. When no box is drawn in "no change" category, values = 100 ± 0%. (B) After washout, 100% of slices exhibited LTP of PS, and the magnitude of LTP was significantly greater than LTP that occurred during tetanus in AP5. After washout, only one slice (in the youngest age group) showed LTD of EPSP. All values are mean ± SE (where error bars are included if $n > 1$ and if SE was sufficiently large to represent graphically).

separation of medial and lateral perforant path afferents by electrophysiologic criteria. Finally, since only the youngest animals showed marked diminution of PS amplitude in response to NMDA receptor blockade with AP5, our data suggest that the NMDA receptor-mediated component of normal synaptic transmission is high early in development and diminishes with maturation.

Considerable anatomic evidence exists that the early postnatal dentate is a morphologically dynamic structure characterized by concomitant growth, regression, and stabilization of key elements. For example, 80% of dentate granule cell neuronogenesis takes place in the first 2 postnatal weeks, yet only about 30% of these neurons are destined to survive until maturity (38). Entorhinal cortical (perforant path) afferents are arranged in laminar fashion from their earliest identification; one autoradiographic study suggested path separation as early as Day 3 (38), although considerable overlap between adjoining laminae is likely. The arrival of these afferents precedes granule cell maturation and hence synapse formation by several days (38). Synaptic number in the suprapyramidal molecular layer undergoes a four-fold increase between Days 11 and 25 (17). Dendritic growth is evidenced by a tripling in width of this layer from Day 4 (80 μm) to adulthood (249 μm) (29) and by the detection of dendrites at the top of the layer during this expansion (36). Dendritic regression is evidenced by a decrease in the number of dendritic segments per neuron between Days 14 and 60 (36). Unlike the principal cells, GABAergic basket cells in the dentate are present and have synaptic connections similar to those in adult animals as early as 5 days postnatally (39).

Our electrophysiologic measurements complement these anatomic observations. For example, the developmental increases in baseline PS amplitude and EPSP slope are likely to reflect parallel increases in granule cell and synapse number. Furthermore, dentate granule cells in the suprapyramidal limb appear to acquire spines and abruptly lose their immature characteristics such as growth cones, varicosities, and filamentous projections at about 7 days (B. Claiborne and S. Jones, personal communication), the earliest age at which we could easily elicit field potentials with adult morphology. The presence of prominent paired pulse depression even at 7–8 days reflects the proximity of basket cells to the granule cell layer and the early maturation of their synaptic connections. The ability to separate lateral from medial paths correlates with the early lamination of entorhinal cortical afferents and the fact that den-

dritic arbors extend to the hippocampal fissure early in development.

The ontogenesis of LTP in the dentate gyrus *in vitro* was first studied by Duffy and Teyler in 1978 (22). Direct comparison with that study is difficult because stimulation was not confined to the medial perforant path, recordings were made in the molecular rather than the granule cell layer, four tetani of lower frequency (15 Hz) but longer duration (10 s) were delivered, chamber type was not specified, and LTP was measured as a change in EPSP amplitude rather than slope. LTP was detected only rarely in young slices (9% at 7 days, 10% at 14 days) and showed a developmental progression in incidence (46% at 30 days, 27% at 60 days, and 80% at 210 days) and magnitude; nevertheless it was shown that several of the youngest slices were capable of demonstrating LTP of a magnitude and duration equal to the average adult tissue. Results of the present study are more consistent with this latter observation since LTP of both EPSP and PS were readily detected throughout development, and in some circumstances were of greater magnitude in the less mature slices.

Our results differ from *in vivo* studies in which LTP of PS was not detected until 14 or 15 days (3, 46) and showed a gradual increase in magnitude with age. The present results enable us to advance to 7 days our previous observations of robust LTP of dentate PS *in vivo* as early as 14 days (44). The inconstant detection and relatively smaller magnitude of synaptic (EPSP) LTP in the submerged slices is similar to results obtained from *in vivo* somatic recordings in which increases in PS amplitude are typically proportionately greater than those in EPSP slope (7, 8). This disparity was particularly evident in our experience in immature dentate gyrus *in vivo* and suggested that the EPSP-to-spike relationship might be developmentally regulated (41, 44). In this context, the EPSP potentiation detected in the interface chamber was unexpectedly large and often accompanied by dramatic increases in EPSP amplitude (see Fig. 4B). Such chamber-specific effects cannot be explained by the obvious technical differences between *in vivo* and *in vitro* preparations (such as the placement of electrodes under visual rather than electrophysiologic guidance, and the placement of the stimulating electrode inside the hippocampal fissure rather than in the angular bundle), but may need to be considered in comparisons of data from different laboratories.

The NMDA dependence of LTP induction in the medial perforant path–dentate gyrus synapse has been documented in adult animals *in vivo* (23) and in hippocampal slices from rats identified as 100 g (10, 37), whose age can be estimated at 32 days (Harlan–Sprague–Dawley Product Guide, 1993) and from 20- to 30-day rats (13). To our knowledge we provide the first documentation of the NMDA dependence of LTP in this pathway in younger animals.

The detection of tetanus-induced LTD in the presence of NMDA receptor blockade was an unanticipated finding and is similar to that recently described in area CA1 in rats 14–16 and 60–65 (but not 29–31) days old (45). In that study, tetanic stimulation of the Schaffer collateral input (six trains, 50 Hz, 2 s, 15-s intertrain interval) in the presence of 25-μm AP5 induced homosynaptic LTD of both PS recorded in the somatic layer and EPSP recorded in the dendritic layer. Whereas stimulus-induced LTD has previously been described in the dentate gyrus, the induction has been both heterosynaptic and NMDA receptor dependent (12, 20). Similarly, homosynaptic low frequency stimulus-induced LTD in CA1 is also NMDA receptor dependent (21). The relationship between the tetanus-induced LTD described here, and the LTD induced in both CA1 and the dentate gyrus by the metabotropic glutamate receptor agonist 1*S*,3*R*-aminocyclopentane dicarboxylic acid (42) remains to be examined. What all of the reported paradigms for induction of homosynaptic hippocampal LTD appear to have in common, however, is that LTD is most readily elicited and most prominently expressed in very young animals.

Our finding that AP5 application caused a larger depression of PS amplitude with decreasing age suggests that the NMDA-mediated component of normal synaptic transmission is greatest in very young medial perforant path–dentate gyrus synapse and decreases with maturation. Burgard *et al.* (10) reported a large (30%) reversible decrease in the medial perforant path-evoked PS amplitude with AP5 application in young adult rats after a 40-min application of 10 μ*M* AP5 in an interface chamber; no attempt was made to study this phenomenon developmentally. Our findings are consistent with observations by others in neocortex (11), hippocampal area CA1 (34), and granule cells of the cerebellum (18). Together with studies showing a transient increase in NMDA receptor density (40) as well as decreased voltage dependence and sensitivity to magnesium blockade in young animals (4, 26, 27, 30, 33), they suggest that this receptor is more likely to be activated during synaptic transmission at a time when developmental plasticity is maximal.

There is increasing evidence that LTP, LTD, and other activity-dependent mechanisms, often but not exclusively (35) NMDA receptor dependent, participate in the refinement of neuronal topography during a "second stage" of brain morphogenesis that begins after initial targeting has taken place (2, 14, 28, 31). Much of this work has been done in developing visual and olfactory systems because structure–function relationships are well understood. The hypothesis that NMDA-mediated activity may also be important as a structuring parameter in developing dentate gyrus receives direct support from the work of Brewer and Cotman (9) who found that NMDA application to cultured dentate granule neurons obtained from 4-day rats stimulated exten-

sive branching of neuronal processes. Our findings indicate that the dentate gyrus *in situ* manifests two forms of activity-dependent synaptic plasticity (NMDA-dependent LTP, and homosynaptic LTD in the presence of AP5) coincident with its most dramatic flux in neuronal elements and raise the possibility that these processes play a role in determining the survival and maturation of neurons and their synaptic connections in this region. The precise mechanisms and morphologic correlates of LTP and LTD and possible developmental changes in NMDA receptor properties in the dentate remain to be examined.

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