In Vivo Recordings of Long-term Potentiation and Long-term Depression
in the Dentate Gyrus of the Neonatal Rat

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Abstract

Previous *in vitro* studies demonstrated that long-term potentiation (LTP) could be elicited at medial perforant path (MPP) synapses onto hippocampal granule cells in slices from 7-day-old rats. In contrast, *in vivo* studies suggested that LTP at perforant path synapses could not be induced until at least days 9 or 10 and then in only a small percentage of animals. Because several characteristics of the oldest granule cells are adult-like on day 7, we re-examined the possibility of eliciting LTP in 7-day-old rats *in vivo*. We also recorded from 8- and 9-day-old rats to further elucidate the occurrence and magnitude of LTP in neonates. With halothane anesthesia, all animals in each age group exhibited synaptic plasticity of the excitatory postsynaptic potential following high frequency stimulation of the MPP. In 7-day-old rats, LTP was elicited in 40% of the animals, and had an average magnitude of 143%. Long-term depression (LTD) alone (magnitude of 84%) was induced in 40% of the animals, while STP alone (magnitude of 123%) was induced in 10%. STP followed by LTD was elicited in the remaining 10%. Data were similar for all ages combined. In addition, the NMDA antagonist CPP blocked the occurrence of LTP at each age, and doubled the percentage of animals expressing LTD alone for all ages combined. These results demonstrate that tetanic stimulation can elicit LTP or LTD at MPP synapses in 7-day-old rats, supporting our premise that at least a portion of the dentate gyrus is functional at this early age.

**Key Words**: granule neurons, development, hippocampus, perforant path, short-term potentiation
Introduction

In adult rats, tetanic stimulation of the medial perforant pathway (MPP) elicits long-term potentiation (LTP) in granule cells of the dentate gyrus both in vivo and in vitro (McNaughton and Barnes 1977; Errington et al. 1987; Abraham and Mason 1988; Burgard et al. 1989; Bronzino et al. 1994; Villarreal et al. 2002). Previous in vivo studies on the development of synaptic plasticity in granule cells indicated that potentiation of the population spike, measured at either 10 minutes or 30 minutes following tetanic stimulation of the perforant path, could not be induced until days 9 or 10, and then in only a small percentage of rats (Wilson 1984; Bekenstein and Lothman 1991b). At days 10 and 11, potentiation of the excitatory postsynaptic potential (EPSP) was reported (Errington et al. 1995). By day 14, most rats exhibited LTP of the population spike, and about 30% showed LTP of the EPSP (Wilson 1984; Trommer and Routtenberg 1990; Bronzino et al. 1994). Whether these results applied to both MPP and lateral perforant path synapses was not clear from the reports. In contrast, in vitro developmental studies demonstrated that LTP at perforant path synapses and, more specifically, at MPP synapses could be elicited as early as day 7 in a small percentage of hippocampal slices (Duffy and Teyler 1978; Trommer et al. 1995). These data suggest that LTP in granule cells can be induced earlier in the slice than in the animal, perhaps because of reduced inhibition in the slice preparation or the anesthetic used for the in vivo recordings.

Recently we found that a small number of the oldest granule cells exhibit adult-like dendritic trees by day 7 (Jones et al. 2003), and previous studies have shown that some granule neurons have adult-like physiological characteristics at this time (DiScenna and Teyler 1994; Trommer et al. 1995; Liu et al. 1996; Ye et al. 2000). Thus, in the present work, we re-
examined the possibility that LTP at MPP synapses could be elicited in rats at 7 days of age. Because the earlier in vivo developmental studies used either urethane or pentobarbital as anesthetics (Wilson 1984; Bekenstein and Lothman 1991b), here we developed the procedures necessary for recording from granule cells in neonatal rats anesthetized with halothane (Park et al. 1992; Fukuda 2000). Halothane is a volatile, nonbarbiturate anesthetic that has been used successfully for in vivo studies of synaptic plasticity in the dentate gyrus of adult rabbits (Thiels et al. 1996; Yeckel and Berger 1998). To our knowledge, halothane has not been used for in vivo studies of synaptic plasticity in developing rodents.

With halothane as the anesthetic, we tested the hypothesis that LTP could be elicited at MPP synapses onto granule neurons in rats between the ages of 7 and 9 days of age. Here we report that all 7-day-old rats (the youngest age at which in vivo recordings could be made), as well as all 8- and 9-day-old animals, exhibited some form of synaptic plasticity following tetanic stimulation of the MPP. LTP, short-term potentiation (STP), and long-term depression (LTD) were observed. Data also showed that LTP in these neonatal rats was dependent on the N-methyl-D-aspartate (NMDA) glutamate receptor, as previously demonstrated for LTP at MPP synapses onto granule neurons in adult rats. Preliminary results were reported in abstract form (O’Boyle et al. 2001)

Materials and Methods

Animals

Sprague-Dawley rats, obtained from Harlan Sprague-Dawley (Indianapolis, IN) or Charles River (Wilmington, MA), were used for all experiments. Recordings were attempted from
postnatal rats between the ages of 6 and 9 days; the day of birth was considered to be postnatal
day 0. No responses were obtained from 6-day-olds (see below), and thus all data reported
here were taken from animals between 7 and 9 days of age and weighing between 12 and 24
grams. The average weights for rats of each age are as follows: 7-day-old rats, 14.5 ± 0.5
grams (mean ± SEM); 8-day-olds, 16.7 ± 0.4 grams; and, 9-day-olds, 19.5 ± 0.6 grams.
All protocols were in accordance with PHS Guidelines, and were reviewed and approved by
the University of Texas at San Antonio Animal Care and Use Committee.

Anesthesia

Anesthesia was induced in all rats with a mixture of 5% halothane (Halocarbon Products,
River Edge, NJ) and oxygen at 2.5 liters/minute (Park et al. 1992). This mixture was supplied
with an Ohio style vaporizer (Model 100H, Surgivet/Anesco, Waukesha, WI) until a gentle
pinch to the footpad did not elicit a response; anesthesia was induced in all animals within 1
minute after the flow of halothane was initiated. To maintain the anesthetized state, a mixture
of 1% to 1.5% halothane and oxygen at 2.5 liters/minute was administered continuously
(Fukada, 2000). Animals were observed closely during the surgeries and recordings to ensure
that regular breathing and skin color were maintained, and that a gentle pinch to the footpad
did not elicit a response.

Recordings

The anesthetized rats were placed on an isothermal pad in a small animal stereotaxic
holder equipped with a neonatal rat adapter (Kopf Model 960). Our preliminary studies using a
rectal thermister probe demonstrated that body temperatures did not differ by more than 2°C
between animals (n = 4) at the time of tetanic stimulation. Bipolar stimulating electrodes were constructed from twisted 0.005” Teflon coated wires threaded through a stainless steel cannula, and were inserted into the angular bundle of the perforant path at AP –4.0, ML +3.0 and DV –1.5 with respect to Bregma. Single wire recording electrodes, constructed using the same wire and cannula, were inserted into the dentate hilar region at AP –1, ML +1.5, and DV –2.5 with respect to Bregma (Wilson 1984). Final placements were determined empirically to yield a satisfactory response (see below). Monophasic stimulating pulses (300-500 µA; 0.3 ms duration; 0.066 Hz) were applied and responses monitored. Stimulus currents and pulse durations were chosen based on previous studies in adult rats (McNaughton et al. 1978) and neonates (Wilson 1984; Bekenstein and Lothman 1991a). Preliminary experiments in 6- and 7-day-old animals indicated that responses could not be elicited in the 6-day-old rats (n = 4), whereas responses were seen in 67% of the 7-day-old rats (n = 15).

The perforant path is divided into lateral and medial components that synapse onto the distal third and the medial third of the granule cell dendritic tree, respectively, and stimulation of each component results in a distinct physiological response in the granule cells in adult rats in vivo (McNaughton and Barnes 1977; McNaughton 1980). Our preliminary experiments (n = 5) demonstrated that the medial perforant path fibers to the granule neurons could be selectively stimulated in rats between the ages of 7 and 9 days. The recording electrode was positioned in the hilar region, while the stimulating electrode was positioned in the angular bundle at the coordinates for the medial perforant path (see above). Stimulus intensity was increased gradually until a maximal excitatory postsynaptic potential (EPSP) was seen at the recording site. In some cases, the position of the recording electrode was adjusted slightly in
order to obtain a maximal response. The stimulation intensity was then reduced to the minimum intensity required to elicit a response. Paired pulses, with an interpulse interval of 30 ms, were applied. Analysis of the field EPSP slopes (see Data Analysis below) indicated that the second response did not facilitate: when compared to the first response, it either showed no change or depressed. The stimulating electrode was then lowered by another 0.5 to 0.9 mm. With the stimulating electrode in this position, the amplitude of the response following a single stimulus remained the same, but paired pulse stimulation resulted in facilitation of the second response. These results are in agreement with data from adult rats showing that paired pulse stimulation of the medial perforant path does not result in facilitation, whereas the same paired pulse stimulation of the lateral perforant path elicits facilitation of the second response (McNaughton and Barnes 1977; McNaughton 1980). These data show that the medial perforant path can be selectively stimulated by the end of the first postnatal week in the rat.

For the experiments reported here, the stimulating electrode was lowered to the medial aspect of the angular bundle and monophasic stimulating pulses applied as described above. The stimulus intensity was increased gradually until a maximal EPSP was seen at the recording site; in some cases, the position of the electrode was adjusted slightly to ensure a maximal response. Next, the stimulation intensity was reduced to the minimum intensity required to elicit a response, and paired pulses, with an interpulse interval of 30 ms, were applied. The lack of paired-pulse facilitation indicated that the stimulating electrode was positioned correctly in the medial perforant path (see above; McNaughton and Barnes 1977). The stimulation intensity was then adjusted to a level that evoked a half-maximal field EPSP at the recording site.
Data collection

Responses to test pulses (300-500 \(\mu\)A; 0.3 ms duration; 0.066 Hz) were collected until a stable baseline of at least 10 minutes in length was obtained. A tetanic stimulus (100 Hz; 1 sec; stimulation intensity set to evoke a maximal EPSP as determined above) was then delivered (McNaughton et al. 1978). Following the tetanus, responses to the test pulses were collected continuously for at least 40 minutes. The tetanic stimulus then was repeated in randomly chosen animals to ensure that the preparation had remained viable. Evoked responses were amplified using a Grass P511 Differential Amplifier (West Warwick, RI), filtered at 0.1 Hz to 1 kHz, digitized at 10,000 Hz, and stored for later analysis using commercially available software (Datawave Systems, Longmont, CO) running on a PC. In addition, the EEG was monitored for at least 1 minute after the stimulating train, and was recorded throughout the experiment. Analysis of the data demonstrated that seizures and afterdischarges did not occur immediately following tetanic stimulation or during the remainder of the recording session.

NMDA receptor antagonist

The highly selective, competitive NMDA antagonist CPP ((R,S)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; Sigma-Aldrich, St. Louis, MO) was dissolved in saline (0.9% NaCl) immediately before use each day. Animals were injected intraperitoneally with either CPP or an equivalent volume of saline (vehicle-treated, control animals) approximately 1.5 hours before they were anesthetized. On any particular day, CPP was administered to one rat from a litter in the morning, while a second animal from the same litter was treated with saline later on the same day; the order was reversed in a number of trials. CPP was given at dosages
of 1, 2 or 3 mg/kg to rats of all three ages. These dosages were chosen because our preliminary studies indicated that the higher doses of CPP (5 and 10 mg/kg) routinely used in adults (Abraham and Mason 1988) caused severe ataxia and sedation in the 7- to 9-day-old rats. Of the rats treated with 1 mg/kg CPP that were used for data, 3 rats were 7 days-of-age, 2 were 8 days-of-age, and 2 were 9 days-of-age. In the 2 mg/kg group used for data, 2 rats were 7 days-of-age, 5 were 8 days-of-age, and 3 were 9 days-of-age. Of those treated with 3 mg/kg and used for data, 3 animals were 7 days-of-age, 5 were 8 days-of-age, and 4 were 9 days-of-age.

**Histology**

At the end of each recording session, the anesthetized rat was sacrificed by decapitation immediately upon discontinuing the halothane. The brain was quickly removed from the skull, frozen in 2-methyl butane, and was sectioned (35 µm) using a freezing microtome (Leica CM3050). Sections were air-dried onto subbed slides, and stained with cresyl violet. Using a 10x objective mounted on a Nikon E600 microscope, we verified the placements of the stimulating and recording electrodes for all animals included in the analyses.

**Data analysis**

The average peak amplitude of the field EPSP for each animal was determined from the peak amplitudes of the individual field EPSPs recorded in the 5 minute window prior to tetanus. The average latency (stimulus to onset) of the field EPSP for each animal was determined from the latencies of 10 individual responses over a 10 minute period (1/minute)
prior to tetanus. Peak amplitudes and onset latencies (mean ± SEM) were compared between age groups using a Student’s $t$ test ($p < 0.05$) to determine statistical significance.

EPSP slope was defined as the maximum slope ($dV/dt$) of the initial portion of the response, and was measured for all individual responses. Because various types of plasticities were elicited in the rats at the three different ages (see Results), each animal was treated as an individual case, and EPSP slopes were compared within each animal. To establish a baseline response, the average slope of the EPSP for each rat was determined for a 10 minute time period immediately preceding the tetanic stimuli. This baseline average was considered to be 100%, and the slopes of each of the individual responses for that animal were expressed as a percentage of this baseline average (normalized slopes). The measures of the normalized slopes of the individual field EPSPs were then averaged for each animal during three 5-minute windows: 1) the 5 minutes preceding tetanus; 2) between 5 and 10 minutes post-tetanus; and, 3) between 30 and 35 minutes post-tetanus.

The criteria for classifying the responses for each animal were based on comparisons between the average normalized slope of the field EPSP in the 5-minute window immediately preceding tetanus and the average normalized slope in each of the post-tetanus windows. If the average normalized EPSP slope in the 30 to 35 minute post-tetanus window was significantly greater than the average EPSP slope preceding tetanus, the response was classified as LTP, regardless of the difference between the average normalized EPSP slope in the 5 to 10 minute post-tetanus window and the average slope preceding tetanus. If the average normalized EPSP slope in the 30 to 35 minute post-tetanus window was significantly less than the average EPSP
slope preceding tetanus, the response was classified as LTD (Clark and Collingridge 1995). If the average slope in the 5 to 10 minute post-tetanus window was significantly greater than the average EPSP slope preceding tetanus, and there was no difference between the 30 to 35 minute post-tetanus value and the pre-tetanus value, the response was classified as STP alone. If the average value in the 5 to 10 post-tetanus window was significantly greater than the baseline and the value in the 30 to 35 minute window significantly less, the plasticity was categorized as STP/LTD. Statistical significance was determined using a Student’s t test (p < 0.05). Once an animal had been categorized as showing one or more forms of the plasticity, animals showing the same type of response were grouped together, and average normalized field EPSP slopes were calculated for each age and each type of plasticity (mean ± SEM). The statistical significance of differences in average normalized slopes between age groups or treatment groups was determined using a Student’s t test (p < 0.05).

Results

Synaptic responses

Because the potential effects of halothane on synaptic responses in neonatal rats had not been examined previously, we were interested in the percentage of animals that responded to low frequency stimulation of the MPP in rats anesthetized with halothane and injected with saline (vehicle-treated control rats). Stimulation of the MPP resulted in field EPSPs in 67% of the 7-day-old rats, 91% of the 8-day-olds, and 83% of the 9-day-olds (Fig. 1). When all ages were combined, stimulation elicited field EPSPs in 81% of the animals. For those 7-day-old control animals in which a stable baseline response was obtained, the average latency of the field EPSP was 16 ms ± 0.9 (Table 1). Although no statistically significant differences in
latencies were found between the age groups (day 7 vs. 8, \( p = 0.81 \); day 7 vs. 9, \( p = 0.21 \); day 8 vs. 9, \( p = 0.32 \)), it is worthwhile to note that there was a slight decrease in the average latency on day 9. This decrease and the wide range of latencies seen in any one age group are to be expected, given the ongoing process of myelination. Myelination of the perforant pathway begins toward the end of the first postnatal week, and many fibers are still unmyelinated on day 9 (Singh 1977). Wilson (1984) reported an average EPSP latency of 11.4 ± 3.8 ms for perforant path responses in 7-day-old rats anesthetized with urethane or pentobarbital, and also showed that latencies decreased with increasing age. It is worth noting that response latencies in young rats may be affected by body temperatures which are slightly lower in neonatal rats than in adults (Schmidt et al. 1987).

In the 7-day-old rats, the average peak amplitude of the field EPSPs was 0.51 mV (Table 1). Average peak amplitudes were compared between the three age groups, and no statistically significant differences were found (day 7 vs. 8, \( p = 0.73 \); day 7 vs. 9, \( p = 0.69 \); day 8 vs. 9, \( p = 0.37 \)). For rats of all ages combined, the average peak amplitude of the field EPSP was 0.51 mV (Table 1). In a small number of rats (3/27), population spikes were observed after tetanus.

**Occurrences of synaptic plasticities**

Field EPSP slopes were used to determine synaptic plasticities and responses were categorized as LTP, LTD, STP or STP/LTD as described in the Methods. All rats with stable baseline responses exhibited one or more forms of synaptic plasticity following the tetanic stimulation. On day 7, 40% of the animals exhibited LTP, and 40% exhibited LTD alone (Fig.
2). Of the animals showing LTP, all except 2 also exhibited STP. Only 10% of the animals showed STP alone and another 10% showed STP followed by LTD. Thus, 20% of the 7-day-old rats exhibited STP (either alone or in combination with LTD) that was not followed by LTP, and 50% showed LTD (either alone or following STP).

The frequency of occurrence of each type of plasticity was recorded in 8-day-old and 9-day-old animals as well (Fig. 2). On day 8, LTP was elicited in a similar percentage of rats as on day 7, and all animals showing LTP also exhibited STP. While the percentages of animals exhibiting LTP were similar on days 7 and 8, only 22% of the 8-day-old rats showed LTD, as compared to the 40% on day 7. The percentage of animals exhibiting STP alone rose to 22% on day 8 while the percentage showing STP followed by LTD remained almost constant at 11%. Therefore, 33% of the 8-day-old rats showed STP either alone or followed by LTD, and 33% demonstrated LTD either alone or following STP. On day 9, the percentages of rats exhibiting the various plasticities were similar to the percentages seen on day 7: 37.5% exhibited LTP (all showed STP also), 37.5% exhibited LTD, 12.5% showed STP alone, and another 12.5% exhibited STP/LTD. Thus, 25% of the 9-day-old rats showed STP, either alone or preceding LTD, and 50% exhibited LTD, either alone or following STP. Taken together, these data show that the percentages of rats exhibiting LTP were similar for all three ages, while the percentage exhibiting LTD was slightly decreased and the percentage exhibiting STP slightly increased on day 8, as compared to day 7 or 9.

For all ages combined (Fig. 3), LTP was induced in 41% of the animals and LTD alone was observed in 33%. STP alone was elicited in 15% of the animals, while STP followed by
LTD was seen in approximately 11%. Thus, the percentage of animals showing STP, either alone or followed by LTD, was 26%, and the percentage exhibiting LTD, either alone or following STP, was 44%. To determine whether an animal’s weight affected the occurrence of LTP or LTD, we compared the average weights of those animals exhibiting LTP to those of the rats exhibiting LTD. Data indicated that there was not a statistically significant difference between the two ($p = 0.88$).

**Magnitudes of synaptic plasticities**

The response magnitudes for each type of synaptic plasticity at each age for the vehicle-treated control rats are given in Table 2. On day 7, the average magnitude of LTP was 143%. It increased slightly to 156% on day 8 (Fig. 4), and then decreased to 129% on day 9. There were no statistically significant differences, however, between any of these values (day 7 vs. 8, $p = 0.61$; day 7 vs. 9, $p = 0.48$; day 8 vs. 9, $p = 0.35$). In the 7-day-old rats, the magnitude of LTD alone was 84%. The magnitude decreased slightly in the 8-day-old rats, and then increased in the 9-day-olds (Fig. 5). Again, these differences were not statistically significant (day 7 vs. 8, $p = 0.06$; day 7 vs. 9, $p = 0.17$; day 7 vs. 9, $p = 0.49$). When LTD was preceded by STP, the magnitude of the LTD component was also less on day 8 than on the other two days. The magnitude of STP alone was similar on days 7 and 8, but decreased on day 9 (Fig. 6). In contrast, when STP was followed by LTD, the magnitude of the STP component was less on days 7 and 8 than on day 9. Because of the small numbers of rats exhibiting either STP or STP/LTD on each day (see Table 2), the statistical significance of these differences could not be evaluated.
For rats of all ages combined, the average magnitude of the LTP was 144%, the average magnitude for LTD alone was 85%, and for STP alone was 122% (Table 2). When STP was followed by LTD, the average magnitude of STP was 110% and the magnitude of LTD was 90%. It is worth noting that the average magnitude of the STP response (either alone or preceding LTD) was not significantly different from the magnitude of the LTP response ($p = 0.20$ for STP alone; $p = 0.10$ for STP/LTD; Student’s $t$ test), whereas Malenka (1991) found that the magnitude of STP was significantly less than the magnitude of LTP at Schaffer collateral synapses onto CA1 pyramidal neurons in adult slices.

**Effects of NMDA receptor antagonist**

Previous reports have demonstrated that the induction of LTP at the MPP-to-granule cell synapse is blocked by NMDA receptor antagonists in adult animals *in vivo* (Errington et al. 1987; Abraham and Mason 1988; Villarreal et al. 2002) and in slice preparations from young and adult rats (Burgard et al. 1989; Trommer et al. 1995). To determine whether NMDA receptors were also involved in LTP induction in neonates *in vivo*, CPP, a competitive NMDA receptor antagonist that crosses the blood-brain barrier, was administered intraperitoneally at 3 dosages to rats at each age about 1.5 hours before the animals were anesthetized.

Results showed that we were able to elicit responses to low frequency stimulation in about the same percentage of CPP-treated rats as in vehicle-treated control animals, and that CPP did not affect response latencies or peak amplitudes. Responses were elicited in 77% of animals treated with CPP (77.3%, 77.8%, and 77.1%, respectively, for the 1 mg/kg, 2 mg/kg and 3 mg/kg doses); as noted above, responses were elicited in 81% of control rats. The average
onset latencies of the baseline EPSPs for CPP-treated rats were 15.0 ms for those treated with 1 mg/kg of CPP, 12.5 ms for 2 mg/kg, and 15.0 ms for 3 mg/kg (Table 3). None of these values was significantly different from the average EPSP latency in control animals (15.7 ms; control vs. 1 mg/kg, \( p = 0.68 \); control vs. 2 mg/kg, \( p = 0.09 \); control vs. 3 mg/kg, \( p = 0.67 \)). Average peak amplitudes of the baseline EPSPs in the CPP-treated animals were 0.83 mV for those treated with the 1 mg/kg dose of CPP, 0.50 mV for the 2 mg/kg dose, and 0.58 mV for the 3 mg/kg dose (Table 3). There was not a statistically significant difference between any one of these values and the average EPSP peak amplitude in control rats (0.51 mV; control vs. 1 mg/kg, \( p = 0.05 \); control vs. 2 mg/kg, \( p = 0.88 \); control vs. 3 mg/kg, \( p = 0.53 \)).

Previous in vivo studies in adult rodents showed that CPP did not affect response amplitudes or slopes following stimulation of the hippocampal CA3 commissural pathway (Hernandez et al. 1994), or field EPSP slopes following stimulation of the perforant path in the dentate gyrus (Abraham and Mason, 1988).

There was a dose-dependent effect of the CPP on the occurrence of synaptic plasticities following tetanic stimulation in the neonatal rats (Fig. 7). At a dose of 1 mg/kg, CPP reduced the percentage of rats exhibiting LTP to 14% as compared to 41% in the control group, slightly decreased the percentage showing LTD to 29% from 33%, and increased the percentage of animals exhibiting STP alone to 29% from 15%. At this dose, CPP also slightly increased the percentage of animals exhibiting STP/LTD to 14% from 11% in the control group. One animal out of a total of 7 treated with the 1 mg/kg dose of CPP did not exhibit any synaptic plasticity following tetanic stimulation. In contrast, all of the control rats showed some form of synaptic plasticity after the same stimulation, as noted above.
At doses of 2 mg/kg or 3 mg/kg, CPP blocked the occurrence of LTP and approximately doubled the percentage of rats displaying LTD alone as compared to vehicle treatment. With 2 mg/kg CPP, 70% of the animals (Fig. 7) exhibited LTD alone as compared to 33% of the vehicle-treated control rats. With 3 mg/kg CPP, 67% of the rats (Fig. 7) showed LTD alone (Fig. 8). When the percentages of rats exhibiting LTD alone at each dose were combined with those showing LTD after STP, a total of 80% of the rats treated with 2 mg/kg CPP exhibited LTD, and 83% of animals treated with 3 mg/kg CPP exhibited LTD. With either dose of CPP, the percentages of rats displaying STP or STP/LTD were similar to the percentages in the control group.

When the average magnitudes of the LTD responses (LTD alone) in the CPP-treated animals at each dose were compared to the average magnitude of the LTD response in control rats of all ages combined (Table 4), no statistically significant differences were found (control vs. 1 mg/kg, $p = 0.72$; control vs. 2 mg/kg, $p = 0.43$; control vs. 3 mg/kg, $p = 0.63$). It is worth noting that one 8-day-old rat treated with 3 mg/kg CPP exhibited short-term depression: the average magnitude of the response at 5 to 10 minutes post-tetanus was significantly less than the pre-tetanus magnitude, whereas the magnitude at 30 to 35 minutes post-tetanus did not differ from the magnitude recorded prior to tetanus. This form of plasticity was not seen in control animals or in any rats treated with the other two doses of CPP.
Discussion

The findings reported here show that tetanic stimulation elicited LTP, LTD and STP in vivo at MPP synapses onto granule neurons in 7-day-old rats that are anesthetized with halothane. LTP was elicited in 40% of the rats at this age and LTD alone was induced in 40%. STP alone or STP followed by LTD was elicited in the remainder of the animals. To our knowledge, these are the first recordings of LTP at MPP synapses in the dentate gyrus at this age in vivo, and the first demonstration of LTD at this synapse in neonatal rats in vivo. These data support the results of in vitro studies demonstrating plasticity at perforant path to granule cell synapses in slices from 7-day-old rats (Duffy and Teyler 1978; Trommer et al. 1995). Furthermore, they support our previous suggestion that at least a portion of the dentate gyrus is capable of adult-like function by the end of the first postnatal week in the rat (Jones et al. 2003).

LTP in the neonatal dentate gyrus

Following tetanic stimulation of the MPP, LTP of the field EPSP was elicited in 40% of the 7-day-old rats, the earliest day at which reliable baseline recordings could be made. These results contrast with data from two previous in vivo studies of synaptic plasticity in the developing dentate gyrus (Wilson 1984; Bekenstein and Lothman 1991b). Bekenstein and Lothman (1991b) anesthetized neonatal rats with urethane and stimulated the perforant path with varying numbers of trains at frequencies between 50 and 333 Hz. LTP of the population spike, measured at 30 minutes post-tetanus, was not induced in 6- to 8-day-old rats but was elicited in a small fraction of rats between the ages of 9 and 10 days following a 333 Hz tetanus. Similarly, Wilson (1984) anesthetized neonatal rats with either pentobarbital or
urethane and stimulated the perforant path with 1-3 high intensity trains of 400 Hz for 100 ms once every 5-10 minutes. Potentiated responses to test stimuli, measured at 10 minutes post-tetanus, were not seen in either the population spike or the field EPSP at day 7, but were noted in the population spike in about 20% of the animals at day 10. By day 14, the same stimulation parameters induced potentiated population spikes in 42% of the animals, and potentiated EPSPs in 11%. Wilson termed these potentiated responses LTP even though they were measured at only 10 minutes post-tetanus. Neither Bekenstein and Lothman (1991b) or Wilson (1984) distinguished between the medial and lateral components of the perforant path; whether the data consisted of responses from one or the other, or both, was not specified.

While there are several methodological differences between the previous in vivo experiments described above and those reported here, including rat strains and stimulation parameters, it is tempting to speculate that our use of halothane instead of pentobarbital or urethane (Wilson 1984; Bekenstein and Lothman 1991b) allowed the induction of LTP at day 7. Pentobarbital has a greater potentiating effect on GABA inhibition than does halothane (Hirota et al. 1998; Wakasugi et al. 1999), and although pentobarbital and urethane do not block the induction of LTP in granule cells in the adult rat (McNaughton 1982; Wilson 1984; Errington et al. 1987; Bekenstein and Lothman 1991b), it is not known if either interferes with LTP induction in neonatal rats. In the presence of halothane, LTP can be induced at MPP synapses in adult rabbits in vivo (Yeckel and Berger 1998), and, as shown here, at MPP synapses in neonatal rats.
Our finding that LTP can be elicited in vivo at MPP synapses in 7-day-old rats anesthetized with halothane supports data from in vitro slice studies. Recording extracellular field potentials, Duffy and Teyler (1978) found LTP at perforant path synapses, as measured by EPSP amplitude, in 9% of slices from 7-day-old Sprague-Dawley rats and 10% in slices from 10-day-old animals. They reported that the magnitude (mean about 110% of baseline EPSP amplitude) and duration (30 min) of the LTP at these ages were equivalent to those seen in slices from adults. In a more recent study, Trommer et al. (1995) elicited LTP at MPP synapses in at least 48% of slices from 7- to 15-day-old rats. The magnitude of the EPSP slope expressed as a percent of baseline varied from 130% to 218% in this age group, depending on the type of recording chamber used.

In addition to inducing LTP, the tetanic stimuli used here elicited STP, either alone or followed by LTD, in 26% of the animals. STP has been reported at MPP synapses in slices from adult guinea pigs and rabbits (Hanse and Gustafsson 1992; Xie et al. 1996), and at the Schaffer collateral synapses onto CA1 pyramidal cells in slices from adult rats (Malenka 1991; Clark and Collingridge 1995). The development of STP in neonatal animals has not been characterized. Although Wilson (1984) reported an enhanced response that he termed STP following tetanic stimulation of the perforant path in intact neonatal rats, this enhanced response was measured at 10 seconds post tetanus and thus was likely a presynaptic phenomenon (Zucker and Regehr 2002) and not analogous to the longer-lasting STP reported here and elsewhere (Malenka 1991). As noted above, Wilson (1984) also characterized a potentiated response measured at 10 minutes post-tetanus in 10-day-old rats; he termed this response LTP, but it is more analogous to the STP measured here at 10 to 15 minutes post-
tetanus. While it is possible that STP is a less stable form of LTP (Malenka 1991; Hanse and Gustafsson 1992; Clark and Collingridge 1995), several lines of evidence suggest that the mechanism underlying STP expression are distinct from those underlying LTP (Schulz and Fitzgibbons 1997). Whether the STP recorded here represents an “early” form of LTP or a distinct type of potentiated response in immature animals is not known.

**LTD elicited following tetanic stimulation**

Following tetanic stimulation of the MPP, homosynaptic LTD alone was elicited in a third of the neonatal rats treated with vehicle. It is of interest that the LTD seen in the present study was elicited by a high frequency tetanus whereas LTD in the mammalian brain is most often elicited by low frequency stimulation (for reviews see Bear and Linden 2001; Kemp and Bashir 2001). There are, however, several reports of high frequency tetanic stimuli eliciting homosynaptic LTD at hippocampal synapses. High frequency stimulation of the MPP in combination with postsynaptic depolarization resulted in either LTP or LTD in slices from 2- to 3-week-old rats, depending on the amount of postsynaptic depolarization (Wu et al. 2001). In addition, Trommer et al. (1995) described an LTD-like response in slices from neonatal rats following high frequency stimulation. In the hippocampus proper, high frequency stimulation of granule cell axons induced homosynaptic LTD in CA3 pyramidal neurons in slices from rats between the ages of 6 and 14 days (Battistin and Cherubini 1994), as well as in intact adult rats (Derrick and Martinez 1996).

Based on the results reported here and on data from previous studies, it appears that homosynaptic LTD at the MPP synapse is also easier to elicit in young rats than in adults, as
reported for homosynaptic LTD in CA1 pyramidal neurons (Dudek and Bear 1993; Kemp et al. 2000; Wasling et al. 2002). Following low frequency stimulation of the MPP, homosynaptic LTD was elicited in 100% of slices from 8- to 13-day-old rats whereas it was induced in only 60% of slices from 27- to 30-day-old rats (Trommer et al. 1996). Other studies have also demonstrated homosynaptic LTD at MPP synapses in slices from juvenile rats between the ages of 2 and 5 weeks (O’Mara et al. 1995; Wang et al. 1997a; Wu et al. 2001). In contrast, homosynaptic LTD is reportedly difficult to induce at this synapse in adult rats (Pavlides et al. 1988; Doyère et al. 1996; Errington et al. 1995; Abraham et al. 1996; Manahan-Vaughan 1998).

While there is a general consensus that homosynaptic LTD is elicited more easily in immature tissue, the mechanisms underlying this age-related difference are not yet known. It is thought, however, that the amount of postsynaptic Ca\(^{2+}\) derived from Ca\(^{2+}\) influx or intracellular release may determine whether LTP or LTD is induced, with a moderate increase in Ca\(^{2+}\) entry favoring LTD over LTP (for reviews see Bear and Linden 2001; Kemp and Bashir 2001). Thus, because not all perforant path fibers have formed synapses on immature granule neurons at the end of the first postnatal week, it is possible that the concomitant reduction in postsynaptic depolarization and calcium influx during a tetanus favored the induction of LTD seen here.

It is worth considering that the use of halothane in the present study may have influenced the percentage of rats exhibiting LTD, perhaps by affecting EPSP amplitude and GABA-mediated inhibition onto the granule neurons (Lukatch and MacIver 1997; Nishikawa and
MacIver 2000) and thus reducing Ca\(^{2+}\) availability following MPP stimulation (Wang et al. 1997b). For example, halothane decreases EPSP amplitudes, increases the decay times and amplitudes of GABA\(_A\) receptor-mediated inhibitory postsynaptic currents (IPSCs), and increases the frequency of miniature IPSCs in adult hippocampal interneurons and pyramidal cells (Lukatch and MacIver 1997; Nishikawa and MacIver 2000). Interestingly, Thiels et al. (1996) showed that LTD at perforant path synapses could be elicited in adult rabbits anesthetized with halothane; we are currently testing the possibility that halothane may facilitate LTD induction in granule cells in adult rats.

**CPP blocks LTP induction and increases the occurrence of LTD**

Data in the present report demonstrate that LTP induction at MPP synapses in intact 7- to 9-day-old rats was blocked by the competitive NMDA receptor antagonist CPP at dosages of 2 or 3 mg/kg. LTP at this same synapse in adult rats *in vivo* is blocked by a higher dose of CPP (10 mg/kg) and by the NMDA receptor antagonist D-2-aminophosphonovalerate (APV) (Errington et al. 1987; Abraham and Mason 1988; Villarreal et al. 2002). Similarly, it is blocked in slices from neonatal and adult rats by APV (Burgard et al. 1989; Trommer et al. 1995). Interestingly, CPP did not block the occurrence of STP at MPP synapses in the present study, suggesting that the induction mechanisms for STP and LTP may differ, and that the primary component of STP at this synapse in rats may be mediated by AMPA receptors (Clark and Collingridge 1995; Xie et al. 1996).

Here we found that homosynaptic LTD at the MPP synapse was not blocked by CPP. Others have suggested that metabotropic glutamate receptors (mGluRs) may mediate
homosynaptic LTD induced by low frequency stimulation at MPP synapses in slices from juvenile rats between 2 and 5 weeks of age (O'Mara et al. 1995; Camodeca et al. 1999). Interestingly, high frequency stimulation of the MPP combined with postsynaptic depolarization in slices from rats in this same age group induced two forms of homosynaptic LTD: one form was blocked by APV and the other was blocked by group I mGluR antagonists (Wu et al. 2001). In slices from neonatal rats, however, homosynaptic LTD induced by low frequency stimulation of the MPP was not blocked by either APV or by a group I/II mGluR antagonist (Trommer et al. 1996).

As noted above, CPP blocked LTP induced by high frequency stimulation, thus reducing the percentage of neonatal rats showing LTP from about 40% to zero. Interestingly, CPP increased the percentage of animals exhibiting LTD alone by a similar amount, from 33% of the total in the controls to 68% in the presence of 3 mg/kg CPP. Thus, CPP appeared to "unmask" LTD. Similar results from in vitro studies have been reported. The NMDA receptor antagonist APV blocked LTP induced by high frequency stimulation and unmasked LTD at MPP synapses in slices from neonatal rats (Trommer et al. 1995) and at Schaffer collateral synapses in slices from young (11 to 18 days) and adult rats (Velíšek et al. 1993; Cummings et al. 1996). Such unmasking could have resulted from a decrease in postsynaptic Ca$^{2+}$ following tetanus in the presence of NMDA receptor antagonists (Cummings et al. 1996).

**Development of the hippocampal formation**

Because granule neurons are generated after hippocampal pyramidal neurons in the rodent (Bayer 1980), there has been a long-standing assumption that the dentate gyrus is a "late-
developing" region of the hippocampal formation. Several lines of evidence suggest, however, that this may not be the case. For example, our work indicates that some granule neurons located in the suprapyramidal blade, the earliest-formed portion of the dentate gyrus, have adult-like dendritic trees as well as axons that reach hippocampal field CA3 by day 7 (Jones et al. 2003; Claiborne et al. 1990; Rihn and Claiborne 1990). Also at this time, granule cell afferents are in their approximate adult locations in the suprapyramidal blade, synapses are present on granule cell dendrites, and the dendritic trees of dentate interneurons are well-developed (Cowan et al. 1980; Seress and Ribak 1990; Seay-Lowe and Claiborne 1992).

Importantly, results presented here demonstrate that LTP and LTD can be induced \textit{in vivo} at MPP synapses onto granule cells in 7-day-old rats, confirming earlier work in slices (Duffy and Teyler 1978; Trommer et al. 1995). At about this same age, LTP and LTD can be elicited in hippocampal CA1 pyramidal neurons and LTD induced in CA3 pyramidal neurons (Harris and Teyler 1984; Dudek and Bear 1993; Battistin and Cherubini 1994). Taken together, these data show that at least the earliest-formed portion of the dentate gyrus becomes adult-like at about the same time as the hippocampus proper, suggesting that the development of the dentate gyrus may not be a rate-limiting step in the maturation of hippocampal function.

\textbf{Acknowledgements}

We thank Ms. Janie Sanchez and Ms. Maribel Sanchez for assistance with the histology, Dr. Barbara Trommer for useful discussions, and Drs. David Jaffe and Timothy Teyler for helpful suggestions on methodology and data analysis. We also gratefully acknowledge the assistance and suggestions of the late Dr. John Sarvey. This work was supported by National Institutes of Health grants GM08194 (B.J.C.), GM60655 (B.J.C.) and DA09183 (B.E.D.).
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### Table 1: Average onset latencies and peak amplitudes of baseline field EPSPs for vehicle-treated rats

<table>
<thead>
<tr>
<th></th>
<th>7 Days (n=10)</th>
<th>8 Days (n=9)</th>
<th>9 Days (n=8)</th>
<th>All Ages (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latencies to Onset (ms)</td>
<td>16.3 ± 0.9 (12.0 - 19.2)</td>
<td>16.9 ± 2.4 (9.5 - 31.8)</td>
<td>13.7 ± 1.9 (9.3 - 26.5)</td>
<td>15.7 ± 1.0 (9.3 - 31.8)</td>
</tr>
<tr>
<td>Peak Amplitudes (mV)</td>
<td>0.51 ± 0.12 (0.18 - 1.42)</td>
<td>0.46 ± 0.07 (0.21 - 0.85)</td>
<td>0.57 ± 0.11 (0.19 - 1.05)</td>
<td>0.51 ± 0.06 (0.18 - 1.42)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Ranges are in parentheses. There were no statistically significant differences between average response amplitudes or latencies for each age group. See text for $p$ values of individual comparisons.
Table 2: Normalized field EPSP slopes following tetanus in vehicle-treated rats

<table>
<thead>
<tr>
<th></th>
<th>7 Days (n=10)</th>
<th>8 Days (n=9)</th>
<th>9 Days (n=8)</th>
<th>All Ages (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTP*</td>
<td>143 ± 13% (n = 4)</td>
<td>156 ± 22% (n = 4)</td>
<td>129 ± 10% (n = 3)</td>
<td>144 ± 9% (n = 11)</td>
</tr>
<tr>
<td>LTD*</td>
<td>84 ± 2% (n = 4)</td>
<td>92 ± 1% (n = 2)</td>
<td>81 ± 5% (n = 3)</td>
<td>85 ± 2% (n = 9)</td>
</tr>
<tr>
<td>STP</td>
<td>123% (n = 1)</td>
<td>128 ± 4% (n = 2)</td>
<td>109% (n = 1)</td>
<td>122 ± 5% (n = 4)</td>
</tr>
<tr>
<td>STP/LTD</td>
<td>106%/82% (n = 1)</td>
<td>105%/101% (n = 1)</td>
<td>120%/88% (n = 1)</td>
<td>110± 5%/90 ± 6% (n = 3)</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*There were no statistically significant differences between the normalized slopes for LTP at each age, or between the slopes for LTD at each age. See text for p values of individual comparisons.
Table 3: Average onset latencies and peak amplitudes of baseline EPSPs for CPP- and vehicle-treated rats

<table>
<thead>
<tr>
<th></th>
<th>1 mg/kg CPP (n=7)</th>
<th>2 mg/kg CPP (n=10)</th>
<th>3 mg/kg CPP (n=12)</th>
<th>Vehicle-treated rats (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latencies to Onset</td>
<td>15.0 ± 2.2 (7.1 - 25.5)</td>
<td>12.5 ± 1.1 (6.2 - 17.1)</td>
<td>15.0 ± 0.7 (10.1 - 18.3)</td>
<td>15.7 ± 1.0 (9.3 – 31.8)</td>
</tr>
<tr>
<td>Peak Amplitudes</td>
<td>0.83 ± 0.22 (0.42 - 2.08)</td>
<td>0.50 ± 0.03 (0.36 - 0.64)</td>
<td>0.58 ± 0.10 (0.10 - 1.13)</td>
<td>0.51 ± 0.6 (0.18 – 1.42)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Ranges are in parentheses. There were no statistically significant differences between average response latencies or amplitudes for CPP-treated animals in each group as compared to vehicle-treated rats. See text for p values of individual comparisons.
Table 4: Normalized field EPSP slopes following tetanus in CPP- and vehicle-treated rats

<table>
<thead>
<tr>
<th></th>
<th>1 mg/kg (n=7*)</th>
<th>2 mg/kg (n=10)</th>
<th>3 mg/kg (n=12)</th>
<th>Vehicle-treated (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTP</td>
<td>111%</td>
<td>---</td>
<td>---</td>
<td>144 ± 9%</td>
</tr>
<tr>
<td></td>
<td>(n = 1)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 27)</td>
</tr>
<tr>
<td>LTD**</td>
<td>83 ± 8%</td>
<td>82 ± 3%</td>
<td>83 ± 3%</td>
<td>85 ± 2%</td>
</tr>
<tr>
<td></td>
<td>(n = 2)</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>STP</td>
<td>113 ± 9%</td>
<td>112 ± 2%</td>
<td>110%</td>
<td>122 ± 5%</td>
</tr>
<tr>
<td></td>
<td>(n = 2)</td>
<td>(n = 2)</td>
<td>(n = 1)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>STP/LTD</td>
<td>111 %/80%</td>
<td>103%/91%</td>
<td>107 ± 5%</td>
<td>110± 5%/90%</td>
</tr>
<tr>
<td></td>
<td>(n = 1)</td>
<td>(n = 1)</td>
<td>(n = 1)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>STD</td>
<td>---</td>
<td>---</td>
<td>90%</td>
<td>(n = 1)</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*In addition to the data reported here, one rat treated with 1 mg/kg showed no change in the normalized field EPSP slope following tetanus.

**There were no statistically significant differences between the normalized slope for LTD at each dosage and that for vehicle-treated rats. See text for p values of individual comparisons.
Figure 1. Baseline field EPSP response recorded in the hilar region of the dentate gyrus to low frequency stimulation of the MPP in a 7-day-old rat. Scale bar: 0.4 mV and 10 ms.
Figure 2. Histogram of percentages of vehicle-treated control rats at each of the three ages exhibiting various forms of synaptic plasticity following tetanic stimulation of the MPP (7 days, n = 10; 8 days, n = 9; 9 days, n = 8).
Figure 3. Histogram of percentages of vehicle-treated control rats of all ages combined (7 to 9 days; n = 27) exhibiting various forms of synaptic plasticity following tetanic stimulation of the MPP.
Figure 4. Tetanic stimulation (upward arrow) of MPP induced LTP in an 8-day-old, vehicle-treated rat. Representative field EPSPs are shown in the upper portion of the figure: left EPSP is a baseline response taken from the time point indicated by the left downward arrow, and right EPSP is a potentiated response taken 30-35 minutes post-tetanus (right downward arrow). Scale bar: 0.2 mV and 10 ms.
Figure 5. Tetanic stimulation (upward arrow) of MPP induced LTD alone in a 9-day-old vehicle-treated rat. Representative field EPSPs are shown in the upper portion of the figure: left EPSP is a baseline response taken from the time point indicated by the left downward arrow, and right EPSP is a depressed response taken 30-35 minutes post-tetanus (right downward arrow). Scale bar: 0.2 mV and 10 ms.
Figure 6. Tetanic stimulation (upward arrow) of MPP induced STP alone in an 8-day-old, vehicle-treated rat. Representative field EPSPs are shown in the upper portion of the figure: left EPSP is a baseline response taken from the time point indicated by the left downward arrow, and right EPSP is a potentiated response taken 5-10 minutes post-tetanus (right downward arrow). Scale bar: 0.2 mV and 10 ms.
Figure 7. Histogram of percentages of rats treated with each of the three doses of CPP or with vehicle (controls) that exhibited various forms of synaptic plasticity following tetanic stimulation of the MPP (1 mg/kg, n = 7; 2 mg/kg, n = 10; 3 mg/kg, n = 12; vehicle-treated controls, n = 27).
Figure 8. Tetanic stimulation (upward arrow) of MPP induced LTD in a 9-day-old rat treated with 3 mg/kg CPP. Representative field EPSPs are shown in the upper portion of the figure: left EPSP is a baseline response taken from the time point indicated by the left downward arrow, and right EPSP is a depressed response taken 30-35 minutes post-tetanus (right downward arrow). Scale bar: 0.2 mV and 10 ms.