Low-frequency stimulation induces long-term depression and slow onset long-term potentiation at perforant path-dentate gyrus synapses in vivo

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Gonzalez J, Morales IS, Villarreal DM, Derrick BE. Low-frequency stimulation induces long-term depression and slow onset long-term potentiation at perforant path-dentate gyrus synapses in vivo. J Neurophysiol 111: 1259–1273, 2014. First published December 11, 2013; doi:10.1152/jn.00941.2012.—The expression of homosynaptic long-term depression (LTD) is thought to mediate a crucial role in sustaining memory function. Our in vivo investigations of LTD expression at lateral (LPP) and medial perforant path (MPP) synapses in the dentate gyrus (DG) corroborate prior demonstrations that PP-DG LTD is difficult to induce in intact animals. In freely moving animals, LTD expression occurred consistently among LPP-DG and MPP-DG responses. Interestingly, following acute electrode implantation in anesthetized rats, low-frequency stimulation (LFS; 900 pulses, 1 Hz) promotes slow-onset LTD at both MPP-DG and LPP-DG synapses that utilize distinct induction mechanisms. Systematic administration of the N-methyl-D-aspartate (NMDA) receptor antagonist (+/-)-cyclopiperidine-6-piperidperazine (CPP; 10 mg/kg) 90 min before LFS selectively blocked MPP-DG but not LPP-DG slow onset LTD, suggesting MPP-DG synapses express a NMDA receptor-dependent slow onset LTD whereas LPP-DG slow onset LTD induction is NMDA receptor independent. In experiments where paired-pulse LFS (900 paired pulses, 200-ms paired-pulse interval) was used to induce LTD, paired-pulse LFS of the LPP resulted in rapid onset LTD of DG responses, whereas paired-pulse LFS of the MPP induced slow onset LTD of DG responses. Although LTD observations were very rare following acute electrode implantation in anesthetized rats, LPP-DG LTD was demonstrated in some anesthetized rats with previously implanted electrodes. Together, our data indicate in vivo PP-DG LTD expression is an inconsistent phenomenon that is primarily observed in recovered animals, suggesting perturbation of the dentate through surgery-related tissue trauma influences both LTD incidence and LTP induction at PP-DG synapses in vivo.

LTD; dentate gyrus; perforant path; slow onset LTD; in vivo

CONTRASTING LONG-TERM POTENTIATION (LTP), long-term depression (LTD) encompasses long lasting decreases in synaptic efficacy induced by low-frequency stimulation (LFS) (Barriónuevo et al. 1980; Dudek and Bear 1992). LTD is observed at many synapses, including the hippocampus (Dudek and Bear 1992; Mulkey and Malenka 1992; Bear and Abraham 1996), amygdala (Wang and Gean 1999; Lin et al. 2000; Heinbockel and Pape 2000), neocortex (Artola et al. 1990; Yoshimura et al. 1991; Kirkwood et al. 1993; Kirkwood and Bear 1994), and cerebellum (Ito 1986; Linden 1994). In addition to erasing memories (Stevens 1990; Siegelbaum and Kandel 1991; Tadaharu 1993; Malleret et al. 2010), preventing synaptic saturation in neural networks (Sejnowski 1977; Churchland and Sejnowski 1992; Rolls 1996), and increasing memory storage capacity (Willshaw and Dayan 1990; Churchland and Sejnowski 1992; Malenka 1994), mounting evidence suggest hippocampal LTD is critical for learning and encoding (Bramwell and Manahan-Vaughn 2001; Kemp and Manahan-Vaughn 2007, 2008), including object-place configuration memory (Kemp and Manahan-Vaughn 2004) and novelty acquisition (Manahan-Vaughn and Bramwell 1999; Lemon and Manahan-Vaughn 2006, 2012; Dong et al. 2012).

Although heterosynaptic LTD is routinely observed in the dentate gyrus (DG) in vivo (Abraham and Goddard 1983; Christie and Abraham 1992a; Doyère et al. 1997), homosynaptic perforant path-dentate gyrus (PP-DG) LTD has proved more elusive to elicit in the intact animal (Errington et al. 1995; Abraham 1996; Abraham et al. 1996; Doyère et al. 1996; Fung et al. 2011). LFS paradigms yielding consistent observations of LTD in CA1 are ineffective in producing DG LTD in vivo (Abraham et al. 1996; Doyère et al. 1996). For instance, some studies observe no DG homosynaptic LTD in vivo in response to LFS (Errington et al. 1995; Abraham et al. 1996), whereas Bramham and Srebro (1987) reported DG homosynaptic LTD in only 5 of out 16 intact animals following LFS. Furthermore, inducing PP-DG homosynaptic LTD in vivo has entailed a range of prerequisite manipulations, such as priming LTD by activating group 2 mGluRs (Manahan-Vaughn 1998) and stimulating DG afferents at an intensity evoking a less than half-maximal response (Klausnitzer et al. 2004; Pöschel and Manahan-Vaughn 2005, 2007).

DG afferents originate from lateral and medial entorhinal cortex, where inputs from these structures traverse the angular bundle via the lateral perforant path (LPP) and medial perforant path (MPP) before, respectively, forming synapses on distal and middle regions of DG granule cell dendrites (Hjorth-Simonsen 1972; Steward 1976; Witter et al. 1989; Witter and Amaral 2004; Wyss 1981). The LPP and MPP comprise physiologically distinct inputs (McNaughton 1980) that, respectively, relay nonspatial and spatial information to the hippocampal formation (Hargreaves et al. 2005; Hunsaker et al. 2007; Yoganarashima et al. 2011). The medial and lateral aspects of the PP were not explicitly isolated in some studies of in vivo DG homosynaptic LTD (Bramham and Srebro 1987; Errington et al. 1995; Abraham et al. 1996; Manahan-Vaughn 1998). This may have enabled mixed LPP and MPP activation. No investigations have sought to compare the relative propensity for LPP or MPP LFS to induce LTD in the DG in vivo during distinct behavioral states, including awake and pentobarbital or urethane-anesthetized states. Here, we compare in vivo homosynaptic LTD expression in LPP-DG and MPP-DG synapses following LFS in awake, freely moving animals or in...
intact animals under pentobarbital or urethane anesthesia. In freely moving animals, LTD expression occurred inconsistently among LPP-DG and MPP-DG responses. Interestingly, following acute electrode implantation in anesthetized rats, LFS (900 pulses, 1 Hz) promotes slow-onset LTP at both MPP-DG and LPP-DG synapses that utilize distinct induction mechanisms. In contrast, LPP-DG LTD was observed in four out of six anesthetized animals with previously implanted electrodes. Together, these data suggest in vivo PP-DG LTD is an inconsistent phenomenon that is primarily observed in animals with previously implanted electrodes, whereas LTP rather than LTD is observed in anesthetized animals following acute surgery. Thus these data suggest that surgery-related tissue trauma influences both in vivo LTD incidence and LTP induction at PP-DG synapses.

MATERIALS AND METHODS

Subjects. The following experiments employed 106 adult male Sprague-Dawley rats (230–735 g; aged 3 to 9 mo old; Charles River Laboratories, Wilmington, MA) individually housed at the University of Texas at San Antonio animal facility under a 12-h light/dark cycle. Rats, aged 100 ± 6 postnatal days (means ± SE), were allowed ad libitum access to food and water before acute electrode implantation. Animals with chronic electrodes were older (165 ± 7 postnatal days old, means ± SE) at the time of experimentation as the stability of these electrodes requires no or minimal skull growth. These animals were fed a restricted diet to prevent excessive weight gain during postoperative recovery. All experimental procedures adhered to the National Institutes of Health Animal Care and Use Guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Texas at San Antonio.

Electrode implantation. Rats were given surgical levels of anesthesia before electrode implantation under stereotaxic guidance. Specifically, acute experiments entailed anesthetizing rats (230–735 g) with either sodium pentobarbital (65 mg/kg; Sigma-Aldrich) or urethane (1.5 g/kg; Sigma-Aldrich) administered intraperitoneally before implanting recording and stimulating electrodes, whereas chronic electrode implantation required sterile surgery in sodium pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. For surgeries involving sodium pentobarbital anesthesia, supplementary injections of pentobarbital-anesthetized rats. To obtain surgical levels of anesthesia. Body temperature was maintained at 37°C via a feedback controlled direct current heating pad. A Teflon-coated, stainless steel recording electrode (0.005 mm in diameter; A-M Systems) was lowered to the hilar region of the DG (A/P −3.5 mm, M/L +2.0 mm, D/V −3.0 mm; Paxinos and Watson 1994; Fig. 1A). Bipolar stainless steel stimulating electrodes were situated in either the MPP (A/P −7.9 mm, M/L +4.2 mm, D/V −2.5 mm; Paxinos and Watson 1994) or LPP (A/P −8.0 mm, M/L +5.0 mm, D/V −2.8 mm; Paxinos and Watson 1994) via placement in the dorsomedial or dorsolateral aspect of the angular bundle, respectively. DG responses were determined by utilizing both stereotoxic coordinates and audiologicization of the CA1 pyramidal cell and DG granule cell layers. LPP-DG and MPP-DG responses exhibit different slope and peak latency characteristics (McNaughton and Barnes 1977; Abraham and McNaughton 1984; Bramham et al. 1991) that facilitate LPP-DG and MPP-DG response isolation. Specifically, LPP-DG responses display a decrease in slope and an increase in peak latency relative to MPP-DG responses (Fig. 1B; McNaughton and Barnes 1977; Abraham and McNaughton 1984; Bramham et al. 1991). Furthermore, MPP- and LPP-DG responses are distinguished on the marked paired-pulse facilitation observed in the LPP when stimulus intervals occur at 30–50 ms (McNaughton 1980; Andreasen and Hablitz 1994).

In animals with permanent indwelling electrodes, the electrodes were attached with gold amphenol pins and stabilized in a head stage with stainless steel screws and dental acrylic. These animals received preoperative doses of atropine (0.1 mg/kg; Sigma-Aldrich) intraperitoneally to prevent respiratory congestion during surgery and an intramuscular injection of Aquacillin (100k units) or Baytril (enrofloxacin; 5 mg/kg) to prevent postoperative infection. Following permanent electrode implantation, rats were given Rimadyl (4 g, BID; BioServ), an analgesic, for 3 days postoperatively. Data collection ensued after a 2-wk recovery period.

Experimental design. Behaving animal experiments were performed during the animal’s light cycle and involved collecting responses evoked with current intensities that produce an approximation of the half-maximal peak slope, as determined by input/output assessments. In these experiments, LPP stimulation intensities ranged from 158 to 395 μA, whereas MPP stimulation intensities ranged from 171 to 495 μA. Rats were allowed to habituate to the recording chamber for at least 30 min before collecting PP evoked DG baseline field excitatory postsynaptic potential (EPSP) responses, thus limiting novelty-dependent facilitation of synaptic plasticity induction and
LPP-DG responses were evoked with a stimulation intensity range of below half-maximal stimulation intensities. A stimulation intensity range of 100–390 onsets LTP. Following a stable 20-min baseline of MPP-DG responses induced high-frequency stimulation-induced LTP and LFS-induced slow thetization animals to compare the LTP expression mechanism mediating PP-DG LFS data. To elucidate the relative propensity for LPP or MPP LFS to induce LTD in the DG of freely moving rats, LFS-induced synaptic plasticity in the DG was compared in animals possessing a chronic stimulating electrode isolated in either the lateral or medial aspect of the angular bundle. Following LTD of the LPP (900 pulses, 1 Hz; n = 6), DG field EPSP slope measures exhibited a sustained decrease relative to baseline, lasting at least 1 h (91.3 ± 2.7%; Fig. 2A). A

<table>
<thead>
<tr>
<th>Condition</th>
<th>LFS Paradigm</th>
<th>LPP/MPP</th>
<th>n</th>
<th>Normalized EPSP 1-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake, freely moving</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>6</td>
<td>91.3 ± 2.7</td>
</tr>
<tr>
<td>Awake, freely moving</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>6</td>
<td>102.9 ± 10.4</td>
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<tr>
<td>Pentobarbital</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>5</td>
<td>109.4 ± 2.5</td>
</tr>
<tr>
<td>CPP + pentobarbital</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>6</td>
<td>118.5 ± 6.6</td>
</tr>
<tr>
<td>Urethane</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>5</td>
<td>120.2 ± 7.1</td>
</tr>
<tr>
<td>CPP + urethane</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>6</td>
<td>100.4 ± 4.7</td>
</tr>
<tr>
<td>Urethane</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>6</td>
<td>123.0 ± 4.2</td>
</tr>
<tr>
<td>CPP + urethane</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>5</td>
<td>110.9 ± 8.3</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>900 pulses, 1 Hz</td>
<td>LPP</td>
<td>5</td>
<td>132.2 ± 7.1</td>
</tr>
<tr>
<td>Low-intensity 900 pulses, 1 Hz</td>
<td>MPP</td>
<td>111.5 ± 5.5</td>
<td></td>
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</tr>
<tr>
<td>Pentobarbital</td>
<td>900 paired pulses</td>
<td>MPP</td>
<td>4</td>
<td>111.8 ± 9.0</td>
</tr>
<tr>
<td>Low-intensity 900 pulses, 1 Hz</td>
<td>MPP</td>
<td>111.8 ± 9.0</td>
<td></td>
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</tr>
</tbody>
</table>

Normalized field excitatory postsynaptic potentials (EPSPs) reflect means ± SE. LFS, low-frequency stimulation; LPP, lateral perforant path; MPP, medial perforant path; CPP, (+/−)-cyclopiperidine-6-piperiperenzine.
repeated-measures one-way ANOVA indicates LFS of the LPP produces a significant reduction in DG field EPSP \([F(1,5) = 10.05, P < 0.05, n = 6; \text{Fig. 2A}]\) in awake, freely moving rats. Four out of six animals expressed homosynaptic LTD following LFS of the LPP, as assessed by significant differences among 5-min baseline and 56- to 60-min post-LFS slopes of individual experiments \((P < 0.05, \text{repeated-measures one-way ANOVA})\).

Given the paucity of prior examinations of the receptors involved in homosynaptic LTD induction at LPP-DG synapses, separate experiments were conducted to evaluate the dependence of LTD on \(N\)-methyl-\(d\)-aspartate (NMDA) receptor activation. Animals were given either CPP (10 mg/kg), an NMDA receptor antagonist, or water vehicle at least 2 h before LFS of the LPP. Vehicle-treated animals \((n = 6)\) demonstrated infrequent LPP-DG LTD expression following LFS, where only three out of six animals showed significant decreases in DG field EPSPs recorded 1 h after LPP LFS \((P < 0.05, \text{repeated-measures one-way ANOVA})\). These data suggest LPP-DG LTD induction was somewhat more reliable in animals that were not pretreated with a vehicle injection. In parallel experiments, CPP-treated animals \((n = 7)\) exhibited DG field EPSPs measuring 94.4 ± 8.4% of baseline 1 h following LFS. Thus subsequent pharmacological analysis of LPP-DG LTD induction was not possible due a lack of significant depression in groupwise comparisons of LPP-DG field EPSPs recorded before and 1 h after LFS in vehicle-treated animals \([F(1,5) = 1.01, P > 0.05, n = 6, \text{repeated-measures one-way ANOVA}]\). Two out of seven CPP-treated animals displayed significant decreases in DG field EPSPs recorded 1 h after LPP LFS \((P < 0.05, \text{repeated-measures one-way ANOVA})\).

LFS of the MPP (900 pulses, 1 Hz) resulted in DG field EPSPs measuring 102.9 ± 10.4% of baseline \([\text{Fig. 2B}; F(1,5) = 0.14, P > 0.05, n = 6, \text{repeated-measures one-way ANOVA}]\) in freely moving rats. Two out of six animals demonstrated significant reductions in MPP-DG field EPSPs recorded 1 h after MPP LFS \((P < 0.05, \text{repeated-measures one-way ANOVA})\). One out of six rats exhibited robust LTP following MPP LFS. Although more animals displayed significant reductions in PP-DG field EPSPs following LPP LFS relative to MPP LFS \((P < 0.05, \text{repeated-measures one-way ANOVA})\), Kolmogrov-Smirnov statistical analysis indicates no significant difference in empirical cumulative probability distributions of LFS-induced alterations in LPP-DG and MPP-DG slopes \((P > 0.05; \text{Fig. 2C})\). Thus physiological differences in LPP and MPP inputs \((\text{McNaughton 1980}; \text{Abraham and McNaughton 1984})\) do not impact the probability and magnitude of decreased PP-DG responses following LFS. Together, these data suggest LTD is induced inconsistently at both LPP-DG and MPP-DG synapses in freely moving animals.

**LFS of the LPP or MPP promotes slow onset LTP in the DG of anesthetized rats.** Evaluations of PP-DG LTP in pentobarbital-anesthetized rats permitted additional analysis of dentate synaptic plasticity in intact animals. Although LTP is generally thought to occur with high-frequency stimulation \((\text{Bliss and Lomo 1973}; \text{Dudek and Bear 1992}; \text{Blaise and Bronzino 2003}; \text{Raymond 2007})\), previous studies demonstrate a slow-rising enhancement of field EPSPs following LFS \((\text{Li et al. 1998}),\)
responses in sodium pentobarbital-anesthetized rats (n = 5; Li et al. 1998, 2001; Lanté et al. 2006a,b; Huang and Kandel 2007; Habib and Dringenberg 2009, 2010a). Similar to these studies, LFS (900 pulses, 1 Hz) of the LPP-induced slow onset LTP of dentate synapses in pentobarbital-anesthetized rats. Following LFS, analysis of dentate field EPSPs indicates a significant change from baseline measuring 109.4 ± 2.5% [F(1,4) = 17.58, P < 0.05, n = 5], repeated-measures one-way ANOVA; Fig. 3, A and B]. Separate experiments similarly resulted in slow onset LTP [118.5 ± 6.6%, F(1,5) = 9.55, P < 0.05, n = 6], repeated-measures one-way ANOVA; Fig. 3, A and B] when the NMDA receptor antagonist CPP (10 mg/kg) was administered at least 90 min before LPP LFS in pentobarbital-anesthetized rats. Consistent with prior reports suggesting LPP-DG LTP occurs via a NMDA receptor-independent mechanism (Bramham et al. 1988, 1991), Newman-Keuls multiple pairwise comparisons characterize a significant increase in DG field EPSP following LFS of the LPP in CPP-treated pentobarbital-anesthetized rats. Furthermore, one-way ANOVA analysis revealed no significant difference between control and CPP-treated dentate field EPSP ratios derived from 56 to 60 min following LPP LFS and the last 5 min of baseline when the NMDA receptor antagonist CPP (10 mg/kg) was administered at least 90 min before LPP LFS in pentobarbital-anesthetized rats. Urethane-anesthetized rats displayed slow onset LTP of DG field EPSPs lasting at least 1 h following LFS of the MPP (Fig. 3, C and D). Dentate field EPSPs measured 120.2 ± 7.1% of baseline [F(1,7) = 9.29, P < 0.05, n = 8], repeated-measures one-way ANOVA] after stimulating MPP fibers with 900 pulses at current intensities evoking a half-maximal response. Supporting previous studies demonstrating NMDA receptor-dependent LTP at MPP-DG synapses (Bramham et al. 1991), dentate field EPSPs exhibited no change relative to baseline when CPP administration preceded LFS of the MPP in pentobarbital-anesthetized rats [100.4 ± 4.7%, F(1,5) = 0.02, P > 0.05, n = 6], repeated-measures one-way ANOVA; Fig. 3, C and D]. Control and CPP-treated dentate field EPSP ratios derived from 56 to 60 min following MPP LFS and the last 5 min of baseline were significantly different [F(1,12) = 5.27, P < 0.05, one-way ANOVA]. These data suggest NMDA receptors facilitate slow onset LTP induction following LFS of the MPP in pentobarbital-anesthetized rats.

Given how urethane anesthesia poses relatively minimal influence on GABA inhibitory synaptic transmission (Maggi and Meli 1986; Sceniak and MacIver 2006), similar LFS experiments were performed in urethane-anesthetized rats to evaluate the possibility that pentobarbital-induced enhancement of GABA synaptic transmission (Schulz and MacDonald 1981; Study and Barker 1981; Twyman et al. 1989; Akaike et al. 1990; Eghbali et al. 2000, 2003; Birnir et al. 2000; Mathers et al. 2007) prevented DG LTD expression in pentobarbital-anesthetized rats. Urethane-anesthetized rats displayed slow onset LTP of DG field EPSPs lasting at least 1 h following LFS (900 pulses, 1 Hz) of the LPP [123 ± 4.2%, F(1,4) = 37.23, P < 0.05, n = 5], repeated-measures one-way ANOVA; Fig. 4, A and B]. Interestingly, LPP-DG slow onset LTP was not significant in additional studies involving CPP administration.
Fig. 4. LFS induces slow onset LTP at LPP-DG and MPP-DG synapses in urethane-anesthe-
ized rats. A: evaluation of normalized LPP-DG fEPSP dV/dt analysis (means ± SE) in control
(n = 5) and CPP-treated (n = 7) urethane-
anesthetized rats. LPP LFS (900 pulses, 1 Hz)
was delivered at stimulation intensities evoking a half-maximal LPP-DG response in both naïve
rats and in animals given CPP at least 90 min
before LFS. Differences in post-LFS-to-base-
line LPP-DG fEPSP ratios among control and
CPP-treated rats were not statistically signif-
icant. B: representative LPP-DG fEPSPs col-
lected during baseline and 56–60 min after LPP
LFS in control (top) and CPP-treated (bottom)
urethane-anesthetized rats. Calibration: 1 mV, 5
ms (top); 0.5 mV, 5 ms (bottom). C: comparison
of normalized MPP-DG fEPSP dV/dt analysis
(means ± SE) in control (n = 5) and CPP-
treated (n = 5) urethane-anesthetized rats. MPP
LFS (900 pulses, 1 Hz) was delivered at stim-
ulation intensities evoking a half-maximal
MPP-DG response in both naïve rats and in
animals given CPP at least 90 min before LFS.
CPP blocked LFS-induced slow onset LTP at
MPP-DG synapses. D: representative MPP-DG
fEPSPs recorded during baseline and 56–60
min after MPP LFS in control (top) and CPP-
treated (bottom) urethane-anesthetized rats.
Calibration: 1 mV, 5 ms.

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at least 90 min before LPP LFS in urethane-anesthetized rats [110.9 ± 8.3%, F(1,6) = 2.01, P > 0.05, n = 7, repeated-
measures one-way ANOVA, Fig. 4, A and B]. In the presence of
CPP, two out of seven urethane-anesthetized animals dem-
strated LPP-DG slow onset LTP following LFS, suggesting
NMDA receptor antagonists did not completely block LTP
induction at these synapses. Nonetheless, the NMDA receptor
sensitivity of slow-onset LTP at LPP-DG synapses in urethane-
anesthetized rats is inconclusive given how control and CPP-
treated LPP-DG field EPSP ratios demonstrated no significant
difference in response to LPP LFS [F(1,10) = 1.54, P > 0.05,
one-way ANOVA].

Urethane-anesthetized animals showed a slow onset LTP of
DG field EPSPs following LFS (900 pulses, 1 Hz) of the MPP.
Specifically, normalized dentate field EPSPs measured 132.2 ±
7.1% of baseline 1 h after LFS of the MPP [F(1,4) = 26.37, P < 0.05, n = 5, repeated-measures one-way ANOVA; Fig.
4, C and D]. In the presence of CPP, LFS (900 pulses, 1 Hz)
of the MPP produced no change in DG field EPSP in
urethane-anesthetized rats [104 ± 9.6%, F(1,4) = 0.25, P > 0.05, n = 5, repeated-measures one-way ANOVA; Fig. 4, C
and D]. One-way ANOVA analysis indicate a significant differ-
eence between control and CPP-treated DG response slope ratios
derived from 56 to 60 min following MPP LFS and the last 5 min
of baseline [F(1,8) = 7.27, P < 0.05]. Thus urethane-anesthetized
rats also exhibit a NMDA receptor dependent slow onset LTP at
MPP-DG synapses following LFS.

Together, in both pentobarbital- and urethane-anesthe-
tized rats, LTD was not induced by LFS. Rather, a slow onset
LTP of DG field EPSPs was observed in anesthetized rats
following LFS of the MPP or LPP. Similar to the receptor
sensitivities of MPP and LPP LTP induced by high-frequency
stimulation (Bramham et al. 1991; Do et al. 2002), LFS-
induced potentiation of MPP-DG synapses occurs via a NMDA
receptor-dependent mechanism while LPP-DG slow onset LTP
is induced in a NMDA receptor-independent manner in pen-
tobarbital-anesthetized animals. Although NMDA receptor an-
tagons reduced the number of LPP-DG slow onset LTP
observations in urethane-anesthetized animals, we were unable
to determine the NMDA receptor sensitivity of LPP-DG slow
onset LTP in these animals as LTP induction was not com-
pletely blocked by NMDA receptor antagonism. In lieu of how
LPP-DG slow onset LTP was induced more reliably in CPP-
treated pentobarbital, relative to CPP-treated urethane, anes-
thetized animals, anesthetic differences may influence the
impact of NMDA receptor antagonists on LPP-DG slow onset
LTP induction in response to LFS.

Prior examinations of PP-DG responses in vivo indicate
baseline slope measures are slow to stabilize under pentobar-
bital or urethane anesthesia, where baselines slowly increased
shortly after electrode implantation (Gilbert and Mack 1999).
As the increases observed here similarly are slow to develop,
we addressed whether these increases may be, rather than LTP,
artifacts from electrode injury. In pentobarbital-anesthetized
animals, we collected a stable 20-min baseline of MPP-DG
responses before recording an additional 75 min of responses,
and then a single 1-h period of collection. Following this 75-min period, we delivered LFS
(900 pulses, 1 Hz) and collected responses for an additional
hour. As shown in Fig. 5, no increases in MPP-DG field EPSPs
were observed over the 75-min period of extended baseline,
where, before LFS, MPP-DG field EPSPs measured 101 ±
0.9% of initial baseline. In contrast, three out of three animals
showed a significant increase in MPP-DG field EPSPs follow-
Previous studies indicate brief stress exposure suppressed homosynaptic LTD at LPP-DG synapses in ex vivo mouse hippocampal slices (Spyrka et al. 2011). Given the disparity of LPP-DG LTD observations in freely moving rats that did not occur in LFS experiments performed in anesthetized animals, we investigated the possibility that stress associated with acute surgery and stereotaxic restraint prevents LTD induction in anesthetized animals. To avoid the effects of acute electrode implantation required for in situ data collection in anesthetized animals (Jankowsky et al. 2000), rats implanted with electrodes 2 wk earlier were anesthetized with sodium pentobarbital (65 mg/kg ip) before subsequent LPP LFS (900 pulses, 1 Hz) in the absence of stereotaxic restraint. On average, LFS of the LPP produced no significant change in dentate field EPSPs [94.9 ± 9.1%, \(F(1,5) = 0.45, P > 0.05, n = 6\), repeated-measures one-way ANOVA; Fig. 6A]. Interestingly, LFS of the LPP induced LTD in four out of six anesthetized rats with previously implanted electrodes (Fig. 6B), as assessed by significant differences among 5-min baseline and 56–60 min post-LFS slopes of individual experiments (\(P < 0.05\), repeated-measures one-way ANOVA). The occurrence of LPP-DG LTD in four out of six experiments was observed in both freely moving and anesthetized rats with previously implanted electrodes. This contrasts with the very rare occurrence of LTD when LFS followed acute electrode implantation in anesthetized animals (4 out of 67 experiments). These data indicate LTD of LPP-DG synapses occurs with higher incidence in animals with previously implanted electrodes, suggesting surgery-related stress or tissue injury sustained during acute electrode implantation impairs LTD induction and concomitantly facilitates LTP induction in the DG.

Low-intensity LFS of the LPP or MPP fails to elicit LTD in the DG of anesthetized rats. Low-frequency afferent stimulation is thought to yield a small-to-moderate increase in intracellular calcium concentration that favors LTD induction (Lisman 1989; Mulkey and Malenka 1992; Artola and Singer 1993; Bear and Malenka 1994; Linden and Connor 1995; Christie et al. 1996; Coussens and Teyler 1996; Yang et al. 1999). Given how LTP induction occurs with large increases in intracellular calcium concentration (Lisman 1989; Artola and Singer 1993; Bear and Malenka 1994; Coussens and Teyler 1996; Yang et al. 1999), slow onset LTP following LFS may reflect a high afferent stimulation intensity that activated a threshold number of afferent fibers enabling sufficient calcium influx to induce LTP. We therefore used a low-intensity LFS paradigm (Klausnitzer et al. 2004; Pöschel and Manahan-Vaughan 2005, 2007) to investigate whether DG LTD induction requires subhalf-maximal afferent stimulus intensities (22–435 μA).

Following LFS (900 pulses, 1 Hz) of the LPP at current intensities evoking field EPSPs characterized as 25% of the maximal field EPSP, dentate field EPSPs measured 111.5 ± 5.5% of baseline (Fig. 7A) in sodium pentobarbital-anesthetized rats (\(n = 6\)). Interestingly, low-intensity LFS of the LPP produced a significant difference in dentate field EPSPs relative to baseline \([F(1,5) = 7.48, P < 0.05\), repeated-measures one-way ANOVA].
Low-intensity LFS (900 pulses, 1 Hz) of the MPP produced no change in DG field EPSP relative to baseline in pentobarbital-anesthetized rats (111.8 ± 9.0%, \( P = 0.05 \), \( n = 5 \), Kruskal-Wallis one-way ANOVA; Fig. 7B). Taken together, these data suggest low-intensity LFS is insufficient to induce LPP- or MPP-DG LTD in anesthetized rats. Paired-pulse LFS of the LPP or MPP does not induce LTD in the DG of anesthetized rats. Previous studies demonstrate LTD induction by prolonged LFS (900 pulses, 1 Hz) occurs with less prevalence in adult animals (Dudek and Bear 1993; Kemp et al. 2000; Milner et al. 2004; Blaise and Bronzino 2003). Interestingly, paired-pulse LFS induces LTD at CA3-CA1 synapses in adult animals (Thiels et al. 1994; Kemp and Bashir 1997, 1999; Kemp et al. 2000). As we are using adult animals, we attempted to induce PP-DG LTD using a 900 paired-pulse LFS paradigm (1,800 stimuli, 200-ms paired-pulse interval) capable of inducing CA3-CA1 LTD in mature animals (Kemp et al. 2000). Stimulating the LPP with 900 paired pulses did not result in LTD of dentate field EPSPs in pentobarbital-anesthetized rats (\( n = 4 \)). Instead, a rapid onset LTP of dentate field EPSPs ensued paired-pulse LFS of the LPP [Fig. 7C; \( F(1,3) = 10.54, P < 0.05 \), repeated-measures one-way ANOVA]. Potentiated LPP-DG responses measured 119 ± 6.7% of baseline field EPSPs and sustained without decay for at least 1 h.

Paired-pulse stimulation of the MPP pathway induced a slow onset LTP of DG field EPSPs in pentobarbital-anesthetized rats (Fig. 7D, \( n = 5 \)). Dentate field EPSPs measured 132.2 ± 10.6% of baseline [\( F(1,4) = 10.93, P < 0.05 \), repeated-measures one-way ANOVA] following paired-pulse LFS of the MPP. The resulting slow onset LTP of MPP-DG synapses persisted at least 1 h without decrement. Together, these data suggest paired-pulse LFS of the LPP or MPP does not induce DG LTD in pentobarbital-anesthetized rats. Instead, PP paired-pulse LFS results in pathway specific variants of LTP in the DG.

Prior LTP saturation of PP-DG synapses occludes LFS-dependent slow onset potentiation in anesthetized rats. LFS induces a slow onset LTP in anesthetized animals (Li et al. 1998, 2001; Lanté et al. 2006a, 2006b; Huang and Kandel 2007; Habib and Dringenberg 2009, 2010a). This potentiation is NMDA receptor dependent at MPP synapses and NMDA
receptor independent at LPP synapses (Bramham et al. 1991). Because this parallels the NMDA dependence and independence of LTP at MPP and LPP synapses when using high-frequency stimulation (Bramham et al. 1991), this suggests the slow onset LTP induced by LFS and LTP induced by high-frequency stimulation employ similar expression mechanisms. If this is the case, the high-frequency stimulation-induced LTP would be expected to occlude the subsequent expression of LFS-induced slow onset LTP (Habib and Dringenberg 2010a). In contrast, if these stimulation paradigms employ distinct LTP expression mechanisms, LTP induced by high-frequency stimulation will exhibit further potentiation following subsequent LFS.

LTP occlusion experiments were performed in pentobarbital-anesthetized animals to compare the LTP expression mechanism mediating high-frequency stimulation-induced LTP and LFS-induced slow onset LTP. Theta burst stimulation of the MPP resulted in a robust LTP measuring 170.5 ± 34.7% of baseline dentate field EPSPs $[F(1,4) = 9.99, P < 0.05, n = 5]$, repeated-measures one-way ANOVA; Fig. 8. Subsequent LFS produced no further increase or decrease in post-TBS dentate field EPSPs (Fig. 8). Following LFS of the MPP, dentate field EPSPs measured 99.2 ± 7.5% of the average field EPSP occurring 5 min before LFS, indicating prior high-frequency stimulation-induced LTP occludes the subsequent occurrence of LFS-induced slow onset LTP in the DG. In addition, one-way ANOVA comparisons of the effects of LFS on dentate field EPSP ratios from pentobarbital-anesthetized rats given no prior TBS ($n = 8$) and those receiving LFS following previous theta burst stimulation ($n = 5$) approached significance $[F(1,11) = 4.41, P = 0.06, \text{one-way ANOVA}]$.

These results demonstrate dentate LTP saturation mediated by theta burst stimulation of the MPP prevents LFS-induced slow onset potentiation in pentobarbital-anesthetized rats, suggesting LTP generated by TBS or LFS share common expression mechanisms, as is suggested for slow-onset LTP seen at other synapses (Habib and Dringenberg 2010a).

**DISCUSSION**

The present study is a systematic effort to evaluate the relative propensity for LPP or MPP LFS to induce homosynaptic LTD in the DG of both behaving rats and in sodium pentobarbital or urethane-anesthetized rats. Our data corroborate prior demonstrations that homosynaptic LTD is difficult to induce in the DG of intact animals (Errington et al. 1995; Abraham 1996; Abraham et al. 1996; Doyère et al. 1996; Fung et al. 2011). Our findings suggest dentate homosynaptic LTD is expressed inconsistently among both LPP-DG and MPP-DG synapses in freely moving animals. Interestingly, LTD was more likely to be observed in freely moving animals and anesthetized rats with previously implanted electrodes, whereas LTD was not observed when LFS followed acute electrode implantation in pentobarbital- or urethane-anesthetized rats. Rather than LTD, our acute experiments in anesthetized rats routinely resulted in a slow-rising LTP of MPP-DG and LPP-DG responses following LFS. Slow onset LTP induced by LFS in animals with previously implanted electrodes was rarely observed. Together, our findings suggest surgery-related tissue trauma produce perturbations that both impair LTD induction and facilitate the occurrence of LTP at PP-DG synapses in vivo.

LTD has traditionally been described as having either a homosynaptic or heterosynaptic origin. Homosynaptic LTD characterizes depression occurring at synapses receiving presynaptic LFS, whereas heterosynaptic LTD emanates at synapses that are not activated by high-frequency stimulation of inputs on the same postsynaptic neuron (Bear and Abraham 1996). Although heterosynaptic LTD is routinely observed in the DG in vivo (Abraham and Goddard 1983; Christie and Abraham 1992a; Doyère et al. 1997), homosynaptic PP-DG LTD has proved more elusive to elicit in the intact animal (Errington et al. 1995; Abraham 1996; Abraham et al. 1996; Doyère et al. 1996; Fung et al. 2011). Interestingly, Abraham et al. (2007) demonstrate heterosynaptic LTD requires presynaptic activity and thus may share similarities with homosynaptic LTD. Thus it remains unclear why homo- but not heterosynaptic LTD is difficult to obtain in vivo. Nevertheless, the difference in the prevalence of hetero- and homosynaptic LTD in the dentate may be explained as follows: as the induction of LTD requires moderate increases in intracellular calcium concentration (Lisman 1989; Mulkey and Malenka 1992; Artola and Singer 1993; Bear and Malenka 1994; Linden and Connor, 1995; Coussens and Teyler 1996; Yang et al. 1999), heterosynaptic LTD prevalence may relate to the propagating calcium waves produced by high-frequency stimulation (Jäger et al. 2002) at converging inputs. In this scenario, strongly stimulated synapses initiate a large intracellular increase in calcium concentration that may induce LTD (Lisman 1989; Artola and Singer 1993; Bear and Malenka 1994; Coussens and Teyler 1996; Yang et al. 1999). In addition, the calcium increase at strongly stimulated synapses may diffuse short distances within the dendrite (Jäger et al. 2002), allowing low-to-moderate intracellular calcium increases at adjacent synapses that may enable basal levels of presynaptic activity to induce “heterosynaptic” LTD (Abraham et al. 2007). Alternatively, feedforward inhibition of granule cell dendrites may limit the incidence of homosynaptic LTD by reducing PP postsynaptic depolarization. Given how presynaptic activity is required for

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**Fig. 8.** High-frequency stimulation-induced LTP occludes LFS-dependent slow onset LTP. Normalized MPP-DG fEPSP dv/dt analysis (means ± SE) depicts the expression of robust LTP following theta burst stimulation (5 trains of 10 ms, 400-Hz theta bursts with 20-s intertrain intervals repeated 3 times every 10 min) in pentobarbital-anesthetized rats ($n = 5$). Potentiated MPP-DG fEPSP exhibited no additional enhancement of fEPSP dv/dt following subsequent 900 pulse, 1-Hz MPP LFS.
LTD resulting from sustained low-frequency afferent stimulation or coincident LTP induction at adjacent afferents (Abraham et al. 2007), homosynaptic LTD may occur under different induction conditions. These variants of homosynaptic LTD may be dissociated in terms of whether LTD requires the co-occurrence of LTP at adjacent afferents. Nonetheless, the possibility that a given synapse expresses a single variant of homosynaptic LTD may explain the inconsistent observations of LTD following low-frequency afferent stimulation. Specifically, LTD expression that co-occurs with LTP induction at neighboring synapses may obscure subsequent LTD induced by low-frequency afferent stimulation.

In the present studies, LFS experiments ensuing acute electrode surgery in anesthetized animals failed to induce homosynaptic PP-DG LTD. Contrasting the dogma suggesting high-frequency stimulation elicits LTP whereas LFS induces LTD (Dudek and Bear 1992; Blaise and Bronzino 2003), our data indicate both pentobarbital- and urethane-anesthetized rats exhibit slow onset LTD (Li et al. 1998, 2001; Lanté et al. 2006a, 2006b; Huang and Kandel 2007; Habib and Dringenberg 2009, 2010a) of DG responses following 900 pulses, 1 Hz LFS of the LPP or MPP. We also demonstrate PP-DG slow onset LTD is not an artifact of a slow rising baseline previously observed in anesthetized animals (Gilbert and Mack 1999). Our findings of stable baselines lasting >90 min suggest DG slow onset LTD reflects an activity-dependent phenomenon that requires LFS. Although Lanté et al. (2006b) show 3- to 5-min short duration LFS is ineffective in eliciting DG slow onset LTD, previous reports demonstrate LFS-induced slow onset LTD occurring at external capsule-basolateral amygdala synapses after 900 pulses, 1 Hz LFS (Li et al. 1998, 2001; Huang and Kandel 2007), Schaffer collateral-CA1 synapses following 3- to 5-min short duration LFS (Lanté et al. 2006a, 2006b) and in response to alternating LFS of medial septal and CA3 commissural CA1 afferents (Habib and Dringenberg 2009, 2010a). Slow oscillations of similar ~1 Hz frequency as these LFS paradigms have been reported in the hippocampus during non-REM sleep (Wolansky et al. 2006; Schall et al. 2008) and have been concomitantly implicated in memory consolidation (Bodizs et al. 2002; Marshall et al. 2006). Thus observations of LFS-induced synaptic potentiation in the hippocampus may suggest endogenous hippocampal slow oscillatory activity comprises a physiologically relevant framework enabling LTP and memory consolidation (Habib and Dringenberg 2009b). Similar to CA1 (Lanté et al. 2006b; Habib and Dringenberg 2010a,b), our data suggest the DG is capable of expressing several, distinct forms of slow onset LTD as evidenced by the presence of both NMDA receptor dependent and independent variants.

AMPA receptors mediate fast glutamatergic neurotransmission via GluA1–4 receptor subunits (Traynelis et al. 2010) that enable ligand gated sodium, potassium, and, depending on the receptor subunit composition, calcium conductance (Swanson et al. 1997). Phosphorylation of GluA1 receptor subunits has been implicated in enhancing AMPA receptor conductance (Derkach et al. 1999; Kristensen et al. 2011; Jenkins and Traynelis 2012) and regulating synaptic plasticity (Lee et al. 2000, 2003, 2010). Previous studies in knockout mice lacking GluA1 receptor subunits suggest paired theta burst stimulation at Schaffer collateral-CA1 synapses results in both a GluA1-dependent, rapid onset LTP and a GluA1-independent, slow onset LTP (Hoffman et al. 2002; Romberg et al. 2009). Specifically, stimulation paradigms resulting in rapid onset LTP may reflect the phosphorylation of GluA1 subunits and the subsequent increase in AMPA receptor conductance (Derkach et al. 1999; Kristensen et al. 2011; Jenkins and Traynelis 2012), augmenting the depolarization kinetics of rapid onset LTP. In contrast, LFS may trigger a GluA1-independent, slow onset LTP via slower mechanisms such as incorporating pre-existing AMPA receptors in the postsynaptic density (Man et al. 2000; Malinow 2003; Hanley 2010). Thus observations of a rapid onset LTD may not preclude the parallel expression of an independent slow onset LTP (Habib and Dringenberg 2009). Nevertheless, the computational and functional differences between rapid and slow onset LTD remain undefined and warrants further study.

Similar to LTP occlusion studies in hippocampal area CA1 (Habib and Dringenberg 2010a), LFS-induced slow onset LTD at MPP-DG synapses is occluded by prior TBS-generated LTD in pentobarbital-anesthetized rats. LFS-induced LTD occlusion by prior HFS-induced LTP is thought to indicate both forms of LTD share a common expression mechanism (Habib and Dringenberg 2010a). However, it should be noted that LTD occlusion may also occur with DG activity-dependent modifications of subsequent plasticity induction efficacy and/or magnitude, a phenomenon known as metaplasticity (Abraham and Bear 1996; Abraham and Tate 1997; Christie and Abraham 1992b; Christie et al. 1995; Philpot et al. 1999; Derrick 2007). Specifically, prior synaptic activity that induces LTP may increase the threshold for a subsequent induction of LTD (Bienenstock et al. 1982; Abraham and Bear 1996; Abraham and Tate 1997; Philpot et al. 1999; Derrick 2007). Because such metaplastic effects would inhibit further potentiation, metaplasticity may limit the utility of LTD occlusion as a means of determining whether LTD generated by different induction protocols share a common expression mechanism.

Although LTD induction by prolonged LFS (900 pulses, 1 Hz) occur with less prevalence in adult animals (Dudek and Bear 1993; Kemp et al. 2000; Milner et al. 2004; Blaise and Bronzino 2003), paired-pulse LFS induces LTD at CA3-CA1 synapses in adult animals (Thiels et al. 1994; Kemp and Bashir 1997, 1999; Kemp et al. 2000). As we utilize adult animals, we attempted to induce PP-DG LTD in adult anesthetized rats with a paired-pulse LFS paradigm effective in eliciting LTD in adult CA1 hippocampal slices (Kemp et al. 2000). Consistent with prior examinations of short, low-frequency trains of paired PP stimuli on DG field EPSPs (Doyère et al. 1996), our data show PP paired-pulse LFS (900 paired stimuli, 200-ms paired-pulse interval) failed to induce homosynaptic LTD in the DG. Instead, paired-pulse LFS of the LPP resulted in rapid onset LTD of DG field potentials, whereas paired-pulse LFS of the MPP induced slow onset LTD of DG field potentials. Thus the subfields of the hippocampal formation demonstrate differences in synaptic plasticity induced by LFS (Abraham et al. 1996; Doyère et al. 1996; Lanté et al. 2006b; Fung et al. 2011).

Afferent stimulation intensity may serve to regulate the number of fibers that produce presynaptic action potentials and, by extension, the amount of postsynaptic depolarization and increase in postsynaptic calcium concentration, where the magnitude of postsynaptic calcium concentration regulates the induction of LTD or LTP at a given synapse (Lisman 1989; Mulkey and Malenka 1992; Bear and Malenka 1994; Coussens and Teyleur 1996; Yang et al. 1999). For instance, low afferent
stimulation intensity may activate fewer presynaptic axons resulting in moderate levels of postsynaptic depolarization and small increases in postsynaptic calcium concentration that result in LTD induction. Thus our observations of LTP following LFS in anesthetized animals may indicate that the postsynaptic depolarization was too great to induce LTD. We thus explored the hypothesis that subhalf-maximal afferent stimulus intensities optimize dentate LTD induction in anesthetized rats by producing decreased levels of postsynaptic depolarization and calcium influx (Lisman 1989; Mulkey and Malenka 1992; Bear and Malenka 1994; Christie et al. 1996; Coussens and Teyler 1996; Yang et al. 1999). Surprisingly, low-intensity LFS of the LPP or MPP failed to induce DG LTD in anesthetized rats, suggesting that even low-intensity LFS is insufficient to induce PP-DG LTD in anesthetized rats. In contrast, homosynaptic LTD was observed, albeit inconsistently, in the DG of awake, freely moving rats following LFS of PP afferents. Specifically, four out of six naive (non-injected) animals displayed significant reductions in DG responses following LFS of the LPP, and two out of six animals exhibited significant reductions in DG responses following LFS of the MPP. Interestingly, homosynaptic LTD induction was somewhat less reliable when a vehicle intraperitoneal injection preceded LFS in freely moving animals. The basis for the lower incidence of LTD expression in vehicle-treated animals vs. noninjected animals is unknown. Nonetheless, the possibility that stress of intraperitoneal injections may have suppressed LTD induction in vehicle-treated animals is unlikely given our observations of LTD in some recovered animals that were given intraperitoneal injections of pentobarbital anesthesia (see below).

Relative to the rare occurrence of LTD following acute electrode implantation in anesthetized rats, the greater incidence of homosynaptic PP-DG LTD in freely moving rats may result from either state-specific differences between anesthetized and behaving animals or the impaired LTD induction associated with surgery-related stress or tissue injury sustained during acute electrode implantation. These alternatives motivated our examinations of LFS-induced synaptic plasticity at PP-DG synapses in anesthetized rats with previously implanted chronic recording and stimulating electrodes. Although significant LTD was not observed in our averaged data obtained from LFS of the LPP in anesthetized rats with previously implanted electrodes, four out of six anesthetized animals exhibited LTD following LFS. These data suggest state-specific differences between anesthetized and behaving animals are unlikely to explain the absence of LTD when LFS experiments immediately followed acute electrode implantation in anesthetized animals. Interestingly, slow onset LTP was rarely observed following LFS in anesthetized animals with previously implanted electrodes. Thus anesthesia effects are unlikely to facilitate the routine expression of LFS-induced slow onset LTP following acute electrode implantation in anesthetized rats. As our data show animals with previously implanted electrodes demonstrate a higher incidence of PP-DG LTD and rarely express slow onset LTP following LFS, it is possible that surgery-related stress or tissue injury sustained during acute electrode implantation may simultaneously impair LTD induction and facilitate LTP expression at PP-DG synapses. However, numerous studies show behavioral stress facilitates LTD induction in the dorsal hippocampus (Xu et al. 1997; Xiong et al. 2004; Yang et al. 2005; Artola et al. 2006; Chauolloff et al. 2007; Titterness and Christie 2008; Maggio and Segal 2009; Niehusmann et al. 2010). Thus stress-related effects of handling, administration of anesthetic, acute surgery, and stereotaxic restraint would be expected to facilitate LTD induction and cannot explain the impaired LTD induction observed in our acute LFS experiments. As stress is known to suppress LTP in the dorsal hippocampus (Foy et al. 1987; Shors et al. 1989, 1990; Diamond and Rose 1994; Shors and Dryver 1994; Garcia et al. 1997; Pavlides et al. 2002; Maroun and Richter-Levin 2003; Vouimba et al. 2004; Xiong et al. 2004; Howland and Wang 2008), our observations of slow onset LTP are unlikely due to stress-related effects of acute surgery and stereotaxic restraint. Alternatively, transient tissue trauma that ensues electrode implantation may encompass a more plausible reason for the diminished occurrence of LTD and the routine expression of slow onset LTP in our acute LFS experiments. Specifically, injury imposed by electrode penetration may result in a local immune response enabling glial release of prostaglandins and proinflammatory cytokines that subsequently induces hyperexcitability and disrupts neural plasticity (Jankowsky et al. 2000; Yirimya and Goshen 2011). In addition to suppressing LTD induction, inflammation-induced hyperexcitability may prime the induction of slow onset LTP following LFS of the PP as with prior studies showing enhanced cellular excitability primes LTP induction (Cohen et al. 1999). Jankowsky et al. (2000) demonstrate proinflammatory cytokine increases are not evident when animals are evaluated 3 wk after permanent electrode implantation. Thus recovery and the eventual decrease release of prostaglandins and proinflammatory cytokine production following tissue recovery in animals with previously implanted electrodes may allow dentate LTD induction.

In vitro techniques and in vivo electrode insertion both generate tissue trauma and proinflammatory cytokines (Jankowsky et al. 2000). Nonetheless, PP-DG homosynaptic LTD is routinely observed in vitro (Trommer et al. 1995; O’Mara et al. 1995; Wang et al. 1997), whereas PP-DG homosynaptic LTD is not readily induced in vivo (Errington et al. 1995; Abraham 1996; Abraham et al. 1996; Doyère et al. 1996; Fung et al. 2011). As LTD is primarily observed in young animals (Dudek and Bear 1993; Kemp et al. 2000; Milner et al. 2004; Blaise and Bronzino 2003), one possible explanation for this disparity may stem from the use of younger animals (aged \( \leq 35 \) postnatal days old) in in vitro investigations (Trommer et al. 1995; O’Mara et al. 1995; Wang et al. 1997) compared with those of in vivo studies (Errington et al. 1995; Abraham et al. 1996; Doyère et al. 1996; Fung et al. 2011). Given prior demonstrations of age-dependent increases in proinflammatory cytokine levels (Takao et al. 1996; Murray and Lynch 1996; Ye and Johnson 1999), the younger tissue used to investigate DG LTD in vitro may have less susceptibility for trauma-related impairment of synaptic plasticity.

In summary, homosynaptic LTD expression in the DG is an inconsistent phenomenon observed primarily in freely moving and anesthetized animals with previously implanted electrodes. In contrast, following surgery and acute electrode implantation, anesthetized rats demonstrate LFS mediated slow-onset LTP at both MPP-DG and LPP-DG synapses that utilize distinct induction mechanisms. Together, these data demonstrate homosynaptic PP-DG LTD is observed more readily in recovered...
animals with previously implanted electrodes compared with animals with acute electrode surgery, suggesting surgery-related tissue trauma influence in vivo LTD incidence and LTP induction at PP-DG synapses. Future studies may evaluate whether homosynaptic LTD induction by LFS at LPP-DG and MPP-DG synapses is modulated by sleep-wake behavioral states, as with synaptic plasticity induced by high-frequency stimulation at PP-DG synapses (Bramham and Srebro 1989).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.G., I.S.M., D.M.V., and B.E.D. performed experiments; J.G. analyzed data; J.G. interpreted results of experiments; J.G. prepared Figs.; J.G. drafted manuscript; J.G., D.M.V., and B.E.D. edited and revised manuscript; J.G., I.S.M., D.M.V., and B.E.D. approved final version of manuscript; B.E.D. conception and design of research.

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