

Alternating Low Frequency Stimulation of Medial Septal and Commissural Fibers Induces NMDA-Dependent, Long-Lasting Potentiation of Hippocampal Synapses in Urethane-Anesthetized Rats

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ABSTRACT: Recent evidence indicates that some synapses exhibit long-lasting synaptic potentiation in response to low frequency (1 Hz) stimulation, similar to long-term potentiation (LTP) following high frequency induction protocols. Here, the authors characterize a form of long-lasting synaptic potentiation in the hippocampal CA1 area following alternating, single pulse stimulation of the medial septum (MS) and hippocampal CA3 commissural fibers (MS-H LTP). In urethane-anesthetized rats, alternating single pulse stimulation of the MS and CA3 (50 pulses each at 0.5 Hz, 1,000 ms interstimulus interval [ISI]) produced gradual increases of field excitatory postsynaptic potential (fEPSP) amplitude in CA1 (~123% of baseline), while MS or CA3 stimulation alone was ineffective. The fEPSP enhancement was long-lasting (>4h) and repeated episodes of alternating MS-CA3 stimulation tended to result in greater levels of potentiation than those elicited by a single episode. Surprisingly, ISIs of 500, 750, and 1,500 ms did not result in significant changes in fEPSP amplitude, while an ISI of 100 ms produced synaptic depression. MS-H LTP was resistant to systemic administration of nicotinic and muscarinic receptor antagonists (scopolamine, mecamylamine), but abolished by systemic MK-801 (0.5 mg/kg, i.p.) or local CA1 application of AP-5 (10 mM), indicative of a critical role of hippocampal NMDA receptors in this effect. Paired-pulse facilitation experiments revealed a gradually developing, significant inverse correlation between fEPSP enhancement and decrease in paired-pulse facilitation ratio, suggesting a role of changes in presynaptic transmitter release. Together, these data demonstrate a novel form of long-lasting synaptic enhancement in CA1 neurons in response to low frequency activity in separate afferent systems, an activity that might mimic some aspects of natural discharge patterns during the acquisition or consolidation of memory processes in hippocampal circuits. © 2008 Wiley-Liss, Inc.

KEY WORDS: LTP; NMDA receptor; cholinergic receptor; paired-pulse facilitation; Schaffer collaterals/commissural fibers

INTRODUCTION

The hippocampus receives diffuse fiber inputs from basal forebrain areas that have been shown to contribute to learning and memory proc-

esses (Hasselmo, 1999; Hasselmo and Giocomo, 2006). The input to the hippocampal formation from the medial septum (MS) is comprised of at least three distinct, parallel neurochemical pathways, consisting of cholinergic, GABAergic and glutamatergic fibers (Dutar, 1977; Dutar et al., 1995; Sotty et al., 2003). While all three fiber systems likely contribute to cellular activity and plasticity of hippocampal neurons, to date, the majority of work has examined the influence of cholinergic and GABAergic inputs to the hippocampus. Cholinergic fibers from the MS to the hippocampus are known to facilitate excitability and long-lasting plasticity (e.g., long-term potentiation, LTP) induction at hippocampal synapses *in vitro* and *in vivo* (Krnjević and Ropert, 1982; Markram and Segal, 1990; Huerta and Lisman, 1993; Auerbach and Segal, 1994; Leung et al., 2003; Ovsepian et al., 2004; Dringenberg et al., 2008), effects that can be mediated by both muscarinic (Huerta and Lisman, 1993; Leung et al., 2003; Ovsepian et al., 2004; Dringenberg et al., 2008) or nicotinic receptor mechanisms (Fujii et al., 1999; Matsuyama et al., 2000; Ji et al., 2001). These and similar investigations have provided support for the notion that hippocampal plasticity/LTP is enhanced in the presence of elevated acetylcholine release.

GABAergic inputs from the MS to the hippocampal formation have also been shown to modulate hippocampal excitability. For example, electrical stimulation of the MS markedly enhances CA1 population spikes evoked by Schaffer collateral/commissural stimulation through a disinhibitory, GABAergic mechanism (Krnjević et al., 1988; Ovsepian, 2006). These indirect influences on pyramidal cell excitability could provide an additional mechanism to modulate long-lasting plasticity at hippocampal synapses.

More recently, electrophysiological and anatomical studies have raised the possibility that septohippocampal projections might also release glutamate as a transmitter substance in the hippocampus (Sotty et al., 2003; Colom et al., 2005; Bland et al., 2007). At present, the role of this putative projection system in the regulation of hippocampal activity has not been examined. Nevertheless, the data summarized above clearly support the notion that different hippocampal afferents originating in the MS play an important role in modulating activity and plasticity processes at hippocampal synapses.

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The majority of studies examining hippocampal plasticity and its modulation by extra-hippocampal systems have used typical LTP protocols that require high frequency stimulation or theta burst stimulation of glutamatergic afferents to induce long-lasting changes in synaptic efficacy (e.g., Larson et al., 1986; Larson and Lynch, 1988; Huerta and Lisman, 1993; Schulz et al., 1994; Leung et al., 2003; Ovsepian et al., 2004). In contrast, low frequency stimulation (LFS) produces minimal or no LTP, or may result in long-term depression (LTD) (Bramham and Srebro, 1987; Christie and Abraham, 1992; Christie et al., 1994; Thiels et al., 1994; Wasling et al., 2002; Massey and Bashir, 2007). However, recent evidence indicates that some synapses can show long-lasting synaptic potentiation in response to LFS (1 Hz). Lanté et al. (2006a,b) showed that, in hippocampal slices, 1 Hz stimulation of the CA1 area produces a slow-onset synaptic potentiation in close proximity to the stimulation site, an effect that was not observed in CA3. Similarly, Huang and Kandel (2007) demonstrated a slow-onset, protein synthesis-dependent potentiation of synaptic efficacy at cortico-amygdala synapses following 1 Hz stimulation, which required coactivation of glutamatergic and neuromodulatory (serotonergic, dopaminergic, and noradrenergic) signals. Collectively, these data suggest that, in specific fiber systems, the initiation of LTP-like processes can occur in the absence of high frequency activation of glutamatergic synapses.

At present, the extent to which lower frequency inputs are effective in inducing synaptic potentiation in different forebrain systems, and the contributions of neuromodulatory signals to such forms of plasticity, are not well understood. Here, we characterize a form of long-lasting hippocampal synaptic plasticity in the CA1 area following alternating, single pulse stimulation (0.5 Hz) of the MS and Schaffer collateral/commissural pathway *in vivo* (MS-H LTP). Surprisingly, MS-H LTP is resistant to both muscarinic and nicotinic receptor antagonists, but is abolished by hippocampal NMDA receptor blockade. These experiments demonstrate that long-lasting synaptic enhancement can be induced by repeated, infrequent coactivation of separate afferent systems to the hippocampal CA1 area *in vivo*.

MATERIALS AND METHODS

Subjects and Surgical Preparation

All experiments were performed on male Long-Evans rats (320–450 g) housed in a colony room (12-h/12-h reverse light cycle) with food and water available *ad libitum*. The procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care (CCAC) and approved by the Queen's University Animal Care Committee (QUACC). Each rat was used for only one experiment.

Experimental procedures were conducted under deep urethane anesthesia (1.5 g/kg, intraperitoneal [i.p.], administered in three 0.5-g/kg doses every 20 min and supplemented with 0.2–0.6 g/kg as required prior to the onset of data collection).

Rats were placed in a stereotaxic apparatus and body temperature was maintained between 36°C and 37°C by means of an electrical heating blanket. An incision was made to expose the skull surface and small skull holes were drilled above the CA3 region (AP, –4.16; ML, –3.0; V –4.0), the contralateral CA1 area (for recordings in stratum radiatum: AP, –4.16; ML, +3.0; V, –3.0; for stratum oriens: AP, –4.30; ML, –3.0; V –2.5), and at bregma point (AP, 0.0; ML, 0.0; V –6.5) to gain access to the MS. Skull holes for ground and reference electrodes (jewellery screws attached to miniature connectors) were made in the bone overlying the prefrontal cortex and cerebellum, respectively. An additional skull hole for a septal stimulation return electrode (jewellery screw) was drilled adjacent to bregma. All stereotaxic measurements were based on the anatomical work of Paxinos and Watson (1998). Final ventral placements of the CA3 stimulation and the CA1 recording electrodes were adjusted to elicit maximal amplitude field excitatory postsynaptic potentials (fEPSPs) and paired-pulse facilitation (100-ms interstimulus interval, ISI) in area CA1 in response to contralateral CA3 stimulation.

Electrophysiology

Stimulation of CA3 (0.2 ms pulses every 30 s, intensity adjusted to yield 50–60% of maximal fEPSP amplitude) was provided by a concentric bipolar electrode (Rhodes Medical Instruments Series 100, David Kopf, Tujunga, CA, USA) connected to a stimulus isolation unit providing a constant current output (PowerLab/16 s system with ML 180 Stimulus Isolator, AD Instruments, Toronto, Canada). MS stimulation was provided through a monopolar electrode (Teflon insulated stainless steel, 125- μ m tip diameter) delivering single, negative constant-current pulses (0.2 ms duration, 1.0 mA).

Typically, fEPSPs were recorded in stratum radiatum of CA1, but some animals had a second electrode placed in stratum oriens. All fEPSPs were differentially recorded (Teflon insulated stainless steel wire, 125- μ m tip diameter) against a reference electrode placed in the bone overlying the cerebellum. The CA1 signals were amplified, filtered (0.3–1 kHz), digitized (10 kHz), and stored for subsequent off-line analysis using the PowerLab system running Scope software (v. 4.0.2).

In a subset of animals, paired-pulse stimuli (0.2 ms pulse duration, 50 ms ISI, every 30 s, intensity adjusted to yield 50–60% of maximal fEPSP amplitude) were used throughout the experiment, rather than single test pulses, as described above.

Experimental Procedures

Prior to formal data collection, input–output curves were established for each animal by recording fEPSPs in CA1 in response to CA3 stimulation between 0.1 and 1.0 mA (0.1 mA increments). Based on these input–output curves, a stimulation intensity eliciting between 50% and 60% of the maximal fEPSP amplitude was chosen for the subsequent experiment. For each rat, 60 initial baseline fEPSPs (every 30 s) were recorded. Subsequently, the MS-CA3 alternating stimulation protocol was delivered, consisting of 50 single pulses to both

the MS and CA3 area, delivered at 0.5 Hz, with the ISI between successive MS and CA3 pulses ranging from 100 to 1,500 ms. Typically, recordings of fEPSPs in CA1 (every 30 s, as in baseline) in response to single pulse CA3 stimulation continued for 2 h following the alternating stimulation protocol. However, in a subset of animals, recordings continued for a total of 4 h. Further, in an additional group of animals, the effect of repeated episodes of alternating MS-CA3 stimulation was examined to assess the saturation point of synaptic potentiation. We also studied the effects of repeated single pulse stimulation (50 pulses at 0.5 Hz, as above) of either the MS or CA3 alone to examine if coactivation of these areas is required to elicit synaptic potentiation.

For experiments assessing the role of cholinergic (muscarinic and nicotinic) and NMDA receptors in synaptic changes elicited by alternating MS-CA3 stimulation (using an ISI of 1000 ms), different groups of rats received scopolamine (5 mg/kg, i.p.), mecamylamine (5 mg/kg, i.p.) or MK-801 (0.5 mg/kg, i.p.). All drugs (obtained from Sigma/Research Biochemicals, Oakville, Ontario, Canada) were dissolved in saline and administered 30 min prior to the commencement of baseline fEPSP recordings. In cases when antagonists produced significant changes to MS-CA3 induced synaptic enhancement, additional experiments were performed to verify that drugs did not exert significant, suppressant effects on baseline (i.e., nonpotentiated) fEPSPs.

Local drug application in CA1 was performed using a reverse microdialysis probe (Mab. 2.14.4; 2-mm active polyethersulfane membrane length, 35-kDa cutoff; S.P.E. Ltd, North York, ON, Canada) placed immediately adjacent to the recording electrode, with the probe tip extending about 1 mm below the tip of the electrode. The dialysis probe was connected to a Hamilton microsyringe driven by a microdialysis pump (CMA/102, CMA Microdialysis, Solna, Sweden) and continuously perfused with artificial cerebral spinal fluid (aCSF) (1.0 μ l/min) composed of (in mM): 118.3 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 NaH₂PO₄, 2.5 CaCl₂, 10.0 glucose, 22.1 NaHCO₃. For the purpose of NMDA receptor blockade, aCSF containing DL-amino-5-phosphonopentanoic acid (AP-5) (10 mM, Sigma/RBI) was applied. Reverse dialysis allows local drug application by means of concentration-driven diffusion of drug molecules through the dialysis probe membrane into the surrounding neural tissue, with estimated drug concentrations outside the membrane reaching approximately 10% of aCSF concentrations (e.g., Oldford and Castro-Alamancos, 2003).

At the end of each experiment, rats were perfused through the heart with 10% formalin, their brains were extracted, and standard histological techniques were used to verify all electrode placements. Data obtained with inaccurate placements were excluded from the data analysis.

Data Analysis

All data are expressed as a mean \pm standard error of mean (SEM). The maximal fEPSP amplitude was analyzed offline by the Scope software. Subsequently, amplitude data were averaged

over 10-min intervals, and these averages were normalized by dividing all data for each rat by the average baseline (pre-MS-CA3 alternating stimulation) amplitude of that animal. Paired-pulse facilitation (PPF) ratio is expressed as the maximum amplitude of the second fEPSP divided by the amplitude of the first fEPSP in a pair. The change in PPF ratio over time was calculated as that PPF average in the last 30 min of the total recording period minus the initial PPF ratio during baseline (prior to alternating MS-CA3 stimulation). For statistical analyses, analyses of variance (analysis of variance (ANOVA)) and, where statistically appropriate, simple effects tests were computed using the software package CLR ANOVA (v.1.1, Clear Lake Research, Inc., Houston, TX). A linear regression test was used for correlational analyses.

RESULTS

Alternating Single Pulse Stimulation of the MS and CA3 Elicits an Increase in fEPSP Amplitude in CA1 (MS-H LTP)

Single pulses applied to area CA3 produced positive-going fEPSPs (latency to peak about 10–11 ms) recorded in the contralateral stratum radiatum of the CA1 area, while fEPSPs recorded in stratum oriens consisted of phase-reversed, negative-going potentials (see Fig. 1). These fEPSP characteristics are equivalent to those previously reported as apical and basilar dendritic fEPSPs on CA1 pyramidal cells (Leung et al., 2003; Ovsepian et al., 2004). The data reported below refer to recordings taken in stratum radiatum, except where noted otherwise.

In rats receiving only CA3 stimulation throughout the experiment ($n = 8$; single test pulses every 30 s plus 50 single pulses at 0.5 Hz during the induction phase), fEPSP amplitude in stratum radiatum remained relatively stable throughout the entire recording period (Fig. 1; average fEPSP amplitude of $105 \pm 4.0\%$ of baseline during the last 30 min of the experiment). However, alternating single pulses applied to the MS and CA3 ($n = 14$; a total of 50 pulses at 0.5 Hz for each, starting with MS and followed by CA3 stimulation, 1,000 ms ISI) produced a gradual, significant increase of fEPSP amplitude over the 2 h period following MS-CA3 stimulation (Fig. 1; average fEPSP amplitude of $123 \pm 4.5\%$ of baseline during the final 30 min). In a subset of these experiments ($n = 4$), the stability of synaptic enhancement was assessed by continuing fEPSP recordings over a 4-h period after induction. In these experiments, enhancement was maintained for the entire recording period (average of 120% of baseline between 3.5 and 4 h following induction; data not shown). In an additional control group, stimulation of the MS (50 pulses at 0.5 Hz; $n = 6$) in the absence of CA3 pulses during induction did not elicit significant changes in fEPSP amplitude (104% of baseline during the last 30 min; simple effect of time, $F_{14,70} = 1.202$, $P = 0.2,942$; data not shown).

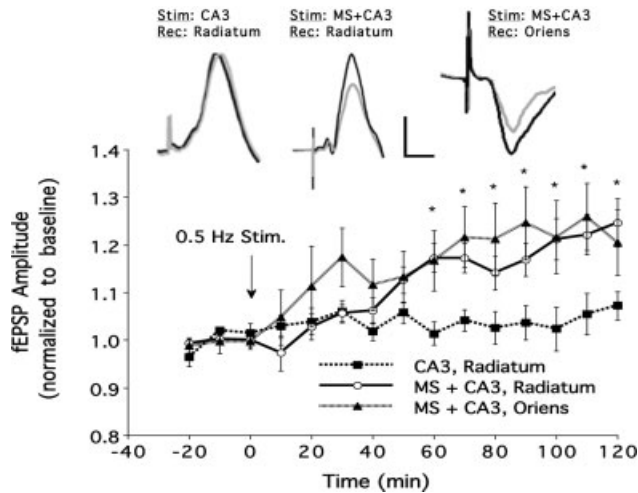


FIGURE 1. The effects of alternating medial septum (MS)-CA3 stimulation on amplitude of field excitatory postsynaptic potentials (fEPSPs) recorded in CA1 of urethane-anesthetized rats. Alternating single pulse stimulation (Alternating Stim., at arrow) of the MS and CA3 area (a total of 50 pulses at 0.5 Hz for both, starting with MS and followed by CA3 stimulation, 1,000 ms interstimulus interval) resulted in a gradual increase in fEPSP amplitude in both strata radiatum ($n = 14$) and oriens ($n = 7$) over the 2 h period following alternating stimulation. Single pulses applied to CA3 in the absence of MS stimulation failed to induce synaptic enhancement in stratum radiatum (50 single pulses to CA3, 0.5 Hz; $n = 8$). Inserts depict typical fEPSPs during baseline (gray) and at the end of the experiment (black) in animals receiving MS and CA3 stimulation (Stim.) for recordings (Rec.) in stratum radiatum (middle), stratum oriens (right), as well as control animals receiving only CA3 stimulation (left, recordings in stratum radiatum). Note the enhanced fEPSP amplitude in rats receiving the alternating protocol (fEPSPs are averages of 10 individual sweeps, calibration is 10 ms and 1 mV). Statistics for MS + CA3, radiatum group vs. CA3, radiatum group: effect of group, $F_{1,20} = 6.2$, $P = 0.02$; effect of time, $F_{14,280} = 8.3$, $P < 0.0001$; group-by-time interaction, $F_{14,280} = 4.9$, $P < 0.0001$; simple effect of time significant for animals receiving MS-CA3 stimulation ($F_{14,350} = 15.0$, $P < 0.0001$) but not for animals receiving CA3 stimulation alone ($F_{14,98} = 1.2$, $P = 0.276$). * denote significant ($P < 0.05$) group differences. Stratum oriens group also showed a significant effect of time, $F_{14,84} = 4.28$, $P < 0.0001$, and the level of potentiation did not differ from that seen in the stratum radiatum group, $F_{1,19} = 0.69$, $P = 0.415$.

A potentiation effect very similar to that in stratum radiatum was also observed for fEPSPs recorded in stratum oriens ($n = 7$), where MS-CA3 stimulation again elicited a gradually developing increase in fEPSP amplitude to a maximum of 123% of baseline during the last 30 min of the experiment (Fig. 1). Thus, these experiments demonstrate that MS-H LTP constitutes a long-lasting form of synaptic potentiation that requires coactivation of medial septal and CA3 fibers to elicit enhancement of synaptic responses of CA1 neurons.

Next, we examined the effects of changing the ISI between alternating MS-CA3 pulses (delivered at 0.5 Hz, as above, all ISIs refer to interval between the initial MS pulse and the subsequent CA3 pulse) during induction. Surprisingly, ISIs of 500 ($n = 4$), 750 ($n = 5$), and 1,500 ms ($n = 6$) did not result in

significant changes of fEPSP amplitude relative to baseline, while an ISI of 100 ms ($n = 5$) produced significant synaptic depression (Fig. 2). Thus, relatively strict temporal requirements apply to the induction of MS-H LTP, with ISIs of about 1,000 ms apparently required in order to elicit this form of synaptic potentiation.

In an attempt to examine whether repeated episodes of alternating MS-CA3 stimulation can elicit greater levels of potentiation than those seen with a single induction episode, we assessed the effect of multiple MS-CA3 induction episodes, delivered at 2 h intervals (Fig. 3; two induction episodes over 4 h, $n = 15$, three induction episodes over 6 h, $n = 5$). Amplitude of fEPSPs averaged over 1.5 to 2 h after each of the first, second, and third induction episodes were 15, 31, and 31%, respectively (Fig. 3, insert). Consequently, two induction episodes resulted in the maximal enhancement of synaptic responses of CA1 neurons.

MS-H LTP Is Dependent on NMDA, But Not Cholinergic Receptor Activation

Systemic administration of the NMDA receptor antagonist MK-801 (0.5 mg/kg, i.p., given 30 min prior to the onset of baseline recordings; $n = 6$) completely prevented synaptic potentiation elicited by MS-CA3 alternating stimulation (Fig. 4). In fact, it appeared as if the alternating stimulation protocol resulted in synaptic depression after MK-801 treatment, even though this effect did not reach statistical significance (see Fig. 4 legend for statistics). Control experiments showed that, in

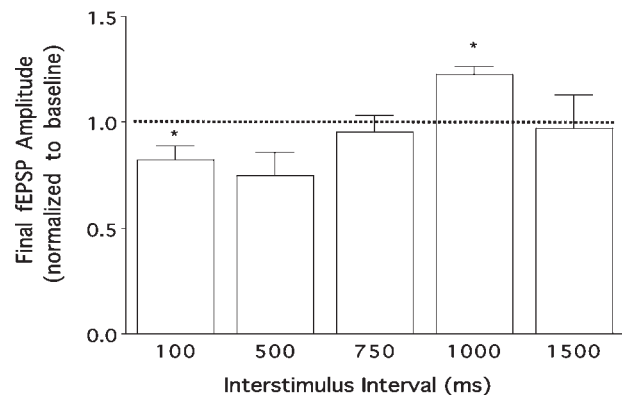


FIGURE 2. The effects of various interstimulus intervals (ISIs) between alternating stimulation of the medial septum (MS) and CA3 area on amplitude of CA1 field excitatory postsynaptic potentials (fEPSPs) averaged between 1.5 and 2 h following the alternating stimulation. ISIs of 100 ms ($n = 5$) and 1,000 ms ($n = 14$) resulted in significant synaptic depression and enhancement, respectively. All other ISIs (500 ms, $n = 4$; 750 ms, $n = 5$; 1,500 ms, $n = 6$) did not induce significant changes in fEPSP amplitude. Statistics: effect of time comparing fEPSP amplitude values averaged over the 30 min baseline period and the last 30 min of recording (i.e., 1.5–2 h following induction), 100 ms, $F_{1,4} = 19.6$, $P = 0.01$; 500 ms, $F_{1,3} = 5.4$, $P = 0.102$; 750 ms, $F_{1,4} = 0.4$, $P = 0.592$; 1,000 ms, $F_{1,13} = 26.8$, $P = 0.0002$; 1,500 ms, $F_{1,5} = 0.04$, $P = 0.856$. * denotes significant changes from baseline fEPSP amplitude.

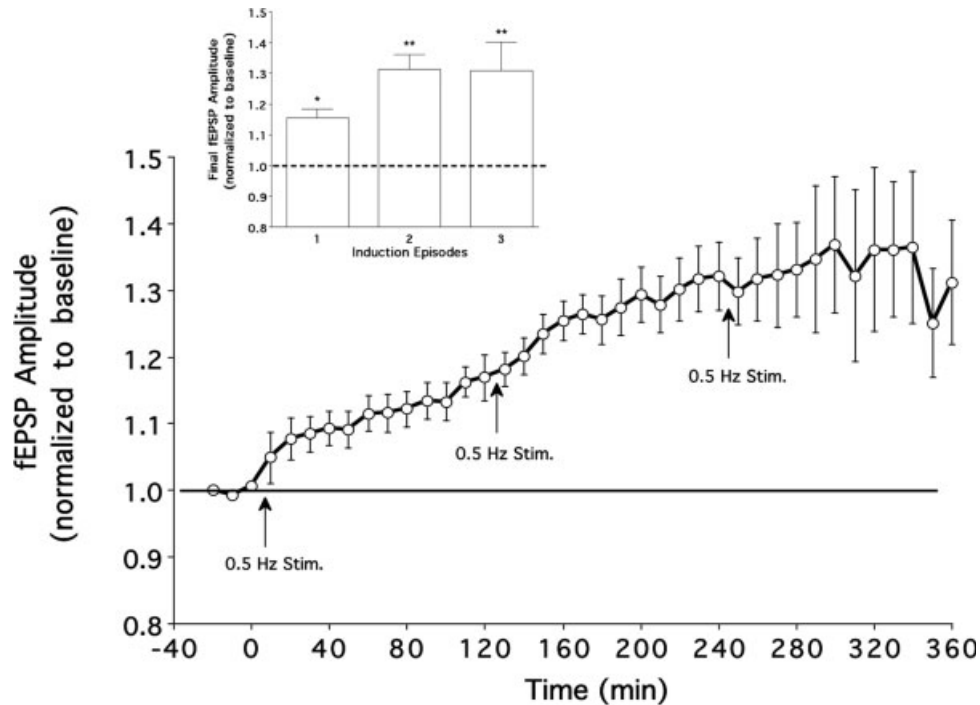


FIGURE 3. The effects of multiple induction episodes of alternating stimulation of the medial septum (MS) and CA3 area on amplitude of CA1 field excitatory postsynaptic potentials (fEPSPs). Repetition of the alternating MS-CA3 stimulation protocol (at arrows) every 2 h (50 MS-CA3 single pulse stimulations, 1,000 ms interstimulus interval, 0.5 Hz per induction) enhanced fEPSP amplitude to 115, 131, and 131% of baseline during the last half

hour following one, two, and three induction episodes, respectively. Statistics: effect of time for 4 h, $F_{26,234} = 13.5$, $P < 0.001$, $n = 15$; 6 h, $F_{38,152} = 8.4$, $P < 0.001$, $n = 5$); Insert shows fEPSP amplitude averaged over 1.5–2 h following each of the three induction episodes; * and ** indicate significant differences ($P < 0.05$) from baseline and baseline plus one induction episode, respectively.

rats receiving only CA3 stimulation without additional MS stimuli ($n = 4$), MK-801 administration did not significantly suppress fEPSP amplitude over time ($F_s < 0.8$, $P > 0.7$ for effects of time, group, and interaction comparing rats receiving only CA3 stimulation without drug treatment vs. MK-801; data not shown). Thus, nonspecific depressant effects on CA3-CA1 transmission during the course of the experiment do not account for the inhibition of synaptic enhancement following MK-801 treatment.

Subsequently, local CA1 application of aCSF ($n = 5$) or aCSF containing DL-AP-V (10 mM, $n = 5$) by means of reverse microdialysis was performed to further characterize the role of NMDA receptors. In the presence of continuous aCSF at the CA1 recording site, alternating MS-CA3 produced a gradual, significant increase of fEPSP amplitude over the 2 h recording period, consistent with the data reported above (Fig. 4; average fEPSP amplitude of 120% of baseline during the final 30 min). In contrast, application of AP-V to CA1 completely blocked the enhancement of fEPSP amplitude following alternating MS-CA3 stimulation (average fEPSP amplitude of 93% of baseline during the final 30 min of the experiment). Application of AP-V resulted in a small, nonsignificant suppression of baseline fEPSP amplitude (97%) relative to aCSF application.

Further experiments were conducted to assess the effects of systemic cholinergic receptor blockade (Fig. 5). Surprisingly,

enhancement of fEPSP amplitude elicited by MS-CA3 alternating stimulation was not significantly affected by administration of mecamylamine (5 mg/kg, i.p., administered 30 min prior to onset of baseline recordings, $n = 9$) or scopolamine (5 mg/kg, same administration regime, $n = 7$). Thus, it appears that muscarinic or nicotinic receptor stimulation is not required for the enhancement of synaptic strength seen in these experiments.

Enhancement of fEPSP Amplitude Is Associated With Decreases in PPF Ratio

In order to assess the relative contributions of presynaptic and postsynaptic changes in fEPSP enhancement following MS-CA3 alternating stimulation, we examined changes in PPF ratio (two test pulses throughout the experiment, 50 ms ISI, $n = 10$) in a subset of animals that received two MS-CA3 induction episodes 2 h apart. In these experiments, fEPSP amplitude increased to 115% and 127% of baseline for the first and second induction episodes, respectively, while PPF ratio was 104% and 93% of the baseline ratio at these time points (Fig. 6A). The raw PPF ratios were 1.89 during baseline, 1.97 following one induction episode, and 1.76 following the second induction episode, calculated as amplitude of the second fEPSP/amplitude of the first fEPSP in a pair (all values are averages over the last 30 min of the 2-h period following each of the two

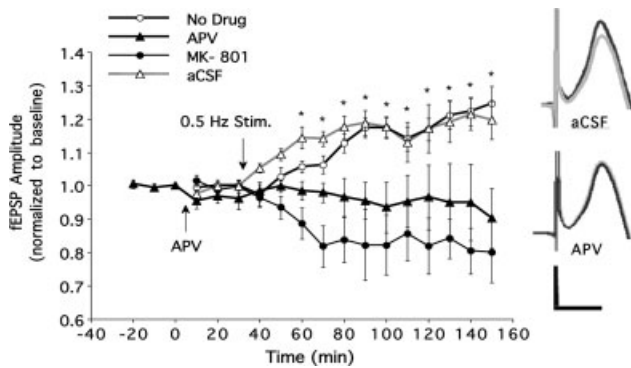


FIGURE 4. The effect of MK-801 and AP-V treatment on changes in field excitatory postsynaptic potential (fEPSP) amplitude following alternating medial septum (MS)-CA3 stimulation. Systemic administration of MK-801 (0.5 mg/kg, i.p., $n = 6$) 30 min prior to onset of baseline recordings prevented synaptic enhancement during the 2-h period following alternating MS-CA3 stimulation (at arrow; 50 single MS-CA3 pulses at 0.5 Hz, 1,000 ms interstimulus interval). No drug group ($n = 14$) is same as in Figure 1. In the presence of artificial cerebrospinal fluid (aCSF, $n = 5$, applied in CA1 by reverse microdialysis), alternating MS-CA3 stimulation resulted in a gradual increase in fEPSP amplitude that was not significantly different from the no drug group. In contrast, local application of AP-V in CA1 (10 mM, $n = 5$) completely blocked the increase in fEPSP amplitude elicited by MS-CA3 stimulation. Inserts depict fEPSPs during baseline (gray) and at the end of the experiment (black) for an animal receiving CA1 application of aCSF (top) or AP-V (bottom). Note the blockade of synaptic potentiation in the rat receiving AP-V (fEPSPs are averages of 10 individual sweeps, calibration is 10 ms and 0.5 mV). Statistics: MK-801 vs. No Drug, effect of group, $F_{1,18} = 16.7$, $P = 0.0007$; effect of time, $F_{14,252} = 1.5$, $P = 0.331$; group-by-time interaction, $F_{14,252} = 7.6$, $P < 0.0001$; simple effect of time for MK-801 treated rats, $F_{14,252} = 1.3$, $P = 0.182$. AP-V vs. aCSF, effect of group, $F_{1,8} = 8.9$, $P = 0.017$; effect of time, $F_{14,112} = 2.4$, $P = 0.860$; group-by-time interaction, $F_{14,112} = 2.4$, $P = 0.005$; simple effect of time for aCSF, $F_{14,56} = 6.8$, $P < 0.0001$; AP-V, $F_{14,56} = 0.2$, $P = 0.998$, * indicates significant ($P < 0.05$) difference between aCSF and AP-V groups.

induction episodes). Correlation analyses of these data revealed that there was no significant relation between fEPSP amplitude and PPF measures after one induction episode (Fig. 6B). However, there was a significant, inverse correlation between the fEPSP enhancement and PPF ratio after the second induction episode, i.e., the final 30 min of the experiment (Fig. 6C). At this time point, changes in PPF ratio accounted for 46% of the total enhancement of fEPSP amplitude induced by alternating MS-CA3 stimulation (see Fig. 6 legend for statistics).

DISCUSSION

The majority of studies examining plasticity in the hippocampal formation have used LTP protocols that require high frequency stimulation of glutamatergic afferents to induce long-lasting changes in synaptic efficacy. Here, we characterize a novel form of long-lasting synaptic plasticity, present in both

strata radiatum and oriens of CA1, elicited by alternating, LFS of the MS and CA3 area (MS-H LTP). MS-H LTP has a slow onset, can be saturated with repeated induction episodes, and appears to be optimal at unusually long (1,000 ms) ISIs between successive MS and CA3 stimuli. Pharmacological experiments demonstrate a critical role of hippocampal NMDA receptor activation, but apparently no critical involvement of cholinergic mechanisms with the induction protocol used here. Interestingly, data from the paired-pulse experiments suggest that MS-H LTP is comprised of two processes, with the initial synaptic enhancement occurring in the absence of changes in PPF ratio, while later enhancement is related to a decrease in PPF ratio. Consequently, initial postsynaptic changes might be followed by changes in transmitter release from terminals of presynaptic CA3 neurons. To the best of our knowledge, these experiments are the first to describe long-lasting enhancement at CA3-CA1 hippocampal synapses using low frequency, alternating stimulation of two separate afferent systems in an intact *in vivo* preparation.

Typically, enhancement of hippocampal synapses is induced by various forms of high frequency or θ -burst stimulation protocols, while LFS (e.g., 1 Hz) generally results in synaptic depression (Malenka and Bear, 2004; Massey and Bashir, 2007; see also Thiels et al., 1994). However, recent evidence has shown that some synapses exhibit long-lasting potentiation in response to LFS protocols. Lanté et al. (2006a,b) showed that,

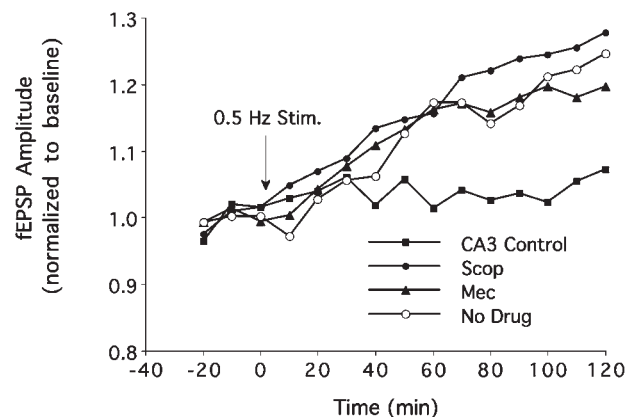


FIGURE 5. The effects of scopolamine and mecamylamine on enhancement in field excitatory postsynaptic potential (fEPSP) amplitude in CA1 following medial septum (MS)-CA3 alternating stimulation. Enhancement of fEPSP amplitude elicited by MS-CA3 stimulation (at arrow, same parameters as in Fig. 1) was resistant to administration of scopolamine (Scop., 5 mg/kg, i.p., $n = 7$) or mecamylamine (Mec., 5 mg/kg, i.p., $n = 9$). Both drugs were administered 30 min before the onset of baseline recordings. Statistics: ANOVA comparing mecamylamine and untreated rats, effect of group, $F_{1,21} = 0.003$, $P = 0.962$, effect of time, $F_{14,294} = 20.2$, $P < 0.001$, group-by-time interaction, $F_{14,294} = 0.4$, $P = 0.962$; scopolamine vs. untreated rats, effect of group, $F_{1,19} = 1.0$, $P = 0.34$, effect of time, $F_{14,266} = 20.1$, $P < 0.001$, group-by-time interaction, $F_{14,266} = 0.5$, $P = 0.922$; simple effects of time significant for untreated rats and rats receiving scopolamine or mecamylamine, $F > 8.0$, $P < 0.001$; No Drug group and CA3 control group are same as in Figure 1; error bars omitted for clarity.

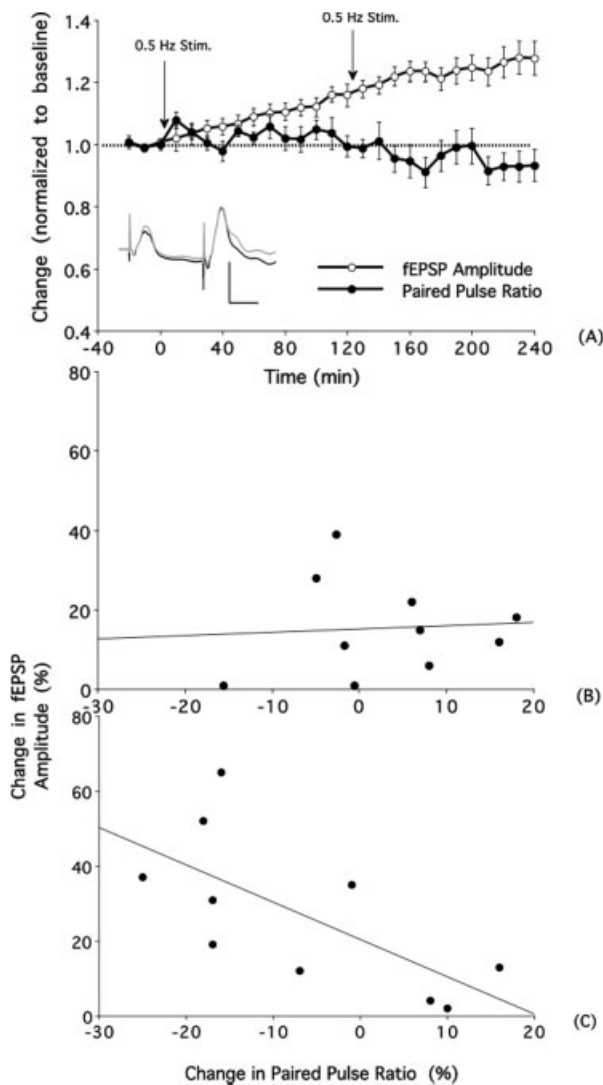


FIGURE 6. Changes in paired-pulse facilitation (PPF) ratio and field excitatory postsynaptic potential (fEPSP) amplitude in CA1 following medial septum (MS)-CA3 alternating stimulation. (A) Two episodes of MS-CA3 stimulation (at arrows, same parameters as in Fig. 1) resulted in a significant enhancement of fEPSP amplitude, and a significant decrease in the PPF ratio ($n = 10$, paired-pulses delivered throughout the experiment, 50 ms interstimulus interval, data are a subset of animals shown in Fig. 3). Inserts depict fEPSPs during baseline (black) and at the end of the experiment (gray). Note the increase in fEPSP amplitude in response to the first stimulation pulse, accounting for the decrease in PPF ratio (fEPSPs are averages of 10 individual sweeps, calibration is 20 ms and 1 mV). (B) Correlation between change in fEPSP amplitude and PPF ratio after one induction episode (values averaged between 1.5 and 2 h following the first induction; PPF ratio change = final PPF ratio minus initial PPF ratio (averaged during baseline)/initial PPF ratio $\times 100$). (C) Correlation between changes in fEPSP amplitude and PPF ratio 1.5–2 h after the second induction episode (data calculated as in panel B). Statistics: (A) fEPSP amplitude, effect of time, $F_{26,234} = 13.5$, $P < 0.001$; PPF ratio, effect of time, $F_{26,234} = 2.2$, $P = 0.001$; (B) The best-fit linear regression line is plotted, $y = 15.065 + 0.079776x$, $r = 0.07$, $r^2 = 0.005$; (C) $y = 20.312 - 0.99828x$, $r = -0.68$, $r^2 = 0.46$, $P = 0.03$.

in hippocampal slices, 1 Hz stimulation of the CA1 area applied for 5 min produced a gradual, slow-onset synaptic potentiation of local CA1 synapses in close proximity to the stimulation site. This enhancement of fEPSPs occurred in area CA1, but not CA3 or dentate gyrus (DG), and was independent of NMDA receptor activation (Lanté et al., 2006a,b). Similarly, Huang and Kandel (2007) described a slow-developing, NMDA-independent form of synaptic potentiation following 1 Hz stimulation of cortico-lateral amygdala fibers in vitro, a form of LTP that requires the coactivation of glutamatergic and various neuromodulatory inputs including serotonin, dopamine and noradrenalin. Together, these findings suggest that long-lasting synaptic potentiation can be elicited by low frequency stimulation, results that are intriguing since low frequency activity mimics some aspects of spontaneous oscillatory patterns present in hippocampal neurons (Vanderwolf, 1969; Buzsáki et al., 1983; Buzsáki, 1989).

Interestingly, while previous work suggests that hippocampal and amygdaloid LTP elicited by LFS stimulation is not dependent on NMDA receptor activation (Lanté et al., 2006a,b; Huang and Kandel, 2007), our findings indicate that MS-H LTP does not occur in the presence of the NMDA receptor antagonist MK-801 (systemic) or AP-5 (locally applied in CA1). Consequently, it appears that multiple receptor and signaling mechanisms can trigger LTP-like synaptic enhancement in response to low frequency inputs. Based on our data, we speculate that the repeated coactivation of septal and commissural inputs constitutes an effective mechanism of NMDA receptor activation in CA1. Electrophysiological and anatomical studies have provided evidence for glutamatergic projections from the MS to the hippocampal formation (Sotty et al., 2003; Colom et al., 2005), which may play a role in cellular synchrony and generation of the hippocampal theta rhythm (Bland et al., 2007) independent of cholinergic and GABAergic projections. Whether this glutamatergic projection system plays a role in the induction of MS-H LTP is unknown, even though this hypothesis is consistent with the lack of effectiveness of high doses of cholinergic (muscarinic and nicotinic) receptor antagonists to suppress this form of synaptic potentiation. Clearly, for the induction protocol used here, engagement of cholinergic fibers between MS and CA1 is not a critical prerequisite for MS-H LTP to occur, even though it is possible that changes to the protocol might reveal potential cholinergic contributions to MS-H LTP. Work by Straube and Frey (2003) has shown that the relative contributions of NMDA and other, neuromodulatory signals to the induction of heterosynaptic LTP depend on strength of the induction protocol, with weaker protocols showing greater dependence on neuromodulatory LTP reinforcement (see also Huang and Kandel, 2007). Whether changes to the stimulation parameters used for the alternating MS-CA3 protocol reveal an involvement of cholinergic mechanisms remains to be determined.

To us, the most surprising finding of these experiments was the fact that unusually long ISIs (1,000 ms) between consecutive septal and CA3 stimuli were required to elicit MS-H LTP. This time window is well beyond those normally assumed to be effective in inducing NMDA receptor-dependent types of

synaptic strengthening such as LTP and spike-time dependent plasticity (Larson and Lynch, 1988; Bennett, 2000; Dan and Poo, 2004; Malenka and Bear, 2004; Bi and Rubin, 2005; Yao and Dan, 2005; see also Massey and Bashir, 2007). However, there are some types of synaptic facilitation that exhibit fairly broad temporal windows for the integration of separate synaptic inputs to trigger NMDA-dependent plasticity. Popescu et al. (2007) characterized a form of heterosynaptic facilitation induced by pairing of corticostriatal and amygdala inputs onto single, striatal medium spiny neurons in vitro. Long-lasting (>30 min) potentiation following repeated pairing of these convergent inputs was NMDA-dependent and also showed a surprisingly broad time window between cortical and amygdaloid stimuli of up to 500 ms. Thus, there is growing evidence that some forms of NMDA-dependent plasticity exhibit highly unconventional temporal dynamics with regard to the integration of synaptic inputs that are effective in eliciting long-lasting potentiation. The fact that we observed significant synaptic depression at short (100 ms) ISIs between MS and CA3 pulses is consistent with other reports showing that low frequency, paired stimulation protocols are an effective means to induce LTD at commissural-CA1 synapse (Thiels et al., 1994).

To date, the relative contributions of presynaptic and postsynaptic mechanisms to different forms of hippocampal LTP continue to remain controversial (Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Nicoll and Malenka, 1999; Maruki et al., 2001; Brecht and Nicoll, 2003; Malenka and Bear, 2004). We analyzed changes in the ratio of PPF, a form of short-term plasticity primarily associated with successively increasing calcium levels in the presynaptic terminal, resulting in an increased probability of transmitter release (Katz and Miledi, 1968; Foster and McNaughton, 1991; Schulz et al., 1994, 1995). A number of in vitro studies have demonstrated a relation between LTP magnitude and changes in PPF ratio, indicative of presynaptic contributions to LTP (Voronin and Kuhnt, 1990; Schulz et al., 1995; Schulz, 1997; Schulz and Fitzgibbons, 1997). Similarly, the data from our paired-pulse analysis indicated that, during the later phases of the experiment, there was an inverse correlation between the change in PPF ratio and the magnitude of synaptic enhancement. This correlation developed gradually during the course of the experiment since it was not present during the first 2 h following the initial induction episode. These data indicate that changes in presynaptic functioning contribute to MS-H LTP. Given that rats used for the PPF experiments received two separate induction episodes, it remains to be determined whether the change in PPF ratios are the result of the repeated alternating stimulation given to these animals, or whether one induction episode is sufficient to engage presynaptic mechanisms. It is of interest to note, however, that the distinct temporal dynamics of changes in PPF ratios noted here fit well with recent evidence regarding the role of presynaptic and postsynaptic plasticity loci in LTP expression. Bayazitov et al. (2007) showed that, for LTP at CA3-CA1 synapses in vitro, presynaptic changes during the early phase are minimal, but become more prominent during the later phase of LTP (>1 h post induction). These experi-

ments also indicate that NMDA receptor activation is crucial for the postsynaptic component, while voltage gated calcium channels are necessary for the expression of the delayed, presynaptic component of 'compound LTP'. Finally, Bayazitov et al. (2007) showed that the cAMP-protein kinase A cascade is necessary for the presynaptic changes associated with compound LTP, either by acting directly in presynaptic terminals to enhance transmitter release, or by influencing the production of a retrograde messenger in the postsynaptic cells. Interestingly, the cAMP-protein kinase A cascade also appears to be critical for plasticity induced by low frequency stimulation (LFS) in the hippocampus (Lanté et al., 2006a) and amygdala (Huang and Kandel, 2007). Whether any of these mechanisms are involved in MS-H LTP remains to be determined.

In summary, the present experiments describe a novel form of synaptic enhancement elicited by alternating, low frequency, single pulse stimulation of CA3 and medial septal fibers. This form of LTP is NMDA-receptor dependent, apparently does not require activation of cholinergic receptors, and may involve temporally distinct, presynaptic and postsynaptic mechanisms following induction. These data reinforce recent findings that long-lasting synaptic enhancement can occur in response to low frequency activity patterns, which might mimic some aspects of natural neuronal oscillations present during phases of acquisition or consolidation of memory processes (e.g., Buzsáki, 1989).

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