Enhanced Synaptic Transmission in CA1 Hippocampus After Eyeblink Conditioning

JOHN M. POWER, LUCIEN T. THOMPSON, JAMES R. MOYER, JR., AND JOHN F. DISTERHOFT
Department of Cell and Molecular Biology, Northwestern University Medical School, Chicago, Illinois 60611

Power, John M., Lucien T. Thompson, James R. Moyer, Jr., and John F. Disterhoft. Enhanced synaptic transmission in CA1 hippocampus after eyeblink conditioning. *J. Neurophysiol.* 78: 1184–1187, 1997. CA1 field potentials evoked by Schaffer collateral stimulation of hippocampal slices from trace-conditioned rabbits were compared with those from naive and pseudoconditioned controls. Conditioned rabbits received 80 trace conditioning trials daily until reaching a criterion of 80% conditioned responses in a session. Hippocampal slices were prepared 1 or 24 h after reaching criterion (for trace-conditioned animals) or after a final unpaired stimulus session (for pseudoconditioned animals); naive animals were untrained. Both somatic and dendritic field potentials were recorded in response to various stimulus durations. Recording and data reduction were performed blind to the conditioning state of the rabbit. The excitatory postsynaptic potential slope was greater in slices prepared from trace-conditioned animals killed 1 h after conditioning than in naive and pseudoconditioned controls (repeated-measures analysis of variance, $F = 4.250$, $P < 0.05$). Associative learning specifically enhanced synaptic transmission between CA3 and CA1 immediately after training. This effect was not evident in the population field potential measured 24 h later.

INTRODUCTION

The hippocampus plays an essential role in the learning of certain spatial and temporal associative tasks, with hippocampal lesions producing severe deficits (Squire 1987). One model of associative learning is eyeblink conditioning, in which a conditioned stimulus (CS) is followed by an unconditioned stimulus (US) that induces an eyeblink. After repeated pairings, the subject exhibits conditioned responses (CRs), blinks after CS onset before US onset. Although the hippocampus is not required for all forms of eyeblink conditioning (Thompson 1986), when the CS and US are separated by a 500-ms trace (no stimulus) interval, hippocampectomized rabbits fail to acquire the eyeblink conditioning task (Moyer et al. 1990; Solomon et al. 1986).

Hippocampal synaptic enhancement may underlie the acquisition of conditioned eyeblink responses. Multiunit studies have demonstrated a learning-dependent increase in the firing of CA1 and CA3 neurons that models the time course and amplitude of the CR and is not seen in unpaired controls (Berger et al. 1976; Solomon et al. 1986). Additionally, perforant-path-evoked field potentials in dentate gyrus are enhanced during delay conditioning (Weisz et al. 1984). In vivo experiments, however, cannot eliminate extrahippocampal factors and localize changes to the hippocampus.

In vitro experiments, which can localize changes to the hippocampus, also suggest that synaptic alterations underlie eyeblink conditioning. High-frequency Schaffer collateral stimulation evoked larger summating excitatory postsynaptic potentials (EPSPs) in CA1 pyramidal neurons from conditioned rabbits (LoTurco et al. 1988). [^{3}H] α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) binding in CA1, CA3, and dentate gyrus subfields is increased after conditioning (Tocco et al. 1992). In the present study, Schaffer collateral evoked synaptic responses in slices from trace-conditioned rabbits are compared with those in naive and pseudoconditioned controls at intervals where maximal postsynaptic changes have been observed.

METHODS

Subjects and surgeries

Animal care was approved by the US Department of Agriculture and managed by Northwestern University's Animal Care and Use Committee. Behavioral treatment and slice preparation methods were identical to those previously described (Moyer et al. 1996; Thompson et al. 1996). Forty-six young (1.5 mo) female rabbits (New Zealand White) received either trace conditioning or pseudoconditioning or were naive. Trace-conditioned rabbits received 80 pairings daily of a 100-ms tone CS, followed by a 500-ms trace interval, then a 150-ms air puff, until reaching a criterion of 80% CRs in a given session. Pseudoconditioned subjects received an equal number of unpaired stimuli for a matched number of training sessions.

Slice preparation

Transverse hippocampal slices (400 μm) were prepared from rabbits 1 or 24 h after training and held in artificial cerebrospinal fluid (aCSF, composition, in mM: 124 NaCl, 26 $NaHCO_3$, 10 D-glucose, 3 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, and 1.24 NaH_2PO_4, pH 7.4, 23°C). All recordings and analyses were performed blind to the animal's conditioning state.

Electrophysiology

Extracellular recordings were made 1–6 h after slice preparation, during the period of optimal slice health (Green and Greenough 1986). Slices with crisp, well-defined cell layers were perfused with 31°C oxygenated aCSF in a submersion slice chamber. Field potentials were evoked by Schaffer collateral stimulation with the use of 75-μm twisted stainless steel bipolar electrodes (250 kΩ) aligned with the end of the dentate gyrus (see Fig. 1). Field potentials were recorded in both stratum radiatum and stratum pyramidale with a 1- to 5-MΩ glass electrode filled with 2 M NaCl, positioned 750 μm from the stimulating electrode. Slices were stimulated (100 μs, 100 μA) every 30 s until evoked potentials stabilized (usually 15–20 min). Slices were discarded if a 200-μs, 100-μA stimulation evoked a somatic field potential with population spike amplitude <1.5 mV or exhibited multiple low-ampli-

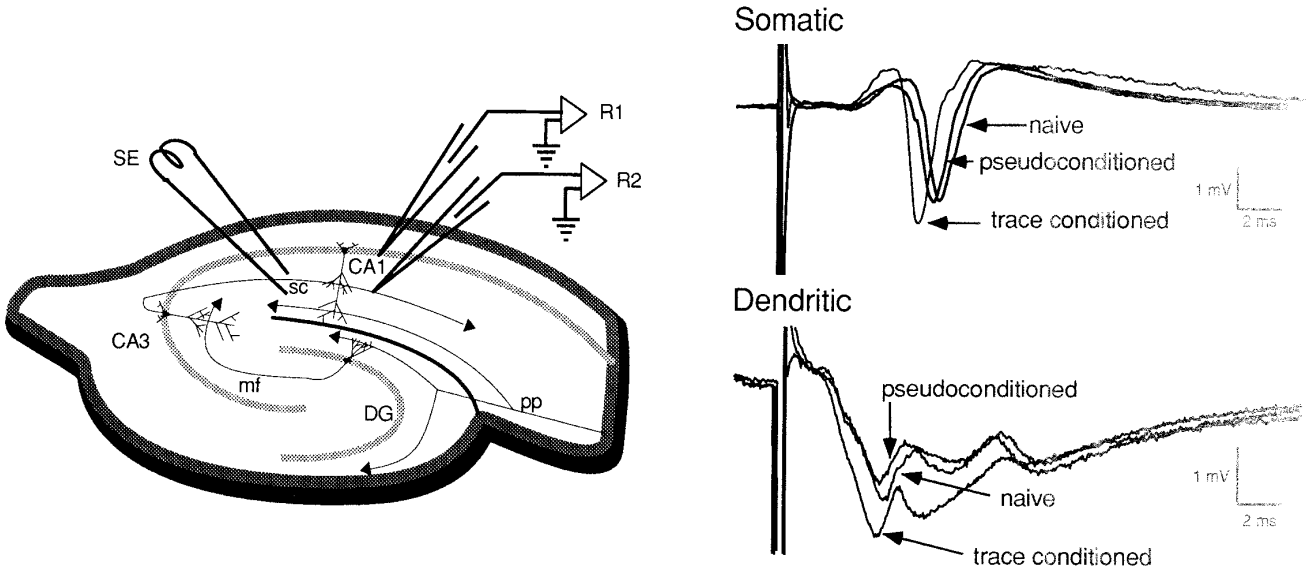

FIG. 1. Diagram of a rabbit hippocampal slice depicting locations of stimulating electrode (SE) and recording electrodes (R1, R2) and representative somatic (R1) and dendritic (R2) field potentials 1 h after conditioning. Schaffer collaterals (sc), perforant path (pp), dentate gyrus (DG), mossy fibers (mf), CA1, and CA3 are labeled for clarity.

tude epileptic form responses. A stimulus-response function was generated by evoking four potentials with 100-μA current at different stimulus durations (ranging from 20 to 600 μs). To control for drift, field potentials were evoked with the use of a fixed 100-μs, 100-μA stimulus both before and after determination of stimulus-response function. Slices were excluded if the pre- versus posttest field potentials differed by $\geq$15%.

Data analysis

Evoked potentials were digitized (10–20 kHz) and analyzed with the use of custom software. Somatic field potentials measured population spike amplitude and latency. The EPSP slope of dendritic field potentials measured synaptic current. Recordings were made from three to five slices per animal. Measurements at each stimulus duration were averaged for a given animal and used in subsequent statistical analysis. Repeated-measures analyses of variance (Abacus Concepts, Statview) were performed with the use of behavioral treatment as a factor. Significant effects ($\alpha = 0.05$) were additionally analyzed with the use of Fisher's Protected Least Significant Difference (PLSD) post hoc tests.

RESULTS

Conditioning specifically enhanced synaptic potentials at intervals shortly after rabbits reached behavioral criterion. The initial EPSP slopes of dendritic potentials from conditioned animals killed 1 h after the final conditioning session were greater [$F(4,41,7) = 3.119$, $P = 0.0250$, see Fig. 2, *A* and *B*] than the slopes from naive (Fisher's PLSD, $P < 0.02$) and pseudoconditioned controls (1 h, $P < 0.005$; 24 h, $P < 0.01$). There were no significant differences between naive and pseudoconditioned control animals (either 1 or 24 h) or between conditioned and control animals killed 24 h after conditioning.

It has been suggested that slices exhibiting the largest responses were likely to be the healthiest, and the most representative of the in vivo condition (Green and Greenough 1986). When data from the slice exhibiting the largest responses were chosen to represent the animal, the results were unchanged [$F(4,41,7) = 2.705$, $P < 0.05$; Fisher's PLSD: trace 1 h, naive, $P < 0.025$; trace 1 h, pseudo 1 h, $P < 0.015$; trace 1 h, pseudo 24 h, $P < 0.02$; trace 1 h: trace 24 h, $P > 0.15$). No significant differences in the population spike amplitude were seen between groups [$F(4,41,7) = 3.119$, $P \geq 0.25$, see Fig. 2, *C* and *D*), although a trend was seen toward a conditioning-associated enhancement of the population spike amplitude 1 h after conditioning. There were no significant differences in spike latency, nor was there any correlation between field measurements and time of recording after slice preparation.

DISCUSSION

These results demonstrate an enhancement of CA1 Schaffer collateral synaptic transmission, intrinsic to the hippocampus, as a result of learning a hippocampally dependent task. The lack of pseudoconditioning effects indicates that the synaptic enhancement was due to a learned association between the CS and US, not simply stimulus presentations. Similar behaviorally induced synaptic potentiation in hippocampus has been reported in vivo in rats during and after exploratory learning (Moser et al. 1994), tone-shock conditioning (Laroche et al. 1987), operant conditioning (Skelton et al. 1987), and environmental enrichment (Sharp et al. 1985), and in vitro after environmental enrichment (Green and Greenough 1986).

Possible mechanisms for the observed synaptic enhancement include changes in the following.

1) Synaptic structure. Synaptic remodeling (e.g., increasing perforated synapses with multiple transmission zones) (Geinisman 1993) could contribute to an enhanced EPSP.

2) Transmitter release or binding. Increased glutamate release from hippocampal slices has been reported after paired tone shock conditioning (Laroche et al. 1987). Additionally, enhanced AMPA (Tocco et al. 1992) and *N*-methyl-

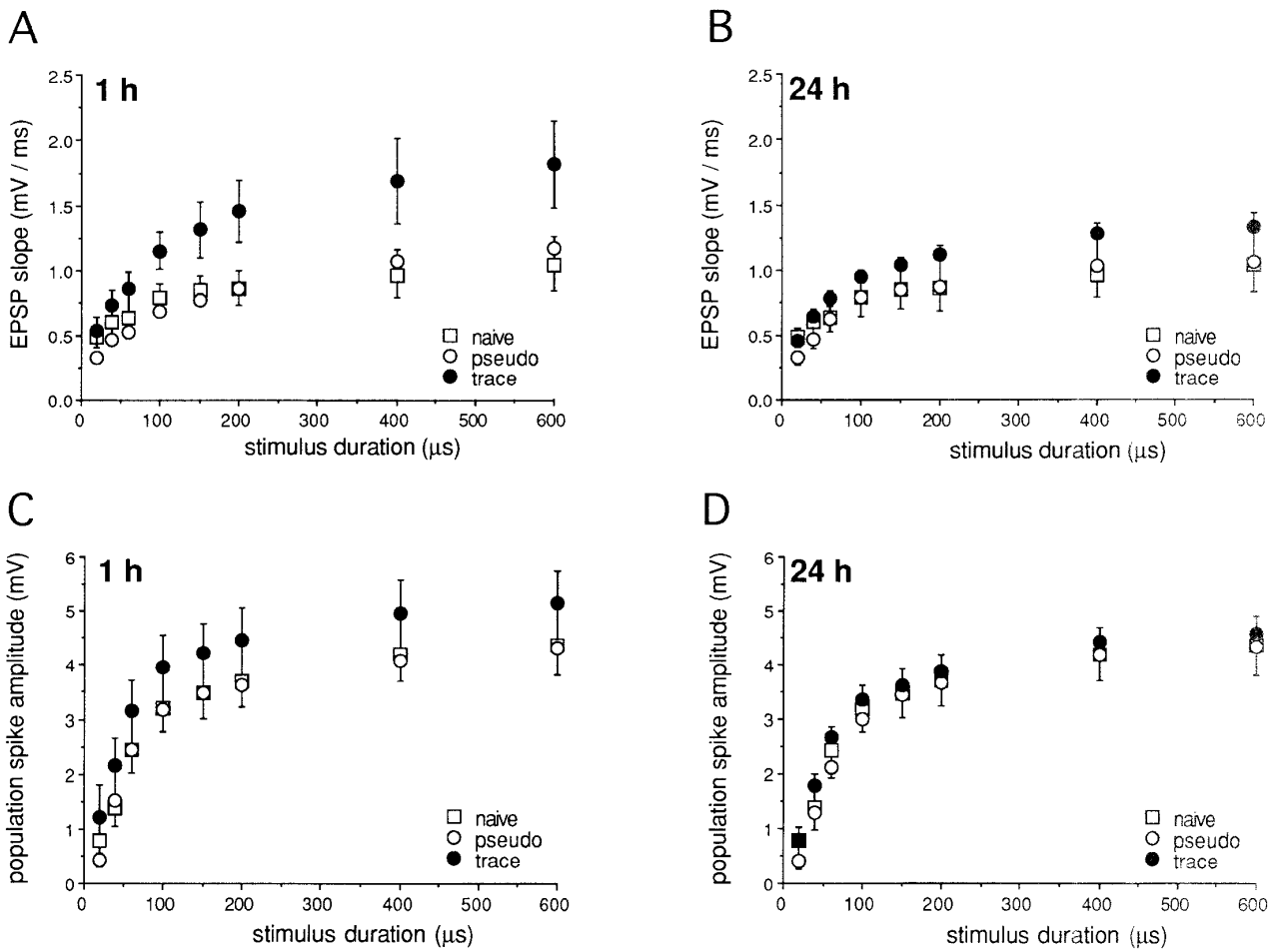

FIG. 2. Effects of conditioning on Schaffer collateral evoked field potentials recorded in CA1. Means ± SE are given for trace-condititioned (1 h, $n = 9$; 24 h, $n = 13$), pseudoconditioned (1 h, $n = 9$; 24 h, $n = 8$), and naive ($n = 7$) animals for each stimulus intensity value. *A*: excitatory postsynaptic potential (EPSP) slope recorded in dendrites was greater in slices prepared from conditioned animals 1 h after conditioning. *B*: no significant differences in initial EPSP slope were seen between conditioning groups 24 h after conditioning. No conditioning effect was seen in population spike amplitude input-output function 1 h (*C*) or 24 h (*D*) after conditioning.

D-aspartate (Stewart et al. 1992) receptor binding has been demonstrated after learning. It is unlikely that the synaptic potentiation observed here is analogous to long-term potentiation (LTP) at the Schaffer collateral CA1 synapses. Unless depotentiated, LTP can last for days (Bliss and Lomo 1973). The synaptic potentiation observed here diminished within 24 h. In addition, we observed no potentiation of the spike beyond that predicted from the enhancement of the EPSP (E-S potentiation) after learning, often present in LTP (Andersen et al. 1980).

3) Postsynaptic conductances. Postsynaptic conductance changes contributing to the observed synaptic enhancement would likely be preferentially localized to the apical dendrites, because similar changes in somatic conductance would further enhance the population spike, and E-S potentiation was not observed. We have previously reported postsynaptic reductions in postburst afterhyperpolarization (AHP) and in spike-frequency accommodation after trace conditioning (e.g., Moyer et al. 1996; Thompson et al. 1996). Unlike the synaptic enhancement seen here, AHP and spike-frequency accommodation changes were maximal up to 24 h after the final conditioning session and persisted for several days. The time course of the current generating the slow AHP in CA1 pyramidal neurons suggests that AHP changes do not directly contribute to short-latency changes in the EPSP slope. However, the AHP reduction may have facilitated the synaptic enhancement. Pharmacological blockade of the AHP can convert the potentiation resulting from a weak tetanus from a short-term to a more sustained potentiation (Sah and Bekkers 1996).

None of the proposed mechanisms are mutually exclusive, and further work is needed to fully examine these and other possibilities. The transient learning-specific enhancement of dendritic field potentials that we observed in CA1 appears to be different both from the previously reported AHP and accommodation changes and from LTP. We hypothesized that the AHP reductions could act as an adjustable gain control, amplifying specific synaptic inputs (Moyer et al. 1996; Thompson et al. 1996). Thus enhancement of the CA1 Schaffer collateral synapses may amplify inputs from CA3 neurons. Our data would be consistent with a process in which generalized synaptic alterations (observable in population field potentials) occurred early during learning, and later became localized to a much more specific pattern or

set of synapses (not observable in population field potentials). The specific pattern of synaptic alterations may include both potentiation and depression that obscure each other in population measures but may play important roles in the learning process.

This work was supported by National Institutes of Health Grants 1F31 MH-10826, R01 DA-07633, R01 MH-5734, R01 MH-52409, and R01 AG-08796.

Present address of J. R. Moyer: Dept. of Psychology, Yale University, New Haven, CT 06520.

Address for reprint requests: J. M. Power: Dept. of Cell and Molecular Biology, Northwestern University Medical School, 303 East Chicago Ave., Chicago, IL 60611-3008.

Received 13 December 1996; accepted in final form 13 March 1997.

REFERENCES

ANDERSEN, P., SUNDBERG, S. H., SVEEN, O., SWAN, J. W., AND WIGSTRÖM, H. Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea pigs. *J. Physiol. (Lond.)* 302: 463–482, 1980.

BERGER, T. W., ALGER, B., AND THOMPSON, R. F. Neuronal subrate of classical conditioning in the hippocampus. *Science* 192: 438–485, 1976.

BLISS, T.V.P. AND LOMO, T. Long-term potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)* 232: 331–356, 1973.

GEINISMAN, Y. Perforated synapses with multiple, completely partitioned transmission zones: probable structural intermediates in synaptic plasticity. *Hippocampus* 3: 417–434, 1993.

GREEN, E. J. AND GREENOUGH, W. T. Altered synaptic transmisssion in dentate gyrus of rats reared in complex environments: evidence from hippocampal slices maintained in vitro. *J. Neurophysiol.* 55: 739–750, 1986.

LAROCHE, S., ERRINGTON, M. L., LYNCH, M. A., AND BLISS, T.V.P. Increase in [^{3}H] glutamate release from slices of dentate gyrus and hippocampus following classical conditioning in the rat. *Behav. Brain Res.* 25: 23–29, 1987.

LOTURCO, J. J., COULTER, D. A., AND ALKON, D. L. Enhancement of synaptic potentials in rabbit CA1 pyramidal neurons following classical conditioning. *Proc. Natl. Acad. Sci. USA* 85: 1672–1676, 1988.

MOSER, E. I., MOSER, M., AND ANDERSEN, P. Potentiation of dentate synapses initiated by exploratory learning in rats: dissociation from brain temperature, motor activity, and arousal. *Learn. Memory* 1: 55–73, 1994.

MOYER, J. R., JR., DEYO, R. A., AND DISTERHOFT, J. F. Hippocampectomy disrupts trace eyeblink conditioning in rabbits. *Behav. Neurosci.* 104: 243–252, 1990.

MOYER, J. R., JR., THOMPSON, L. F., AND DISTERHOFT, J. F. Trace eyeblink conditioning increases CA1 excitability in a transient and learning specific manner. *J. Neurosci.* 16: 5536–5546, 1996.

SAH, P. AND BEKKERS, J. M. Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. *J. Neurosci.* 16: 4537–4542, 1996.

SHARP, P. E., MCNAUGHTON, B. L., AND BARNES, C. A. Enhancement of hippocampal field potentials in rats exposed to a novel, complex environment. *Brain Res.* 339: 361–365, 1985.

SKELTON, R. W., SCARTH, A. S., WILKIE, D. M., MILLER, J. J., AND PHILLIPS, A. G. Long-term increases in dentate cell responsivity accompany operant conditioning. *J. Neurosci.* 7: 3081–3087, 1987.

SOLOMON, P. R., VANDER SCHAAF, E. R., THOMPSON, R. F., AND WEISZ, D. J. Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behav. Neurosci.* 100: 729–744, 1986.

STEWART, M. G., BOURNE, R. C., AND STEELE, R. J. Quantitative autoradiographic demonstration of changes in binding to NMDA-sensitive [^{3}H]glutamate and [^{3}H]MK801, but not [^{3}H]AMPA receptors in chick forebrain 30 min after passive avoidance training. *Eur. J. Neurosci.* 4: 936–943, 1992.

SQUIRE, L. *Memory and Brain.* New York: Oxford Univ. Press, 1987.

THOMPSON, L. T., MOYER, J. R., JR., AND DISTERHOFT, J. F. Transient changes in excitability of rabbit CA3 neurons with a time course appropriate to support memory consolidation. *J. Neurophysiol.* 76: 1836–1849, 1996.

THOMPSON, R. F. The neurobiology of learning and memory. *Science.* 233: 941–947, 1986.

TOCCO, G., ANNALA, A. J., BAUDRY, M., AND THOMPSON, R. F. Learning of hippocampal-dependent conditioning task changes the binding properties of AMPA receptors in rabbit hippocampus. *Behav. Neural Biol.* 58: 222–231, 1992.

WEISZ, D. J., CLARK, G. A., AND THOMPSON, R. F. Increased responsivity of dentate granule cells during nictitating membrane response conditioning in rabbit. *Behav. Brain Res.* 12: 145–154, 1984.