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An Analysis of the Expression Locus of Long-term Potentiation in Hippocampal CA1 Neurons*

Shane M. Roach, *Student Member*, Ude Lu, *Member*, Dong Song, *Member*, and Theodore W. Berger, *Fellow, IEEE*

***Abstract*— Long-term potentiation (LTP) has long been understood as an increase in the potency of a synaptic connection between two neurons. In this study, we combine a previously developed two-stage cascade model with electrophysiological recordings of rat hippocampal CA1 pyramidal cells both before and after LTP to analyze linear and nonlinear contributions of pre and post-synaptic partners to the strengthening of their synaptic connectivity. The result suggests that the major nonlinear expression locus of LTP exists in the post-synaptic side. Additionally, the report reveals that LTP should be understood not only in the traditional view as a change in the magnitude of communication between two cells, but also as a change in their temporal coding properties of information exchange.**

I. Introduction

Under certain stimulation conditions, the synaptic pathway between neurons can be reinforced indefinitely such that a postsynaptic neuron will exhibit stronger responses to presynaptic stimulation (Fig. 1). This phenomenon is known as long-term potentiation (LTP) and has been shown to exist at many synapse types and have varying modes of expression [1], [2]. Along with other forms of synaptic plasticity, LTP is widely held to be the basis for learning and memory in the brain [3]. LTP has been the focus of great interest and study since its discovery and large advances towards understanding the molecular and functional mechanisms behind it are continually being made. It has also been the center of an extended debate as to which component of the synapse is responsible for the potentiating effect. As new techniques shed ever finer light on the contributing mechanisms from both sides of the synapse, the surplus of information has revealed no unifying theory of LTP agency, and it is quite possible that different circuits employ separate pre or postsynaptic modes of LTP expression [4]. Thus a nonparametric method to separate and quantify LTP contribution would be highly useful for characterizing individual synapses.

A second area of application for such a method would be the nonlinear effects of LTP. A common approach has been to view LTP as simply an increase in magnitude of the postsynaptic response. This perspective considers only the changes observed for isolated, single pulse responses. Neurons and synapses, however, encode information via a temporal transformation from incoming to outgoing spike-trains [5], and thus it is essential to look at higher-order temporal effects such as paired- and triple-pulse effects in order to understand the full consequence of synaptic plasticity.

This study applies a previously reported two-stage cascade model to the widely studied CA3 Schaffer collateral to CA1 pyramidal cell synapses of the hippocampus in order to divide presynaptic expressions of LTP from postsynaptic contributions in a quantifiable way. The cascade model utilizes a non-parametric modeling methodology that takes higher-order effects into consideration and provides intuitive representations of single-, paired-, and triple-pulse responses.

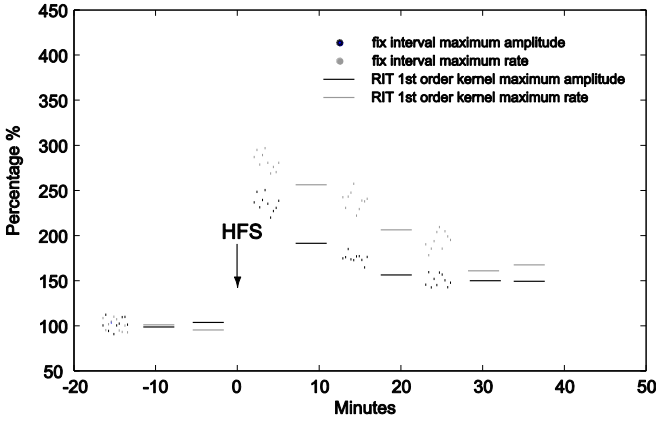

Figure 1. Single pulse responses show an increase in magnitude due to high frequency stimulation in hippocampal CA1 cells.

II. Materials and Methods

A. Electrophysiology

Whole-cell patch clamp recordings were performed on 400 μm thick hippocampal slices from four-week-old Sprague-Dawley rats. Slices were prepared in iced sucrose cutting solution using standard procedures. In addition, the

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Shane M. Roach is with the Department of Neuroscience, University of Southern California, Los Angeles, CA 90089 USA (phone: 213-740-8063; fax: 213-740-8061; e-mail: shaneroa@usc.edu).

Ude Lu is with the Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089 USA (e-mail: ulu@usc.edu).

Dong Song is with the Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089 USA (e-mail: dsong@usc.edu).

Theodore W. Berger is with the Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089 USA (e-mail: berger@bmsr.usc.edu).

Schaffer collaterals were surgically separated from the CA3 cell bodies to prevent spontaneous firing of CA3 axons. Slices were sustained for recording with a perfusion of room-temperature artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 Glucose, 1 $MgSO_4$, 2 Ascorbic Acid, and 2 $CaCl_2$ with a pH of 7.4, osmolarity of 295 mOsm/L, and aerated with a 95% O_2, 5% CO_2 gas mixture. During recording, the same ACSF was used with an addition of 20μM picrotoxin to block inhibitory $GABA_A$ channels.

A bipolar stimulating electrode with a minimal gap was placed on the surface of the stratum radiatum over the Schaffer collaterals using visual cues. A whole-cell patch was achieved on CA1 pyramidal cells using 4 MΩ tip resistance glass micro pipettes with an internal solution of (in mM): 150 K-SO_3, 10 HEPES, and 2 Mg-ATP with a pH of 7.3 and an osmolarity of 290 mOsm/L. The input-output properties of the patched neuron were calibrated via increasing amplitude, isolated stimulations in five second intervals. Once the IO curve was established, the stimulation intensity with a 3 mV peak excitatory post-synaptic potential (EPSP) was utilized for the duration of the protocol in accordance with the two-stage cascade model requirements.

B. LTP Induction and Recording Protocol

In order to utilize the two-stage cascade model, a given cell's EPSP and excitatory post-synaptic current (EPSC) for the same random-interval train (RIT) stimulation must be recorded both before and after LTP induction. The stimulation protocol outlined in Fig. 2 was applied in order to achieve this goal. For EPSP recordings, the current was clamped at baseline leakage levels (cells with leakage > 200 nA were rejected) and a Poisson distributed random-interval train (RIT) of 400 spikes with a 2 Hz mean frequency and 10-4500 ms inter-spike interval (ISI) was evoked in the Schaffer collaterals. EPSCs were recorded with the voltage clamped at -15mV below threshold and the same RIT that was recorded for the previous EPSP was repeated under voltage clamp. Fixed interval stimulation trains of five seconds were run in between RIT recordfing sets in order to monitor the neuron health and verify baseline leakage and resting voltage levels.

After recording EPSPs and EPSCs for two different RITs, LTP was induced via high frequency stimulation (HFS) of 100 Hz for 1 second. Five-second-fixed-interval trains were run every 10 minutes until 30 minutes had elapsed after LTP induction in order to monitor LTP stabilization. Once potentiation had stabilized, the post-LTP RITs were run observing the same protocol as the pre-LTP RITs. An extra set of data for each RIT was obtained if the cell maintained leakage levels for the duration of the recording.

C. Two-Stage Cascade Model Analysis

In order to separate and quantify the pre and post-synaptic contributions to LTP, we employed a previously developed two-stage cascade input-output model [6]. This model utilizes two sets of third-order Volterra kernels to capture the nonlinear dynamics of synaptic transmission. The first kernel set uses EPSC data to estimate the impulse response in the form of vesicle release strength. The second kernel set then predicts EPSP waveforms from the release

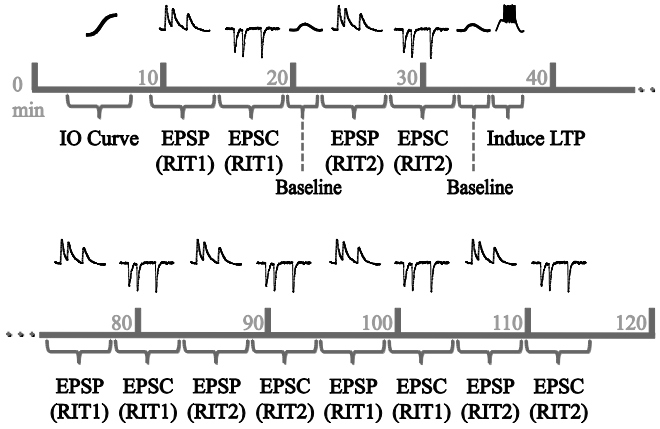

Figure 2. Alternating whole-cell current and voltage clamp recordings were made during two different RIT sets and then repeated after LTP induction for a minimum of four recordings per RIT.

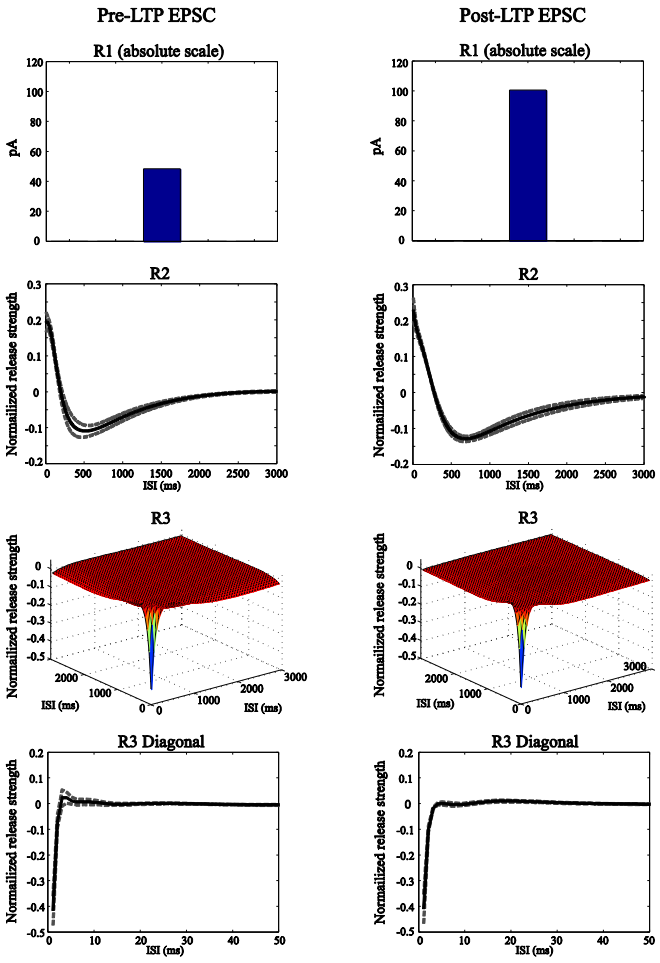

Figure 3. Response functions demonstrating the single-pulse (R2), paired-pulse (R2), and triple-pulse (R3) effects on EPSC amplitude representing presynaptic vesicle release strengths.

strength scalars given in the first stage.

The Volterra kernels can then be used to calculate response functions that provide a natural physiological representation of 1) isolated pulse responses for first order functions, 2) paired-pulse effects for second order functions, and 3) triple-pulse nonlinear dynamical effects for third order response functions [7–9].

III. Results

A. Response Functions of Pre and Post-synaptic Contributions Before and After LTP

Fig. 3 shows the first, second, and third order response functions for the presynaptic vesicle release strengths estimated from EPSC recordings before and after LTP. The first row represents vesicle release strengths for an isolated stimulus (R1). The paired-pulse effect (R2) demonstrate a 20% facilitation in EPSC amplitude at minimal ISIs, no facilitation beyond 200 ms ISIs, and a peak depressive effect of 10% for ISIs between 200 and 2000 ms. The triple-pulse effect (R3) shows the only significant change is strictly depressive in nature for ISIs shorter than 2 ms.

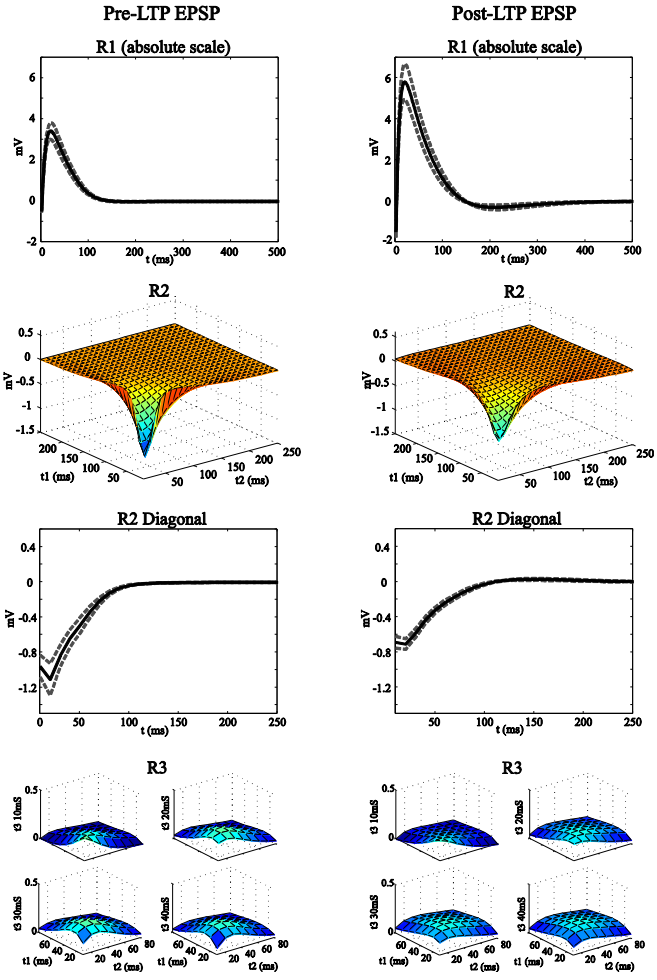

Figure 4. Response functions demonstrating the single-pulse (R2), paired-pulse (R2), and triple-pulse (R3) effects on EPSP waveforms representing postsynaptic mechanisms.

The results for the second stage of the model which represent postsynaptic mechanisms are shown in Fig. 4. These response functions are one order higher than those of Fig. 3 as the output is a continuous waveform EPSP generated from the scalar vesicle release strength input. The left hand column is comprised of response functions prior to LTP induction and the right hand column is generated from potentiated responses. The overall trend for the R2 functions is that prior action potentials with an ISI of 100ms or less depress the second action potential by up to 1 mV. The R3 functions demonstrate only a facilitative effect that peaks at 0.2 mV for ISIs of 20 to 40 ms.

B. LTP Induced Nonlinearity Change

Pre-LTP response functions were subtracted from post-LTP response functions in order to view the change in nonlinear dynamics due to LTP for both pre and postsynaptic data sets. The left column of Fig. 5 shows pre-LTP R2 subtracted from post-LTP R2 and pre-LTP R3 subtracted from post-LTP R3 for presynaptic effects. Similarly, the right hand column shows the difference for the postsynaptic effects.

A paired T-test was performed (Fig. 6) against the normalized nonlinearity change functions to determine significance (p value=0.05). For presynaptic mechanisms, no significant change was found for either R2 or R3. Postsynaptic mechanisms also showed no significant change

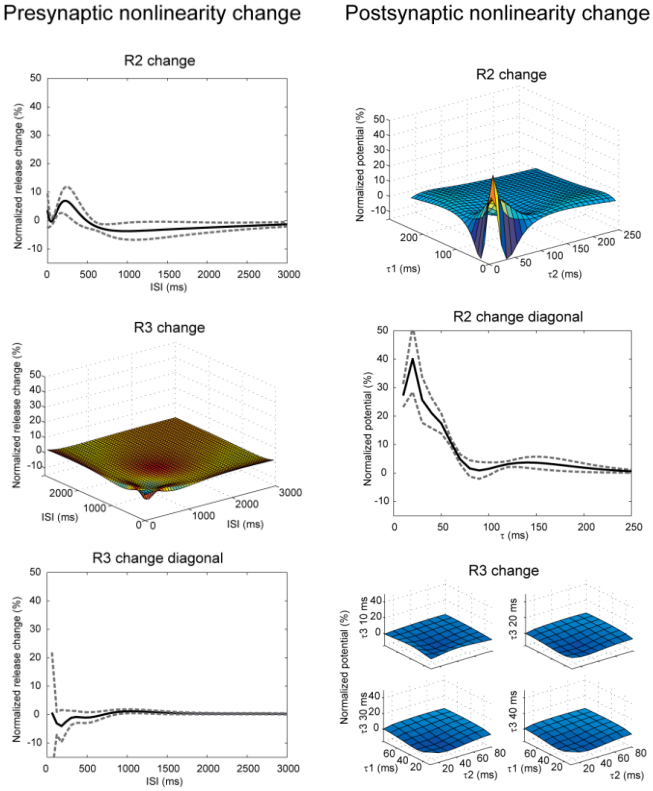

Figure 5. Nonlinearity change in pre and postsynaptic mechanisms due to LTP obtained by subtracting pre-LTP functions from post-LTP functions.

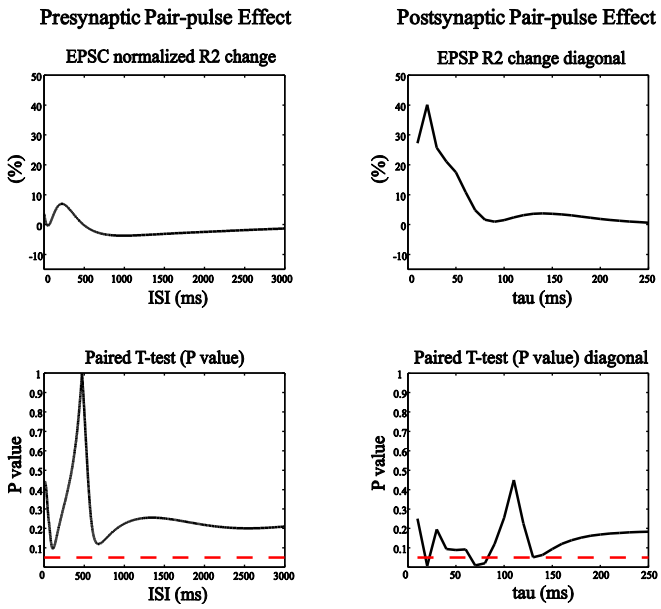

Figure 6. Paired T-test of normalized pre and postsyaptic R2 change shows no significance for presynaptic mechanisms in R2 but a significant change in poststynaptic R2 within the first 50 ms.

in R3. R2, however, demonstrated a significant change for ISIs of less than 50 ms.

The T-test results suggest that the nonlinearity expression locus for LTP in rat hippocampal CA3 Schaffer collateral to CA1 pyramidal cell synapses is postsynaptic.

IV. Discussion

The application of the two-stage cascade model to pre- and post-LTP data provides two new insights. First, it gives a quantitative value to pre and postsynaptic contributions to the potentiation of the synaptic pathway. By taking the difference of these values before and after LTP instantiation we see that changes in nonlinear dynamics, specifically the paired-pulse effects, occur postsynaptically.

Second, through the response functions we are able to see intuitively how LTP alters higher-order effects on both sides of the synapse. The response functions provide evidence that LTP does not only increase the magnitude of a neuron's responsiveness to stimulation, but that potentiation also changes the way neurons encode the information transmitted to them across the synapse.

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