Primming-Induced Shift in Synaptic Plasticity in the Rat Hippocampus

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Wang, Hongyan and John J. Wagner. Primming-induced shift in synaptic plasticity in the rat hippocampus. J. Neurophysiol. 82: 2024–2028, 1999. The activity history of a given neuron has been suggested to influence its future responses to synaptic input in one prominent model of experience-dependent synaptic plasticity proposed by Bienenstock, Cooper, and Munro (BCM theory). Because plasticity of synaptic plasticity (i.e., metaplasticity) is similar in concept to aspects of the BCM proposal, we have tested the possibility that a form of metaplasticity induced by a primming stimulation protocol might exhibit BCM-like characteristics. CA1 field excitatory postsynaptic potentials (EPSPs) obtained from rat hippocampal slices were used to monitor synaptic responses before and after conditioning stimuli (3–100 Hz) of the Schaffer collateral inputs. A substantial rightward shift (5-fold) in the frequency threshold between long-term depression (LTD) and long-term potentiation (LTP) was observed <1 h after primming. This change in the LTD/P crossover point occurred at both primmed and unprimmed synaptic pathways. These results provide new support for the existence of a rapid, heterosynaptic, experience-dependent mechanism that is capable of modifying the synaptic plasticity phenomena that are commonly proposed to be important for developmental and learning/memory processes in the brain.

INTRODUCTION

Network theories employing activity-dependent changes in synaptic strength are prominent partners with experimental approaches to investigating the mechanisms underlying learning and memory at the molecular and cellular levels in the nervous system. A key feature of the network "learning rule" described by Bienenstock, Cooper, and Munro (1982) (BCM theory) is the concept of a sliding modification threshold, \( \theta_M \), the value of which takes into account the prior activity of the postsynaptic neuron. This concept is crucial for the preservation of a dynamic response range of the neuron to synaptic stimulation, and it previously has been suggested that \( \theta_M \) may correspond to the long-term depression (LTD)/long-term potentiation (LTP) crossover point in conditioning frequency-response experiments (Dudek and Bear 1992). If so, then a growing group of phenomena in which prior activity affects the subsequent induction of synaptic plasticity (i.e., metaplasticity) (for review, see Abraham and Bear 1996) may provide examples of shifting \( \theta_M \). One such example of metaplasticity involves the use of a high-frequency stimulation (HFS) "primming" protocol that results in the subsequent facilitation of LTD induction (Holland and Wagner 1998; Wexler and Stanton 1993). In this report, we describe the effects of HFS primming on a number of conditioning frequencies delivered to both homosynaptic and heterosynaptic pathways in the CA1 region of the rat hippocampal slice preparation. Our experimental results are in good agreement with several fundamental predictions of the BCM type of learning rule.

METHODS

Male Sprague-Dawley rats (45–80 days old) were anesthetized deeply by Halothane inhalation before decapitation. The brain was removed from the cranium, and transverse hippocampal slices (500 \( \mu \)m) were cut in ice-cold saline using a vibratome. The CA3 region was removed surgically immediately after slice dissection. Slices were submerged in a recording chamber and continuously perfused with saline saturated with 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \) at \( \sim 1 \text{ ml/min} \). The recording chamber and perfusion saline were then warmed to 30°C for the duration of the experiment. The slices were incubated for \( \sim 1 \) h in the chamber before an experiment was begun. The saline contained (in mM) 120 \( \text{NaCl} \), 3 KCl, 1.5 MgCl\(_2\), 1 NaH\(_2\)PO\(_4\), 2.5 CaCl\(_2\), 26 NaHCO\(_3\), and 10 glucose.

Extracellular recording electrodes were filled with 300 mM NaCl and placed in the s. radiatum of CA1. The field excitatory postsynaptic potential (fEPSP) population responses were evoked with a bipolar stimulating electrode (Kopf Instruments) placed on either the CA3 or the subicular side of the recording electrode in the stratum radiatum. Stimulation parameters consisted of single square waves of 40–90 \( \mu \text{A} \) of 0.3-ms duration. Data were digitized at 10 kHz and analyzed with pCLAMP 6 software (Axon Instruments). The initial slope of the population EPSP was measured by fitting a straight line to the first millisecond of the EPSP immediately after the fiber volley. Stimulus response curves were performed at the beginning of each experiment. Pulses of an intensity that gave 40–60% of the maximum response were given at a frequency of 0.05 Hz for the remainder of the experiment. All stimulation protocols were performed at the test pulse intensity, and when two synaptic pathways were monitored, their independence was evaluated as previously described (Wagner and Alger 1995). The conditioning protocol consisted of 600 pulses at the indicated frequencies of stimulation that was repeated after a 10-min interval. Primming was induced using two sets of three HFS (100 Hz/1 s) trains given at an interval of 20 s, with 15 min between sets (Holland and Wagner 1998). Homosynaptic primming (as in Fig. 2) was performed in the presence of \( \text{D,L-2-amino-5-phosphononovaleric acid (D,L-APV; 100 \mu M)} \) to prevent LTP. Heterosynaptic primming of an independent pathway (as in Fig. 3) was performed in the absence of APV. For clarity, the responses during conditioning/primming protocols are not illustrated in the figures.

Synaptic responses were quantified by averaging the EPSP slopes from 15 consecutive responses at baseline frequency 25–30 min after the completion of the conditioning protocol and dividing this value by the average of the 15 EPSP slopes from 5 min before beginning conditioning stimulation. Values for nonconditioned groups were obtained by monitoring the baseline response for an equivalent amount of time. Unless otherwise noted, the n values reported represent slices taken from different animals for a given experimental group (e.g., \( n = 6 \) is 6 slices obtained from 6 different animals).
RESULTS

The extracellular field EPSP was monitored in the CA1 stratum radiatum of the hippocampal slice in response to Schaffer collateral stimulation. Two episodes of 600 pulses delivered at the indicated frequencies were administered at an interval of 10 min. This protocol has been used previously, at a frequency of 1 Hz, to study the induction of homosynaptic LTD in this preparation (Holland and Wagner 1998; Kerr and Abraham 1995).

Activity-dependent plasticity is dependent on the frequency of conditioning

We have tested the effects of varying the frequency of stimulation, while keeping the number of episodes and pulses delivered constant. As shown in Fig. 1A, 3-Hz stimulation had no consistent effect on baseline responses in naïve (i.e., control) slices prepared from 45- to 80-day-old rats (92 ± 7%, n = 8 slices from 7 animals) when compared with nonconditioned control slices (98 ± 4%, n = 4 slices). In contrast to this slight depression, delivering the same number of pulses at 10 Hz resulted in a potentiation of the synaptic response (122 ± 13%, n = 6 slices from 5 animals, Fig. 1B). Stimulation at 30 Hz (141 ± 12%, n = 7 slices from 4 animals, Fig. 1C) and 100 Hz (117 ± 7%, n = 6 slices, Fig. 1D) resulted in significant amounts of potentiation. We observed significantly less potentiation at 100 Hz compared with that observed after the 30-Hz protocol (an “inverted U-shaped” relationship) (cf. Christie et al. 1995). This effect is not a predicted

**FIG. 1.** Activity-dependent changes in synaptic responses vary according to the frequency of the conditioning protocol. A–D: summary plots of CA1 field excitatory postsynaptic potential (EPSP) slopes measured at a baseline frequency of 0.05 Hz (mean ± SE, in some cases the error bars are smaller than the symbols). Effects of conditioning protocols at the indicated frequencies (3–100 Hz) are illustrated. Insets: average of 10 consecutive sweeps obtained at the indicated times, vertical scale is 2 mV, horizontal scale is 50 ms. E: summary comparisons at 25–30 min postconditioning from the preceding experiments. Bars are the means ± SE, *, significant difference when compared with the nonconditioned (i.e., 0.05 Hz), control group (P < 0.05, ANOVA followed by Dunnet’s post hoc tests).
result from BCM and is possibly due to the induction of depo-
tentiation during the relatively long (6-s) 100-Hz trains (Abraham
and Huggett 1997). These results, and the statistical comparisons
with the control, nonconditioned (i.e., the 0.05-Hz baseline test
frequency) slice group are summarized in Fig. 1E.

**Homosynaptic priming results in an experience-dependent
shift in synaptic plasticity**

Similar experiments were interleaved with those described
above in which the slice was “primed” via a high-frequency
stimulation protocol prior to the delivery of 3- to 100-Hz condi-
tioning protocols (Fig. 2, A–D). We previously have observed that
this form of HFS priming significantly facilitates the induction of
LTD after 1-Hz conditioning (Holland and Wagner 1998; Wagner
and Alger 1995). As shown in Fig. 2A, priming enhanced the
depression observed after 3-Hz conditioning (80 ± 3%, n = 7
slices from 6 animals), such that a significant amount of LTD was
present compared with primed slices that did not experience
conditioning stimulation (103 ± 7%, n = 4 slices). Priming
altered the response to 10-Hz conditioning in an even more
striking manner, qualitatively as well as quantitatively. A signif-
icant depression occurred after 10-Hz stimulation of primed slices
(78 ± 3%, n = 5 slices, Fig. 2B), whereas the same conditioning
protocol resulted in a potentiation in unprimed control slices (see

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**FIG. 2.** Homosynaptic priming results in an experience-dependent change in the subsequent synaptic responses to conditioning
stimulation. A–D: summary plots of CA1 field EPSP slopes measured at a baseline frequency of 0.05 Hz (mean ± SE, in some cases
the error bars are smaller than the symbols). At 15 and 30 min the pathway is primed via HFS. Postpriming effects of the
conditioning protocols at the indicated frequencies (3–100 Hz) are illustrated. E: summary comparisons at 25–30 min postcondi-
tioning from the preceding experiments. Bars are the means ± SE. *, significant difference when compared with the nonconditioned
(i.e., 0.05 Hz), primed group (P < 0.05, ANOVA followed by Dunnet’s post hoc tests).
Heterosynaptic priming results in an experience-dependent change in the subsequent synaptic responses to conditioning stimulation. A–D: summary plots of CA1 field EPSP slopes measured at a baseline frequency of 0.05 Hz (mean ± SE, in some cases the error bars are smaller than the symbols). *, independent synaptic pathway is primed (data not shown) that evokes a short-lived heterosynaptic depression. Postpriming effects of the conditioning protocols at the indicated frequencies (3–100 Hz) are illustrated. E: summary comparisons at 25–30 min post conditioning from the preceding experiments. Bars are the means ± SE, *, significant difference when compared with the corresponding frequency group of the control results from Fig. 1. (P < 0.05, unpaired t-tests). F: both homosynaptic (□) and heterosynaptic (□) priming results in a shift in the long-term depression (LTD) and long-term potentiation (LTP) crossover point ($\theta_{LTP}$) when compared with the effects of (3–100 Hz) conditioning stimulation in unprimed controls (●). Data at the 1-Hz frequency point are taken (with permission) from an earlier publication (Holland and Wagner 1998) and are illustrated for comparative purposes.

Fig. 1B). Priming also significantly altered the response to 30-Hz stimulation. The amount of potentiation observed in controls after 30-Hz stimulation was attenuated in the primed slices (108 ± 6%, n = 5 slices, Fig. 2C). When a 100-Hz conditioning protocol was tested in primed slices, a significant amount of potentiation occurred (121 ± 6%, n = 5 slices, Fig. 2D). These results, and the statistical comparisons with the primed, unconditioned (i.e., the 0.05-Hz baseline test frequency) slice group are summarized in Fig. 2E.

**Heterosynaptic priming results in an experience-dependent shift in synaptic plasticity**

The results illustrated in Fig. 2 demonstrate that the priming of a given synaptic pathway can alter the subsequent response to conditioning stimulation of the same pathway (i.e., a homosynaptic effect). We also tested for any heterosynaptic actions in a third group of slices by first priming one synaptic input and then applying a 3- to 100-Hz conditioning protocol to a second, inde-
dependent input (Fig. 3, A–D). As we have observed previously using 1-Hz conditioning (Holland and Wagner 1998), the priming of heterosynaptic inputs significantly shifts the synaptic plasticity response in a manner analogous to that observed for the homosynaptically primed pathway. Thus both 3- and 10-Hz conditioning resulted in the occurrence of depression (79 ± 3%, 86 ± 2%, n = 5 slices each, Fig. 3, A and B, respectively). Also, consistent with our previous findings (Holland and Wagner 1998), the depression was homosynaptic because the potentiated synaptic responses of the primed pathway were not significantly affected after conditioning (data not shown). As was the case with homosynaptic priming, the potentiation observed in controls after 30-Hz conditioning was not present in the heterosynaptically primed, the potentiation observed in controls after 30-Hz conditioning was not present in the heterosynaptically primed slices (101 ± 5%, n = 5 slices from 4 animals, Fig. 3C). When a 100-Hz conditioning protocol was tested in heterosynaptically primed slices, a significant amount of potentiation occurred (131 ± 7%, n = 5 slices from 4 animals, Fig. 3D). These results and the statistical comparisons with the unprimed control data at the corresponding frequencies from Fig. 1 are summarized in Fig. 3E.

**Discussion**

Our findings in this report provide strong experimental evidence for the existence of a nonlinear, sliding modification threshold rule governing synaptic plasticity in the hippocampal slice preparation of the type envisioned by Bienenstock, Cooper, and Munro (1982). Although experimental results in favor of a BCM-like learning rule have been steadily accumulating in recent years, support has been lacking in at least two critical areas (for review, see Abraham and Tate 1997; Bear 1996): First, the BCM model employs a synaptic modification threshold (θM) analogous to the θ_{LTP} “crossover” point in our data, see Fig. 3F), the value of which is predicted to be the same at all modifiable synapses of the neuron at any given point in time. Although the relative position of θM is not fixed and is allowed to shift according to the prior postsynaptic activity of the neuron, its value must be the same at all modifiable synapses (i.e., primed and unprimed synapses). By employing a range of conditioning frequencies at nonprimed control, homosynaptically primed, and heterosynaptically primed synaptic pathways, we tested for evidence of this heterosynaptic feature of the BCM model. Any change in the position of the LTD/P crossover point elicited by our HFS priming protocol should be evident at inactive (unprimed) synapses as well as active (primed) ones. This is the result we obtained when the effects of heterosynaptic priming were assessed. As can be seen in Fig. 3F, a significant shift in the LTD/P crossover point has occurred after priming that is expressed at both primed and unprimed synaptic inputs (□ and □, respectively), compared with the control frequency-response curve (■). Furthermore this rightward shift after priming was quantitatively comparable for both active (~5-fold) and inactive (~7-fold) synapses. This finding is consistent with a uniform value of θM at all modifiable synapses of the neuron, irrespective of their individual histories of activity.

A second area of question involves practical matters related to the temporal aspect of changing the value of θM. It has been suggested that perhaps a shift in θM would not occur in an in vitro preparation or that the required duration of stimulation exceeds the typical viability of the slice preparation (Bear 1996). The possibility also has been offered that stimulation patterns that emulate the large-scale effects of sensory deprivation (as in the case of dark rearing, for example) are required to observe a shift in the value of θM (Abraham and Tate 1997). However (as underscored by Bear 1996), if a means of attaining rapid shifts in the modification threshold was not possible, the use of the sliding modification threshold would be greatly diminished. If the rapid changes (e.g., seconds/minutes) in synaptic strength such as those exemplified by typical LTP/D induction protocols are potentially of physiological relevance, then a concomitantly paced sliding of θM also must be able to take place to prevent saturation of depression or potentiation. Evidence for a shift in θM has been obtained by investigating the effects of sensory deprivation in vivo (during a period of weeks) on synaptic plasticity in slices from visual cortex (Kirkwood et al. 1996), and significant reversal occurred after 2 days of light exposure. Although we have not yet systematically explored the minimum temporal relationship between priming and conditioning stimulation in our experiments, it is clear from Fig. 3F that the LTD/P crossover point can shift significantly by 45 min after the initiation of priming. Thus the HFS priming protocol we have employed provides an example of how the value of θM might be changed rapidly in a manner more compatible with other means of inducing synaptic plasticity in an in vitro slice preparation. The results are consistent with the existence of a BCM-like mechanism at work in the CA1 region of the hippocampus.

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