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Dissection of Bidirectional Synaptic Plasticity Into Saturable Unidirectional Processes

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O'Connor, Daniel H., Gayle M. Wittenberg, and Samuel S.-H. Wang. Dissection of bidirectional synaptic plasticity into saturable unidirectional processes. *J Neurophysiol* 94: 1565–1573, 2005. First published March 30, 2005; doi:10.1152/jn.00047.2005. In populations of synapses, overall synaptic strength can undergo either a net strengthening (long-term potentiation) or weakening (long-term depression). These phenomena have distinct induction pathways, but the functional outcome is usually measured as a single lumped quantity. In hippocampal CA3-CA1 synapses, we took two approaches to study the activity dependence of each phenomenon in isolation. First, we selectively blocked one process by applying kinase or phosphatase inhibitors known, respectively, to block potentiation or depression. Second, we saturated depression or potentiation and examined the activity dependence of the converse process. The resulting unidirectional learning rules could be recombined to give a well-known bidirectional frequency-dependent learning rule under the assumption that when both pathways are activated kinases dominate, resulting in potentiation. Saturation experiments revealed an additional process in which potentiated synapses can be locked at high strength. Saturability of the components of plasticity implies that the amount of plasticity contributed by each pathway depends on the initial level of strength of the synapses. Variation in the distribution of initial synaptic strengths predicts a form of metaplasticity and can account for differences in learning rules observed under several physiological and genetic manipulations.

INTRODUCTION

Synaptic learning rules describe the relationship between neural activity patterns and the resulting changes in synaptic strength. Such persistent modifications of synaptic strength are widely believed to underlie functional changes such as learning in the CNS (Hebb 1949). Increases and decreases in synaptic strength—long-term potentiation and long-term depression (LTP and LTD)—are present in a wide variety of excitatory synapses and have been the focus of 30 years of intensive research (Bliss and Collingridge 1993; Malenka and Nicoll 1999; Sanes and Lichtman 1999).

Populations of synapses can undergo either LTP or LTD, thus making plasticity a bidirectional phenomenon (Dudek and Bear 1992). LTP and LTD proceed by separate pathways with LTP depending on kinase activity (Malenka et al. 1989; Malinow et al. 1989) and LTD depending on phosphatase activity (Mulkey et al. 1993). LTP and LTD can reverse one another (Dudek and Bear 1992; Mulkey and Malenka 1992), indicating a common final target of expression.

We reasoned that if the pathways leading to LTP and LTD were sufficiently independent, it should be possible to measure depression and potentiation as separate processes (Fig. 1*A*). A separation that occurs at downstream steps would have the advantage of preserving the activity dependence with which the mechanisms are activated in the naïve preparation. We studied hippocampal CA3-CA1 synapses, in which the neurotransmitter glutamate can act on AMPA-type, *N*-methyl-D-aspartate (NMDA)-type, and metabotropic receptors. Neural activity leading to plasticity acts through elevations in intracellular calcium, which activates both kinases and phosphatases. We used kinase and phosphatase inhibitors to reveal processes that could generate unidirectional potentiation or depression rules (Fig. 1*B*). We were also able to obtain similar rules by saturating potentiation or depression. The component rules could be mathematically recombined to form the original frequency-dependent learning rule. Taken together, these data account for how saturable potentiation- and depression-inducing processes combine to determine bidirectional plasticity.

METHODS

Slice preparation

Transverse hippocampal slices (300 μm thick) from Sprague-Dawley rats (P14–P21) were cut in ice-cold artificial cerebrospinal fluid (ACSF) comprising (in mM) 126 NaCl, 3 KCl, 1 NaH_2PO_4, 25 D-glucose, 25 $NaHCO_3$, 2 $CaCl_2$, and 1 $MgCl_2$ saturated with 95% O_2-5% CO_2, incubated at 34°C for 10–15 min, and transferred to a room-temperature interface chamber for ≥1 h before recording. For recordings slices were transferred to an immersion-type recording chamber and perfused at 2–4 mL/min with ACSF heated to 27.5–32°C except as indicated.

Extracellular field potential electrophysiology

For field potential recording the stimulation and recording electrodes were glass pipettes (1–12 MΩ when filled with 1 M NaCl or ACSF) placed >100 μm apart. The stimulus intensity (40–85 μA, 0.1 ms) was set to give responses between 1/2 and 2/3 of the maximal response size (Dudek and Bear 1992). Test stimuli were given at 0.03 Hz. Experiments started with 10–20 min of baseline followed by a conditioning protocol of three trains of 100 pulses at 0.5–100 Hz with 5 min of test pulses between trains. Paired-pulse facilitation was measured using an interpulse interval of 40 ms.

Drugs

To block LTP, K252a (Calbiochem) was prepared as a stock solution (1 mM in DMSO), stored at −20°C, and, on the day of the experiment, diluted to 10 μM with oxygenated ACSF containing Fast

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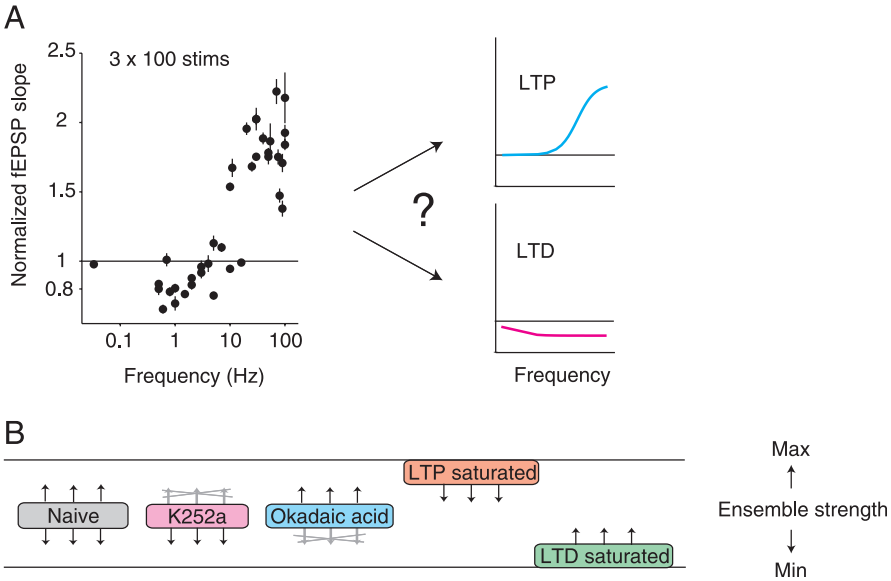

FIG. 1. Separating bidirectional plasticity at the CA3-CA1 synapse into isolated processes of potentiation and depression. *A, left*: normalized excitatory postsynaptic potential (EPSP) slope measured after stimulation (3 × 100 pulses, separated by 5 min) at various frequencies. Low frequencies produce long-term depression (LTD) and high frequencies produce long-term potentiation (LTP). *Right*: hypothetical LTP and LTD curves that combine to give the naïve learning rule at left. *B*: illustration of the strategies used to isolate potentiation and depression processes. The kinase inhibitor K252a blocks LTP induction, leaving only depression. The phosphatase inhibitor okadaic acid blocks LTD induction, leaving only potentiation.

Green FCF (Sigma) as a tracer. K252a solution was pressure ejected (3–6 psi of 95% O_2-5% CO_2) through a 49 μm ID Kapton capillary tube placed near the recording site. Application of equal concentrations of DMSO and Fast Green FCF without K252a did not affect LTP (data not shown). KN-62 (LC Labs) was prepared as a 20 mM stock solution in DMSO, stored at −20°C, and bath applied at a final concentration of 10 μM (0.05% DMSO). To block LTD, slices were preincubated in 1 μM okadaic acid (LC Labs; in 0.1% DMSO, prepared fresh daily) for 1–3 h before recording. Preincubation of slices in 0.1% DMSO alone did not affect LTD (data not shown). To measure the acute effects of okadaic acid on basal transmission and paired-pulse facilitation, okadaic acid was applied at a final concentration of 1 μM (DMSO 0.1%, to match the incubation experiments) to a recirculating bath (23–24°C). 0.1% DMSO alone had no effect on either basal transmission or paired-pulse facilitation (data not shown). [(+)-Alpha-methyl-4-carboxyphenylglycine] (MCPG) and DL-APV (Sigma) were prepared as stock solutions, kept at −20 and +4°C, respectively, and diluted to their final concentrations in ACSF. 6-Methyl-2-[phenylethynyl]-pyridine (MPEP; Sigma) was prepared as a stock solution in either water (pH with NaOH) or DMSO, kept at −20°C, and diluted with ACSF. For experiments without MCPG or MPEP, DL-APV was applied in the bath at 200 μM. APV and MCPG were applied together through the Kapton capillary tube at 1 and 5 mM, respectively. APV and MPEP were applied together either through the capillary at 1 mM and 100–250 μM, respectively, or in the bath at 200 and 25 μM.

Data acquisition and analysis

Extracellular signals were amplified with either an Axopatch-1D or a WPI DAM 80, filtered at 1–3 kHz, acquired with a either a Digidata 1320A and pClamp 8 or a NI-6052E and custom MATLAB software, and analyzed using pClamp 8 and MATLAB. The slope of the initial 0.5–2.0 ms of the fEPSP was measured using least-squares regression. Frequency-plasticity curves were obtained by least-squares fits to the function $y(x) = 1 + A\{1 - 1/[1 + (x/B)^C]\}$, with C constrained to ≤1.5. Errors for time series plots are SE across experiments by time point. Errors on the frequency-plasticity curves are SE computed using standard error propagation methods. Errors in the text are SE unless otherwise indicated.

Baseline measurements were taken in the 10 min prior to conditioning. In LTP and LTD experiments, changes from baseline were measured using the 5-min period from 40–45 min after the last stimulus. Example fEPSPs shown in the figures represent the mean of all sweeps from these baseline and measurement periods. LTP was saturated using 4 TBS episodes (at 0.05 Hz), 5-min test pulses, 1 TBS episode, 5-min test pulses, 1 TBS, 10-min test, 1 TBS, 10-min test, 1 TBS, 20-min test. One TBS episode comprised 10 bursts at 5 Hz, where each burst was five pulses at 100 Hz. LTD was saturated using 500 pulses at 1 Hz, 5-min test pulses, 500 at 1 Hz, 5-min test pulses, 500 at 1 Hz, 10-min test, 500 at 1 Hz, 10-min test, 500 at 1 Hz, 20-min test. LTP- and LTD-saturated "baselines" used a period 18–20 min after the last saturation stimulus.

RESULTS

To determine how kinase-dependent potentiation and phosphatase-dependent depression mechanisms could account for the observed overall activity-dependent bidirectional learning rule (Dudek and Bear 1992), we used extracellular field recording to monitor total synaptic strength. The data reported here come from a total of 245 experiments: 146 experiments with no drugs present, 67 experiments in the presence of a kinase or phosphatase inhibitor, 25 experiments in the presence of glutamate receptor blockers in addition to kinase or phosphatase inhibitor, and 7 experiments using various combinations of drugs.

Under naïve (no drug) conditions, stimuli at 100 Hz led to potentiation of 103 ± 13% (n = 3) from baseline, and stimuli at 0.5–2 Hz led to depression of 19 ± 3% (n = 10) from baseline, consistent with previous reports. We applied the same stimulus protocols in the presence of drugs that selectively block the induction of either LTP or LTD (Fig. 2, *A* and *B*, *right*). The kinase inhibitor K252a blocks LTP induction by potently inhibiting calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC). K252a does not affect LTD induction (Matthies et al. 1991; Wang et al. 1997; Wyllie and Nicoll 1994) and may gate depression processes by blocking cAMP-dependent protein kinase A (PKA), an enzyme that can interfere with LTD induction by activating a phosphatase inhibitor protein (Blitzer et al. 1998). In the presence of K252a, trains of stimuli (3 30- to 100-Hz trains of 100 pulses each) that normally induce potentiation (87 ± 6% increase from baseline, n = 15 experiments) led instead to a depression of 21 ± 4% decrease from baseline (n = 8).

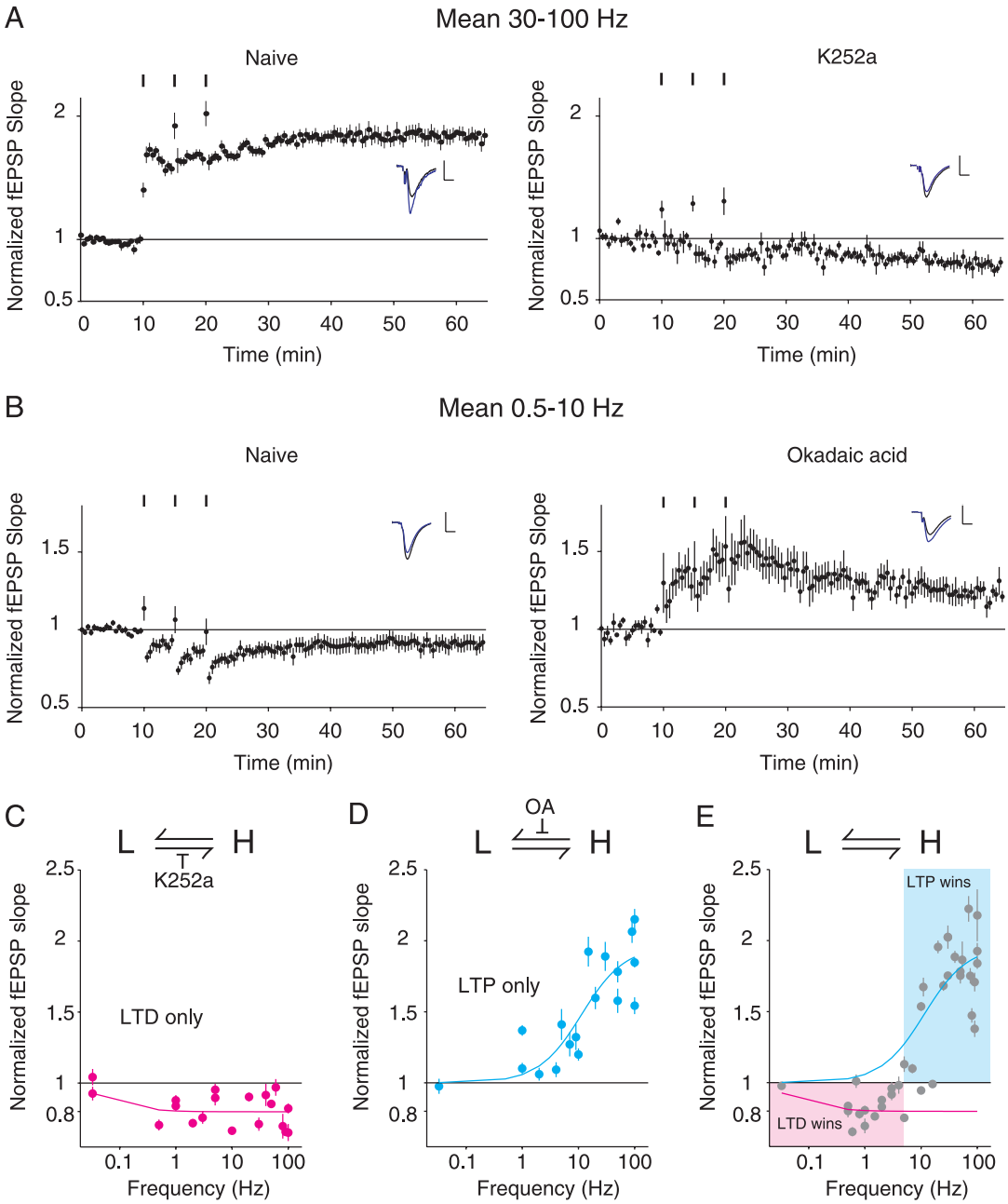

FIG. 2. Blocking the induction of LTP or LTD unmasks the converse competing process. *A*: the kinase inhibitor K252a blocks LTP induction, leaving depression. Vertical black lines indicate 3 conditioning trains of 100 pulses each. Scale bars 0.5 mV, 5 ms. *B*: the phosphatase inhibitor okadaic acid blocks LTD induction, leaving potentiation. Scale bars 0.5 mV, 5 ms. *C* and *D*: frequency-plasticity curves in slices treated with K252a (19 experiments) and okadaic acid (18 experiments). The leftmost points on the curves were obtained by continuing test pulses at 0.03 Hz. *E*: the same curves superimposed on data from untreated slices (38 experiments).

Conversely, okadaic acid, a specific inhibitor of protein phosphatases 1 (PP1) and 2A (PP2A), blocks LTD induction while sparing LTP induction (Mulkey et al. 1993; O'Dell and Kandel 1994). In slices preincubated with 1 μM okadaic acid, low-frequency stimuli (3 trains 5 min apart, each containing 100 pulses delivered at 0.5–10 Hz) that normally produced a slight depression (decrease of 9 ± 5%, n = 18; Fig. 2*B*) led instead to a 23 ± 5% potentiation (n = 8; different from no-drug case, $P < 10^{-3}$, 1-tailed t-test). Potentiation induced by stimulation at 100 Hz in okadaic acid-treated slices (increase of 85 ± 18%, n = 3) was blocked by APV (2 ± 3% change from baseline, n = 4, $P < 0.01$, 1-tailed t-test). The amount of potentiation in okadaic acid was similar to the native case (P = 0.4 compared with 100-Hz LTP, 2-tailed test). Thus blocking the induction of LTP or LTD unmasks the converse process.

Our initial results with kinase and phosphatase blockers suggested that enzymes responsible for both potentiation and depression are activated by a wide range of stimulus frequencies. We therefore measured the frequency dependence of these potentiation and depression processes. In the presence of K252a, stimulation at ≥0.5 Hz led to depression, with maximal depression occurring uniformly over a range of 1–100 Hz (Fig.

2*C*). After treatment with okadaic acid, trains at frequencies of ≥5 Hz led to potentiation (Fig. 2*D*). Thus at frequencies of ~5 Hz and higher, processes that could lead to either LTP or LTD are activated.

We tested whether the frequency-dependent learning rules measured in the presence of kinase or phosphatase inhibitor matched naïve data quantitatively. Curves fitted to the K252a and okadaic acid data reached levels that closely matched the maximal levels of LTD and LTP seen in untreated slices (Fig. 2*E*): at low frequencies, the untreated slice data overlap with the depression-only curve, whereas at high frequencies, the data quantitatively match the potentiation-only curve.

To test for multiple possible sites of K252a action in unmasking high-frequency-dependent depression, we tested several glutamate receptor mechanisms. First, we tested whether LTD in K252a requires NMDA receptor activity, which under naïve conditions is needed for the induction of 1-Hz LTD (Dudek and Bear 1992). In the naïve condition, APV was sufficient to block plasticity induced by 100-Hz stimulation (3 ± 5% increase from baseline, $n = 4$; Fig. 3*A*). However, in the presence of K252a, 100-Hz stimulation could now trigger depression even in APV (24 ± 7% decrease from baseline, $n = 4$; Fig. 3*A*). This result was specific to high-frequency activation: in K252a and APV, 1-Hz stimulation did not evoke LTD (8 ± 12% increase from baseline, $n = 5$; Fig. 3*A*). Taken together these experiments indicate that one action of K252a is to unmask an activity-dependent but NMDA receptor-independent route to depression.

As a test for the involvement of CaMKII in unmasking depression, the more specific CaMKII blocker KN-62 (Tokumitsu et al. 1990) was used. In KN-62 (10 μM), 100-Hz stimulation led to no change in synaptic strength (2 ± 7% increase from baseline, $n = 4$; Fig. 3*A*) (Bortolotto and Collingridge 1998). This difference may be due to the relative nonspecificity of K252a: KN-62 blocks the calcium-dependent form of CaMKII but not protein kinase C or cAMP-dependent protein kinase (Tokumitsu et al. 1990), whereas K252a blocks all three of these enzymes (Kase et al. 1987). Depression in K252a was blocked by the combination of APV with either the metabotropic glutamate receptor (mGluR) antagonist MCPG [(+)-alpha-methyl-4-carboxyphenylglycine; 3 ± 8% increase from baseline, $n = 6$; Fig. 3*A*] or the mGluR5-selective antagonist MPEP (6-methyl-2-[phenylethynyl]-pyridine; 1 ± 15% decrease from baseline, $n = 6$; data not shown). Thus the actions of K252a on targets other than CaMKII, such as protein kinase C or cAMP-dependent protein kinase, are required to unmask an mGluR-dependent form of LTD.

Blockade of potentiation with K252a unmasks a depression that can be triggered by high frequencies but is not seen under normal conditions. This could be either because under normal conditions potentiation events outnumber depression events (superposition of different synaptic populations) or because depression and potentiation compete within individual synapses that contain both mechanisms. In the presence of okadaic acid, the maximum amount of potentiation is the same as in the naïve case, suggesting that superposition does not take place. When both drugs were applied, high-frequency stimulation led to no net plasticity (0 ± 10%, $n = 3$; Fig. 3*A*), suggesting that these drugs together are sufficient to block activity-dependent plasticity mechanisms. Taken together, these results are consistent with models (Castellani et al. 2001; Lisman and Zhabotinsky 2001; Matsushita et al. 1995) in which potentiation and depression mechanisms compete directly within individual synapses.

Confidence in the frequency threshold for these pharmacologically identified depression- and potentiation-only rules is limited by the possibility that these drugs cause a change in the effectiveness of the stimuli (for instance by altering excitation-secretion coupling), thus changing induction conditions and therefore shifting the curves. We therefore measured the effect of these drugs on two measures of synaptic transmission, single-shock response size and paired-pulse facilitation (Fig. 3, *B* and *C*). Any change would shift the potentiation-only rule by an unknown amount depending in part on whether the change was pre- or postsynaptic. As an assay of possible presynaptic effects we measured paired-pulse facilitation (PPF) (Murthy et al. 1997; however, see Rozov and Burnashev 1999). In experiments in which okadaic acid was washed in, PPF went from 2.0 ± 0.1 before wash-in to 3.5 ± 0.5 ($n = 4$, measured 50–60 min after wash-in; Fig. 3*B*). When these data were pooled with

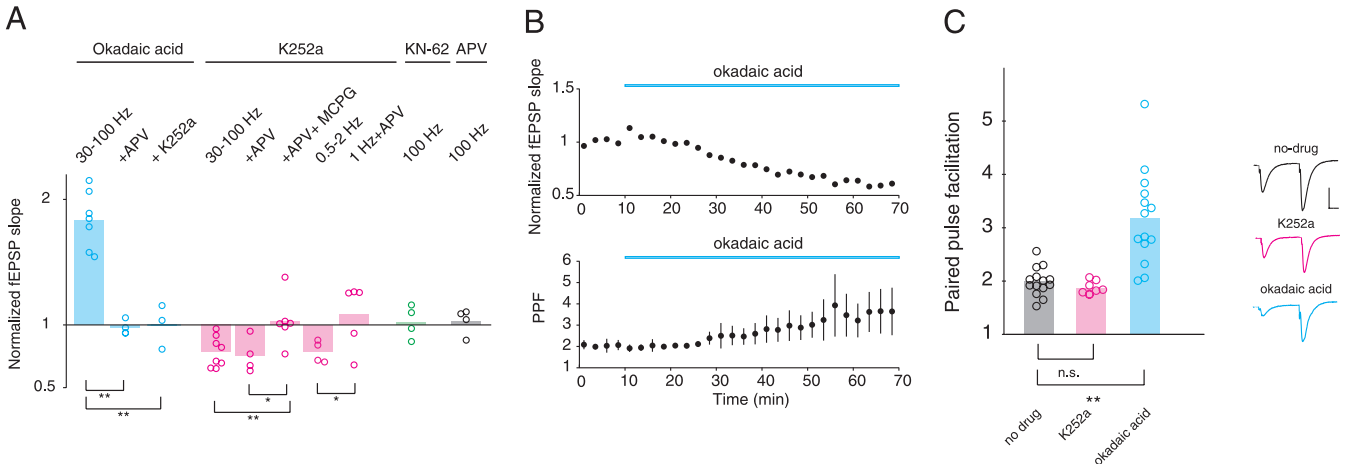

FIG. 3. Pharmacology of okadaic acid and K252a effects. *A*: plasticity in the presence of various drugs. One asterisk, significance at the $P < 0.05$ level; 2 asterisks, significant at the $P < 0.01$ level for 1-tailed *t*-test. *B*: okadaic acid wash-in (horizontal cyan line) causes a reduction in response amplitude (*top*) with a parallel increase in paired-pulse facilitation (*bottom*). *C*: paired-pulse facilitation (no drug, gray) is similar unaffected by K252a (magenta), but is increased after treatment with okadaic acid (cyan). Each point shows the mean of 20–60 responses from a single experiment. Example sweeps are averages of 20 responses. Scale bars 0.5 mV, 10 ms.

data from slices incubated in okadaic acid and control slice data, PPF was 2.0 ± 0.1 in naïve slices (n = 14) and 3.2 ± 0.2 in okadaic acid (n = 14; Fig. 3*C*). This difference in PPF ($P <$ 0.0001, 2-tailed t-test) indicates that the release probability may be decreased by okadaic acid. Consistent with a decrease in release probability, okadaic acid decreased the average single-shock response by 36 ± 12% (n = 4; $P < 0.05$, 1-tailed t-test). Measurements of the relationship between PPF and release probability from single terminals (Murthy et al. 1997) indicate that this change corresponds to up to a twofold decrease in release probability in okadaic acid. Using this to correct for the efficacy of presynaptic stimulation in triggering glutamate release would imply a slight downward shift in the threshold for potentiation. This leaves unaffected the conclusion that K252a-independent depression and okadaic acid-independent potentiation are both triggered by an overlapping and wide range of frequencies. Application of K252a had little effect on either baseline synaptic transmission (19 ± 20% increase from baseline, n = 6; not different from 0%, P = 0.4) or PPF (1.9 ± 0.04, n = 8; not different from naïve case, P = 0.25; Fig. 3*C*).

We next tested whether component rules with similar frequency dependence could be obtained by saturating either LTP or LTD. LTD was saturated using prolonged stimulation at 1 Hz, which led to a decrease of 35 ± 3% (n = 23) below baseline. After saturation of LTD, synapses were subsequently able to undergo potentiation with stimulation at frequencies of ≥5 Hz. At the highest frequencies (30–100 Hz), three trains of 100 pulses separated by 5 min produced a potentiation that matched the amount seen in naïve synapses (Fig. 4, *A* and *B*), indicating that low-strength synapses can potentiate independent of how long they have been at low strength.

We performed the converse experiment of measuring the plasticity in a population of fully potentiated synapses (Fig. 4, *C* and *D*). LTP was saturated by delivering multiple episodes of theta burst stimulation (TBS), which gave a 129 ± 11% (n = 27) increase from baseline. After saturation of LTP, depotentiation was observed at frequencies well above 0.5 Hz (Fig. 4*D*), indicating that depression mechanisms can be triggered by a wide range of stimulus frequencies. However, synaptic strength was not brought to levels as low as was reached starting from naïve synaptic populations. Presented initially, 0.5- to 2-Hz activation caused synaptic strength to normally reach a level of 19 ± 3% below initial baseline (n = 10). In contrast, 20 min after LTP was saturated to 128 ± 17% above baseline, low-frequency trains only depotentiated to 99 ± 27% above baseline (n = 3; Fig. 4*C*). Thus of a maximum observed range of synaptic strengths of 19 + 128 = 147%, only 29% depotentiation occurred.

Resistance to depotentiation has been studied as a slowly developing process that shares a common trigger with LTP (Stäubli and Chun 1996; Stäubli and Scafidi 1999). However, in cultured synapses, resistance to depotentiation develops immediately after recent unsilencing (Montgomery and Madison 2002), suggesting that lock-in and LTP may be under separate regulation. We therefore characterized the activity-

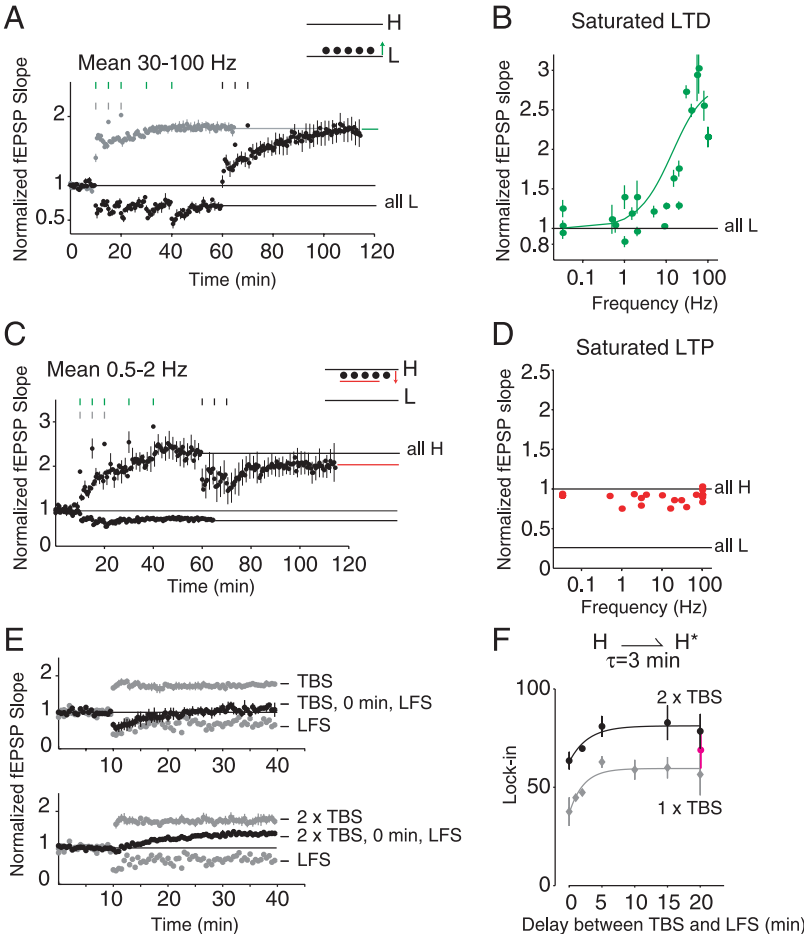

FIG. 4. Frequency-dependent learning rules after saturation of LTD or LTP. *A*: LTD is fully reversible. LTP induction protocols (gray and black marks) drove synapses to the same final level (gray symbols) even after saturation of LTD (black symbols and horizontal green line; trains given at green marks). *B*: frequency-plasticity curve after saturation of LTP. *C*: LTP reversal is prevented by "lock-in" at some synapses. Starting from saturated LTP (trains, green ticks), low-frequency trains (black ticks) gave only 1/5 as much depression (ending in horizontal red line) as expected from saturated LTD in naïve slices (lower data, trains at gray ticks). *D*: frequency-plasticity curve after saturation of LTD. *E*: variable lock-in under conditions of LTP saturation. LTP was induced with 1 episode (*top*) or 2 episodes (*bottom*) of theta-burst stimulation (TBS), followed immediately by 900 LFS (1 Hz). Black data show experiments where both TBS and LFS were given (time during LFS omitted). Gray data show result of giving either TBS alone (top of each panel) or LFS alone (bottom of each panel). Two TBS episodes lead to a similar amount of potentiation as 1 TBS episode, but more lock-in (*bottom* vs. *top*). *F*: time course of lock-in. Summary of lock-in vs. time showing LTP obtained 30 min after either 1 (gray) or 2 (black) TBS episodes and 1-Hz LFS. The magenta circle shows lock-in after 2 TBS episodes in the presence of 100 μM picrotoxin. H* represents the locked-in state.

dependence of lock-in. LTP was induced with one or two episodes of TBS (Fig. 4, *E* and *F*) followed by a depotentiation protocol delivered at different times. The amount of lock-in was measured 30 min after the end of the depression protocol and defined as one minus the amount of depotentiation divided by the difference between LTP alone and LTD alone. Lock-in was observed immediately after LTP induction (25 experiments) and increased over the next 10 min, reaching 0.57 ± 0.11 for the longest delays ($n = 7$; Fig. 4*F*). Two rounds of TBS (21 experiments) led to more lock-in (0.79 ± 0.09 at the longest delay, $n = 7$; Fig. 4*F*) even though the amount of LTP was similar (1 round, 77 ± 10% above baseline, $n = 4$; 2 rounds, 78 ± 10% above baseline, $n = 4$; Fig. 4*E*). Maximal lock-in following two rounds of TBS was similar when inhibition was blocked with 100 μM picrotoxin (0.69 ± 0.09; Fig. 4*F*), suggesting that plasticity of the inhibitory network does not contribute to lock-in. The recruitment of additional lock-in in the absence of additional LTP suggests that lock-in can be modified independently of potentiation.

Saturable model for bidirectional plasticity

Because synaptic plasticity is saturable (Petersen et al. 1998; Yang and Faber 1991), the amount of possible increase or decrease in any given synapse would depend on its starting condition. The contribution of potentiation and depression mechanisms to the overall learning rule would be expected to depend on the starting condition of the synaptic population. To illustrate the effect of saturable synaptic plasticity, we modeled an extreme case in which synapses do not change by graded amounts (Coussens and Teyler 1996) but instead take only two values of strength, denoted L and H (Fig. 5*A*; see APPENDIX A). Potentiation is represented by the transition from L to H, and depression and depotentiation are represented by the transition from H to L. This two-level assumption is a simplified representation of the fact that LTP and LTD are known to be saturable processes. Synaptic plasticity may indeed be composed of discrete transitions: LTP reportedly has all-or-none properties (Petersen et al. 1998), as does LTD (O'Connor et al. 2004). If synapses make transitions among multiple discrete strength states (Lee et al. 2000) or is even continuous (Montgomery and Madison 2002), the effect of synapse saturability on total measured synaptic strength will still be similar to the phenomena seen in our simplified model.

In this model, graded changes in synaptic strength represent the sum of many transitions in an ensemble. In this formulation, the kinase and phosphatase blocker experiments measure the probability of a state transition. Kinase inhibitor unmasks a depression that is equal over a frequency range of 1–100 Hz. This is consistent with the idea that biochemical pathways for depression are activated by frequencies above a depression threshold and not only at low frequencies (Artola and Singer 1993). Phosphatase inhibitor yields a potentiation that is identical to naïve potentiation over a frequency range of 10–100 Hz. Whether a transition occurs is determined first by whichever probability is greater within a given synapse, and second by whether that transition is possible. This model is sufficient to regenerate the naïve learning rule (Fig. 5*B*).

DISCUSSION

Our findings can be used to re-interpret existing knowledge of learning rules. Learning rules at CA3-CA1 synapses exhibit great complexity, including rate-dependent plasticity (Dudek and Bear 1992), depotentiation (Barrionuevo et al. 1980; Bashir and Collingridge 1994; Fujii et al. 1991; Huerta and Lisman 1996; O'Dell and Kandel 1994; Stäubli and Lynch 1990), and metaplasticity (Abraham and Bear 1996). The saturable nature of synaptic strength implies that a population of synapses can give different learning rules depending on the starting distribution of synaptic strengths. For instance, transgenic mice in which the observed learning rule is potentiation-only (Huh et al. 2000; Migaud et al. 1998) may represent conditions in which synapses are all at their saturated low strength. Consistent with this interpretation, depotentiation still occurs in these mutants (Boulanger and Shatz 2002; Zeng et al. 2001), the learning curve is shifted leftward (Huh et al. 2000; Migaud et al. 1998; Zeng et al. 2001), and the amount of saturated LTP differs from wild-type LTP by an amount comparable to the maximal amount of wild-type LTD (Huh et al. 2000; Migaud et al. 1998). This mirrors studies in adult rats in which synapses cannot undergo de novo depression but still show a degree of depotentiation following LTP (Bashir and Collingridge 1994; Fujii et al. 1991; Stäubli and Lynch 1990). Finally, the observation in brain slices from adult animals that LTD is often difficult to induce, but depotentiation still occurs (Wagner and Alger 1995), could be explained if synapses shift toward low-strength states with age.

Our results suggest a novel means of obtaining a sliding threshold for plasticity (Bienenstock et al. 1982) (Fig. 5*B*), one form of metaplasticity (Abraham and Bear 1996). A sliding threshold is expected for any plasticity mechanism in which

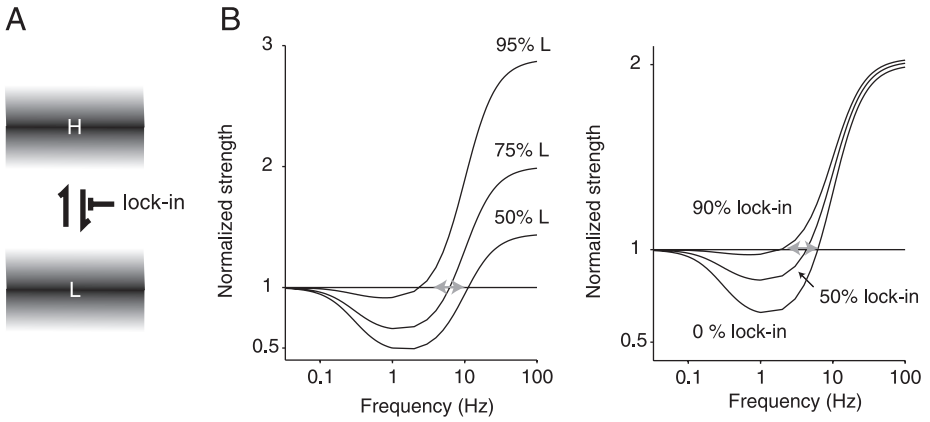

FIG. 5. A minimal model for saturating bidirectional plasticity. *A*: synapses can move between states of low strength (L) and high strength (H). The H-to-L transition (depression/depotentiation) can be blocked by activity and/or recent potentiation. *B*: the model predicts the naïve learning rule. Our data predict 2 distinct forms of metaplasticity that should arise as the fraction of synapses with low strength varies (*left*) or as the fraction of synapses resistant to depression/depotentiation varies (*right*).

synaptic potentiation and depression are saturable (Yang and Faber 1991). As illustrated in our model, redistribution of synaptic strengths changes the shape of the learning rule. One hallmark of this form of metaplasticity would be that a rightward shift in the frequency dependence of the threshold between LTP and LTD would be associated with a decrease in the amount of saturating LTP and an increase in the amount of saturating LTD. This would differ from more generally accepted forms of metaplasticity in which induction mechanisms may be modified (Kirkwood et al. 1996; Philpot et al. 2003). Our model also shows that resistance to depotentiation will cause a shift in the frequency threshold of LTP and LTD, in addition to a reduction in the maximal level of depression (Fig. 5*C*). In particular, if more potentiated synapses are resistant to depotentiation then the LTP/LTD frequency threshold will move to the left.

Kinase block unmasked a phosphatase-dependent LTD, an effect that had not been seen in several previous studies at CA3-CA1 synapses using broad-spectrum kinase inhibitors (Huber et al. 1995; Malenka et al. 1989; Malinow et al. 1988). Two of these studies (Malenka et al. 1989; Malinow et al. 1988) were done in animals of unspecified age and before the general acceptance by investigators of LTD as a phenomenon; the third study (Huber et al. 1995) was done using adult rats, in which LTD is not prominent.

The amount of LTD in the presence of kinase blocker K252a was monotonic with respect to stimulus frequency, reaching a plateau of ~20% depression. This suggests that maximal depression may be limited by a common pathway such as phosphatase activation (Mulkey et al. 1994). However, induction requirements are more variable at the level of cell surface receptors. NMDA-R LTD and mGluR-LTD pathways co-exist in CA1 neurons, and different conditions can emphasize one or the other (Oliet et al. 1997). Extracellular-stimulation 1-Hz LTD has been demonstrated to require NMDA-type glutamate receptor activity (Dudek and Bear 1992). In contrast, in the presence of the kinase blocker K252a, our 100-Hz LTD can proceed with NMDA receptors blocked and requires the activity of metabotropic, G-protein-coupled glutamate receptors. The unmasking of this form of LTD indicates that 100-Hz activation may normally suppress LTD induction mechanisms via kinases. For instance, negative regulation of mGluR pathways can occur via protein kinase C (Swartz et al. 1993), and cAMP-dependent kinase mediates phosphorylation and activation of inhibitor-1 protein, which inhibits protein phosphatase-1 (Blitzer et al. 1998). Activity above a certain threshold level may be sufficient to activate phosphatase-dependent LTD by multiple routes, so long as these routes are not blocked by kinase-dependent pathways. Understanding the relative contribution of NMDA-R and mGluR activation to LTD induction requires the use of methods that explore presynaptic, postsynaptic, and inhibitory (Steele and Mauk 1999) neural activity patterns more systematically.

By a simple mass action argument, we would expect high stimulus frequencies to activate both potentiation and depression mechanisms. Our data suggest that at high stimulus frequencies, an additional veto process is activated that prevents depression from occurring. We have found that block of a K252a-sensitive target other than CaMKII eliminates this effect, allowing a form of LTD to proceed. In saturation experiments, we observe lock-in, in which depotentiation of potentiated synapses is suppressed. Block of depotentiation has been observed to depend on cAMP-dependent kinase (Otmakhova and Lisman 1998). Taken together, these observations suggest that kinase activity can either make potentiation mechanisms dominant over depression mechanisms in the case of a single tetanus or longer-lasting in the case of successive plasticity protocols. On even longer time scales, a requirement for PKA has been noted for induction of LTP to multiple tetani (Huang and Kandel 1994) and for the transition to late phase LTP (L-LTP) (Frey et al. 1993; Nguyen and Kandel 1997). These observations are unified by a basic need to prolong the stability of potentiated synapses and suggest that on a variety of time scales, this stabilization can depend on kinases such as PKA.

In our model the suppression of the depression mechanism by high-frequency activity can be represented as a regulatory step in which the H-to-L transition is inhibited. This step can account for lock-in whether synaptic strength is continuous or discrete. If synapse strength varies continuously, then lock-in would be interpreted as a change in individual synapses' availability to depress/depotentiate. Alternately, if synaptic strength varies discretely then it may be that after potentiating, an individual synapse can undergo a further transition into a locked-in state. The discrete-synapse scenario is supported by unitary synaptic recordings done using patch-clamp recording, in which depression and depotentiation events are of similar size (unpublished results).

Our results indicate that at CA3-CA1 hippocampal synapses, bidirectional plasticity can be pharmacologically separated into kinase- and phosphatase-dependent components. The functional properties of these components can account for the learning rule observed in the naïve state by assuming that kinase activation is able to "trump" phosphatase activation. Because plasticity is saturable, the contribution of each of these components to the amount of net plasticity would depend on the starting state of the synaptic population, thus making the shape of the learning rule malleable. Saturability also implies that synapses are heterogeneous with respect to plasticity. A simple form of saturability is described in our model, which includes just two levels of strength per synapse. Although this assumption was extreme, the model could still reconstitute the observed learning rules. The same equations can also describe a model in which individual synapses vary in strength continuously but are saturable. In this case, the total synaptic strength reflects competition between kinases and phosphatases within individual synapses. Resolution of whether individual synapses change in strength continuously or discretely requires further experiments under unitary recording conditions (O'Connor et al. 2004; Petersen et al. 1998).

APPENDIX: MODEL OF SATURABLE BIDIRECTIONAL PLASTICITY

To model the effects of saturation on bidirectional plasticity, we constructed a simplified two-level synaptic model. This represents the simplest form of saturating plasticity; more elaborate synaptic models with multiple discrete or continuous strengths would yield similar results. The total strength of a population of synapses was modeled as the weighted sum $p_L g_L + p_H g_H$, where p_L and p_H are the fraction of synapses in low (L) and high (H) states ($p_L + p_H = 1$) and g_L and g_H are the average strength of depressed and potentiated synapses.

The initial fraction of synapses in the L and H states, p_L^o and p_H^o, can be estimated from the maximum possible potentiation (Δw_+, when LTP is saturated and $p_L = 0$ and $p_H = 1$) and depression (Δw_-, when LTD is saturated and $p_L = 1$ and $p_H = 0$), which impose the constraint $p_L^o/p_H^o = \Delta w_+/\Delta w_-$. LTP saturated at 229% of baseline and LTD saturated at 65% of baseline, corresponding to $\Delta w_+ = 1.29$ and $\Delta w_- = 0.35$. Thus fitting to a two-state model, the ratio p_L^o/p_H^o is ~4. The ratio of mean maximum and minimum synaptic strengths is given by $\gamma = g_H/g_L = (1 + \Delta w_+)/(1 - \Delta w_-)$; for our data this number is $\gamma = 2.29/0.65 = 3.5$.

A simplified model of bidirectional plasticity was constructed in which the constants r_{UP} and r_{DOWN} represent probabilities of potentiation and depression. (If individual synapses vary continuously, these *r's* can also represent analog rate constants.) Potentiation consisted of transitions from low strength to high strength. The probability of UP events was determined by phosphatase block experiments (Fig. 2*D*) and was empirically well described by the function $r_{UP}(f) = 0.9f^2/(f^2 + f_{UP}^2)$, where $f_{UP} = 10$ Hz. The DOWN process is completely determined by kinase block experiments (Fig. 2*C*) and is empirically well described by the function $r_{DOWN}(f) = f^2/(f^2 + f_{DOWN}^2)$, where $f_{DOWN} = 0.3$ Hz.

The number of upward transitions for a conditioning frequency f was modeled as the fraction of synapses in the low state multiplied by the probability of an upward transition, $p_L\, r_{UP}(f)$. The amount of LTP at high stimulus frequencies is the same in naïve slices and in okadaic acid (phosphatases blocked), suggesting that LTP induction mechanisms suppress LTD induction mechanisms. This suggests that LTP and LTD are not additive, consistent with a competitive model rather than superposition. The number of downward transitions can therefore be modeled phenomenologically as the product $p_H\, r_{DOWN}(f)\, [1 - r_{UP}(f)]$, which expresses the idea that downward transitions occur only when the DOWN process is activated and the UP process is not activated. These downward transitions are also limited to the fraction of potentiated synapses that are not locked in. Letting k be this locked-in fraction, the number of downward transitions is then $p_H\, r_{DOWN}(f)\, [1 - r_{UP}(f)](1 - k)$.

The net changes to the populations p_L and p_H after a bout of activity are then $\Delta p_H = -\Delta p_L = p_L^o\, r_{UP}(f) - p_H^o\, r_{DOWN}(f)\, [1 - r_{UP}(f)]\, (1 - k)$. These changes lead to a relative change in synaptic strength $\Delta w = \Delta p_H\, (\gamma - 1)/(p_L^o + \gamma\, p_H^o)$, where the beginning synaptic strength is normalized to 1. In the two-state case, p_H^o can be replaced with $(1 - p_L^o)$ and Δw can be reduced to

$$\Delta w = \frac{p_L^o[r_{UP} + r_{DOWN}[1 - k + r_{UP}(k - 1)]] - r_{DOWN}[1 - k + r_{UP}(k - 1)]}{\gamma - p_L^o(\gamma - 1)}(\gamma - 1)$$

This model makes the following assumptions: potentiation and depression are processes that are separable pharmacologically; the synaptic ensemble is composed of single synapses at which plasticity is saturated (though the model results will be similar in a continuous framework); and when potentiation and depression compete, potentiation wins.

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