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β-adrenergic signaling broadly contributes to LTP induction

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Abstract:
Long lasting forms of long-term potentiation (LTP) represent one of the major cellular mechanisms underlying learning and memory. One of the fundamental questions in the field of LTP is why different molecules are critical for long lasting forms of LTP induced by diverse experimental protocols. Further complexity stems from spatial aspects of signaling networks, such that some molecules function in the dendrite and some are critical in the spine. We investigated whether the diverse experimental evidence can be unified creating a computational model of multiple signaling pathways with distinct spatial compartments. Our results show that the combination of activity of several key kinases can predict the occurrence of long lasting forms of LTP for multiple experimental protocols. Furthermore, our analysis suggests that activation of the beta adrenergic receptor either via canonical (Gs-coupled) or non-canonical (Gi-coupled) pathways underpins most forms of long-lasting LTP.

Impact statement: Neuromodulation, specifically beta adrenergic receptor switching to inhibitory G protein signaling, plays a pivotal role in both electrically-induced and chemically-induced forms of long term synaptic potentiation

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Abstract

Long lasting forms of long-term potentiation (LTP) represent one of the major cellular mechanisms underlying learning and memory. One of the fundamental questions in the field of LTP is why different molecules are critical for long lasting forms of LTP induced by diverse experimental protocols. Further complexity stems from spatial aspects of signaling networks, such that some molecules function in the dendrite and some are critical in the spine. We investigated whether the diverse experimental evidence can be unified creating a computational model of multiple signaling pathways with distinct spatial compartments. Our results show that the combination of activity of several key kinases can predict the occurrence of long lasting forms of LTP for multiple experimental protocols. Furthermore, our analysis suggests that activation of the beta adrenergic receptor either via canonical (G_s-coupled) or non-canonical (G_i-coupled) pathways underpins most forms of long-lasting LTP.

Introduction

Synaptic plasticity is one of the cellular mechanisms underlying learning and memory. In the hippocampus, long-term potentiation (LTP) has been implicated not only in acquisition, consolidation and retrieval of spatial memories, but also contextual fear extinction [Sweatt, 2009]. Several neuromodulatory systems contribute to both synaptic plasticity and fear memory [O'Dell et al., 2015], including pathological memory retention such as post-traumatic stress disorder (PTSD). One of the most potent regulatory systems is the noradrenergic system, which is activated by arousal, emotion and stress. Experimental evidence shows that norepinephrine is elevated in the hippocampus in mouse models of PTSD [Wilson et al., 2014, Kao et al., 2015]; however, its contribution to long term plasticity is unclear and this lack of knowledge hinders the development of treatments for fear memory disorders.

Numerous experiments investigating long-lasting LTP have revealed the requirement for a plethora of signaling molecules (reviewed in Giese and Mizuno [2013], O'Dell et al. [2015]). Experimental protocols that induce long-lasting LTP activate diverse signaling pathways, which may interact competitively or cooperatively. For example, long-lasting LTP evoked by multiple trains of high-frequency electric stimulation requires protein kinase A (PKA) only if the inter-train interval is greater than 60 s [Woo et al., 2003, Kim et al., 2010]. These networks of signaling pathways may converge on common targets, such as extra cellular regulated kinase (ERK), which is required for most forms of long-lasting LTP [English and Sweatt, 1997, Winder et al., 1999, Thomas and Huganir, 2004, Gelinas et al., 2008]. Alternatively, some components of those signaling pathways are location specific and function in restricted spatial compartments such as spines or dendritic submembrane. Those observations pose the key question of whether this diversity of mechanisms can be explained by collectively considering the combined molecular network.

Another type of unexplained diversity of mechanisms underlying induction of long-lasting LTP is introduced by neuromodulation. To date, β-adrenergic (β-AR) activation has been considered essential for only a subset of experimental protocols, usually for weak electric stimulation. Conversely, commonly used βAR antagonist, such as propranolol, do not affect long-lasting LTP elicited by strong electric stimulation.

The idea that βAR activation is not essential for long-lasting forms of LTP was undermined by recent experiments suggesting that conventional βAR antagonists do not block all downstream signaling pathways. Though βARs typically are coupled with stimulatory G protein (G_s), phosphorylated βARs decouple from G_s and couple with inhibitory G protein (G_i). Both G_s-activated and βAR coupled to G_i-activated signaling pathways converge on a common target, ERK [Luttrell et al., 1996, Della Rocca et al., 1997, Martin et al., 2004], which is required for long-lasting LTP. The ability of propranolol to recruit ERK [Kahsai et al., 2011], suggest that long-lasting LTP evoked by strong stimulation with or without propranolol might require βAR signaling to ERK. This hypothesis is supported by recent experiments showing that a complete βAR antagonist blocks long-lasting LTP induced by strong electric stimulation [Havekes et al., 2012]. Therefore, βAR activation might play a pivotal role for many forms of long-lasting LTP.

To investigate whether the diverse experimental evidence can be unified by considering activation of multiple signaling cascades and address the role of βAR activation in occurrence of long-lasting LTP, we develop a spatial, mechanistic model of signaling pathways underlying induction of long-lasting forms of LTP. We evaluate spatio-temporal dynamics of key kinases that activate molecular pathways reported to play an essential role in long-lasting forms of LTP. We show that the combined elevation of several molecules in the spine and in the dendrite can predict the induction of long-lasting LTP, and, we demonstrate that activation of the βARs is essential for all forms of LTP. These findings may help unravel the contribution of the noradrenergic system to learning and memory and help with the development of treatments for fear and anxiety disorders.

Results

Our goal was to explain the diverse literature on molecular dependence of long-lasting forms of LTP induction. We evaluated whether the spatio-temporal dynamics of molecular signaling pathways can explain and predict which stimulation patterns produce long-lasting LTP. We constructed a model of signaling pathways (Fig. 1) that regulate long-lasting forms of LTP in hippocampal CA1 pyramidal neurons in NeuroRD [Oliveira et al., 2010] using the morphology of a dendrite with one spine (Fig. 2). We simulated seven experimental paradigms (Table 1), four of which elicit long-lasting forms of LTP, one of which results in E-LTP, and two of which cause no lasting change in synaptic efficacy. Our goal was to derive an equation to explain the outcomes

and molecular dependence of the seven paradigms. In deriving the equation, we concentrated on the activity of molecular species that are implicated in spine-specific and dendrite-specific changes and accompany long term plasticity.

Spine and dendrite molecular signatures required to predict plasticity

We quantified the spatio-temporal dynamics of molecular species that are known to play a role in the induction of long-lasting forms of LTP, including PKA [Abel et al., 1997, Winder et al., 1999, Woo et al., 2003, Gelinas and Nguyen, 2005], calcium-calmodulin-dependent protein kinase II (CaMKII) [Sanhueza et al., 2011] and exchange protein directly activated by cAMP (Epac) [Gelinas et al., 2008]. These molecules were activated either by calcium pathways or by the βAR coupling either to G_s or G_i. We devised two equations that we called 'signatures' to predict the occurrence of long-lasting LTP. The first one evaluated combined activity of key molecular species in the spine, the second one evaluated combined activity of key molecular species the dendrite. We assumed that if the experimental protocol enhanced activity of key molecular species in the spine, then spine specific changes would be induced and, similarly, if the experimental protocol enhanced activity of key molecular species in the dendrite, then dendrite specific changes would be induced. To evoke long-lasting forms of LTP both spine specific and dendrite specific changes needed to be induced.

The spine molecular signature trace (referred to as the spine signature) evaluates the initiation of plasticity processes in the spine by calculating time dependent increases in CaMKII, Epac, and PKA activity in the spine:

$$\mathrm{S_{spine}}(t) = \frac{\Delta\mathrm{pCaMKII}(t)}{\max \Delta\mathrm{pCaMKII}} + \frac{\Delta\mathrm{Epac}(t)}{\max \Delta\mathrm{Epac}} + \frac{\Delta\mathrm{PKA}(t)}{\max \Delta\mathrm{PKA}} \tag{1}$$

where $\Delta\mathrm{Epac}(t)$ is the fold increase in cAMP bound Epac, $\Delta\mathrm{pCaMKII}(t)$ is fold increase in phosphorylated CaMKII, $\Delta\mathrm{PKA}(t)$ is the fold increase in phosphorylation of PKA targets. Max ΔX is a normalization value equal to the maximum activation of molecular specie X among all the investigated paradigms. If the spine signature exceeds its threshold for more than $10\,s$, spine-specific changes are induced.

The dendritic signature represents spatially non-specific plasticity processes, and takes into account molecular species: PKA, Epac and CaMKII:

$$\mathrm{S_{dendrite}}(t) = \frac{\Delta\mathrm{Epac}(t)}{\max \Delta\mathrm{Epac}} + \frac{\Delta\mathrm{pCaMKII}(t)}{\max \Delta\mathrm{pCaMKII}} + \frac{\Delta(\mathrm{pI1}(t) + \mathrm{pPDE4}(t))}{\max \Delta(\mathrm{pI1} + \mathrm{pPDE4})} + \frac{\Delta\mathrm{G_i}(t)}{\max \Delta\mathrm{G_i}}, \tag{2}$$

For the dendritic signature, the PKA activity is subdivided into two terms: inhibitory G protein ($\mathrm{G_i}(t)$) which represents phosphorylated β2AR, and other phosphorylated PKA targets: I1 and PDE4. We have subdivided the PKA activity into these two parts to evaluate the role of G_s-G_i switching (and β-arrestin) in

synaptic plasticity, and also to evaluate the role of novel β2AR antagonists. $\Delta(\mathrm{pI1}(t) + \mathrm{pPDE4})$ represents PKA phosphorylation of other phosphoproteins not included in the model for LTP induction. If the dendrite signature exceeds its threshold for more than $10\,s$, dendrite-specific changes are induced.

To induce long-lasting forms of LTP, both the spine- and dendrite-specific changes must be induced.

Molecular signatures explain both electrically and chemically induced LTP

The first question addressed was whether a single set of empirically derived thresholds could predict the outcome of seven different experimental protocols without a change in models parameters. Fig. 3 and 4 show that indeed there is a range of thresholds for both the spine and dendritic signature, which allows for predicting long-lasting forms of LTP. Furthermore, two of the experimental protocols (Fig. 4) demonstrate that separate spine and dendritic signatures are needed. Specifically, the signatures for HFS, which does not produce a long-lasting form of LTP, exceed the spine threshold but not the dendritic threshold. Also the signatures for ISO alone exceeds the dendritic threshold but not the spine threshold. In summary our model with spine and dendritic signatures correctly predicts the plasticity induction for all seven protocols, whereas a single signature would have given wrong predictions.

Another question we investigated was how bath application of ISO, which activates the βARs, transforms weak electric stimulation into a paradigm that evokes long-lasting forms of LTP. Fig. 4 reveals that indeed ISO transforms HFS and LFS into long-lasting LTP. The spine signature for HFS crosses the spine (Fig. 4A) but dendritic signature (Fig. 4D) for ISO+HFS is considerably greater than for HFS alone (Fig. 4B). These signatures suggest that HFS alone can induce the spine-specific changes required for plasticity, but that ISO is required to activate processes in the dendrite or soma required for long-lasting forms of LTP. In contrast to HFS, ISO enhances both the spine and dendrite signature for LFS, suggesting that LFS alone is insufficient to induce either spine-specific or dendrite-specific changes.

Molecular signatures explain PKA dependence

We evaluated the molecular dependence of LTP by examining distinct molecular components of the spine and dendrite plasticity signature. Prior research revealed that ISO+HFS requires Epac, but not PKA [Gelinas et al., 2008], whereas ISO+LFS requires PKA [Winder et al., 1999]. The model shows that both ISO+LFS and ISO+HFS need cAMP activated molecules provided by ISO: either PKA (Fig 5C) or Epac (Fig 5B), because CaMKII activity is too small (Fig 5A). For ISO+LFS (but not ISO+HFS), PKA is specifically needed to exceed the threshold, because Epac is too small.

To validate the model and further evaluate the PKA dependence and temporal sensitivity of long-lasting forms of LTP, we performed an additional set of simulations. We simulated all paradigms inducing long-

lasting forms of LTP in the presence of specific PKA inhibitors. Bath application of PKA inhibitors was simulated by eliminating activity of the PKA catalytic subunit. As blocking PKA activity lowers NMDA receptor currents by 20% [Skeberdis et al., 2006], calcium influx was lowered by 20% for these simulations. For all simulated paradigms, we calculated both spine and dendritic molecular signatures and used the same thresholds determined for the previous set of simulations.

Consistent with experiments [Woo et al., 2003, Gelinas et al., 2008], blocking PKA lowers either the spine (Fig. 6C) or dendritic (Fig. 6D) signature below threshold for all PKA-dependent forms of plasticity. Blocking PKA activity lowers the spine signature for ISO+LFS, but not that of the other protocols. Blocking PKA lowers the dendritic signature for 4xHFS-80s, so that it no longer crosses the threshold. Collectively, the model correctly predicts that blocking PKA will block long-lasting LTP induced by both 4xHFS-80s, and ISO+LFS, but will not block long-lasting LTP produced by 4xHFS-3s or ISO+HFS.

Examination of molecular components of the signatures helps to further understand the role of PKA and Epac in long-lasting forms of LTP. Blocking PKA reduces PDE4 activity [MacKenzie et al., 2002], which increases cAMP and Epac activity. The increase in Epac is sufficient to compensate for lack of PKA for both ISO+HFS and 4xHFS-3s (Fig. 6B). Epac does not compensate for PKA for the ISO+LFS case, because the low calcium influx with LFS does not activate sufficient CaMKII (Fig. 6A) compared to ISO+HFS.

β2AR is a critical PKA target for induction of long-lasting forms of LTP

PKA phosphorylation of β2AR has been suggested to be critical for hippocampus-dependent learning and long-lasting forms of LTP [Havekes et al., 2012]. PKA-mediated G_s-G_i switching is potentially relevant for all forms of long-lasting forms of LTP because electric stimulation is accompanied by a release of norepinephrine from locus coeruleus neuron terminals [Milusheva et al., 1994]. Though propranolol does not block long-lasting LTP induced by 4xHFS-80s [Swanson-Park et al., 1999], this does not rule out β2AR involvement as propranolol is an incomplete antagonist that allows some ERK recruitment [Kahsai et al., 2011].

This experimental evidence raises the critical question of whether βAR activation is required for all long-lasting forms of LTP. To answer this question we simulated the response to novel βAR antagonists in combination with electrical stimulation. We simulated 4xHFS-80s with bath applied propranolol (does not stimulate cAMP production but allows some ERK recruitment [Kahsai et al., 2011]) or ICI-118,551 (a complete antagonist [Kahsai et al., 2011]). We also simulated LFS or HFS preceded by bath application of carvedilol, an antagonist that does not stimulated cAMP production, but does allow ERK [Kahsai et al., 2011].

Fig. 7 shows that βAR is necessary for induction of long-lasting LTP even though propranolol does not block its induction. Simulations reveal that ICI-118,551, but not propranolol reduces the dendritic

signature to below the threshold, demonstrating the importance of non-canonical pathways activated by the βAR for induction of long-lasting forms of LTP. βAR stimulation is not required to elevate cAMP in the spine, as the signature crossed the threshold in the presence of ICI-118,551, because calcium elevation in the spine is sufficient to produce enough cAMP (Fig. 7A, C). These simulations make the experimentally testable prediction that ICI-118,551 will block long-lasting LTP induced by 4xHFS-80s and suggest that βAR activation is required for ERK recruitment.

Fig. 8 shows a different aspect of βAR signaling. Spine and dendritic molecular signatures show that carvedilol can not substitute for ISO when paired with both HFS and LFS. The spine signatures of both the Carvedilol+HFS and Carvedilol+LFS are lower than for weak stimulation. Carvedilol+HFS spine signature crosses the spine threshold, showing that calcium influx due to HFS alone produces a high calcium concentration that activates both CaMKII and cAMP pathways in the spine, without the need for additional βAR stimulation. In contrast, stimulation paradigms, such as LFS, that yield low calcium concentration need additional G_s stimulation from canonical βAR-activated pathways. As a consequence, the spine signature (Fig. 8A) for carvedilol+LFS does not cross its threshold. In the dendrite, G_i recruited by carvedilol binding to βAR is too low to compensate for the absence of G_s and thus the dendritic signatures for both carvedilol+HFS and carvedilol+LFS do not cross the threshold (Fig. 7D). The model makes the experimentally testable prediction that carvedilol will not support long-lasting LTP induced by either HFS or LFS.

E-LTP

A spatial approach allowed us to monitor changes in the phosphorylation of the AMPA receptor subunit GluA1 (AMPAR) in the PSD (Fig. 2). We monitored AMPAR phosphorylation (pAMPAR) because it is correlated with E-LTP [Benke et al., 1998]. HFS, ISO+HFS, 4xHFS-3s, 4xHFS-80s each cause five-fold increases in phosphorylation of AMPA receptors resembling E-LTP (Fig. 9A), whereas ISO and ISO+LFS cause minor increases in phosphorylation of AMPA receptors (Fig. 9B). Thus, though explaining E-LTP was not a goal of the model, an emergent property was that the model correctly predicts the development of E-LTP.

Stimulation of segregated spines helps preserve spatial specificity

A question of major importance for information processing is which events triggered by synaptic plasticity are spatially specific. Recent experiments using glutamate uncaging at single spines suggest that uncaging induced structural plasticity is spine specific[Matsuzaki et al., 2004]. On the other hand, some molecules,

such as Ras, can diffuse into nearby spines, reducing the threshold for LTP at those spines [Harvey et al., 2008, Govindarajan et al., 2011]. In addition to spatial specificity, other experiments suggest that stimulation of multiple spines may either cooperate with each other [Zhai et al., 2013] or compete for resources [Govindarajan et al., 2011]. Thus, the next set of simulations investigated whether electrically induced synaptic plasticity exhibits spatial specificity, i.e., what is the extent of diffusion of key molecules to adjacent spines. We used a 20 μm dendrite with 8 dendritic spines, applied 4xHFS-80s and evaluated stimulation of two adjacent spines (1.5 μm apart) and two non-adjacent (8 μm apart), i.e. separated, spines.

Both stimulation of separated and adjacent spines produce spine and dendritic signatures that exceed the threshold, and thus are able to induce L-LTP. Fig. 10C shows that the dendritic signature exceeds the threshold throughout the dendritic branch. In contrast, Fig. 10B reveals some degree of spatial specificity in the spine signature. Statistical analysis shows that for both adjacent and separated spine stimulation, the molecular signatures of stimulated spines is greater than the molecular signature of unstimulated spines (GLM, stimulus spacing and stimulation as factors, $F(2, 61) = 163$, $F > .0001$; factor stimulation: $P < 0.0001$, factor spacing: $P = 0.623$. For both adjacent and separated spine stimulation, the duration of the spine signature above threshold of stimulated spines is significantly greater than 10 s (t-test, $T(7) < 0.0001$ for both adjacent and separated spine stimulation). In contrast, spine signatures of unstimulated spines are not above threshold for greater than 10 s t-test, $T(7) = 0.9$ for upper threshold, 0.06 for lower threshold for separated spine stimulation; $T(7) = 0.79$ for upper threshold, 0.016 for lower threshold for adjacent spine stimulation. For both adjacent and separated stimulation protocols, the CaMKII and Epac of the non-stimulated spines is lower than that of the stimulated spine, which is consistent with the gradients observed experimentally [Lee et al., 2009].

Robustness of results

The ability to predict long-lasting forms of LTP does not depend on the precise details of the molecular signatures; instead the LTP predictions are similar for a range of thresholds, and for slight variations in the signature equations. The kinase-to-phosphatase balance, evaluated by the molecular signatures, is thought to control direction of synaptic plasticity [Lisman et al., 2005]. There are at least two ways of assessing this balance: either measuring the quantity of phosphorylated targets of kinases and phosphatases (1), or assessing a ratio of kinase activity to phosphatase activity. Importantly, LTP predictions of our model are similar when the spine molecular signature evaluates the ratio of kinases (CaMKII and PKA) to phosphatases (PP1 and PP2B) (not shown).

The figures show a threshold range to demonstrate that the model makes the same predictions for any

threshold value between the upper and lower thresholds, and does not require a precisely set threshold. To further assess robustness of our results, we evaluated individual simulations (realizations of paradigms), that were executed with different random seeds. Supporting information Tables S1 and S2 show that, despite variability in the timecourse, the signatures for each realization of the long-lasting LTP eliciting paradigms cross their thresholds for more than 10 s uninterrupted. Further analysis (Tables S1 and S2) reveals that these results are statistically significant. In addition lowering the spine threshold range and increasing the time the spine signature remains over the threshold to 15 s, does not significantly change the number of individual simulations that exceed the spine threshold (data not shown).

Discussion

To predict long-lasting forms of LTP we developed a stochastic reaction-diffusion model of a dendrite with spines. We looked at activity of the key molecular species during the first 10 min following plasticity induction, because long-lasting LTP is blocked by protein kinase inhibitors applied during or immediately after induction of LTP [Huang and Kandel, 1994, Huber et al., 1995]. We devised a set of molecular signatures: one in the spine and one in the dendrite, that predict induction of long-lasting forms of LTP. We demonstrated that two molecular signatures can explain the results of a large number of experimental protocols. Additional simulations revealed the complex role of the βAR activation in long-lasting forms of LTP. The spatial aspect of these simulations was critical, as a single molecular signature that calculated a spatial average of molecular activity was unable to predict the induction of all forms of long-lasting forms of LTP. Moreover, the Pearson correlation coefficients for all simulations using the single-spine model show that spine and dendritic signatures (time spent above threshold) are not correlated across all induction paradigms and trials ($N = 64$, $P > 0.15$).

Separate molecular signatures in the spine and in the dendrite represent distinct phenomena. Two signatures can be viewed as corresponding to synaptic tagging and capture [Frey and Morris, 1997, Redondo et al., 2010]. Synaptic tagging involves labeling of specific dendritic spines that are to undergo long term plasticity, and capture implies that a spatially non-specific signal induces synthesis of plasticity related proteins (PRPs), and in some cases, initiates transcription [Redondo and Morris, 2011]. PRPs are synthesized locally or trafficked up the dendrite and captured by tagged spines to stabilize synaptic strength. Crossing the threshold by the spine molecular signature can be viewed as setting the tag and crossing the threshold by the dendritic molecular signature corresponds to sending the signal initiating the synthesis of PRPs.

In constructing the spine molecular signature, we evaluated molecules that are implicated in synaptic tagging, AMPA receptor insertion, actin remodeling and structural plasticity [Matsuzaki et al., 2004, Okamoto

et al., 2004, Nishiyama and Yasuda, 2015, Patterson et al., 2010, Yang et al., 2008]. Blocking CaMKII activity [Sajikumar et al., 2007, Ramachandran and Frey, 2009, Redondo et al., 2010] has been shown to block tagging, and CaMKII also is implicated in the actin remodeling underlying structural plasticity [Shen et al., 1998, Khan et al., 2011, 2012] by triggering SynGAP dispersion from synaptic spines [Araki et al., 2015]. PKA is required for synaptic tagging [Duffy et al., 2001, Navakkode et al., 2004, Young et al., 2006, Huang et al., 2006] and is implicated in structural plasticity. PKA modulates the activity of LIM kinase [Lamprecht and LeDoux, 2004, Nadella et al., 2009], which phosphorylates (and inhibits) cofilin allowing for actin polymerization. Cofilin-mediated actin dynamics regulates spine morphology and AMPAR trafficking during synaptic plasticity [Chen et al., 2007, Gu et al., 2010]. Epac anchors in the PSD [Penzes et al., 2011] and triggers changes to spine cytoskeleton via Rap1 activation [Penzes and Cahill, 2012]. Interestingly, synapses stimulated by HFS while blocking PKA activity fail to be tagged [Huang et al., 2006], whereas ISO+HFS stimulation while blocking PKA still yields L-LTP [Gelinas et al., 2008]. Our simulations suggest that this seemingly contradictory result arises from the difference between the amount of Epac provided by HFS alone versus ISO+HFS. The plausibility of the spine signature is evident from its time course, which is comparable to the dynamics of molecular activation measured using live cell imaging [Nishiyama and Yasuda, 2015].

The molecular signature in the dendrite takes into account molecules that play a role in synthesis of PRPs. Both PKA and Epac activate ERK via Rap1 regulation [Vossler et al., 1997, Lin et al., 2003, Wang et al., 2006, McAvoy et al., 2009]. Also, PKA phosphorylation of β2AR can produce ERK activation by switching the β2AR coupling from G_s to G_i [Luttrell et al., 1996, Della Rocca et al., 1997, Martin et al., 2004], though this has not been directly demonstrated in neurons. ERK has been shown to be critical in L-LTP [Winder et al., 1999, Kelleher et al., 2004, Thomas and Huganir, 2004, Gelinas and Nguyen, 2005, Gelinas et al., 2007, Ajay and Bhalla, 2007] and the synthesis of PRPs [Sajikumar et al., 2007]. Both PKA and ERK can phosphorylate CREB, a molecule directly implicated in transcription. CaMKII is required for regulation of protein synthesis via phosphorylation of cytoplasmic polyadenylation element binding protein [Atkins et al., 2004, 2005] in hippocampal plasticity, but see [Sajikumar et al., 2007, Ramachandran and Frey, 2009]. Though both spine and dendrite signatures incorporated the same molecules, they have different downstream targets in the spine and in the dendrite. Thus the two molecular signatures set the stage for future models that incorporate control of actin dynamics in the spine and ERK activation in the dendrite.

Several other models have evaluated the molecular dependence and temporal sensitivity of L-LTP induction. Jain and Bhalla [2014] developed the most comprehensive model of signaling pathways leading to transcription of mRNA, demonstrating that different temporal stimulation patterns could recruit different mRNAs. In agreement with their results, our simulations showed that different stimulation patterns pro-

duced different patterns of elevation of various kinases. It would be quite interesting to couple our dendritic model to downstream modules of the Jain and Bhalla [2014] model to evaluate control of transcription by L-LTP stimulation patterns. Several other models investigated synaptic tagging and capture [Clopath et al., 2008, Barrett et al., 2009, Smolen et al., 2012] at hippocampal CA3-CA1 synapses. All of these models were able to predict various aspects of the synaptic tagging and capture hypothesis. Nonetheless, these models used simplified and abstract equations for activation of key kinases and phosphatases; thus it is not clear how well they could extrapolate to alternative stimulation patterns. A model by Smolen et al. [2014] also used streamlined equations for activation of key kinases and phosphatases, but included a model of histone deacetylation, which regulates transcription [Kuo and Allis, 1998]. That model suggested that promoting histone acetylation while simultaneously slowing cAMP degradation could help in restoring L-LTP, which is impaired in mouse models of Rubinstein-Taybi syndrome, a condition resulting in lower levels of CREB binding protein, which reduces transcription.

Our simulations of a dendrite with multiple spines are consistent with the spatial specificity of homo- and heterosynaptic plasticity suggested by imaging of spine morphological plasticity. Stimulation of two spines on the same branch produces a dendritic signature that crosses the threshold along the entire branch, regardless of the spatial configuration of those stimulated spines. This result is consistent with [Zhai et al., 2013], showing that one train of 5 Hz stimulation applied to two spines on the same branch saturates ERK activation in that branch. During these simulations, spine signatures of the unstimulated spines are elevated, although lower than these of the stimulated spines. This observation is consistent with the gradients observed experimentally [Lee et al., 2009]. Furthermore, the increase in signature of non-stimulated spines is consistent with the observation of a reduced LTP threshold heterosynaptically [Harvey et al., 2008]. It is, however, also possible that not all spines will exhibit potentiation due to competition for resources, as in [Govindarajan et al., 2011]. Our model does not take into account this competition, but such a model would allow only the spines with the highest signatures to capture PRPs, and thus non-stimulated spines with lower signatures would not exhibit LTP. The agreement between these simulations and experiments suggests the model could be used to predict the spatial pattern of LTP in response to in vivo like stimulation patterns.

We evaluated AMPAR phosphorylation by CaMKII and PKA as an indicator of E-LTP, and found agreement between our simulations and experimental results [Lee et al., 2000, 2003, 2010]. The brief duration of the AMPAR phosphorylation in our model is likely due to absence of AMPAR re-cycling mechanisms [Esteban et al., 2003]. Previous work has shown AMPAR recycling contributes to bistability [Hayer and Bhalla, 2005], and insertion of a phosphorylated AMPAR may protect it from dephosphorylation. Alternatively, AMPAR phosphorylation may only be a trigger for insertion, and the time course of E-LTP may reflect the removal of AMPARs in the synapse.

Induction of long-lasting LTP initiates a cascade of complex molecular interactions; therefore signaling pathway modeling is a useful approach to facilitate understanding of this complexity. In addition to confirming the plasticity outcome and molecular dependence for numerous LTP induction protocols, our model makes several experimentally testable predictions. Our model reveals that βAR signaling through non-conventional pathways is necessary in the dendrite, therefore ICI-118,551, a complete βAR antagonist, is predicted to block long-lasting LTP induced with 4xHFS-80s. Moreover, both conventional (G_s-activated) and non-conventional (G_i-activated) pathways are required for ISO+LFS to produce long-lasting LTP, therefore we predict, that bath application of carvedilol, a βAR antagonist, will not induce long-lasting LTP. Though our model focuses on βAR signaling, CA1 neurons express dopamine receptors, which have been implicated in some forms of long-lasting LTP [Huang and Kandel, 1995]. If such receptors are shown to undergo switching of G_s to G_i coupling, then these receptors also may contribute to a plethora of long-lasting forms of LTP. In summary, our model suggests that the non-linearity of signaling pathway interactions may explain why experimentally blocking any of the molecules included in our signature can disrupt long-lasting LTP.

Materials and Methods

To investigate how temporal pattern of synaptic activation determines which signaling pathways are activated, we employed a multi-compartmental, stochastic reaction-diffusion model of calcium and cAMP activated signaling pathways (Fig. 1). The model was adapted from an existing model of a CA1 hippocampal pyramidal neuron [Kim et al., 2011]. The pathways included calcium-calmodulin activated molecules, such as calcineurin (PP2B) and phosphodiesterase 1B (PDE1B), cAMP activated molecules: Epac and PKA, and interactions between calcium and cAMP pathways via Inhibitor-1 (I1). The previous model [Kim et al., 2011] was modified by adding neurogranin (Ng) [Kubota et al., 2007], a calmodulin buffer, implicated in LTP and learning [Zhabotinsky et al., 2006, Zhong et al., 2015]. Most importantly we added several pathways downstream of β2AR [Daaka et al., 1997] to the CA1 hippocampal pyramidal neuron model. Norepinephrine binds to stimulatory G protein (G_s) coupled β2ARs [Tang et al., 1991], which are expressed in CA1 [Hillman et al., 2005]. The activated α subunit of G_s ($G_{\alpha s}$GTP) synergistically enhances cAMP production by calcium-calmodulin bound adenylyl cyclase 1 (AC1). Elevations in cAMP, produced by either prolonged stimulation of β2AR or increases in intracellular calcium, activate PKA, which can phosphorylate β2AR. There are four sites of heterologous phosphorylation [Sibley and Lefkowitz, 1985] on the β2AR [Liggett et al., 1989], whose phosphorylation leads to alternative G protein coupling. In the model, a single phosphorylation event decouples the β2AR from G_s, and the fully phosphorylated β2AR then binds inhibitory G protein (G_i). The β2ARs are phosphorylated in a cooperative and distributive manner [Chay et al., 2015], which yields

an ultrasensitive response [Thomson and Gunawardena, 2009, Ferrell and Bhatt, 1997, Dushek et al., 2011]. Note that both G_i [Luttrell et al., 1996, Della Rocca et al., 1997, Martin et al., 2004] and β-arrestin [Shenoy et al., 2006] have been implicated in ERK recruitment to the pβ2AR; thus, in our model the G_i binding to pβ2AR could alternatively represent β arrestin binding. Model parameters are presented in Supporting information Table S3.

The morphology of the model comprised one spine attached to a 2 μm dendrite or 8 spines attached to a 20 μm long dendrite with 0.6 μm diameter (Fig. 2). In all cases, the dendrite and spines were subdivided into voxels for purpose of simulation. The layer of voxels immediately adjacent to the membrane was considered the submembrane domain. AC (type 1 and 8), PKA holoenzyme, G proteins and the β2ARs were localized and anchored both in this submembrane domain and the spine head. The diffusible molecules included cAMP, ATP, calcium, all forms of calmodulin (CaM), CaMKII, β2AR agonists and antagonists, I1 and Epac. Their diffusion constants are listed in Supporting information Table S4.

We used a stochastic model, as many molecular populations are small. In such case activations fluctuate greatly about the mean within such small compartments [Gillespie, 1977]. Similarly, diffusion of second messenger molecules out of the spines and along the thin dendrites should be described using stochastic equations. The model was implemented using an efficient mesoscopic stochastic reaction-diffusion simulator NeuroRD [Oliveira et al., 2010], version 2.1.10, because the large numbers of molecules in the morphology described (Fig. 2) made tracking individual molecules in microscopic stochastic simulators computationally expensive. Model simulations used a time step of 2.9μs. Based on results from our prior studies, simulations were repeated four times using a different random seed. To determine whether the combination of stimulation and βAR ligand would induce L-LTP or not, we analyzed the duration above threshold using SAS (version 9.4, SAS Institute, NC). We applied the procedure TTEST to each condition to evaluate whether the duration above threshold was significantly greater than 10 s. To investigate correlation between the spine and dendritic signatures we calculated Pearson correlation coefficient by applying procedure CORR.For the multi-spine simulations, we used the SAS procedure GLM to perform a two-way analysis of variance using condition (adjacent or separate) and stimulation (spine was stimulated or not) as factors. All model simulation files are available from modelDB (`https://senselab.med.yale.edu/ModelDB/showModel.cshtml?model=190304` access code JJSzmek).

Stimulation Paradigms

Different forms of LTP are evoked by different stimulation patterns [Lisman et al., 2005]; thus, we performed simulations using seven, well characterized, stimulation paradigms (Table 1). Four of them experimentally

elicit L-LTP, one results in E-LTP and the remaining two stimulation paradigms do not produce LTP, though one (LFS) elicits brief depression. Electric stimulation of Schaeffer collaterals results in activation of post-synaptic NMDA receptors and action potentials, thus each stimulation pulse was simulated in the model as calcium injection both into the spine to represent NMDA receptors, and into the dendrite to represent activation of voltage dependent calcium channels. Electric stimulation in the hippocampus is accompanied by NE release [Milusheva et al., 1994], which was modeled as ligand influx. Bath applied ISO was simulated by injecting sufficient ISO to produce a 1μM concentration.

Several data sources were used to adjust calcium pulse amplitudes for the other stimulation paradigms. To stimulate calcium influx during 100 Hz trains of electric stimulation (HFS), we used release probabilities from [Kim et al., 2012] which provides changes in the amplitudes of calcium pulses in the spine during high frequency trains. We assumed that amplitudes of consecutive calcium pulses in the dendrites are uniform, because they result from full height action potentials. To calculate absolute amplitudes of calcium pulses, we constrained calcium concentration in the spine and in the dendrite to match experimental data [Scheuss et al., 2006]: 10 μM in the spine and 2 μM in the dendrite. This pattern of calcium pulses was used in all stimulation paradigms using trains of HFS: 1 train of 100 Hz (HFS), four trains of 100 Hz given 3 sec apart (4xHFS-3s), four trains of 100 Hz given 80 sec apart (4xHFS-80s) and bath applied isoproterenol followed by 1 train of 100 Hz (ISO+HFS). For the 5 Hz (LFS) stimulation paradigm, spine calcium pulses were of the same amplitude, and equal to the amplitude of the first pulse of the HFS train [Scheuss et al., 2006].

In order to estimate the temporal pattern and amplitude of neuromodulation elicited by electric stimulation, we first fitted a model (Eq. 3) describing vesicle release [Tsodyks et al., 1998] to voltammetric responses of norepinephrine release in the rat Ventral Bed Nucleus Stria Terminalis following electric stimulation of noradrenergic projection pathways [Park et al., 2009]. Using the tuned vesicle release model (Supporting information Table S8) we estimated norepinephrine release for stimulation patterns in Table 1. The spatial distribution of norepinephrine during and following release was in agreement with a spatial gradient of neuromodulators [Dreyer et al., 2010], namely a high concentration in the spine release site (1 μm) and lower at the dendrite.

$$\begin{aligned}
\frac{\mathrm{d}R}{\mathrm{d}t} &= \frac{1-R-E}{\tau_\mathrm{r}} - uR\delta(t-t_\mathrm{AP}) \\
\frac{\mathrm{d}E}{\mathrm{d}t} &= -\frac{E}{\tau_\mathrm{i}} - uR\delta(t-t_\mathrm{AP}) \\
\frac{\mathrm{d}u}{\mathrm{d}t} &= -\frac{u}{\tau_\mathrm{f}} + U_\mathrm{SE} * (1-u)\delta(t-t_\mathrm{AP})
\end{aligned} \tag{3}$$

Amplitude of neurotransmitter release in response to single AP = $A_\mathrm{SE}E$

PKA phosphorylates NMDA receptors, which increases the amplitude of calcium influx through these receptors [Skeberdis et al., 2006, Murphy et al., 2014]. This enhancement of NMDA medicated calcium influx has been observed with bath application of ISO. Thus, for the case of ISO+LFS, calcium influx was increased by 50% [Liebmann et al., 2009]. Inhibition of PKA lowers NMDA medicated calcium influx by 20% [Skeberdis et al., 2006], thus calcium influx was lowered by 20% for those simulations.

We modeled propranolol (1 μM; Swanson-Park et al. [1999]) ICI-118,551 (100 nM; Havekes et al. [2012]) and carvedilol (10 μM [Tzingounis et al., 2010]) by allowing it to bind the β2AR [Smith and Teitler, 1999] (Table S3), and then both propranolol- and carvedilol-bound β2AR was able to bind with G_i and form a target representing ERK activation. Binding affinity was constrained so that carvedilol produces one third the G_i bound β2AR compared to that of propranolol [Kahsai et al., 2011].

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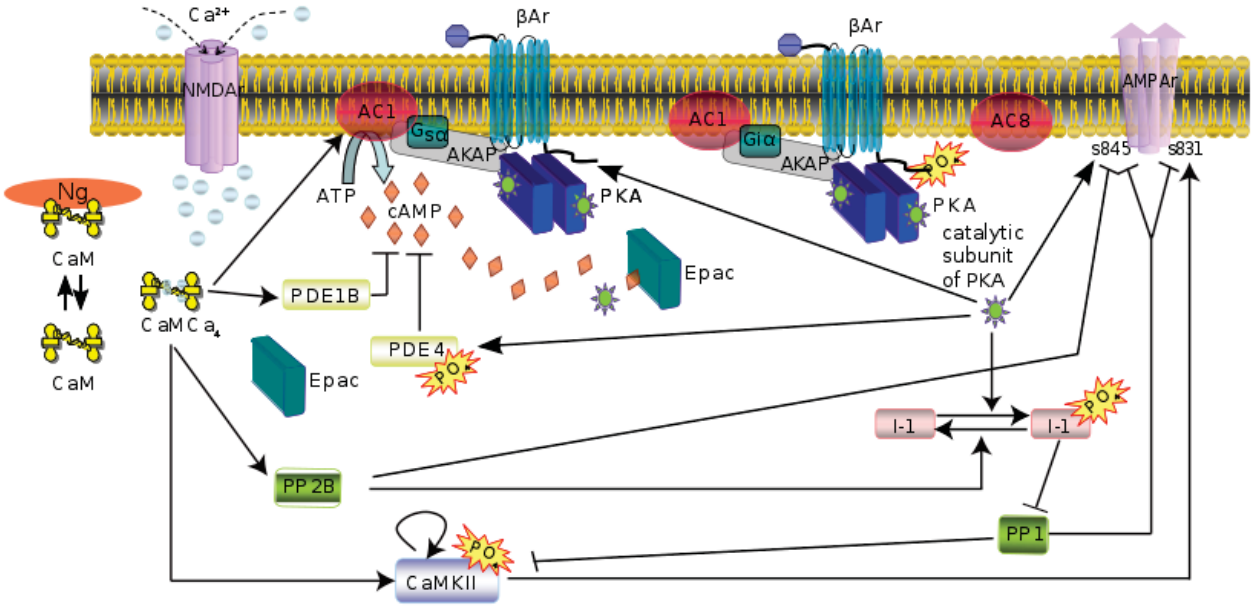


Figure 1. Diagram of postsynaptic signaling pathways. Each arrow is modeled with one or more bimolecular or enzyme reactions. Diffusion is not illustrated in this diagram.

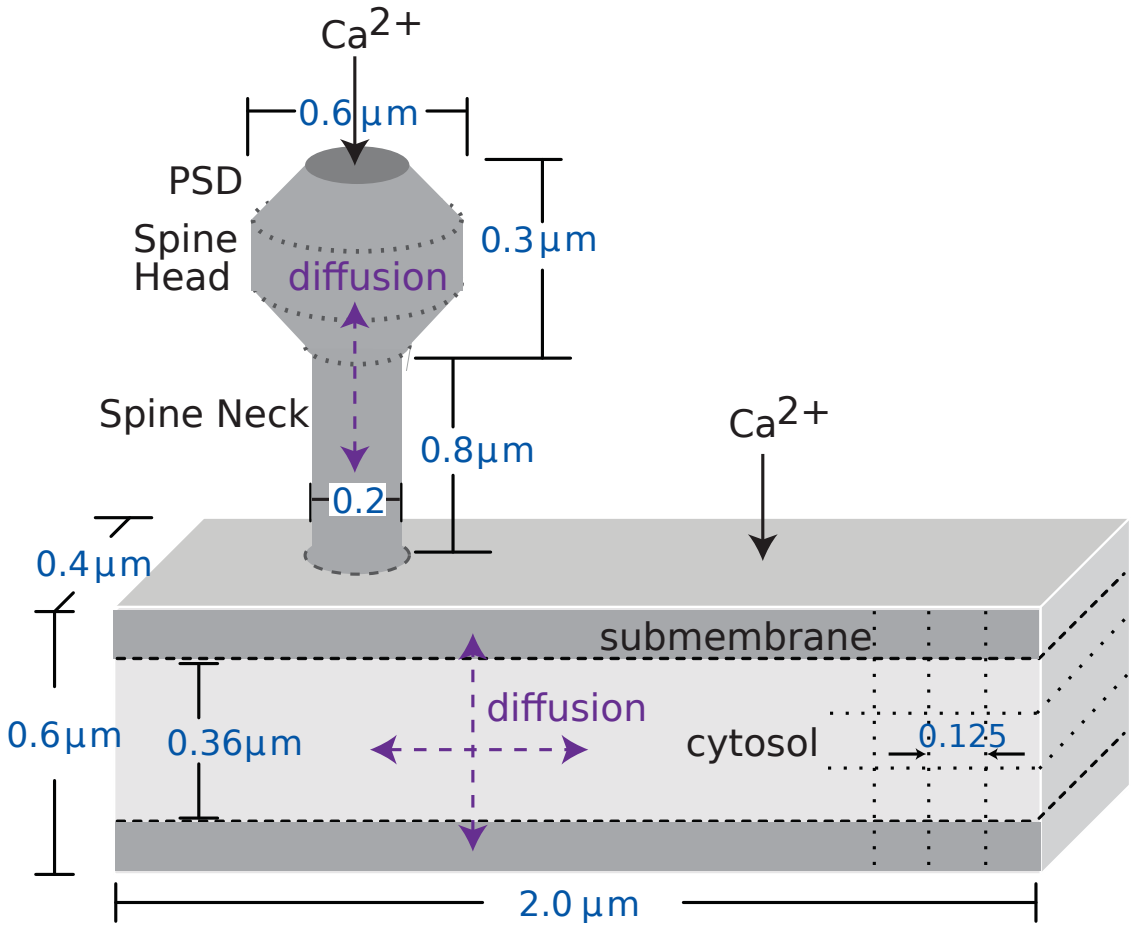


Figure 2. Morphology of dendrite with attached spine and location of calcium influx in the model. Dendritic subvolumes are cuboids, whereas the spine subvolumes are either cylindrical or conical, as portrayed. Dotted lines show part of the subvolumes. These subvolumes adjacent to the top and bottom surface of the dendrite are considered submembrane subvolumes. Other dendritic subvolumes are part of the cytosol. Calcium injection in a focal dendritic region represents influx through voltage dependent calcium channels. Calcium injection in the PSD represents influx through NMDA receptors and voltage dependent calcium channels in the spine. Diffusion is two-dimensional in the dendrite and one-dimensional in the spine, with reflective boundary conditions.

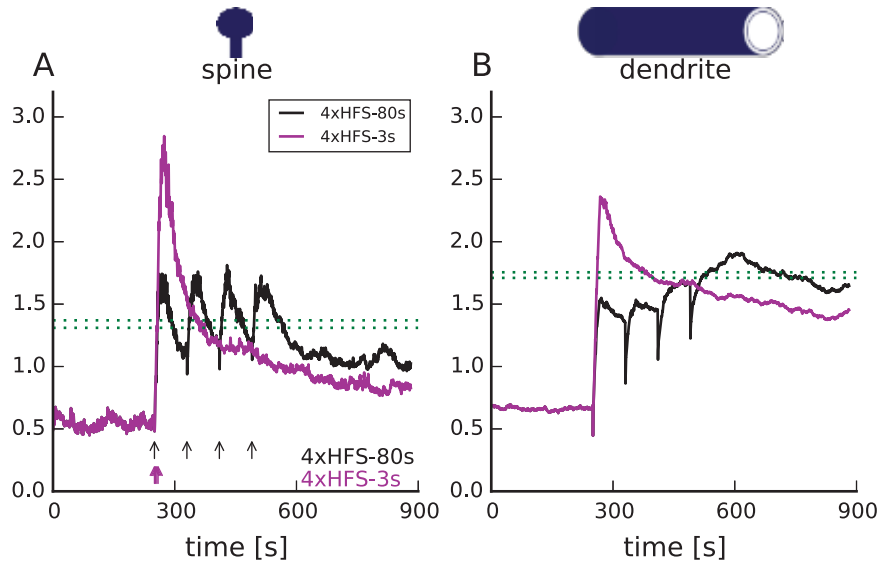


Figure 3. The molecular signatures correctly predict that both 4xHFS-80s and 4xHFS-3s will elicit a long-lasting form of LTP. Both spine (A) and dendritic (B) signatures of both paradigms cross their respective thresholds. For all panels dashed green lines represent the range of threshold, which correctly predicts the plasticity outcome.

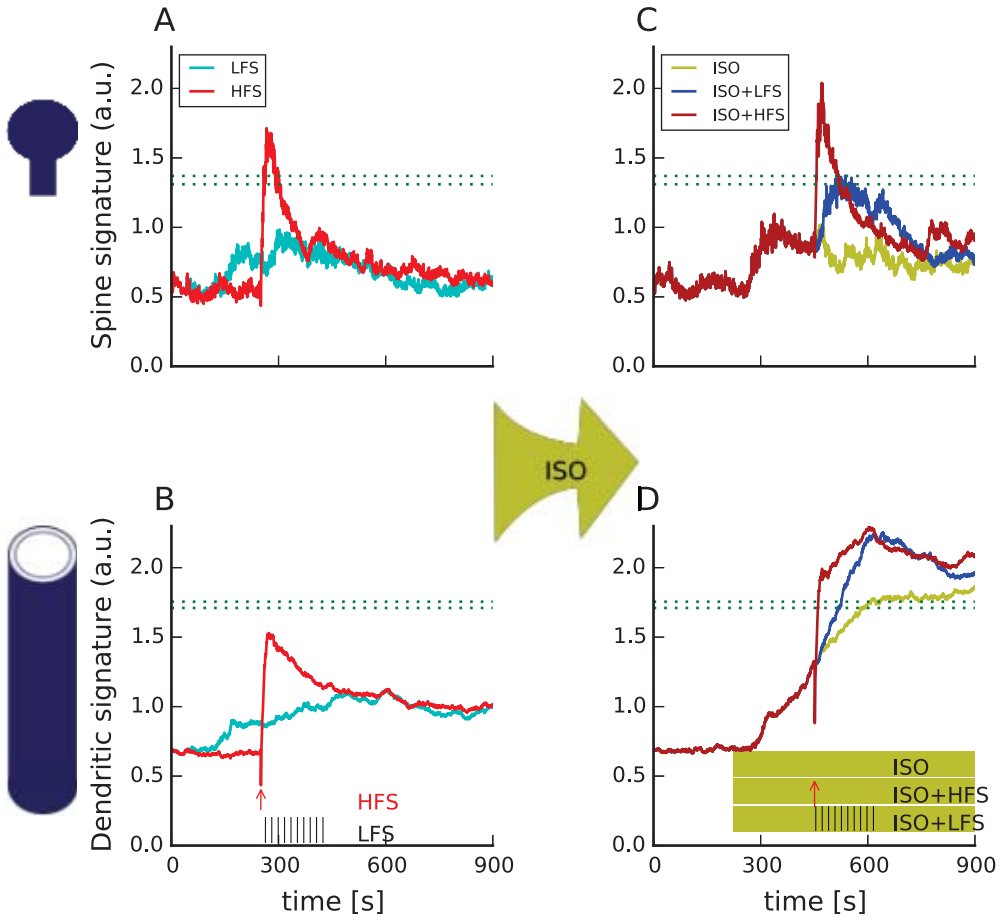
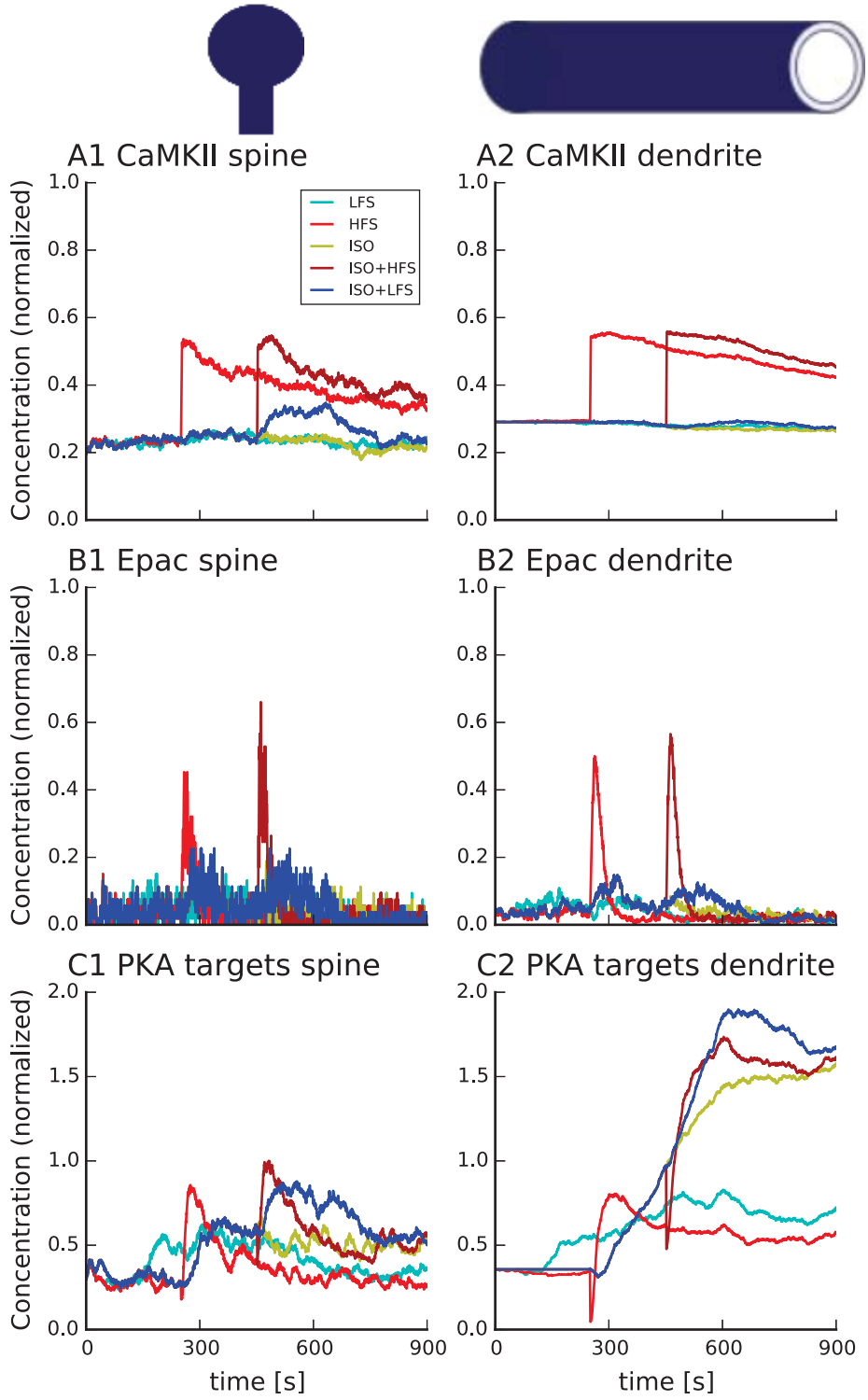


Figure 4. The molecular signatures correctly predict βAR activation will transform weak stimulation (LFS, HFS) into a long-lasting form of LTP. The effect of the βAR activation is visible mainly in the dendrite (B, D), where it elevates the dendritic signature above the threshold for both ISO+LFS and ISO+HFS. It also provides necessary cAMP elevation to allow the spine signature (A, C) of ISO+LFS to cross the threshold. For all panels dashed green lines represent the range of threshold, which correctly predicts the plasticity outcome.



458

459 **Figure 5.** Traces of molecular components of the signature reveal that LFS, but not HFS, requires PKA
460 for the signature to exceed the threshold for induction of long-lasting LTP. Legend in panel A1 applies to all
461 panels. (A) CaMKII activity in the spine (A1) and dendrite (A2),(B) Epac activity in the spine (B1) and
462 dendrite (B2), (C) PKA activity in the spine (C1) and dendrite (C2). PKA is not required for long-lasting
468 LTP induced with ISO+HFS because ISO increases Epac strongly for HFS.

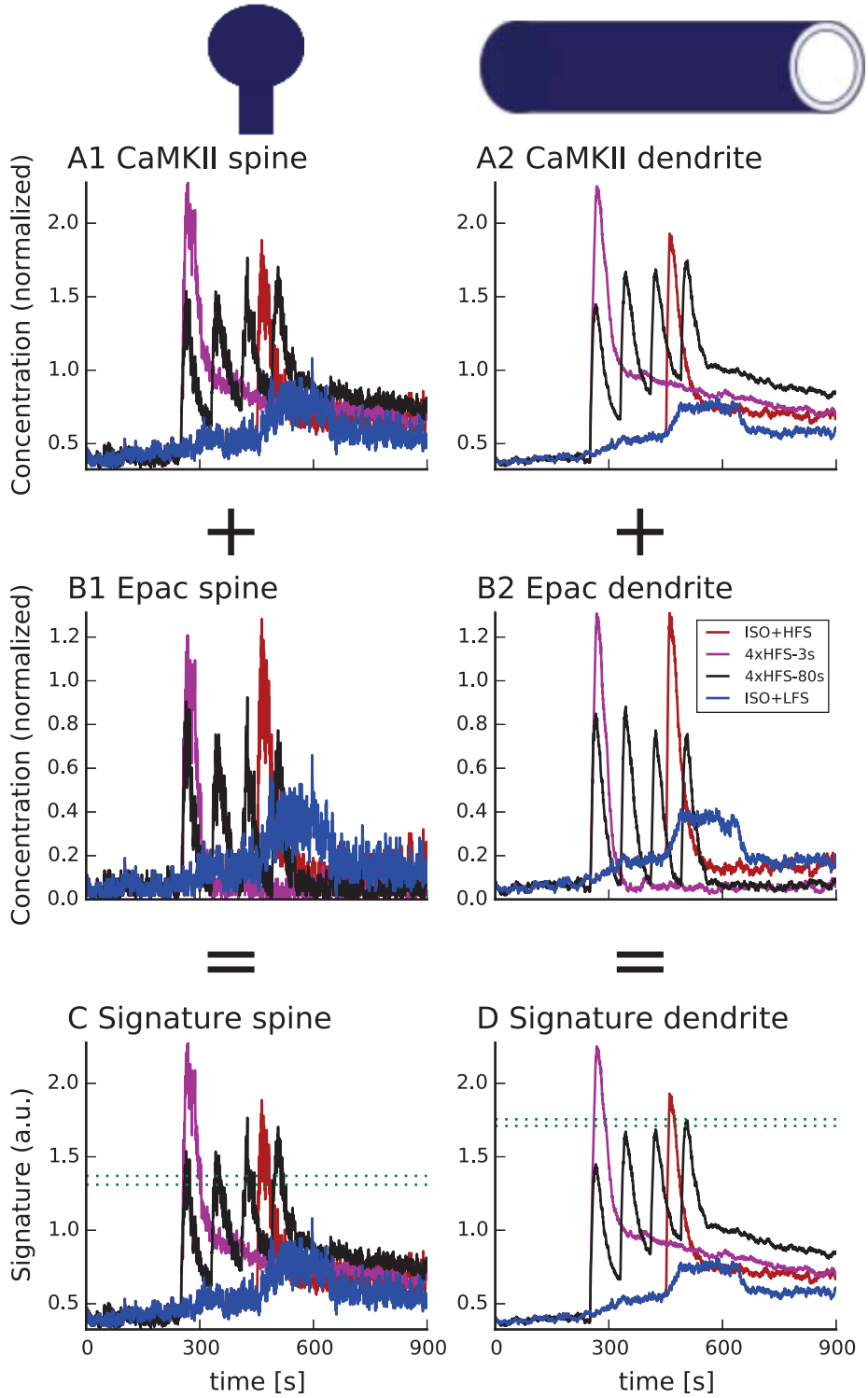
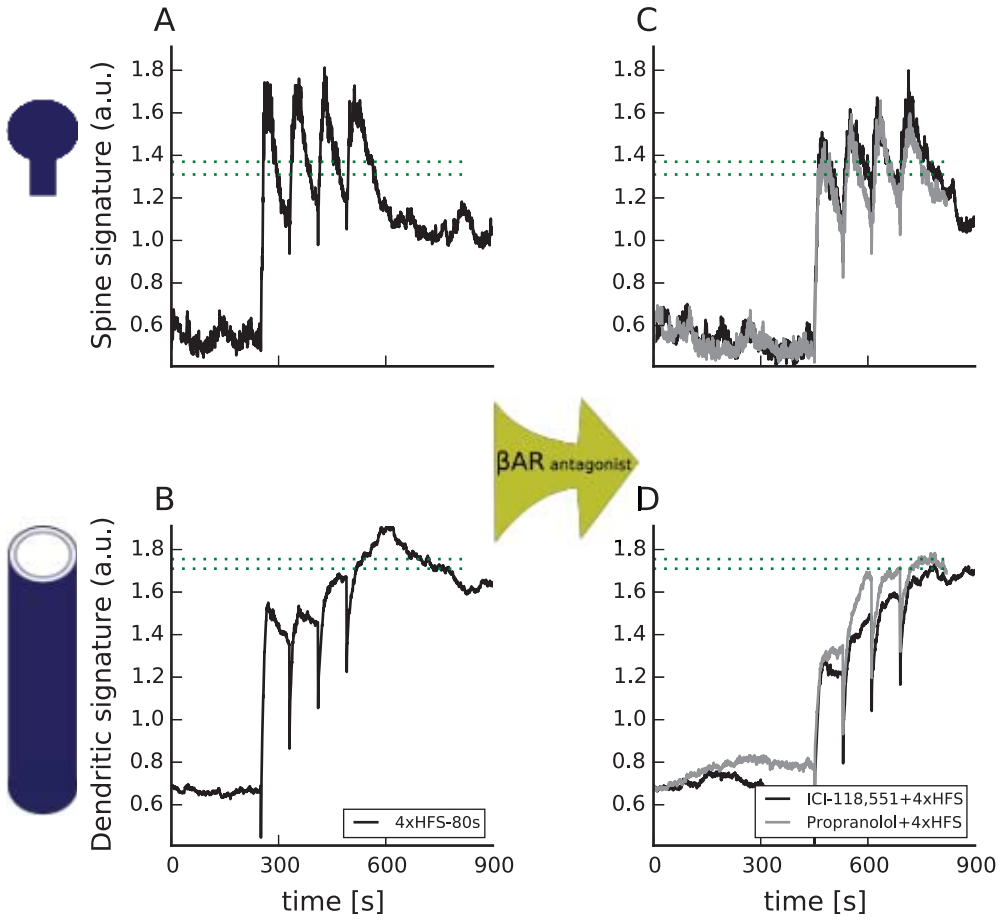


Figure 6. Molecular signatures in the spine (C) and dendrite (D) predict that inhibiting PKA blocks long-lasting forms of LTP for PKA-dependent stimulation paradigms. As seen in panels B1 and B2, in the presence of PKA inhibitors, an elevation in Epac activity compensates for PKA for 4xHFS-3s and ISO+HFS. PKA inhibition slightly lowers CaMKII in the spine (A1) and dendrite (A2). Legend in panel B2 applies to all panels. The dashed green lines represent the range of threshold, which correctly predicts the plasticity outcome.



473

474 **Figure 7.** βAR activation is critical in the dendrite, but not in the spine for HFS. Inhibition of βAR
475 activation by ICI-118,551, a β2AR antagonist, blocks a PKA-dependent long-lasting form of LTP (4xHFS-
476 80s). Propranolol (C, D, gray trace), which recruits some ERK [Kahsai et al., 2011], does not abolish
477 long-lasting LTP [Swanson-Park et al., 1999]. (A,B) show spine and dendritic signatures of 4xHFS-80s in
478 control conditions. (C,D) show effect of ICI-118,551 or propranolol. The dashed green lines represent the
479 range of threshold which correctly predict the plasticity outcome.

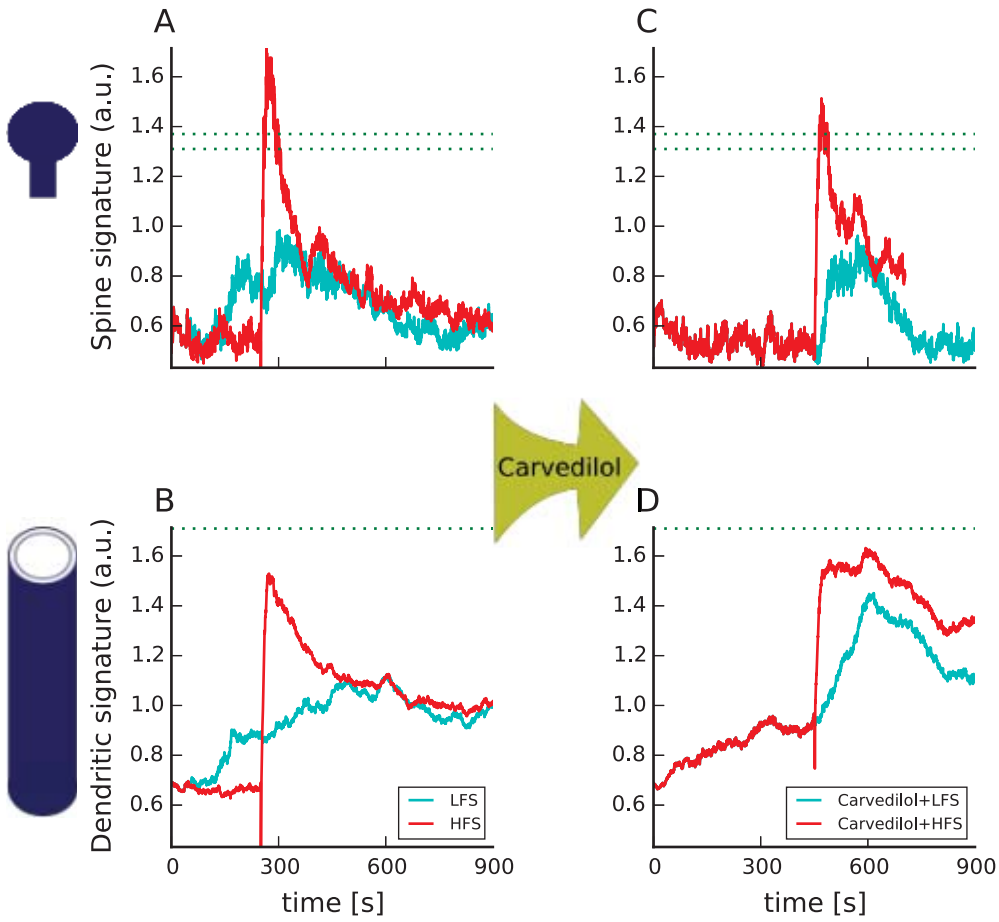


Figure 8. Molecular signatures of experimental protocols eliciting long-lasting forms of LTP using novel β2AR antagonist demonstrate the role of non-canonical βAR-activated pathways in induction of long-lasting LTP. For HFS, calcium influx due to electric stimulation is sufficient to elevate the spine signature above threshold (A). The spine signature (A,C) predicts that carvedilol will not support a long-lasting form of LTP elicited using HFS and LFS. Dendritic (B,D) signatures of weak electric stimulation are elevated after bath applying carvedilol, transforming HFS but do not cross the threshold. The dashed green lines represent the range of threshold which correctly predict the plasticity outcome.

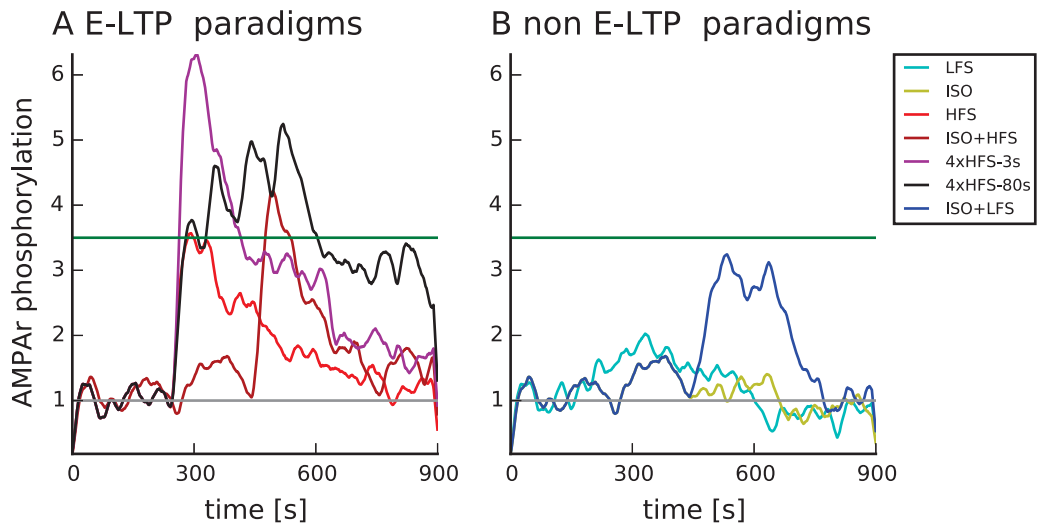


Figure 9. Changes in AMPA receptor phosphorylation (pAMPAR) caused by stimulation paradigms from Table 1 correlate with induction of E-LTP. Both panels show phosphorylation at Serine 845, Serine 831 or both, relative to the steady state phosphorylation. (A) Stimulation paradigms that elicit E-LTP experimentally. HFS, ISO+HFS, 4xHFS-3s, 4xHFS-80s elicit E-LTP. (B) Stimulation paradigms that do not elicit E-LTP experimentally. Green horizontal line depicts E-LTP threshold. Grey horizontal line depicts no change.

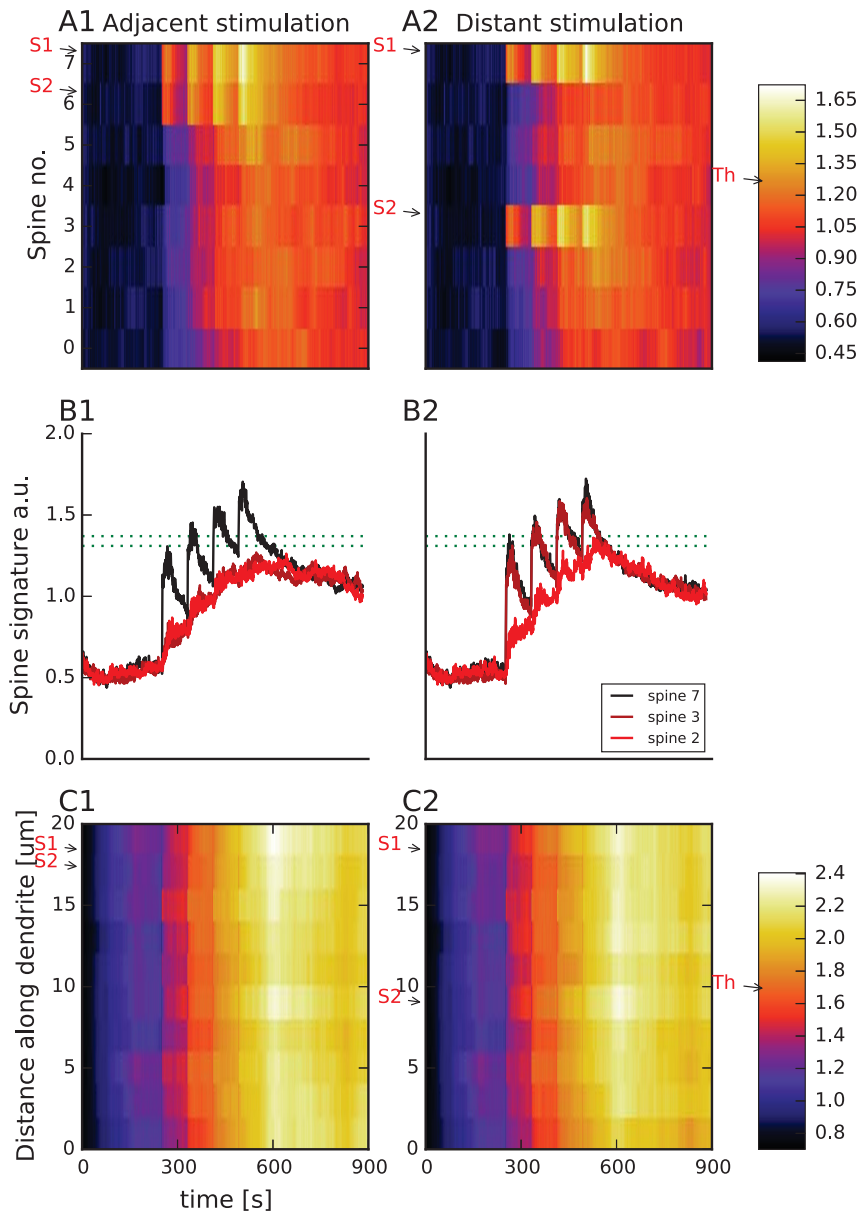
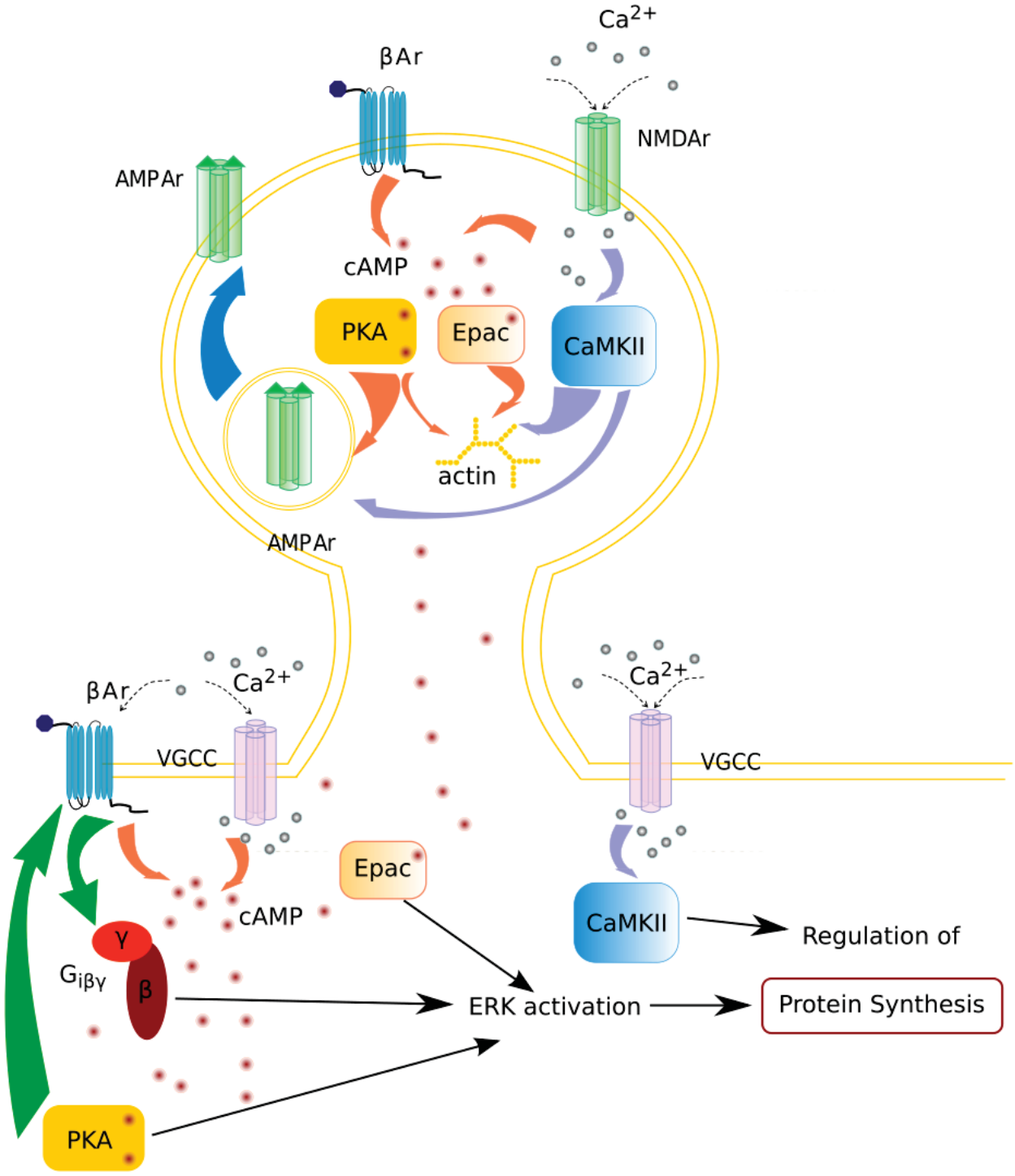


Figure 10. The spine signature exhibits spatial specificity, whereas the dendritic signature exceeds the threshold when two spines are stimulated, whether the spines are adjacent or separated. (A) A color plot of spine signature shows that only a few of the non-stimulated spines have a signature that exceeds the threshold. Arrow labeled Th shows threshold of the spine signature on the colorbar. A1: With stimulation of two adjacent spines, only the stimulated spines and an adjacent spine will exhibit LTP. A2: When separate spines are stimulated, the stimulated spines and and also several nearby spines will exhibit LTP. Arrows labeled S1 and S2 show position of stimulated spines. (B) Time course of molecular signature for three spines (spine 2, 3 and 7), showing the difference in spine signature for stimulated and non-stimulated spines. (C) Molecular signatures in the dendrite. Arrow labeled Th shows threshold of the dendritic signature on the colorbar. Arrows labeled S1 and S2 show position of stimulated spines. B1: Spines 6 and 7 are stimulated. B2: Spines 3 and 7 are stimulated.



511

512 **Figure 11.** Schematic diagram depicting core mechanisms underlying stabilization of the synaptic strength
513 that accompanies long-term synaptic plasticity. In the dendrite βAR activation is required for paradigms
514 with low Ca^{2+}, e.g. LFS. In the dendrite βAR activation either by G_S coupling or by switching to G_i
516 coupling is needed for dendrite specific changes.

Table 1. Experimental protocols and their characteristics. n.a. stands for not applicable. Protocol provides the abbreviation that is used throughout the article. Description gives brief explanation of experimental protocol. Outcome indicates experimentally observed outcome of the protocol: E-LTP, a long-lasting form of LTP, both or no change. Molecular dependence lists which molecules were experimentally shown essential.

Protocol	Description	Outcome	Molecular dependence
LFS	1800 s of 5 Hz	brief depression [Gelinas and Nguyen, 2005, Winder et al., 1999]	n.a.
ISO	bath applied 1 µM of isoproterenol	no change [Gelinas and Nguyen, 2005, Winder et al., 1999]	n.a.
HFS	1 s of 100 Hz (1 train)	E-LTP [Duffy et al., 2001]	CaMKII [Huang and Kandel, 1994]
4xHFS-3s	4 trains of HFS with 3 s inter-train interval	E-LTP, long-lasting LTP	CaMKII [Sanhueza et al., 2011]
ISO+HFS	bath applied 1 µM of isoproterenol 10 minutes before HFS	E-LTP, long-lasting LTP [Gelinas et al., 2008]	Epac, ERK [Gelinas et al., 2008]
4xHFS-80s	4 trains of HFS with 80 s inter-train interval	E-LTP, long-lasting LTP	PKA [Woo et al., 2003, Zhang et al., 2011], ERK [English and Sweatt, 1997]
ISO+LFS	bath applied 1 µM of isoproterenol 10 minutes before LFS	long-lasting LTP [Gelinas and Nguyen, 2005, Winder et al., 1999]	PKA, ERK [Winder et al., 1999]