Review

Homer 1a and mGluR5 phosphorylation in reward-sensitive metaplasticity: A hypothesis of neuronal selection and bidirectional synaptic plasticity

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Abstract

Drug addiction and reward learning both involve mechanisms in which reinforcing neuromodulators participate in changing synaptic strength. For example, dopamine receptor activation modulates corticostriatal plasticity through a mechanism involving the induction of the immediate early gene Homer 1a, the phosphorylation of metabotropic glutamate receptor 5 (mGluR5)'s Homer ligand, and the enhancement of an NMDA receptor-dependent current. Inspired by hypotheses that Homer 1a functions selectively in recently-active synapses, we propose that Homer 1a is recruited by a synaptic tag to functionally discriminate between synapses that predict reward and those that do not. The involvement of Homer 1a in this mechanism further suggests that decaminutes-old firing patterns can define which synapses encode new information.

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Abbreviations: Arc, activity-regulated cytoskeleton-associated protein; D1R, dopamine receptor 1; ERK, extracellular signal-regulated kinase; EVH1, Ena/VASP Homology 1; H1a, Homer 1a; HFS, high-frequency stimulation; IEG, immediate early gene; KCl, potassium chloride; LFS, low-frequency stimulation; LTP, long-term potentiation; mGluR5, metabotropic glutamate receptor 5; mGluR5pS, mGluR5 with phosphorylated S1126; NMDAR, NMDA receptor; PIN1, peptidyl-prolyl cis-trans isomerase NIMA-interacting 1; PRP, plasticity-related protein; SIC, slow inward current

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1. Introduction

Animals experience many events in the course of a day, yet preferentially encode those that have special salience. The basis for this selectivity can be related generally to "reinforcement pathways" that release neuromodulators including dopamine, noradrenaline, or serotonin in specific brain areas in response to an experience. Drug addiction can be conceptualized as a form of learning that usurps these neuromodulatory reward pathways (Hyman et al., 2006; Luscher and Malenka, 2011). How do neuromodulators influence memory formation and how does their misappropriation by drugs of abuse lead to addiction? As we will describe here, neuromodulators evoke intracellular signaling events that create conditional states of enhanced synaptic plasticity, which can alter synaptic drive and create long-lasting associations between neurons. These associations underlie reward learning and memory. We describe newly discovered biochemical events in this pathway that are time-dependent and conditional in ways that suggest parallels with theoretical models of learning. We begin with a biochemical model that is built upon the function of a class of proteins encoded by cellular immediate early genes (IEGs) and highlight the events that create such conditionality. We then speculate on how these molecular events might relate to physiological and theoretical models of learning.

2. Biochemical model describes contingencies for neuromodulator-dependent synaptic plasticity

2.1. Metabotropic glutamate receptors, Homer, and cocaine addiction

Dr. Conquet’s lab at GlaxoSmithKline first reported that metabotropic glutamate receptor type 5 (mGluR5) plays a role in the behavioral responses to cocaine (Chiamulera et al., 2001). Since then, pharmacological manipulation of mGluR5 has similarly been shown to influence self-administration of cocaine and other drugs of abuse (Kenny et al., 2003; Paterson and Markou, 2005). mGluR5 is a group 1 metabotropic glutamate receptor that activates phospholipase C to generate the intracellular second messengers diacyl glycerol (which activates protein kinase C) and inositol triphosphate (which releases Ca$^{2+}$ from intracellular stores). In addition to these canonical signaling outputs, group 1 mGluRs can regulate signaling pathways that control activation of PI3 kinase, tyrosine kinase and phosphatase, and elongation factor 2 kinase, generate endocannabinoids and D-serine, and activate membrane ion channels (Nicoletti et al., 2011). Given this diversity of function, it is perhaps surprising that mGluR5 deletion mice (mGluR5$^{-/-}$) are viable and fertile, and possess many baseline behavioral responses that allow them to survive in a lab setting. However, mGluR5$^{-/-}$ mice show several behavioral endophenotypes relevant to addiction including reduced locomotion in response to cocaine, reduced cocaine motor sensitization, and reduced cocaine self-administration (Bird and Lawrence, 2009). The finding that mGluR5 is involved in the behavioral responses to cocaine was unexpected, as cocaine was known to influence dopamine levels and receptors, but not directly affect glutamate receptors. How then is mGluR5 involved in responses to cocaine?

One lead toward answering this question came from studies of genes whose mRNAs are rapidly up-regulated in response to brain activity. Classical studies reported more than 50 years ago that de novo RNA and protein synthesis are required for animals to establish long-term memory (Davis and Squire, 1984). Importantly, protein and RNA synthesis are required only during a brief time window ~3 h immediately following a salient experience (Montarolo et al., 1988). This suggested that it may be possible to discover critical events that underlie memory by identifying mRNAs and encoded proteins that are up-regulated during this time window and examining their synaptic function.

Homer 1a (H1a) is an example of a gene that is rapidly transcribed in brain neurons in response to neuronal...
activating stimuli, including cocaine (Brakeman et al., 1997). H1a is a member of a family of gene products generated by three different genes, termed Homer 1, 2, and 3 (Xiao et al., 1998; Tu et al., 1998). All of the Homer gene products share a conserved protein domain at their N-terminus called the EVH1 (Ena Vasp Homology 1) domain. H1a is generated from the Homer 1 gene by a mechanism that involves activity-dependent transcript termination within an intron (Bottai et al., 2002). This mechanism prevents splicing of the intron, and the intron becomes an exon that encodes the shorter C-terminus of the H1a protein. Homer 1 is additionally notable in that intron 1 is large (~40 kb). Because of the finite processivity rate of transcription by Pol II, it takes ~20 min from the time of a stimulus for H1a mRNA to appear in the neuronal soma (Vazdarjanova et al., 2002). This contrasts with 2–3 min for the de novo transcription of Arc mRNA (Guzowski et al., 1999). Despite the complexity of H1a’s transcriptional induction, H1a and Arc are both closely tied to physiological synaptic activity; these two gene products can be monitored in coordination to identify neurons of the hippocampus and cortex that are engaged in experience-dependent information processing (Vazdarjanova et al., 2002).

Homer’s EVH1 domain binds the consensus sequence PPXXF (Tu et al., 1998). mGluR5’s C-terminus contains a Homer binding site (TPPSPF), as do regions of the IP3 receptor, the ryanodine receptor, Shank, and dynamin 3 (Tu et al., 1999). These proteins are likely co-functional within the synapse. H1a is unique within the Homer gene family; it lacks the C-terminal coiled-coil and leucine zipper region that mediates dimerization or tetramerization of all other Homer molecules (Hayashi et al., 2009; Xiao et al., 1998). These longer forms of Homer (in forebrain Homer 1c and Homer 2 are the major forms; Homer 3 predominates in cerebellum) can form crosslinked complexes, connecting mGluR5 with downstream signaling partners. H1a lacks the dimerization domain and therefore acts as a “dominant negative” that can disrupt crosslinking, freeing mGluR5 from crosslinked complexes. mGluR5 signaling might be modulated in response to neuronal activity through the dynamic expression of H1a and consequent disruption of these complexes (Xiao et al., 2000).

Evidence that Homer proteins contribute to behavioral responses to cocaine came with studies that observed genetically deleting Homer 1 or Homer 2 (Szumlinski et al., 2004). Interestingly, naïve Homer1−/− or Homer2−/− mice exhibit neurochemical changes in the striatum that are typically seen in wildtype mice upon repeated administration of cocaine (Szumlinski et al., 2004). An emergent model suggested Homer crosslinking restricts mGluR5 function to inhibit an mGluR5-dependent cocaine-induced plasticity (Szumlinski et al., 2006).

2.2. D1R activation induces mGluR5 phosphorylation: a de novo binding site for PIN1

To test the hypothesis that interruption of Homer binding to mGluR5 creates a condition that mimics or enhances cocaine sensitization, we created a knock-in mouse that incorporates a single amino acid mutation of mGluR5 (TPPPSR; mGluR5R/R) that markedly reduces Homer EVH1 binding (Tu et al., 1998). By disrupting Homer binding to mGluR5, we hoped to mimic Homer 1−/− and Homer 2−/− animals’ responses to cocaine. However, mGluR5R/R mice showed normal neurochemical parameters, normal locomotor responses to cocaine, and normal motor sensitization (Park et al., 2013). Thus, interruption of Homer-dependent crosslinking by mutation of mGluR5 did not produce the same effect as deleting crosslinking Homer. A different model was needed.

An alternate model for the role of Homer binding to mGluR5 emerged with the discovery that the Homer binding site in mGluR5 is phosphorylated by kinases that target proline-adjacent serine or threonine (proline-directed kinases) (Orlando et al., 2009). Since proline-directed ERK kinases are activated by dopamine receptor (D1R) stimulation (Gerfen, 2003; Gerfen et al., 2008; Kim et al., 2006; Valjent et al., 2004), we reasoned that mGluR5 might be phosphorylated by ERK following D1R stimulation by cocaine (Koob and Bloom, 1988; Koob, 1992). Indeed, cocaine administration or direct D1R stimulation induced mGluR5 phosphorylation in an ERK-dependent manner (Fig. 1, steps 2 and 3) (Park et al., 2013). These findings suggest a model whereby addictive drugs and natural rewards enhance dopamine levels (Carelli and Wightman, 2004; Luo et al., 2011), stimulate D1Rs, and activate ERKs to phosphorylate mGluR5; this phosphorylation could mediate some of the behavioral effects of cocaine.

Phosphorylation of the Homer binding site in mGluR5 has two important effects: it increases the affinity of Homer EVH1 binding and creates a binding site for a prolyl isomerase (Fig. 1, steps 3 and 5) (Park et al., 2013). These effects are revealed in dynamic structural studies conducted by Dr. Kerns’ lab at Brandeis that used NMR to determine the conformation and binding affinity of synthetic phosphopeptides and Homer 1 EVH1. A prior crystal structure of the EVH1 domain bound to the mGluR5 sequence (TPPPSF) showed that the alignment of phenylalanine’s aromatic side chain along the aromatic surface of EVH1 is energetically important to the mGluR5-Homer interaction (Beneken et al., 2000). The cis conformation of the S-P bond is required to create a tight hairpin turn between phenylalanine and the trigonal prism of the peptide’s N-terminus. Phosphorylated forms of the mGluR5 peptide enable an increase in the cis conformation of the S-P bond, allowing a near 40-fold increase in EVH1 binding affinity (Park et al., 2013).

A second consequence of mGluR5’s phosphorylation is that it creates a binding site for the prolyl isomerase PIN1 (Fig. 1, step 5) (Park et al., 2013). Proline’s side chain is chemically bonded to the amino acid backbone, which reduces rotation around this bond. Rotation is important in protein folding during synthesis and for functional properties of certain proteins. Phosphorylation of the S-P bond in the Homer binding site of mGluR5 further slows spontaneous isomerization such that prolyl isomerases become essential to allow isomerization on a time scale of sec−1 (Fig. 1, step 6) (Lu et al., 2007; Lu and Zhou, 2007). PIN1 is a member of the parvulin family of prolyl isomerases and is unique in that it requires phosphorylation of its substrate for its binding and catalytic activity. We therefore hypothesized that PIN1 could be part of a mechanism involving cocaine-induced phosphorylation of mGluR5’s Homer ligand and subsequent
interactions. NMR studies confirmed that PIN1 binds phosphorylated mGluR5 peptide sequence and accelerates isomerization of the pS–P bond by >1000-fold (Park et al., 2013). We tested the hypothesis that phospho mGluR5-PIN1 interactions are enhanced by cocaine administration and found that in mice lacking all forms of Homer, cocaine induces a rapid increase of mGluR5 phosphorylation and a parallel increase of PIN1 binding to mGluR5. However, in wildtype mice, cocaine induced phosphorylation of mGluR5 but did not result in a detectable increase in PIN1 binding. This suggested that cross-linking Homer might compete with PIN1 for interaction with mGluR5’s Homer ligand, thereby restricting PIN1 binding even when mGluR5 is phosphorylated. Biochemical studies confirmed this hypothesis; they also showed that the addition of H1a greatly enhances PIN1-mGluR5 interactions despite the presence of crosslinking Homer. In this model, H1a competes effectively with crosslinking forms of Homer (Fig. 1, step 4) and permits PIN1 to bind in the presence of cross-linking Homer (Fig. 1, step 5; compare Fig. 2A (H1a present and PIN1 binding) to Fig. 2C (long-form Homer, no PIN1 binding). NMR and biochemical studies indicated that enhanced PIN1 binding only occurs when H1a reaches stoichiometric equivalence to crosslinking forms of Homer. Since crosslinking forms of Homer are very abundant at excitatory synapses, and total cellular cross-linking Homer is in stoichiometric excess to H1a even at the peak of its induced expression (Park et al., 2013), this model requires that newly synthesized H1a be able to accumulate at specific synapses. This property of H1a to accumulate at synapses has been demonstrated (Okada et al., 2009). The consequence of H1a accumulation is the facilitated binding of PIN1 to phosphorylated mGluR5. Operationally, this means that PIN1 binding depends on both mGluR5 phosphorylation and the induction of H1a. Thus, cocaine administration enhances PIN1 binding and facilitates isomerization selectively for those mGluR5 complexes that, by virtue of their subcellular localization and presence within recently active neurons, are associated with H1a. We therefore inferred that only the small fraction of total brain mGluR5 that interacts with synaptic H1a can bind PIN1 following cocaine-induced phosphorylation, suggesting that the increased mGluR5-PIN1 interaction might be undetectable in total brain lysate. We specifically tested the hypothesis that the joint action of mGluR5 phosphorylation and H1a are required for cocaine’s behavioral effects, supporting a model in which PIN1-mediated isomerization is central (Park et al., 2013).

2.3. H1a/mGluR5pS/PIN1 contributes to the in vivo effects of cocaine

To assess the relevance of mGluR5 phosphorylation to the behavioral effects of cocaine, we generated another mGluR5 genetic substitution model that expresses an mGluR5 mutant that cannot be phosphorylated at the Homer binding site (AAPPF, mGluR5AA/AA) and is consequently unable to bind PIN1. We compared the effect of repeated cocaine administration in different genetic models that included H1a+/+ (+), mGluR5+/+ (+), mGluR5AA/AA, and PIN1+/+ (+) mGluR5+/+. We monitored motor sensitization, the process whereby repeated cocaine exposure results in enhanced motor responses to the drug. Cocaine motor sensitization was reduced or absent in H1a−/−, mGluR5AA/AA, and PIN1+/− mGluR5+/+, but was intact in mGluR5+/+ (Park et al., 2013). Thus, the ability of cocaine to sensitize motor response appears to depend jointly on mGluR5 phosphorylation and H1a, which together enable PIN1 interactions. Accordingly, cocaine motor sensitization
depends on mGluR5-Homer interactions only in as much as they enable or inhibit the mGluR5-PIN1 interaction. Ongoing studies will assess the role of mGluR5-PIN1 in cocaine self-administration and cocaine seeking behaviors.

2.4. mGluR5-PIN1 mechanism in synaptic plasticity; requirement for H1a induction creates a temporal contingency

How might the mGluR5-PIN1 interactions influence synapses to enable cocaine motor sensitization? Motor sensitization involves an enhancement of synaptic input to the striatum of cocaine-exposed mice (Pascoli et al., 2012). Slice electrophysiology experiments suggest how cocaine exposure might induce this enhancement through D1R and mGluR5 signaling (Centonze et al., 2006). Long-term potentiation (LTP) of striatal responsivity can be induced by standard high-frequency stimulation (HFS) of cortical afferents and subsequently reversed by low-frequency synaptic stimulation (LFS). Both the potentiation and depotentiation of these responses depend on NMDA receptors (NMDAR). Interestingly, depotentiation can be blocked by treatment of striatal slices with D1R agonists prior to induction of LTP or by prior repeated cocaine administration in vivo (Centonze et al., 2006). In fact, the details of the necessary conditions for motor sensitization correspond closely with those that prevent depotentiation (Park et al., 2013). The enhancement of total synaptic input onto the striatum could therefore occur as a consequence of the prevention of depotentiation following D1R stimulation by cocaine. Nevertheless, it is worth noting that the mechanisms mediating potentiation following cocaine exposure might independently prevent depotentiation. Either way, D1R stimulation can be thought to produce a metaplastic
state that enhances synaptic strength and consequently enhances motor responses to cocaine.

Given that locomotor sensitization depends on mGluR5 phosphorylation, H1α induction, and PIN1, we investigated the possibility that these events are also involved in D1R’s ability to block depotentiation. To determine if the potentiating metaplastic effect of D1R is mediated by phosphorylated mGluR5 and H1α, we examined acute striatal slices from mGluR5AA/AA, mGluR5R/R, and H1α−/− mice. LTP and depotentiation were intact in all three mouse lines, but D1R activation failed to block depotentiation only in the mGluR5AA/AA and H1α−/− mice. Thus, H1α induction and phosphorylation of mGluR5 are both required for D1R to exert its metaplastic effect. Moreover, the response to D1R agonist was different in mGluR5R/R mice compared to littermate wildtype mice in an interesting way. In wildtype slices, D1R agonist must be applied ~30 min prior to the depotentiation stimulus for it to block depotentiation. However, in mGluR5AA/R mice, D1R agonist could be applied immediately before the depotentiation stimulus and still block depotentiation. In wildtype but not mGluR5AA/R mice, H1α is required to compete with cross-linking forms of Homer to enable PIN1 binding to phosphorylated mGluR5. However, because mGluR5F1128R does not bind Homer, PIN1 interactions are possible immediately upon phosphorylation without the induction of H1α. This finding implies that D1R stimulation can induce both mGluR5 phosphorylation and H1α synthesis (the two events necessary for PIN1 binding) but at different times; pretreatment is needed in wildtype mice to allow sufficient time for H1α to be synthesized. Thus, the potentiating metaplastic effect of D1R stimulation depends not only on the phosphorylation of mGluR5 but on the presence of H1α, induced by events that are several 10 s of minutes (decamines) old. Restated, time constraints indicate how D1R stimulation may metaplastically alter synaptic strength: the mGluR5–PIN1 mechanism will not have an effect in synapses that do not have decamines-old histories of activity.

2.5. PIN1 isomerization of mGluR5 enhances coupling to NMDA type glutamate receptors

By what mechanism might mGluR5–PIN1 interactions enable potentiating metaplasticity? Stimulation of the mGluR5–PIN1 interaction in striatal cultures enhanced the slow inward current (SIC) that normally follows mGluR5 activation (mGluR5-SIC) (Park et al., 2013). In conditions that enable mGluR5–PIN1 interactions, treatment of cells with D1R agonist resulted in a rapid ERK-dependent increase in the amplitude of the mGluR5-SIC with D1R-expressing neurons. In particular, striatal cultures lacking Homer (Homer1−/−, 2−/−, 3−/−), lacking mGluR5–Homer interactions (cultures prepared from mGluR5R/R), or in which H1α was introduced by virus or induced through KCl application, all exhibited an enhanced mGluR5-SIC following ERK stimulation. By contrast, cultures in which H1α was not induced (wildtype, untreated) or could not be induced (H1α−/−, w/wo KCl treatment) exhibited normal baseline mGluR5-SICs that were not enhanced by ERK stimulation. Similarly, cultures in which mGluR5 phosphorylation could not be induced (mGluR5AA/AA mutants, w/wo H1α virally introduced) also exhibited normal baseline mGluR5-SICs that were unaffected by ERK stimulation. Thus, as with cocaine locomotor sensitization and the potentiating metaplastic effect of D1R stimulation, mGluR5-SIC potentiation also requires both mGluR5 phosphorylation and H1α. Furthermore, amplification of mGluR5-SIC could be inhibited by two different classes of PIN1 inhibitors and was absent in cultures generated from H1α−/− mice. It is therefore reasonable to conclude that the mGluR5–PIN1 interactions enabled by the phosphorylation of mGluR5 and the induction of H1α are critical to all three of these phenomena.

There is even further evidence that the mGluR5-SIC is related to potentiating metaplastic effects of D1R stimulation and induction of locomotor sensitization following cocaine exposure. Across the many listed conditions of striatal culture, pharmacological investigations revealed that both the normal and the enhanced mGluR5-SIC are mediated by an NMDAR current (Park et al., 2013). All of these findings point to one integrated model of cocaine’s behavioral effects. D1R stimulation causes mGluR5 phosphorylation, which in combination with induced H1α, enables mGluR5–PIN1 interactions to create the potentiating metaplastic condition of enhanced NMDA currents (Fig. 1). When these NMDARs are stimulated, their enhanced current facilitates an overall potentiation of synaptic input onto the striatum. The enhanced synaptic input into the striatum mediates the animal’s sensitized response to cocaine.

While it is plausible to envision mechanisms connecting an enhanced NMDAR current to the behavioral effects of cocaine, we do not yet understand how mGluR5 isomerization might enable this enhanced NMDAR current. A biochemical explanation of the effect of mGluR5–PIN1 interactions on NMDARs awaits future investigations.

3. The H1α/mGluR5pS/PIN1 mechanism describes an overlap between reward learning and synaptic tagging, and predicts novel features of neuronal selection

The H1α/mGluR5pS/PIN1 mechanism therefore describes a set of molecular events that cause synaptic plasticity. Importantly, potentiation occurs through this mechanism as a consequence of the coordinated activity of the neuron, its synaptic input, and reward. The temporal relationship between combinations of these events is the topic of theoretical models of learning and plasticity. The H1α/mGluR5pS/PIN1 mechanism therefore elaborates and merges these theoretical models. It suggests that reward learning depends upon the synaptic recruitment of plasticity related proteins (PRPs) whose global synthesis is induced by decamine-old patterns of neuronal firing. It also suggests that reward learning occurs selectively on synapses experiencing coincident input and reward in neurons that are sufficiently active in their networks.
3.1. The H1a/mGR5pS/PIN1 mechanism detects the co-occurrence of glutamatergic, neuromodulatory, and neuronal events and depends on reactivation over decamines

Stated specifically, H1a/mGR5pS/PIN1 potentiating metaplasticity can be realized as a consequence of four events: (1) neuronal firing induces H1a (Fig. 3A-α), after which (2) synaptic activity, when closely incident with (3) D1R stimulation, elevates pERK levels (Fig. 3A-β). High pERK causes phosphorylation of mGluRS5 that endures until (4) synaptic reactivation occurs decamines after (1) (Fig. 3A-γ). Synaptic reactivation in the context of mGR5pS and H1a enables or preserves potentiation (Fig. 3A-δ and ζ). Notably, event (1) happens at the neuron-level, but (2), (3), and (4) co-occur in select synapses (Figs. 3A-S1 and ζ). Upon reactivation of previous potentiated synapses by LFS, the H1a/mGR5pS/PIN1 potentiating metaplasticity mechanism protects synapses meeting these conditions from depotentiation (Centonze et al., 2006; Otmaiko and Lisman, 1998; Park et al., 2013). While necessary for blocking depotentiation, this mechanism might also operate to enhance synaptic strength in other contexts. Indeed, stimulation of D1Rs or other neuromodulator receptors can cause potentiation after normally benign or depressing synaptic activity (Shen et al., 2008; Pawlak et al., 2010). These phenomena all serve as examples of potentiating metaplasticity in which neuromodulators bias some synapses towards potentiation.

Models of synaptic plasticity that reference subsets of these four neuronal and synaptic events have been previously proposed, including those that invoke a “synaptic eligibility trace,” a “synaptic tag,” or a process of neuronal selection influenced by patterns of reactivation. The H1a/mGR5pS/PIN1 mechanism suggests how these distinct models might be integrated and extended. It provides an example of how neuronal firing, synaptic glutamate, neuromodulation, and reactivation can all interact to allow a network to encode reward-related experiences. In particular, the H1a/mGR5pS/PIN1 mechanism integrates the “synaptic eligibility
trace” and “synaptic tag” models. While synaptic tags recruit H1a to all active synapses, those synapses whose activity is uncorrelated with reward are downregulated through H1a’s agonism of mGluR5 (Hu et al., 2010), whereas those whose activity correlates with reward can exhibit potentiation through enhancement of NMDAR current (Park et al., 2013). Further, a requirement for H1a in metaplasticity provides a mechanistic explanation for neuronal selection favoring neurons with recent activity (Rogerson et al., 2014) and suggests that decaminites-old activity may be of particular importance.

### 3.2. The “synaptic eligibility trace” model describes glutamatergic and neuromodulatory contingencies; “synaptic tags” further relate neuronal events to synaptic plasticity

The earliest models of learning and plasticity highlighted the hypothesis that potentiation should occur when postsynaptic neuronal firing is coincident with presynaptic input (Magee and Johnston, 1997). Although these models have clear experimental support and identifiable molecular mechanisms (Cotman and Monaghan, 1988), they do not explain how the association between more temporally distant events —like a cue followed by a reward a second later—might occur. Further, because an outcome and its predictor may be separated in time, it can be a challenge to identify which of the many preceding events are predictors. To solve this “distal reward problem,” and the related “credit assignment problem,” reinforcement learning theoreticians proposed that an “eligibility trace” is initiated by the first event (cue) and decays until the second (reinforcing) event occurs (Foster et al., 2000; Izhikevich, 2007). The cue’s value is augmented in proportion to the remaining trace, or “protoweight” (Gavornik et al., 2009). In this way, value is readily assigned to cues that predict reward at temporal delays. Over many iterations, this process can ultimately separate random cue-reward coincidences from robust predictions (Izhikevich, 2007). A cellular version of this general conception imagines that cue-related glutamatergic input initiates a decaying synaptic state or signal (an eligibility trace) in the activated synapse (Shoulav and Gavornik, 2011). Upon encountering reward—communicating neuromodulators, the remaining trace is converted into a new synaptic weight. Through this mechanism, synapses that reliably receive input before reward are strengthened until more distal reward relationships are learned. Computational models of synaptic eligibility trace have replicated the neural findings in cue-reward learning paradigms (Gavornik et al., 2009; Shuler and Bear, 2006).

However, the synaptic eligibility trace model presumes changes in synaptic strength occur instantaneously and therefore does not address the biological mechanisms of synaptic plasticity or their limitations. In particular, several forms of synaptic plasticity depend on de novo synthesis of PRPs in the hours following their induction by sufficient neuronal firing (Barondes, 1965; Agranoff et al., 1965; Flexner et al., 1965). But how can a neuron-wide event like transcription enable synapse-specific plasticity? In other words, how do nascent proteins exert their effects on only the right synapses? To explain how proteins that are transcribed in the nucleus might act selectively on synapses undergoing plasticity, a “synaptic tag” was imagined (Frey and Morris, 1997). In this model, glutamatergic input tags recently active synapses; this tag then selectively recruits nascent PRPs.

The eligibility trace model can be extended to include this process. Synaptic activity initiates a decaying protoweight, which upon reward, is converted into a provision weight. Such a provision weight is only realized as an actual change in synaptic strength upon interaction with PRPs. We propose that pERK and mGluR5 phosphorylation represent such provision weights; they interact with the PRP H1a to enable potentiation of rewarded synapses. Notably, high pERK activity results from the coactivation of NMDARs and D1Rs (Valjent et al., 2004; Sarantis et al., 2009) or mGluR and D1R (Voulalas et al., 2005), detecting the coincidence of glutamatergic input and reinforcement by dopamine (Cahill et al., 2014). cAMP is coordinately activated with ERK in many models, and as expected of such a provision weight model, the cAMP-dependent effects of dopamine on synapses depend on the relative timing of glutamate and dopamine release. The optimal response occurs when dopamine is delivered within the first second of glutamate release; however, no effect is observed when dopamine is delivered two seconds after glutamate release (Yagishita et al., 2014).

### 3.3. Synaptic tags recruit H1a to punish unreinforced active synapses but enhance reactivated, reinforced active synapses

Like H1a/mGluR5pS/PIN1 potentiating metaplasticity, synaptic tag-related plasticity describes a synaptic event that depends on IEG induction and PRP synthesis. In fact, because it was known to be synthesized de novo following neuronal activity and to interact with mGluR5 at the synapse, the IEG H1a was one of the first candidate PRPs (Kato et al., 2001). Consistent with its hypothesized interaction with a theoretical synaptic tag, H1a was more recently shown to be selectively recruited to recently active synapses (Okada et al., 2009). The emerging knowledge of H1a’s role in synaptic plasticity therefore provides new insight into the interface between synaptic activity, synaptic tagging, and reward learning.

In conditions that do not induce pERK or mGluR5pS, H1a downregulates synaptic strength (Hu et al., 2010). Three types of synapses can therefore be described: those without sufficient synaptic activity to recruit H1a, those with synaptic activity that is not coincident with reward, and those with synaptic activity coincident with reward. Because it is not recruited there, H1a will not affect the synaptic strength of inactive synapses (Fig. 2B). In contrast, H1a downregulates the synaptic strength of active, but unrewarded synapses in which pERK and mGluR5pS are not abundant (Fig. 2C). Finally, H1a enables potentiation of synapses whose activity is coincident with reward, in which pERK and mGluR5pS levels are high (Fig. 2A). This model modifies an earlier view that IEGs homeostatically downregulate all synapses (Hu et al., 2010; Shepherd et al., 2006). If H1a’s synaptic recruitment and downregulatory function is proportional to the level of recent synaptic input (given little pERK activity), this might help explain why activity-driven downregulation has been
observed to scale with initial synaptic strength (Turrigiano and Nelson, 2004). In other words, scaling of synaptic strength could be explained by the observation that H1α is induced in active neurons and recruited to synapses in proportion to their synaptic input.

Interestingly, another IEG, Arc, has also been shown to exert a synapse-specific effect: it accumulates preferentially in inactive synapses (Okuno et al., 2012). In contrast to H1α, Arc’s function has not been shown to depend on a synapse’s reward history and has only been shown to decrease synaptic strength (Shepherd and Bear, 2011). Additionally, there is no evidence that Arc transcription is necessary decaminutes before the induction of the forms of plasticity for which it is required (Park et al., 2008). In fact, Arc-dependent mGluR-LTD can be expressed without any specific protocol that induces Arc transcription decaminutes beforehand. As discussed in Section 3.4, the requirement for H1α induction decaminutes before the time of plasticity induction might inform neuronal selection, restricting plasticity to neurons with sufficient decaminutes-old firing rates to have induced H1α. It is currently unknown if Arc might similarly contribute to neuronal selection.

In summary, H1α is a PRP induced by neuronal activity; pERK and mGR5pS are provisional weights, induced by reinforced synaptic activity. In neurons with sufficient activity to induce H1α, unreinforced active synapses might exhibit low pERK and low mGR5pS and undergo reductions in synaptic strength. Reinforced active synapses will exhibit high mGR5pS and enable potentiation upon reactivation.

### 3.4. H1α-dependent metaplasticity might direct neuronal selection to neurons with decaminutes-old activity

Like all PRPs hypothesized to be recruited by synaptic tags, H1α is only induced by sufficient neuronal activity. This means synaptic input’s ability to modify synaptic strength depends on the recent firing history of its parent neuron. Notably, H1α synthesis takes more than 15 min following its induction (Vazdarjanova et al., 2002).

While the coordinated activity of a neuron and its synapses has been repeatedly hypothesized to influence synaptic plasticity, the H1α/mGR5pS/PIN1 mechanism further entails a novel requirement for its realization: sufficient neuronal activity decaminutes prior to synaptic activity (Figs. 3 and 4). For a synapse with a synaptic tag and mGR5pS provisional weight to realize synaptic change, it must belong to a neuron that exhibited sufficient activity decaminutes earlier.

This requirement is further entailed by the H1α/mGR5pS/PIN1 potentiating metaplasticity mechanism’s dependence on reactivation: an enhanced NMDAR current is converted into a change in synaptic strength only by glutamatergic activation of NMDARs in synapses that already have H1α and mGR5pS from previous events—H1α from sufficient

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**Fig. 4** – The requirement for decaminute-old neuronal activity filters for synapses on select neurons within networks. We propose that the H1α/mGR5pS/PIN1 mechanism may mean that the synapses that learn an event only belong to neurons with significant decaminute-old activity. (1) Many synapses experience NMDAR activation, depicted by green asterisks. (2) Only some of these synapses have previously experienced input-reward pairings sufficient to activate pERK, depicted in pink. (3) Only some of these synapses belong to neurons with sufficient recent firing, of which (4) fewer have decaminutes-old activity sufficient for H1α to be present in the synapse at the time of NMDAR stimulation. (5) Subsequent changes in the activity of these neurons will influence their downstream targets.
decaminute-old neuronal activity and mGR5pS from more recent input-reward pairing.

Consider the following illustration: imagine equivalent corticostriatial synapses that receive D1R stimulation milliseconds after HFS (causing pERK and mGluR5 phosphorylation) and several seconds before LFS (causing the synaptic reactivation on which metaplasticity operates). A synapse belonging to a neuron that fired frequently more than 30 min ago (sufficient time for H1a synthesis) would remain potentiated, whereas a synapse belonging to a neuron that had not fired or had only fired 5 min prior (insufficient time for H1a synthesis), would become depotentiated. In the second case, H1a would not yet be present near synapses. Potentiating metaplasticity mediated by the H1a/mGR5pS/PIN1 mechanism would therefore only be realized in neurons reactivated over decaminute-long timeframes. The H1a/mGR5pS/PIN1 model therefore suggests a novel constraint on how reward influences synaptic strength: the metaplastic effect of neuromodulators like dopamine might only matter in neurons repeatedly activated over decaminutes. Such a constraint has not previously been applied to models of reward-related learning and could produce novel results.

Interestingly, the prior activity of neurons within a network has been shown to influence synaptic plasticity and the network’s subsequent encoding of its experiences (Rogerson et al., 2014; Yiu et al., 2014). Although many neurons within the lateral amygdala show similar initial responses to a cue prior to its pairing with an aversive shock, only some of these responses will become potentiated by cue-shock pairings. The process by which some of many possible neurons come to encode an experience has been termed “neuronal allocation” or “neuronal selection.” Presumably, which neurons learn this cue-shock pairing is relevant for how the network encodes its experiences more generally (Ghosh and Chattarji, 2015). Although many neurons in the network report the cue, only some of them express augmented responses. The fear response will become more likely in conditions that activate the selected neurons and comparatively less likely in conditions activating cue-responsive neurons that did not undergo plasticity. Thus, the cue-shock pairing might be better understood as a more complex condition-cue-shock pairing in which the conditions are defined by the subset of neurons that undergo plasticity due to their prior activity. The network might therefore partially encode experience through the environmental features that are repeated over decaminutes and not those that are more uniquely observed at the time of reward. The dependence of reward plasticity on IEG PRPs within synapses might therefore entail a mechanism by which those contextual features that are robust enough to be repeated over decaminute timeframes are selectively incorporated into a network’s memory.

Consistently, artificial induction of neuronal activity by experimenters can bias the neuronal selection process towards the activated neurons when activity precedes cue-shock pairing, but not when it occurs afterwards (Rogerson et al., 2014; Yiu et al., 2014). Although the hypothesis that decaminute-old activity and H1a (or other PRP IEGs) contribute to neuronal selection is novel and has theoretical appeal, it has yet to be demonstrated. The precise temporal features of the activity required for neuronal selection have not been investigated in detail, and the effect of decaminutes-old activity compared to more recent neuronal firing has not been demonstrated. Nevertheless, such a model predicts that prior induction of H1a would be a particularly good predictor of which neurons come to encode an experience. The coordinated imaging of H1a induction and changes in response of a neural network during a behavioral learning paradigm could provide confirmation of the broader role H1a might serve in neuronal selection and the subsequent dimensionality of a neural network. We note that the mGluR5K mouse does not require H1a for neuromodulator-dependent potentiating metaplasticity and may be useful to test the role of mGR5pS/PIN1 in neuronal selection.

Interestingly, H1a might not be required to enable neuromodulator-dependent potentiating metaplasticity in diseases where mGluR5-Homer interactions are abnormal. For example, in mouse models of Fragile X syndrome, mGluR5 exhibits reduced interactions with long-form Homer (Ronesi, et al., 2012) and may be less dependent upon H1a for neuromodulator-induced PIN1 binding. Similarly, age-related memory impairment involves a decrease in mGluR5-long-form Homer interactions (Menard and Quirion, 2012). Disruptions in long-form Homer function have also been proposed to contribute to schizophrenia (Szumlinski et al., 2005; Spellman, et al., 2011). H1a’s proposed role in neuronal selection suggests that in these cases, reward experience might be encoded much more promiscuously and much less specifically, perhaps altering many of the basic ways in which these nervous systems represent the world. A disruption of the proposed neuronal selection mechanism might explain cognitive dysfunction in these cases.

4. Conclusions

In conclusion, pERK and mGluR5 phosphorylation may serve as provisional weights, detecting the coincidence between glutamatergic synaptic input and reinforcement by dopamine. mGR5pS may be realized as potentiating metaplasticity in neurons in which H1a is induced by sufficient neuronal activity. H1a, recruited to active synapses, might depress unrewarded active synapses and potentiate reactivated rewarded ones. This process will selectively occur within neurons based on their decaminutes-old activity histories, filtering the encoding of new experiences into those features that persist over this timeframe. The H1a/mGR5pS/PIN1 mechanism therefore enables a novel synthesis of systems-level and cellular models of learning.

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