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Roles for *Arc* in metabotropic glutamate receptor-dependent LTD and synapse elimination: Implications in health and disease

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Abstract

The *Arc* gene is robustly transcribed in specific neural ensembles in response to experience-driven activity. Upon induction, *Arc* mRNA is transported to dendrites, where it can be rapidly and locally translated by activation of metabotropic glutamate receptors (mGluR1/5). mGluR-induced dendritic synthesis of *Arc* is implicated in weakening or elimination of excitatory synapses by triggering endocytosis of postsynaptic AMPARs in both hippocampal CA1 and cerebellar Purkinje neurons. Importantly, CA1 neurons with experience-induced *Arc* mRNA are susceptible, or primed for mGluR-induced long-term synaptic depression (mGluR-LTD). Here we review mechanisms and function of *Arc* in mGluR-LTD and synapse elimination and propose roles for these forms of plasticity in *Arc*-dependent formation of sparse neural representations of learned experience. We also discuss accumulating evidence linking dysregulation of *Arc* and mGluR-LTD in human cognitive disorders such as intellectual disability, autism and Alzheimer's disease.

Keywords

Arc/Arg3.1; dynamin; mGluR5; long-term synaptic depression; synapse elimination

Arc/Arg3.1 is one of the most strongly induced immediate early genes in response to experience and neural activity and evidence indicates that *Arc*-induction marks neuronal ensembles that encode learned behaviors [1–7]. Deletion of *Arc* in mice leads to deficits in many forms of learning and memory, as well as experience-dependent plasticity of circuits [5, 8–11]. Thus, it is hypothesized that experience-dependent induction of *Arc* in a neuron, or network of neurons, leads to plasticity circuits that mediate memory of that experience [3–5, 11]. *Arc* has unique qualities among the immediate early genes that make it well suited to cause plasticity of circuits; its mRNA is rapidly transported to dendrites [12] where it is locally translated in response to glutamate [13–16] and it encodes a protein that affects synapse function directly [17–19]. An accumulating body of work implicates dendritic *Arc*

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translation in synaptic weakening, or long-term depression (LTD) [13, 14, 20], and synapse elimination in response to activation of Group 1 metabotropic glutamate receptors (mGluR1/5) [21, 22]. Here we will review the roles of *Arc* in these related forms of synaptic depression and evidence for their dysfunction in human cognitive disease. We also attempt to integrate roles for mGluR-LTD and/or synapse elimination with recent results revealing roles for *Arc* in formation of sparse neural representations of learned experience [5].

***Arc* mRNA is rapidly translated in dendrites in response to Group 1 metabotropic glutamate receptors**

The robust transcriptional and translational regulation of the *Arc* gene and mRNA make it an ideal candidate to couple experience-dependent activation of neuronal circuits to synaptic plasticity within these circuits. For example, in hippocampal CA1 neurons, which encode a memory for place or a spatial environment, *Arc* is induced rapidly, within 30 s, in response to a novel environment [23]. Once *Arc* is induced in neurons, its mRNA is promptly transported to dendrites [12, 24, 25] where it can be rapidly translated, within seconds to minutes, in response to activation of glutamatergic synapses [13–16]. A specific agonist for Group 1 metabotropic glutamate receptors (mGluR1/5), DHPG, is sufficient to induce *Arc* translation [13–15], but glutamate-induced dendritic *Arc* translation relies on both NMDA receptors and mGluR1/5 [16], which may be more relevant *in vivo*. Glutamate or DHPG induces remarkably fast (~15s) translation of *Arc* in dendrites as observed by imaging newly synthesized *Arc* that was tagged with a bright, rapidly decaying *Gaussia*-Luciferase (Gluc) [16] or Venus [15]. Interestingly, *Arc* translation did not occur primarily at synapses, but appeared to be coordinated within a dendritic segment. Although the *Arc* 3'UTR enhances dendritic trafficking of *Arc* mRNA [26], the coding region of *Arc* mRNA is sufficient for glutamate-stimulated local translation [16]. This result, combined with the fact that glutamate stimulates *Arc* translation in seconds, and occurs in the presence of translation initiation inhibitors, suggested that glutamate stimulates ribosomal movement, or elongation, onto *Arc* mRNA that is already initiated and ribosomes may be stalled on *Arc* mRNA in dendrites [16]. In support of this idea, mGluRs potently activate elongation factor 2 kinase and phosphorylation of elongation factor 2 which regulates translational elongation and is necessary for mGluR-induced *Arc* translation [13]. Furthermore, *Arc* mRNA interacts with Fragile X Mental Retardation Protein (FMRP), an RNA binding protein implicated in ribosomal stalling and processivity [27, 28] that is necessary for mGluR-induced translation of *Arc* [15, 29–31].

Dendritic translation of *Arc* is necessary for an mGluR1/5-induced long-term synaptic depression

A major function of *Arc* is to weaken synapses by stimulating endocytosis of postsynaptic ionotropic AMPA subtype receptors (AMPA) and reducing their surface and synaptic expression [16–18]. *Arc* contributes to multiple forms of activity-induced synaptic weakening; including homeostatic downscaling of synapses [19], mGluR-LTD [13, 14, 20] and synapse elimination [21, 22]. If and how these different forms of *Arc*-dependent synaptic weakening interact, if they affect the same synapses or utilize the same molecular

mechanisms (e.g. endocytosis of AMPARs), but are induced in diverse ways is unclear at present and discussed below. For this review, we focus on forms of Arc-dependent synaptic weakening and elimination that require mGluR1/5.

Brief activation of mGluR1/5 (minutes) leads to a LTD of excitatory and inhibitory synaptic transmission in multiple brain regions, which are expressed through different pre- or postsynaptic loci [32, 33]. The most well characterized forms of mGluR-LTD that is mediated postsynaptically at excitatory synapses, occurs through removal of postsynaptic AMPARs in cerebellar Purkinje (Pkj) neurons and hippocampal CA1 neurons and requires Arc [33–35]. Arc is also necessary for an activity-dependent elimination of inputs onto both Pkj and CA1 neurons, which are mGluR1 or mGluR5 dependent, respectively, suggesting that LTD mechanisms contribute to synapse elimination [36] and these are conserved roles for Arc across distinct brain regions.

In CA1 neurons, brief activation of mGluR1/5 with either the selective agonist, DHPG, or synaptic stimulation (paired-pulse low-frequency (1 Hz) stimulation) induces LTD that requires new protein synthesis from pre-existing mRNA and is mediated by postsynaptic endocytosis and decreases in surface AMPAR subunits GluA1 and GluA2 (reviewed [33, 34]). While *de novo* protein synthesis is not required to trigger endocytosis, and decreases in surface of AMPARs, it is required to maintain decreases in surface AMPARs and the persistent increases in endocytosis rates that accompany LTD [14, 37]. mGluR-LTD is deficient in area CA1 of acute hippocampal slices from *Arc* KO mice and postsynaptic inhibition of new Arc translation with antisense oligonucleotides blocks LTD, as well as DHPG-induced decreases in surface AMPARs and persistent increases in AMPAR endocytosis rates [13, 14]. These results suggest that Arc levels are rate limiting for AMPAR endocytosis and mGluR-induced increases in local Arc concentration enhance endocytosis rates which maintain decreased surface AMPARs and synaptic depression [14]. In contrast to this view, recent data demonstrated that DHPG induced a rapid synthesis of Arc, which is then followed by ubiquitination and degradation of Arc by the proteasome, resulting in a long-term decrease in Arc protein levels (> 1hr) [38]. This result suggested that increases in Arc levels trigger LTD, but do not maintain it. Of note, in cultured forebrain or hippocampal neurons, *Arc* levels are induced and remain elevated for an hour after DHPG suggesting that there is little Arc degradation in cultured neurons after DHPG [13, 31] where persistent increases in AMPAR endocytosis rates are observed to accompany LTD. Remarkably, if proteasomal degradation is blocked with proteasome inhibitor, mGluR-LTD no longer requires new protein synthesis [38]. Although these manipulations are not specific for Arc, it supports the view that new Arc synthesis is not absolutely required for mGluR-LTD and mGluRs trigger posttranslational modifications of Arc, or proteins in complex with Arc to cause LTD. Such mechanisms may include tyrosine dephosphorylation of GluA2, which is implicated in mGluR-LTD [39] and phosphorylation of Arc by ERK [40], a protein kinase required for mGluR-LTD [41].

In cultured cerebellar Purkinje (Pkj) neurons, Arc is necessary mGluR-LTD of granule cell inputs but is specifically required for a transcription-dependent “late-phase” of mGluR-LTD, occurring >1 hr after induction [35]. The late-phase of mGluR-LTD also requires the transcription factor, Serum Response Factor (SRF), and its binding to the *Arc* promoter [35].

mGluR-LTD inducing stimulation (e.g. DHPG) can induce both transcription and translation of *Arc* in cultured hippocampal or cortical neurons [13, 42], the former of which likely occurs through regulation of SRF [43]. In contrast to Pkj neurons, mGluR-LTD in area CA1 of acute hippocampal slices is independent of transcription and relies on rapid translation of *Arc*, within 20–30 minutes, from preexisting mRNA [14, 44]. New *Arc* transcription may be required for mGluR-LTD in cultured Pkj cells because *Arc* levels are very low in this culture preparation, whereas, as discussed below, CA1 neurons in acute hippocampal slices express *Arc* transcripts induced *in vivo* [20]. An interesting possibility is that mGluR-LTD in acutely prepared cerebellar slices may depend on rapid *Arc* translation from preexisting mRNA, as observed in CA1. In support of this idea, protein synthesis inhibitors rapidly block mGluR-LTD in Pkj neurons in acute slices [45].

Novelty-induced *Arc* primes CA1 neurons for mGluR-LTD

The requirement for *Arc* in an acute form of synaptic plasticity like mGluR-LTD, suggested that experience-induced *Arc* induction causes or facilitates mGluR-LTD onto activated neurons and may be a mechanism by which *Arc* causes plasticity of select circuits activated by salient experience. To determine if *Arc* induction *in vivo* results in LTD, we used two different *Arc*-GFP transcriptional reporter mice (*Arc*^{GFP} knockin or BAC-*Arc*^{GFP}), which express destabilized GFP when the *Arc* gene is induced [9, 46], and allowed visualization and targeting of live neurons with recent *Arc* induction for measurements of synaptic strength and plasticity [20]. Exposing *Arc*GFP reporter mice briefly (5 min) to a novel environment, induced GFP expression in a subpopulation (~40%) of CA1 pyramidal neurons, consistent with measurements of endogenous *Arc* mRNA [20, 47]. Recording from *Arc*GFP(+) CA1 neurons in acute hippocampal slices of novelty exposed mice did not detect differences in baseline excitatory synaptic function in comparison to neighboring GFP(–) neurons. However, activation of mGluR1/5 onto *Arc*GFP(+) neurons induced a robust mGluR-LTD, whereas, surprisingly, GFP(–) neurons had little or no mGluR-LTD. Therefore, *Arc* induction by brief exposure to novelty does not induce LTD, but facilitates or “primes” neurons for subsequent LTD in response to mGluR1/5 activation. Results indicated that mGluR-LTD priming occurs in *Arc*GFP(+) neurons because they express more dendritic *Arc* mRNA and display robust mGluR-induced dendritic synthesis of *Arc* protein in comparison to their GFP(–) neighbors. A model based on this data proposes that *Arc* mRNA is induced by novelty and transported to dendrites, but may be translationally suppressed (Fig. 1). Subsequent activation of mGluR1/5 derepresses translation of dendritic *Arc* mRNA, increases dendritic *Arc* levels and induces mGluR-LTD. In support of this model, *Arc* and other mRNAs in dendrites are likely translationally suppressed [31, 48–50] and glutamate induces *Arc* translation in dendrites within seconds [16]. Priming of mGluR-LTD by novelty-induced *Arc* provides a cellular mechanism for the known facilitation of LTD by exploration of novel objects or environments [20, 51–59]. If novel environment exposure is required to observe robust mGluR-LTD, then why is mGluR-LTD observed in many slice studies without first exposing animals to novelty? Indeed, the standard procedures of mouse handling prior to acute slice preparation, which include removing an unanesthetized mouse from its home cage and transporting to the lab, are sufficient to induce *Arc* and observe robust mGluR-LTD. Anesthesia of mice in their home cage prevented *Arc* induction during

transportation to the lab and resulted in a small magnitude mGluR-LTD in population field potential recordings in slices [20]. Thus, “standard handling” protocols of mice for slice physiology experiments may constitute a novel, or stressful, experience for the mouse since stress, like novelty, can induce *Arc* in CA1 [60] and enhance mGluR-LTD [61].

Arc and activity-dependent, developmental synapse elimination

In addition to *Arc*'s role in rapid and transient weakening of excitatory synaptic transmission, such as LTD, *Arc* is also necessary for activity-dependent and developmental pruning or elimination of excitatory synapses onto cerebellar Pkj and hippocampal CA1 neurons [21, 22]. Activity-dependent synapse elimination is a process by which experience and learning remove redundant, or inappropriate connections to form and refine circuit connections for optimal mature function. A well-established model of developmental synapse elimination is the climbing fiber (CF) to Pkj neuron synapse in the cerebellum [62, 63]. At birth, multiple CF axons innervate Pkj neurons, and during the first few postnatal weeks, one CF input becomes progressively strengthened, whereas other CF inputs are weakened and ultimately eliminated by the 3rd postnatal week, as measured by evoked CF EPSCs [64]. Activity of Pkj neurons and/or their inputs, specifically mGluR1, NMDARs or P/Q type voltage-dependent Ca²⁺ channels (VDCCs) are necessary for CF input elimination and their blockade results in multiply CF innervated Pkj neurons [62, 63, 65, 66]. Conversely, optogenetically increasing firing rates of Pkj neurons in organotypic cultures accelerates CF input elimination by activating of P/Q VDCCs and inducing *Arc* transcription [21]. Knockdown of P/Q VDCCs or *Arc* in Pkj neurons prevents activity-accelerated CF input elimination in cultures and developmental CF elimination *in vivo* [21, 65]. Overexpression of *Arc* itself is insufficient to eliminate CF inputs in the absence of activity, indicating that other activity-dependent processes function together with *Arc* to confer CF input elimination [21].

Like its role in Pkj neurons, *Arc* is required for synapse elimination in CA1 hippocampal neurons [22]. Although synapse elimination in hippocampal CA1 neurons has been less well characterized in comparison to climbing fibers, insight into this process was made when it was discovered that expression of the activity-dependent transcription factor Myocyte-Enhancer Factor 2 (MEF2) induced a rapid (<24 hr) elimination of excitatory synapses onto hippocampal neurons [67, 68]. Four MEF2 proteins (MEF2A-D) belong to the MEF2/MADS box family of transcription factors [69, 70] and MEF2A and MEF2D are expressed in CA1 neurons. In response to neuronal depolarization and Ca²⁺ influx, MEF2 activates transcription of target genes, including *Arc* [67, 71]. A consensus binding sequence for MEF2 is present in an upstream enhancer of the *Arc* gene, that confers robust activity-dependent transcription and thus is termed the synaptic activity response element (SARE) [72]. MEF2A/D bind to the *Arc* SARE and this binding is necessary for activity-dependent induction of the *Arc* gene. CREB and SRF also have binding sites in the SARE that are required for activity-induced *Arc* revealing a combinatorial interaction of 3 transcription factors that are necessary for full induction of *Arc* [72]. Overexpression of a constitutively active form of MEF2 (MEF2^{VP16}) in organotypic hippocampal slice cultures causes a rapid, within 12–16 hr, and robust (30–50%) elimination of dendritic spines, synaptic markers and depresses evoked EPSC amplitudes as well as the frequency of spontaneous, miniature (m)

EPSCs [67, 68] which is consistent with a functional synapse elimination. MEF2^{VPI6} induces transcription of *Arc* and fails to eliminate functional synapses or spines in CA1 neurons of *Arc* KO mice [22].

To determine the physiological activity patterns that regulate MEF2 transcriptional induction of *Arc* in neurons and whether such patterns eliminate synapses through MEF2 and *Arc*, recent work demonstrated, similar to Pkj neurons [21], that optogenetically driving individual CA1 neurons to fire in bursts at 3 Hz, resulted in a MEF2A/D-dependent induction of *Arc* and an *Arc*-dependent functional and structural synapse elimination [73, 74]. Interestingly, the duration of postsynaptic bursting (1 vs. 24 hr) induced distinct forms of synaptic depression that both required *Arc*. Relatively brief periods of postsynaptic bursting (1 hr) selectively depressed AMPA receptor (R) synaptic transmission, or silenced excitatory synapses, whereas more prolonged (24 hr) firing depressed both AMPAR and NMDAR EPSCs and eliminated spines, indicative of a synapse elimination. Both synapse silencing and elimination required *de novo* transcription and *Arc*, but only synapse silencing in response to brief activity bursts required MEF2A/D [74]. This surprising finding indicates that activity and MEF2A/D-induced *Arc* silence synapses without eliminating them and identifies, perhaps an *Arc*-dependent intermediate stage of synapse elimination. Furthermore, longer durations of postsynaptic activity likely engage other transcription factors, besides MEF2A/D, to induce *Arc* and eliminate synapses. Acute, exogenous re-expression of *Arc*, postsynaptically and cell autonomously, in *Arc* KO CA1 neurons rescues activity or MEF2^{VPI6}-induced synapse silencing or elimination of spines and functional synapses, respectively [22, 74]. However, overexpression of *Arc* alone is insufficient to silence or eliminate synapses suggesting that other MEF2-induced genes are required [22]. One candidate is *Protocadherin 10* (*Pcdh10*), a MEF2 target gene that functions in synapse elimination to degrade the synaptic scaffold, PSD-95 [75]. Degradation of PSD-95 is necessary for AMPAR endocytosis during LTD [76], thus *Pcdh10*-dependent degradation of PSD-95 may be necessary for *Arc* to trigger AMPAR endocytosis and synapse elimination in response to MEF2 activation.

Like novelty-induced priming of mGluR-LTD, MEF2-induced synapse elimination requires a coordinated transcriptional and dendritic translational control of *Arc*. Pharmacological block or genetic deletion of mGluR5 prevents MEF2-induced elimination of spines and functional synapses [22]. One role of mGluR5 in MEF2 triggered synapse elimination is to stimulate synthesis of *Arc* protein in dendrites. mGluR5 antagonists do not interfere with MEF2-induced transcription of *Arc* or increases in dendritic *Arc* mRNA, but block MEF2-induced increases in dendritic *Arc* protein. Culturing hippocampal neurons in microfluidic chambers allowed selective blockade of mGluR5 on dendrites, but not the soma, which was sufficient to block MEF2-induced increases in dendritic *Arc* protein and synapse elimination [22]. Therefore, this data suggests that that two forms of neural “activity” are required to induce synapse elimination, one in the form of action potentials to trigger Ca²⁺ influx in the cell soma and transcription of *Arc* via MEF2, and perhaps other transcription factors. Synaptic stimulation of mGluR5 is then required to translate dendritic *Arc* mRNA into protein for synapse elimination. As in LTD, mGluR5 may also regulate posttranslational modifications of AMPARs [39] that may function together with *Arc* to stimulate endocytosis. Roles for mGluR5 in synapse elimination in forebrain are suggested by

findings of increased frequency of spontaneous excitatory synaptic events (mEPSCs) and/or dendritic spines in CA1 neurons [22] as well as layer 2/3 and 4 neocortical neurons; with cell autonomous deletion of mGluR5 [77, 78] or decreased spines in Nucleus Accumbens with mGluR5 positive allosteric modulators [79]. Whether Arc also functions in synapse elimination in these brain regions is yet to be determined. Similarly, in Pkj neurons, mGluR1 is necessary for CF input elimination [66], although a role for mGluR1 in regulation of Arc synthesis in Pkj neurons is unknown. The common roles of mGluR1/5 and Arc in mGluR-LTD and synapse elimination in Pkj and CA1 neurons suggest that mGluR-LTD mechanisms, such as AMPAR endocytosis, may be an initial trigger for long-term synapse elimination as at other synapses [36, 80]. In support of this idea, structural synapse elimination is a consequence of repeated episodes of LTD induction by mGluR or NMDAR activation [81–83].

Molecular Mechanisms of Arc regulation of AMPAR endocytosis

As stated, an important mechanism underlying LTD is the removal of AMPARs from the post-synaptic surface, effected primarily by increasing the rate of endocytosis. A potential mechanism to explain Arc's role in AMPAR endocytosis was provided by Chowdhury et al. [18], who showed that Arc binds directly to two elements of the endocytic machinery, dynamin and endophilin, and by the more recent finding of DaSilva et al. [84] that Arc also interacts with the clathrin adaptor protein, AP-2 (reviewed in [85]). Dynamins are ~100 kDa GTPases that self-assemble around the necks of invaginating vesicles and promote membrane scission in a GTPase-dependent manner [86, 87]. Mammals express three forms of dynamin: dynamin 2 is ubiquitously expressed; dynamins 1 and 3 are particularly abundant in neurons. All three forms of dynamin share a common domain structure, including an N-terminal GTPase domain, a central phosphoinositide-binding pleckstrin homology (PH) domain flanked by two “stalk” domains, and a C-terminal proline-rich domain (PRD). In the three-dimensional structure, the two stalk domains fold back and interact with each other to form a four-stranded helix involved in dimerization, tetramerization, and higher-order dynamin oligomerization. Dynamin self-assembly, either on biological membranes or, *in vitro* on the surface of liposomes, stimulates its GTPase activity from a basal rate of approximately 1–10 min⁻¹ for unassembled (tetrameric) dynamin to maximal rates greater than 200 min⁻¹ for polymers. We confirmed, using pure proteins, that Arc interacts with dynamins 2 and 3 [88]. We further showed that Arc increases the rate and extent of dynamin self-assembly, and promotes assembly-dependent GTPase activation of dynamins 2 and 3 to levels of approximately 90 min⁻¹ and 120 min⁻¹, respectively. Interestingly, binding of Arc to dynamin 1, which is largely pre-synaptic and functions in synaptic vesicle recycling [87], was nearly undetectable and Arc had no effect on the enzymatic or assembly properties of this isoform. Dynamins 2 and 3 are found in both pre- and postsynaptic nerve terminals.

By analyzing a series of truncation and deletion mutants, Chowdhury et al. [18] identified a segment comprising amino acids 195–214 of Arc as a critical dynamin-binding determinant. We observed that deletion of this segment weakened, but did not completely abolish, the Arc-dynamin interaction [88], suggesting that additional dynamin-binding sites in Arc remain to be identified. Chowdhury et al. [18] also identified the PH domain as the most

likely Arc-interacting determinant in dynamins. Structural studies revealed that dynamins can exist in “closed” and “open” conformations [89–91]. In the closed conformation, the PH domain is folded back onto the stalk, thereby acting as an intra-molecular suppressor of dynamin self-assembly and activation. In the open conformation, the PH domain is extended away from the stalk and is now free to associate with phosphoinositides and to polymerize on the membrane surface. By interacting with the PH domain, Arc may disrupt the autoinhibitory stalk-PH domain interface. It is important to note that the stalk-binding and phosphoinositide-binding residues are on opposite surfaces of the PH domain. Thus, dynamin binding to Arc and to membranes are not mutually exclusive.

Endophilins comprise a family of five (endophilins A1–3 and B1–2) ~40 kDa proteins (endophilins A1–3 and B1–2) that contain N-terminal BAR (Bin/amphiphysin/Rvs) domains and C-terminal SH3 domains connected by variable linker regions. Dimerization of their BAR domains create crescent-shaped structures that induce and/or stabilize membrane curvature [92]. Endophilins interact with dynamin PRDs via their Src homology 3 (SH3) domains, and these interactions promote dynamin oligomerization *in vitro* and in cells [93]. Arc interacts via residues 89–100 with the BAR domains of endophilins A2 and A3 (Fig. 2), which are abundant in post-synaptic compartments, but it does not interact with endophilin A1, which is predominantly localized to pre-synaptic nerve terminals. Importantly, surface expression of GluR1 was significantly reduced in neurons upon overexpression of full-length Arc but not Arc- 91–100 (defective endophilin binding) or Arc- 195–214 (defective dynamin binding) [18].

A deeper understanding of the physical properties of Arc in live neurons is likely to provide valuable insights into its role in synaptic plasticity. For example, it will be important to know whether Arc self-assembles in neurons, as it does *in vitro* [88, 94]. If so, Arc may function as a scaffold to recruit and/or stabilize endocytic proteins, including dynamin and endophilin, on clathrin-coated pits. The mechanism and function of Arc’s interaction with the actin cytoskeleton also remains to be elucidated. In this context, we note that dynamin has an endocytosis-independent role in the regulation of actin polymerization [95] and that endophilin A2 controls a newly discovered form of clathrin-independent but actin- and dynamin-dependent endocytosis termed fast endophilin-mediated endocytosis (FEME) [96, 97]. Perhaps also relevant is the finding that direct binding of endophilins A2 and A3 (but not A1) to oligophrenin 1, a BAR domain-containing Rho GAP (GTPase activating protein), promotes mGluR-induced LTD and the reduction of surface AMPARs [98]. Arc and oligophrenin 1 bind to distinct sites on endophilins, potentially allowing for simultaneous interactions.

Arc-dependent synaptic weakening may contribute to the formation of sparse neural representations of learned experience

As discussed, *Arc* is rapidly and robustly induced in select neuronal populations during salient experience, such as novelty or context-dependent fear and in response to learning [3, 4]. Using the *Arc* gene regulatory elements to optogenetically silence *Arc*-induced hippocampal neuron populations during learning inhibits memory formation [6]. These

results suggest that these Arc-induced neuronal ensembles mediate learned behavior. If or how Arc-dependent synaptic weakening (LTD or synapse elimination) contribute to plasticity of these neuronal populations during learning is unclear. Arc-dependent synaptic weakening may mediate formation of a sparse neural representation of an experience that develops during learning. For example, during exploration of a novel environment a subset (~40%) of hippocampal CA1 neurons fire with some spatial specificity and induce *Arc* [1, 99, 100]. Re-exposure to the same environment re-induces Arc in the same overlapping population of CA1 suggesting that these Arc+ neurons are activated by and encoding the new environment [1] (Fig. 1). As the environment becomes familiar with repeated exposures over days, the average population firing rate of CA1 neurons declines, as well as the number of CA1 neurons that are active [99] and induce *Arc* [47]. Interestingly, CA1 neurons that are less spatially-tuned and with lower firing rates (<12 Hz) show reduced activity upon repeated exposures, whereas more active neurons (>12 Hz firing rate) develop increased firing rates. Therefore, during familiarity or habituation to a novel environment, an initially diffuse ensemble of activated CA1 neurons is sculpted into a sparse, precise network of highly spatially-tuned CA1 place cells that form the neural representation for that environment [99]. *Arc* induction may tag and bind together CA1 neurons that were active in response to a specific environment. The CA1 neurons in the network are then primed for plasticity of their synaptic inputs by virtue of the fact that they have *Arc* mRNA in their dendrites. With repeated exposure to the same environment, neurons that are less spatially tuned and fire at lower rates (<10Hz); rates that promote LTD [101], would be expected to have weakening of active synaptic inputs carrying sensory information about that environment. The *Arc*-dependent LTD priming may contribute to the reduced excitation of those neurons with subsequent environment exposure and generation of a sparse CA1 representation of that environment. In a test of this model, *Arc*GFP reporter mice were first exposed to a novel environment to mark novelty-induced CA1 neuron ensembles and then repeatedly exposed to the same environment over next few hours and acute brain slices prepared to determine if LTD occurred with repeated experience or learning of a new environment [20]. *Arc*GFP(+) CA1 neurons in “repeat environment exposure mice” had depressed excitatory synaptic transmission, in comparison to GFP(-) neighbors and mGluR-LTD was occluded or blocked. This result contrasts with the enhanced mGluR-LTD on ArcGFP+ neurons in mice exposed once to a novel environment [20] and suggest that repeated environment exposure depresses synaptic transmission onto CA1 neurons through a mechanism shared with mGluR-LTD. Such a depression of synaptic transmission with repeated environment exposure may contribute to habituation or familiarity of a novel environment. In support of this idea, mGluR5, Arc and LTD mechanisms such as AMPAR endocytosis are required for behavioral habituation to novel environments [8, 56, 102, 103].

More direct support for a role of *Arc* in the formation of sparse or consolidated neural representations of learned experience, comes from recent work by Wang and colleagues in the motor cortex [5]. Using Arc-GFP knockin transcriptional reporter mice, they imaged *Arc* induction in ensembles of higher order motor cortical neurons (M2) *in vivo* during acquisition of a motor learning task (e.g. walking on a rotating rod; rotarod). The first day of rotarod training increased the number and fluorescence intensity of Arc-GFP(+) M2 neurons in comparison to home caged mice. Repeated, daily rotarod training for the next 2 days, re-

induced *ArcGFP* in a similar, overlapping population of M2 neurons suggesting that motor learning induces *Arc* in a specific motor cortical neuron ensemble [5]. Like CA1 neuron ensembles during novelty learning, motor learning causes *ArcGFP*(+) M2 cortical neuron ensembles to consolidate into a sparse network of highly active *ArcGFP*(+) neurons that mediate the learned skill. Specifically, of the *ArcGFP*(+) neurons induced on the first rotarod training day, some were either “dismissed”, or not re-activated, on subsequent training days and others were “retained” or consistently activated on repeated training days (Fig. 3). Whether a *ArcGFP*(+) neuron was dismissed or retained was related to the level of *ArcGFP* induction on Day 1 of rotarod training. Neurons strongly activated or with elevated levels of *ArcGFP* on Day 1 were more likely to be retained upon subsequent rotarod training days, whereas neurons with lower levels of *ArcGFP* on Day 1 were more likely to be dismissed (Fig. 3). Repeating experiments in the homozygous *ArcGFP* transcriptional reporter knockin mouse (which does not express *Arc* protein), revealed that *Arc* itself is necessary for consolidation of the neural ensemble and for motor learning [5]. As observed in CA1 neurons with repeated novelty, low levels of *ArcGFP* induction in M2 neurons may prime these neurons for *Arc*-dependent LTD of active inputs during rotarod training and contribute to their “dismissal” from the ensemble during subsequent training sessions (Fig. 3). In summary, *Arc* induction occurs in a neuron population with varying levels of activity in response to a learning experience and facilitates formation or consolidation of a sparse, highly active neural ensemble that mediates learning of a new motor task. Evidence that an *Arc* specific neural ensemble mediates learning comes from experiments that utilized the *Arc* SARE to inactivate synapses onto *Arc*-induced neurons in the frontal cortex which erased an acquired rotarod learning skill [7].

If or how *Arc* contributes to the maintenance of the synaptic connectivity onto strongly activated neurons that remain active during repeated environment exposure or repeated motor training is unclear. Recent work demonstrated that *Arc* is necessary for an mGluR1/5, protein synthesis-dependent LTP in CA1 neurons, which requires extremely high frequencies of presynaptic stimulation (~200 Hz) [104]. These results suggest that the highest frequencies of synaptic activation or firing and strong *Arc* induction may prime neurons for LTP, or are necessary to promote *Arc* dependent strengthening and may contribute to retaining highly active neurons in an *ArcGFP*+ motor cortical neuron ensembles with repeated motor learning.

Roles for *Arc*-dependent synaptic weakening in neurodevelopmental disorders

Due to the strong links of *Arc* with learning and memory in healthy animals, perhaps it is not surprising that alterations in *Arc* and *Arc*-dependent synaptic plasticity are associated neuropsychiatric diseases, especially those accompanied by cognitive dysfunction. Although mutations in *Arc* itself are not prevalent in these diseases, dysregulation of *Arc* mRNA, protein levels or mutations in regulators or interacting proteins of *Arc* are linked with intellectual disability, autism [13, 29, 31, 104–108], Alzheimer’s disease [109–111], depression [60] and schizophrenia [112–117]. As discussed below, alterations in mGluR-LTD and synapse elimination are associated with these diseases, and some evidence suggests

this is mediated by dysregulation of Arc. The earliest example comes from rodent models of Fragile X Syndrome, *Fmr1* knockout (KO), the most common inherited form of intellectual disability and leading genetic cause of autism [27, 118] where mGluR-LTD levels in CA1 are enhanced and independent of new protein synthesis [34, 119–123]. Although mGluR-LTD is independent of new protein synthesis in *Fmr1* KO mice, it requires Arc, suggesting that mGluR-LTD in *Fmr1* KO is mediated by preexisting Arc protein [13]. FXS is caused by loss of function mutations in the *FMR1* gene which encodes an RNA binding protein Fragile X Mental Retardation Protein (FMRP) [27, 118]. FMRP binds *Arc* mRNA and is colocalized with *Arc* mRNA and translation in dendrites [16, 29, 124]. FMRP suppresses translation of other mRNA targets and likely Arc [27, 31]. Consequently, in the FXS mouse model, *Fmr1* KO, Arc protein levels are elevated in dendrites and unresponsive to mGluR stimulation [15, 31]. Results from rescue experiments with FMRP and phosphorylation site mutants of FMRP suggested that a Ser500 phosphorylated FMRP functions to suppress Arc translation in dendrites [31]. In response to mGluR1/5 stimulation, FMRP is dephosphorylated by PP2A [125–127] which derepresses dendritic *Arc* translation and promotes LTD [31]. Therefore, mGluR-LTD in the *Fmr1* KO mouse may be independent of new Arc protein synthesis because Arc levels are elevated to a level to support LTD [31]. This idea is supported by data that inhibition of Arc protein degradation, with proteasome inhibitors, leads to elevated Arc protein levels and mGluR-LTD that is independent of new protein synthesis [38]. Because of the similar effects of proteasome inhibitors and *Fmr1* deletion on mGluR-LTD, an interesting possibility is that Arc degradation, in addition to its regulated translation, may be deficient in *Fmr1* KO neurons. Remarkably, like mGluR-LTD, mGluR1/5 and Arc-dependent LTP is independent of new protein synthesis in *Fmr1* KO CA1 neurons, implying that elevated dendritic Arc levels can support LTP or LTD without the need for *de novo* synthesis [104]. This result proposes that posttranslational modifications of synaptic proteins caused by LTP or LTD inducing stimulation determine the direction of synaptic strength changes in response to local Arc synthesis. The findings of altered mGluR-LTD in FXS may be relevant clinically because genetic or pharmacological reduction of mGluR5 is able to rescue phenotypes in FXS animal models, including behavioral phenotypes (reviewed in [128–131]). Whether mGluR5 antagonism will be useful as a therapeutic in humans has yet to be demonstrated [132, 133].

A striking commonality among distinct mouse models of autism and intellectual disability is the protein synthesis-independence of mGluR-LTD; first shown in *Fmr1* KO mice, but recently demonstrated to occur in mouse models of Rett Syndrome [134, 135], 16p11.2 microdeletion [136] and *Syngap* haploinsufficiency [137]. Since mGluR-LTD levels are normal in Rett and 16p11.2 mouse models, it is the uncoupling of LTD from synaptically-regulated synthesis of new proteins in dendrites, including Arc, that correlates with pathology. Based on the model of the role of dendritic Arc synthesis in experience-dependent priming of mGluR-LTD (Fig. 1), and its role in consolidation of neural ensembles during learning (Figs. 3), one may predict that in these forms of autism and ID, that there may be an abnormal consolidation of Arc+ neural ensembles during learning which may lead to cognitive deficits.

Like too much Arc may give rise to enhanced mGluR-LTD in FXS, reduced mGluR-LTD, too little Arc or dysregulation of Arc is associated with other genetic causes of autism and

ID, such as Tuberous Sclerosis Complex Syndrome (TSC). TSC results from loss of function mutations in either TSC1 or TSC2 which form a complex and regulates signaling to mTORC1 [138]. *Tsc2*^{+/-} mice have reduced mGluR-LTD, reduced levels and protein synthesis rates of Arc in hippocampal area CA1 [139]. Similarly, cell autonomous deletion of *Tsc1* or a dominant negative mutant of TSC2 result in reduced mGluR-LTD [140, 141]. Remarkably, enhancing mGluR5 activity, using a positive-allosteric modulator, rescues behavioral phenotypes in *Tsc2*^{+/-} mice [139]. These results, together with the successful rescue of FXS phenotypes with mGluR5 antagonism, suggest that optimal levels of mGluR5 activity, and perhaps mGluR-LTD, are critical for normal cognitive function. Whether mGluR5 therapeutic strategies restore normal Arc levels in TSC or FXS, has not been reported. The cellular mechanisms by which Tsc1/2 regulate Arc levels is unclear. *Tsc2*^{+/-} neurons have overall reduced protein synthesis rates [139], which may lead to reduced Arc protein. Conversely, cultured *Tsc1* KO neurons are hyperexcitable which leads to enhanced steady state levels of Arc mRNA and protein, but reduced activity-induced Arc transcription [142, 143]. *In vivo*, one would expect this to lead to blunted experience-induced Arc in relevant neurons which may contribute to the reduced mGluR-LTD observed with *Tsc1* deletion in hippocampal slices *ex vivo*.

Arc is also dysregulated in a mouse model of Angelman Syndrome, a neurodevelopmental disorder with symptoms of autism and intellectual disability caused by lack of expression of the maternal copy of *UBE3A*, a E3 ubiquitin ligase [144]. In AS mice, Ube3A KO, synaptic Arc levels are elevated which results in decreased synaptic AMPAR expression and reduced synaptic transmission in cultured neurons [105]. Ube3a regulates ubiquitination of Arc and promotes its degradation [105]. Although Ube3A may not directly ubiquitinate Arc, it also inhibits transcription of Arc [107, 145]. In either mechanism, Ube3A inhibits Arc levels. Although elevated levels of Arc are found in both FXS and AS, mGluR-LTD is deficient in AS mice [146], in contrast to FXS where mGluR-LTD levels are elevated. The deficient mGluR-LTD in AS mice may be because synaptic transmission is depressed and mGluR-LTD mechanisms are saturated or “occluded”. Alternatively, or in addition, a deficit in mGluR5 signaling and association with scaffolding proteins is observed in AS mice which may prevent normal mGluR5 signaling to LTD mechanisms [146, 147]. The findings in FXS, TSC and AS indicate that mGluR-LTD phenotypes are not always predicted from steady state Arc levels, but abnormal levels of Arc and mGluR-LTD are associated with cognitive disorders.

Arc, mGluR5 and LTD in age-related cognitive ability and Alzheimer’s disease

In addition to neurodevelopmental disorders, dysregulation of Arc and Arc-dependent mGluR-LTD is implicated in age related-cognitive function and Alzheimer’s disease (AD) [148]. mGluR-LTD is enhanced in areas CA1 and CA3 in aged (2-year-old) rats that have maintained cognitive performance in comparison to their counterparts experiencing age-related memory impairment [149, 150]. Although the correlation of Arc expression with successful cognitive aging in individual animals is unknown, experience-dependent induction of Arc in CA1 and other hippocampal areas declines with age [151, 152]. While

maintaining the ability to express normal levels of mGluR-LTD promotes cognition in aging, excessive or unregulated LTD is associated with dementia and Alzheimer's disease (AD). Accumulating evidence indicates that AD is a disease of synapse loss or failure which may be caused in part by unregulated or excess LTD or synapse elimination mechanisms [153–159]. The pathogenic, soluble amyloid β ($A\beta$) peptides inhibit LTP, as well as promote LTD-like decreases in postsynaptic AMPA and NMDARs and eventually lead to synapse loss [157–159]. $A\beta$ -induced synaptic depression requires activation of mGluR5, and NMDARs in some cases, and prevents or occludes subsequent mGluR-LTD suggesting that $A\beta$ is activating an mGluR-LTD mechanism to cause synaptic pathology [153, 157–159]. Furthermore, $A\beta$ peptides bind to mGluR5 and stimulate signaling [160] and mGluR5 antagonists reverse AD-related brain pathology and cognitive impairment in mouse models [161–163]. Surprisingly, whether *Arc* is necessary for amyloid β -induced synaptic depression or loss has not been reported. There are also alterations in basal and activity-induced *Arc* expression in AD animal models and humans, likely reflecting altered circuit function in these models (reviewed in [148]). The combination of excess synaptic depression, due to $A\beta$ accumulation, together with abnormal experience-regulated induction of *Arc*+ neural ensembles likely contribute to the devastating cognitive deficits associated with AD.

Although the role of *Arc* in AD-associated synaptic weakening is unclear in AD, *Arc* may contribute directly to AD pathogenesis by facilitating the activity-dependent generation of Amyloid β ($A\beta$). Sequential cleavage of APP by β -secretase (BACE1) and γ -secretase yields $A\beta$ peptides that assemble and accumulate in AD. *Arc* binds directly to presenilin 1 (PS1), the catalytic subunit of the γ -secretase complex, and disruption of the *Arc*-PS1 interaction prevents activity-dependent increase in $A\beta$ [109]. *Arc* had previously been shown to play a similar role in generating the transcriptional regulator, NICD (Notch intracellular domain), by γ -secretase cleavage of Notch [164]. Interestingly, *Arc* does not affect the rate of internalization of APP, BACE-1, or γ -secretase from the plasma membrane. Thus, *Arc*'s role in APP processing may not involve its interactions with either dynamin or AP-2, which function primarily in endocytic budding from the plasma membrane. Instead, *Arc* and PS1 co-localize in dendritic puncta that contain endocytic markers, including EEA1 (early endosomes) and Rab11 (recycling endosomes) [109]. APP itself colocalizes with *Arc* and endophilin 3 in early (Rab5-positive), late (Rab7-positive), and especially recycling (Rab11-positive) endosomes. Importantly, the extent of colocalization of APP and γ -secretase in the same endocytic structures was significantly reduced in neurons from *Arc* knockout mice. These results suggest a model wherein *Arc* assists in the sorting of γ -secretase to APP-containing endosomes [109]. The finding that *Arc* induction can generate $A\beta$, together with studies showing that $A\beta$ contributes to *Arc* induction raises the possibility of an *Arc*-mediated positive feedback mechanism in amyloidogenesis [109, 148].

Concluding Remarks

Arc is a fascinating gene and has captured the interest of neuroscientists for decades due to its links to learning and memory. The precise experience-dependent induction of *Arc* in select neural ensembles combined with the transport of *Arc* mRNA to dendrites provides mechanisms to control plasticity of specific synaptic inputs onto these neural ensembles

during learning. Recent work has surprisingly revealed roles of Arc in synaptic weakening mechanisms such as LTD and synapse elimination suggesting important roles for these forms of plasticity in learning and memory. The accumulating evidence of alterations in mGluR-LTD and Arc with diseases of cognition further support their key roles. Challenges for the future include linking what we know about Arc-dependent synaptic plasticity mechanisms to the plasticity of experience-induced, Arc+, neural ensembles and how this mediates learning, as well as understanding the cellular and molecular mechanisms by which Arc is necessary for so many diverse forms of synaptic plasticity. Such basic understanding of these processes will be necessary to know how mGluR-LTD and Arc dysregulation contribute to cognitive disease.

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Highlights

- mGluR1/5-induced dendritic synthesis of Arc causes LTD and synapse elimination
- Arc stimulates endocytosis of postsynaptic AMPA receptors and dynamin assembly
- Experience-induced Arc primes CA1 neurons for mGluR-induced LTD
- Dysregulation of mGluR-LTD and Arc synthesis are linked with neuropsychiatric disease

Brief novel experience → Arc induction in select CA1 neurons

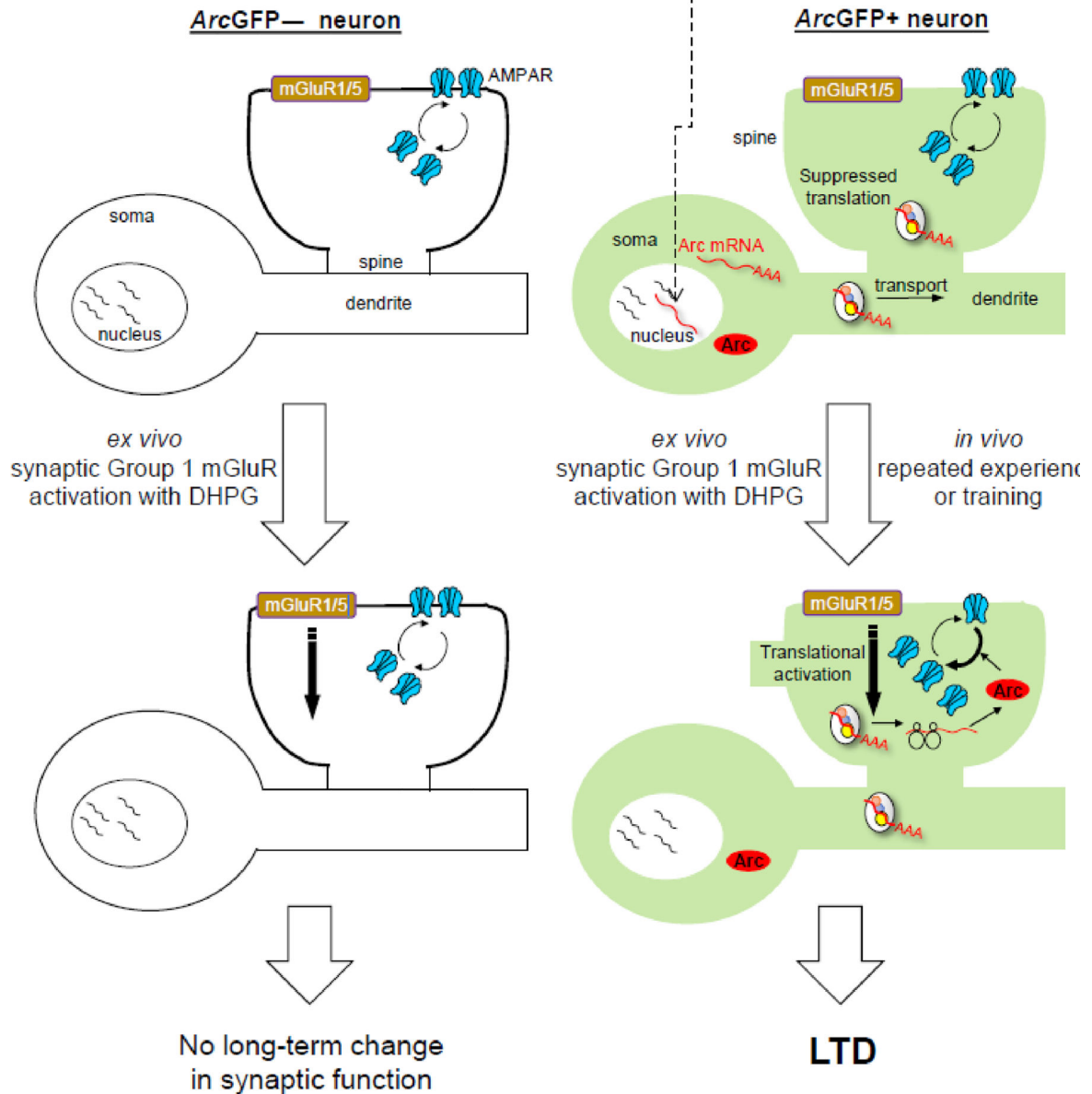


Figure 1. Proposed model of mGluR-LTD priming by novelty-induced Arc in hippocampal CA1 neurons based on data from [20]

A novel experience induces transcription of *Arc* mRNA in a select population of CA1 neurons (*ArcGFP⁺*). After induction, *Arc* mRNAs are transported to dendrites where evidence suggests it is translationally suppressed [31, 48, 50]. *Ex vivo* activation of group 1 mGluRs (in slices) activates *Arc* translation in dendrites, of those neurons with recent *Arc* mRNA induction (*ArcGFP⁺*), but not neighboring *ArcGFP⁻* neurons [13, 14]. *Arc* protein increases the endocytosis rate of AMPA receptors, causing long-term synaptic depression only in *ArcGFP⁺* neurons. Repeated experience of the same environment reactivates synapses on the same neurons initially activated when the environment was novel and *Arc*

mRNA was induced (ArcGFP+) [1] which is proposed to suppress synaptic transmission onto these neurons *in vivo* through a similar mechanism as LTD. Modified from [20].

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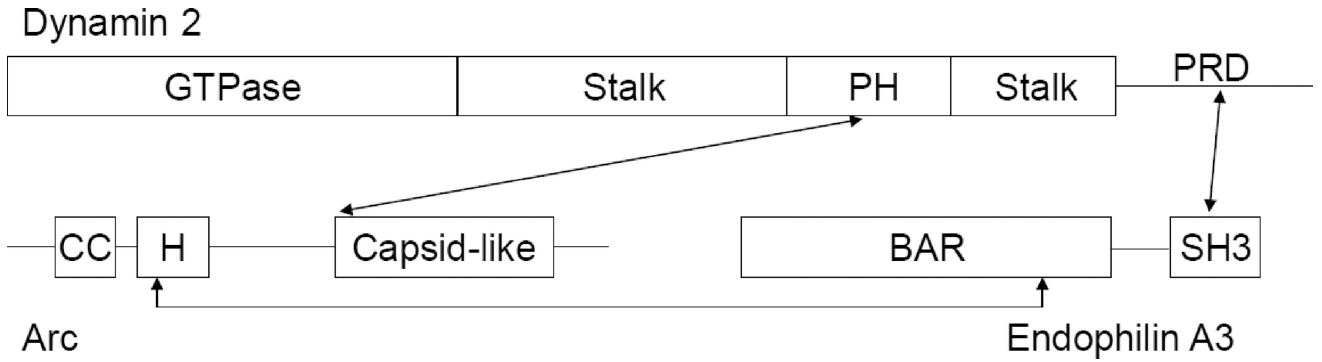


Figure 2. Scheme showing the interaction sites of Arc with Endophilin 3 and Dynamin 2
 Based on secondary structure predictions, Arc is divided into two domains connected by an extended intrinsically disordered region. The N-terminal half contains two stretches predicted to fold into α -helices, the first (~residues 30–60) with coiled-coil (CC) forming potential, the second (~residues 85–130; designated “H” in the figure) containing potential amphipathic segments. The C-terminal half of Arc contains a segment (residues 217–362) that is structurally similar to the retroviral Gag capsid [165]. The domains of dynamin and endophilin are described in the text.

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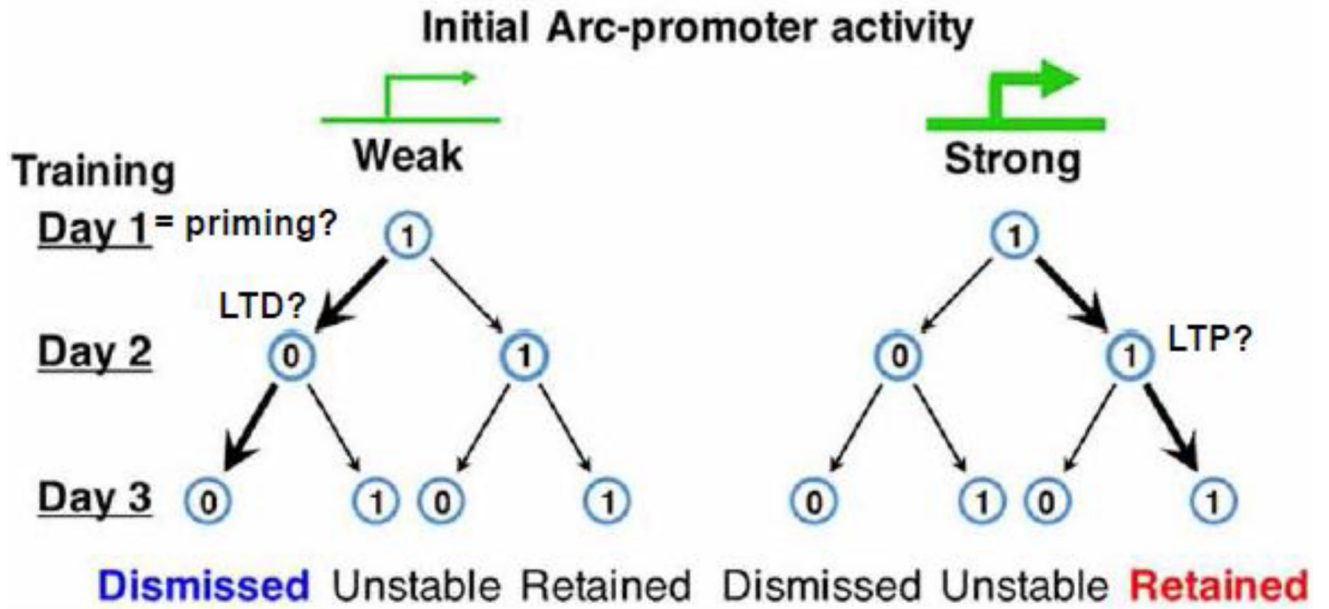


Figure 3. Potential role of experience and *Arc* dependent priming of LTD in refinement of neural ensembles with repeated experience or training

Cao et al, [5] demonstrated that repeated training on a motor task resulted in a sparse neural ensemble of highly active neurons. Motor cortical neurons in M2 induce *Arc* on day 1 of training (“1”), as imaged with a GFP transcriptional reporter *in vivo* which may prime these neurons for synaptic plasticity during repeated training days. Neurons with initially weak levels of *Arc* promoter activity may be primed for LTD which may cause them to be inactive “0” or “dismissed” on subsequent days of training. Neurons with stronger levels of *Arc* promoter activity on Day 1 may be more likely to undergo LTP and to “retain” their strong neuronal activity during subsequent days of training. (Figure is modified from [5]).