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Experience-induced *Arc/Arg3.1* primes CA1 pyramidal neurons for mGluR-dependent long-term synaptic depression

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SUMMARY

A novel experience induces *Arc/Arg3.1* gene as well as plasticity of CA1 neural networks. To understand how these are linked, we briefly exposed GFP reporter mice of *Arc* transcription to a novel environment. Excitatory synaptic function of CA1 neurons with recent *in vivo Arc*-induction (*ArcGFP+*) was similar to neighboring non-induced neurons (*ArcGFP-*). However, in response to group 1 mGluR activation, *ArcGFP+* neurons preferentially displayed long-term synaptic depression (mGluR-LTD) and robust increases in dendritic Arc protein. mGluR-LTD in *ArcGFP+* neurons required rapid protein synthesis and *Arc* suggesting that dendritic translation of Arc underlies the priming of mGluR-LTD. In support of this idea, novelty exposure increased *Arc* mRNA in CA1 dendrites and promoted mGluR-induced translation of Arc in hippocampal synaptoneuroosomes. Repeated experience suppressed synaptic transmission onto *ArcGFP+* neurons and occluded mGluR-LTD *ex vivo*. mGluR-LTD priming in neurons with similar *Arc* activation history may contribute to encoding a novel environment.

INTRODUCTION

Salient experience activates rapid induction of immediate early genes (IEGs) in select neuronal populations and brain regions (Miyashita et al., 2008). IEGs are required for learning and the synaptic plasticity paradigms of long-term potentiation (LTP) and depression (LTD) (Shepherd and Bear, 2011). From this work, it is hypothesized that experience-induced IEGs lead to plasticity of synaptic inputs selectively within an activated neural network and this mediates encoding of memories or learning in response to that experience (Miyashita et al., 2008; Shepherd and Bear, 2011). Although numerous studies

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Conflicts of Interest: None

over the past 2 decades have visualized IEG induction as a means to identify neural networks activated by experience (Miyashita et al., 2008), little is known of how *in vivo* induction of IEGs with experience affects synaptic function or interacts with synaptic plasticity mechanisms.

An IEG whose induction is strongly correlated with learning and salient experience is Activity-regulated cytoskeletal-associated protein, *Arc* or *Arg3.1*. *Arc* is induced in a select population of hippocampal neurons in response to exploration of a novel environment (Guzowski et al., 1999; Miyashita et al., 2008) and hippocampal inhibition of *Arc* translation impairs consolidation of long-term memory (Guzowski et al., 2000). *Arc* protein weakens synaptic transmission by stimulating endocytosis of AMPA subtype glutamate receptors (reviewed in (Shepherd and Bear, 2011)). Interestingly, upon induction, *Arc* mRNA is rapidly transported to dendrites (Steward et al., 1998) and in CA1 neurons, *Arc* is translated in dendrites with activation of Group 1 metabotropic glutamate receptors (mGluRs) and leads to long-term synaptic depression (mGluR-LTD) (Park et al., 2008; Waung et al., 2008). These results suggest that mGluR-LTD may be selectively induced in neuronal populations with experience-induced *Arc*. Importantly, abnormal mGluR-LTD in CA1 is associated with cognitive disorders such as Fragile X Syndrome (FXS), autism, Alzheimer's disease and age-related memory loss (Auerbach et al., 2011; Bozdagi et al., 2012; Huber et al., 2002; Lee et al., 2005; Li et al., 2009). As a result, mGluR dysfunction is causally linked with disease phenotypes in animal models and patients with FXS and autism (Krueger and Bear, 2010). Despite the strong association of mGluR-LTD with cognitive disorders, the role of hippocampal mGluR-LTD in cognition or experience-dependent hippocampal plasticity is unknown.

Here we utilized transgenic mice harboring a fluorescent reporter of *Arc* transcription, briefly exposed them to novelty and examined the consequences of *in vivo*, experience-dependent induction of *Arc* in CA1 on synaptic function and plasticity. Our results indicate that experience-dependent, *in vivo* induction of *Arc* primes individual CA1 neurons for mGluR-LTD. Furthermore, we present evidence that the mechanism of LTD priming involves mGluR-induced, rapid translation of dendritic *Arc* mRNA. This work reveals a novel metaplasticity of synaptic function (Abraham, 2008) that may contribute to the formation of a CA1 neural network representation of a salient experience.

RESULTS

To identify neurons with recent *Arc* induction, we used transgenic mice expressing a bacterial artificial chromosome (BAC) with the *Arc* promoter driving destabilized (4 hr ½ life) GFP (*ArcGFP*-BAC) (Grinevich et al., 2009). *ArcGFP* faithfully reports endogenous *Arc* induction in response to experience and neuronal activity (Grinevich et al., 2009). As observed with *in situ* hybridization for *Arc* mRNA (Guzowski et al., 1999; Miyashita et al., 2008), exposure of *ArcGFP*-BAC mice to a brief (5-min) novel experience increased the number of *ArcGFP*+ CA1 neurons and intensity of GFP in individual cell soma (Fig.S1A–D). In novelty-exposed mice, somatic levels of *Arc* protein were elevated in *ArcGFP*+ neurons as compared to neighboring *ArcGFP*– neurons (Fig.1A), and highly correlated with soma GFP levels ($p < 0.0001$, Fig.S1E), confirming the reliability of the *ArcGFP* reporter. To examine the effects of *in vivo* *Arc* induction on excitatory synaptic function we performed simultaneous whole-cell recordings of miniature (m) and evoked EPSCs from neighboring *ArcGFP*+ and *ArcGFP*– CA1 pyramidal neurons in acute hippocampal brain slices from novelty-exposed *ArcGFP*-BAC mice (Fig.1B). Based on the known function of *Arc* (Shepherd and Bear, 2011), we expected to observe depressed synaptic transmission in *ArcGFP*+ neurons, in comparison to their *ArcGFP*– neighbors. Contrary to our hypothesis, mEPSC frequency and amplitude and evoked EPSC amplitude were similar between

ArcGFP+ and *ArcGFP-* neurons (Fig.1B). EPSC kinetics, passive membrane properties, action potential threshold and firing frequency in response to depolarizing current injections were also similar between *ArcGFP+* and *ArcGFP-* neurons (Fig. 1C; S1F; Table-S1).

In vivo Arc induction does not detectably affect synaptic function. Therefore, we next examined if *ArcGFP+* and *ArcGFP-* neurons were differentially susceptible to induction of synaptic plasticity, such as mGluR-LTD (Park et al., 2008; Waung et al., 2008). mGluR-LTD can be induced with synaptic activation of mGluRs, using paired-pulses of low frequency (1 Hz) synaptic stimulation (PP-LFS), or chemically, using the group 1 mGluR selective agonist, (*RS*)-3,5-Dihydroxyphenylglycine (DHPG). LTD induced by either protocol requires mGluRs, rapid protein synthesis and Arc (Huber et al., 2000; Park et al., 2008; Waung et al., 2008). Delivery of PP-LFS to Schaffer collateral axons acutely depressed evoked EPSCs onto both *ArcGFP+* and *ArcGFP-* neurons (Fig. 1D). However, a persistent LTD was observed in *ArcGFP+* neurons ($61\pm 3\%$ of baseline 30-min after PP-LFS; $n = 6$ cells; $p < 0.0001$) but not in *ArcGFP-* neurons ($92\pm 5\%$; $n = 6$; Fig. 1D,E). As observed with PP-LFS, brief DHPG application ($100\mu\text{M}$; 5-min) caused an acute depression in both *ArcGFP+* and *ArcGFP-* neurons but a persistent LTD only occurred in *ArcGFP+* neurons ($78\pm 4\%$ of baseline 30-min after DHPG; $n = 9$; $p < 0.01$; *ArcGFP-*; $95\pm 6\%$; $n = 9$; Fig.1D,E). DHPG-induced LTD is associated with decreases in mEPSC frequency, but not amplitude, which relies on postsynaptic AMPAR endocytosis and Arc (Moult et al., 2006; Waung et al., 2008). Consistent with these reports, DHPG ($50\mu\text{M}$; 5-min) induced a persistent depression of mEPSC frequency in *ArcGFP+* neurons ($69\pm 7\%$ of baseline; $n = 13$; $p < 0.05$), but not in *ArcGFP-* neurons ($124\pm 15\%$ of baseline; $n = 10$; Fig.1F). LTD magnitude of evoked EPSCs and mEPSCs was positively correlated with the GFP intensity of the soma of recorded CA1 neurons (Fig. S1G). DHPG equally affected input resistance and holding current of *ArcGFP+* and *ArcGFP-* neurons (Fig.S1H-I), indicating that *ArcGFP-* neurons possess functional mGluRs, but are specifically deficient in LTD.

Evidence indicates that mGluR-LTD relies on dendritic translation of Arc mRNA (Huber et al., 2000; Park et al., 2008; Waung et al., 2008). Therefore, we hypothesized that mGluR activation stimulated translation of dendritic Arc mRNA in neurons that have recently undergone Arc transcription. Fluorescent *in situ* hybridization (FISH) for Arc mRNA in conjunction with immunohistochemistry for GFP and β -tubulin (a neuron and dendritic marker) in novelty-exposed *ArcGFP-BAC* mice indicated that *ArcGFP+* CA1 pyramidal neurons contained greater dendritic Arc mRNA than neighboring *ArcGFP-* neurons ($304\pm 47\%$ of *ArcGFP-*; $p < 0.01$ Fig. 2A,B; S2A). Sections incubated with a sense riboprobe for Arc displayed little or no signal (Fig. S2B). To determine if mGluR-stimulation differentially regulated Arc protein levels in the soma or dendrites of *ArcGFP+* and *ArcGFP-* neurons we performed immunohistochemistry for Arc, GFP, and NeuN (neuronal cell bodies; Fig.1A) or β -tubulin (neuronal cell body and dendrites; Fig.2C,D) in hippocampal slices of novelty-exposed *ArcGFP-BAC* mice treated with vehicle or DHPG. Arc protein levels were elevated in *ArcGFP+* (NeuN+) soma as compared to *ArcGFP-* (NeuN+) neighbors ($437\pm 45\%$ of *ArcGFP-*; $p < 0.0001$). Surprisingly, this ratio was unaffected by DHPG treatment ($380\pm 31\%$ of *ArcGFP-*; Fig.1A). Similar results were obtained if we measured a ratio of Arc-to- β tubulin intensity in the soma (Fig. 2D). Arc/ β tubulin protein levels in the dendrites of vehicle-treated *ArcGFP+* neurons were slightly, but not significantly, elevated in comparison to neighboring *ArcGFP-* neurons ($177\pm 12\%$ of *ArcGFP-*; $p = 0.09$; Fig.2D). However, after DHPG treatment Arc/ β tubulin levels in the dendrites of *ArcGFP+* neurons were enhanced in comparison to dendrites from *ArcGFP-* neurons ($292\pm 29\%$ of *ArcGFP-*; $p < 0.0001$). Importantly, DHPG caused an increase in Arc levels in the dendrites of *ArcGFP+* ($p < 0.0001$), but not *ArcGFP-* neurons (Fig.2C,D) suggesting a mechanism for priming of mGluR-LTD in *ArcGFP+* neurons.

Our results suggest that experience-induced *Arc* mRNA is transported to CA1 dendrites and translated upon mGluR stimulation. To test this idea more quantitatively, we used quantitative (q) real-time (RT) PCR to measure *Arc* mRNA in microdissected CA1 dendritic regions (*str. radiatum*) of acute slices prepared from home cage-anesthetized mice or mice exposed to brief (5 min) novelty. Because *Arc* mRNA is not expressed in glia or inhibitory neurons in CA1 (Vazdarjanova et al., 2006), the primary source of *Arc* mRNA in these microdissected regions should be from CA1 pyramidal cell dendrites. Three hours after brief novelty, qRT-PCR revealed a 4-fold increase in *Arc* mRNA in CA1 dendritic regions (Fig. 2E). To determine if novelty exposure promoted dendritic translation of *Arc*, we prepared synaptoneurosomes from CA3-CA1 hippocampal regions from cage-anesthetized or novelty-exposed mice and ³⁵S Met incorporation into *Arc* was measured after immunoprecipitation (Waung et al., 2008). The specificity of ³⁵S Met incorporation into *Arc* was confirmed using mice with genetic deletion of *Arc* (*ArcGFP-KI*^{-/-}; described below; Fig. S2C) (Wang et al., 2006). Although basal *Arc* translation was not affected by novelty exposure (novelty+vehicle), DHPG stimulated *Arc* translation in synaptoneurosomes from novelty-exposed, but not cage-anesthetized mice (Fig. 2F). The enhanced mGluR-stimulated *Arc* translation at synapses of novelty exposed mice would be expected to lead to enhanced mGluR-LTD, which is exactly what we observed in population field (f) EPSP measurements in CA1 of acute slices from novelty-exposed mice in comparison to cage anesthetized mice (Fig. 2G; S2I). Together these results indicate that novelty experience increases *Arc* mRNA in CA1 dendrites, which in turn, promotes mGluR-stimulated dendritic *Arc* translation and LTD.

We hypothesized that enhanced DHPG-induced translation of *Arc* in dendrites underlies the priming of DHPG-induced LTD in *ArcGFP*⁺ neurons. In support of this hypothesis, the protein synthesis inhibitor anisomycin (25 μM) blocked DHPG-induced LTD of mEPSC frequency in *ArcGFP*⁺ neurons (Fig.3A, S3C). To test if *Arc* itself is required, we employed another *Arc* reporter mouse with GFP knocked in to the endogenous *Arc* locus (*ArcGFP-KI*) and the homozygous mice (*ArcGFP-KI*^{-/-}) report *Arc* promoter activation with GFP, but do not produce *Arc* protein (Wang et al., 2006). Because novelty induces IEGs in addition to *Arc*, such as *Homer1a*, in similar CA1 neuronal populations (Vazdarjanova et al., 2002), these experiments will determine whether the *Arc* gene itself is required for the priming of LTD in *ArcGFP*⁺ neurons. We first confirmed that *ArcGFP*⁺ neurons in novelty-exposed *ArcGFP-KI* mice were primed for mGluR-LTD. For these experiments we used heterozygous *ArcGFP-KI*^{+/-} mice which display normal DHPG-induced LTD as measured by extracellular recordings of population EPSPs in CA1 (Fig.S3A). LTD is normal in *ArcGFP-KI*^{+/-} (het) mice likely because *Arc* protein levels are ~80% of wildtype (Wang et al., 2006). As observed in *ArcGFP-BAC* mice, mEPSC frequency, amplitude (Fig.S3B), kinetics and passive membrane properties (Table S1) were similar between *ArcGFP*⁺ and *ArcGFP*⁻ neurons in slices from novelty-exposed *ArcGFP-KI*^{+/-} mice. Furthermore, DHPG application to *ArcGFP*⁺ neurons induced LTD of mEPSC frequency and had no effect on mEPSCs of *ArcGFP*⁻ neurons (Fig.3B), thus confirming the priming effect of *in vivo Arc* induction on LTD in an independent mouse line. In *ArcGFP-KI*^{-/-} (*Arc* KO) mice, *ArcGFP*⁺ neurons responded acutely to DHPG application, as measured by input resistance and holding current (Fig. S3D), but did not display LTD of mEPSC frequency (Fig. 3C). Consistent with previous reports (Plath et al., 2006), no differences were detected in baseline mEPSC frequency (*ArcGFP*⁺ neurons of: *ArcGFP-KI*^{+/-}=0.17±0.04, *ArcGFP-KI*^{-/-}=0.12 ± 0.04, p=0.13; n=8, 11 cells) or kinetics or passive membrane properties (Table S1) between heterozygous *ArcGFP-KI*^{+/-} and homozygous *ArcGFP-KI*^{-/-} (*Arc* KO) mice.

Arc induction with brief novelty primes *ArcGFP*⁺ neurons for LTD upon subsequent mGluR stimulation. We hypothesized that repeated exposure to the same, initially novel, experience would reactivate synapses onto *ArcGFP*⁺ neurons and cause LTD *in vivo*. In

support of our hypothesis, repeated exposure to the same environment activates the same network of CA1 neurons (Guzowski et al., 1999; Thompson and Best, 1990) and mGluR5 is required for synaptic depression triggered by prolonged novelty (Popkirov and Manahan-Vaughan, 2010; Qi et al., 2012). To test our hypothesis, we repeatedly exposed *ArcGFP-BAC* mice to the same novel environment (5-min exposure, every 20 min for 4 hours), prepared acute slices and examined synaptic function in *ArcGFP+* and *ArcGFP-* CA1 neurons. Since we were unable to selectively probe synapses activated by repeated experience, we assayed all active synapses on an *ArcGFP+* neuron by measuring mEPSCs. In contrast to brief *in vivo* experience (Fig. 1B), multiple repeated experiences resulted in depressed mEPSC frequency in *ArcGFP+* compared to neighbor *ArcGFP-* neurons (Fig 3D). There were no differences in mEPSC amplitudes, kinetics or passive membrane properties after repeated experience between *ArcGFP+* cells and a) *ArcGFP-* cells or b) *ArcGFP+* cells in the brief novelty condition (Table S1). If such *in vivo* synaptic suppression in *ArcGFP+* cells occurred via mechanisms common to mGluR-LTD, we would predict that DHPG-induced LTD would be decreased or occluded *ex vivo*. Consistent with this prediction, DHPG activated mGluRs but had no effect on mEPSC frequency of *ArcGFP+* neurons from mice with repeated experience (Fig. 3E, S3E). These results suggest that repeated experience suppresses synaptic function *in vivo* selectively on neurons with recent *Arc* induction and this occurs through a mechanism in common with mGluR-LTD.

DISCUSSION

Here we demonstrate that *in vivo* *Arc* induction after a brief novel experience primes CA1 neurons for LTD in response to subsequent activation of group 1 mGluR receptors. *In vivo* *Arc* induction during brief novelty does not affect baseline synaptic transmission nor excitability, but enhances subsequent induction of synaptic plasticity and therefore may represent a novel form of metaplasticity (Abraham, 2008). We hypothesize that LTD priming by *in vivo* *Arc* induction may play a role in targeted suppression of a subset of CA1 neurons previously activated by the same experience and contribute to the formation of a neural network representation of that experience.

Our results suggest a model wherein *Arc* mRNA is induced by salient experience in select CA1 neurons and is then transported to dendrites, where it remains translationally-suppressed until mGluR activation translates *Arc* protein and induces LTD (Fig. 3F). Additional support for this model includes: 1) mGluRs stimulate dendritic *Arc* translation 2) mGluR-LTD relies on translation in dendrites and specifically translation of *Arc* (Huber et al., 2000; Park et al., 2008; Waung et al., 2008) 3) mGluR-LTD priming requires rapid *de novo* protein synthesis as well as *Arc* and 4) brief novelty induces *Arc* mRNA in select CA1 neurons (Guzowski et al., 1999), increases *Arc* mRNA levels in CA1 dendrites and enhances mGluR-triggered translation of *Arc* at synapses and LTD (Popkirov and Manahan-Vaughan, 2010; Qi et al., 2012). Our data suggests that repeated experience of the same environment causes synaptic weakening of CA1 neurons with dendritic *Arc* mRNA through a mechanism similar to mGluR-LTD (Fig. 3F). Similarly, an mGluR5-dependent synaptic depression occurs *in vivo* in response to prolonged environment exposure (Popkirov and Manahan-Vaughan, 2010; Qi et al., 2012). Since re-exposure to the same environment is known to prune the network of responsive CA1 neurons (Karlsson and Frank, 2008), one possibility is that re-exposure related mGluR-LTD in *Arc+* CA1 neurons contributes to targeted suppression of a subset of neurons activated by the first novel experience, leading to a sparser representation of the original experience.

ArcGFP(+) cells allow single cell assay of recent salient experience-activated network

Arc activation marks specific networks activated by experience (Miyashita et al., 2008). With *ArcGFP* mice, we cannot be certain that the GFP was specifically induced with novel

object exploration. However, cage-anesthetized mice had low ArcGFP+ expression and Arc transcription is not induced by spontaneous firing of cells, or place cell activation without behavioral relevance (Fletcher et al., 2006; Miyashita et al., 2009). These results, combined with the short (2–4 h) half-life of ArcGFP, suggests that ArcGFP+ neurons in our study were at least activated *in vivo* by recent, salient experience. Studies have observed robust mGluR-LTD in slices from rodents that were not subjected to novel object exploration (Auerbach et al., 2011; Huber et al., 2002; Huber et al., 2000; Moulton et al., 2006; Park et al., 2008). How are these studies reconciled with our findings that novelty primes mGluR-LTD? In most slice physiology labs, including our own, mice or rats are not habituated to human handling, and on experiment day animals are removed from their home cage, transported to the lab and anesthetized there. This “standard handling” procedure itself may constitute a brief novel experience since it induces Arc in CA1 neurons (Fig. S2D–G), and enhances mGluR-LTD in comparison to cage-anesthetized mice (Fig. S2H,I).

Translational suppression of dendritic Arc mRNA as a metaplasticity mechanism

We observed that after brief novelty, CA1 as a whole, and Arc-activated neurons in particular, have greater levels of dendritic Arc mRNA, but not Arc protein. Such translational suppression of dendritic Arc mRNA may explain lack of differences in baseline synaptic function between ArcGFP+ and ArcGFP– neurons. Many dendritic mRNAs are translationally suppressed during transport to achieve localized synaptic protein expression (Besse and Ephrussi, 2008). Arc’s 3’UTR harbors an intron and dendritic Arc mRNA is associated with the exon junctional complex (EJC) (Giorgi et al., 2007). Since the EJC is typically removed upon a pioneer round of translation, its association with dendritic Arc mRNA suggests that much of dendritic Arc mRNA has not been translated. The RNA binding protein, Fragile X Mental Retardation Protein (FMRP) may translationally suppress dendritic Arc at steady state (Napoli et al., 2008; Niere et al., 2012). Upon group 1 mGluR-stimulation FMRP is dephosphorylated, altered in its protein interactions, ubiquitinated and degraded which may result in translational de-repression of Arc mRNA and LTD (Napoli et al., 2008; Narayanan et al., 2007; Niere et al., 2012). In addition to FMRP, mGluRs stimulate signaling pathways that lead to translational activation and that are necessary for LTD (Waung and Huber, 2009).

A model for experience-induced Arc and mGluR-LTD priming in the formation of CA1 neural representation of a novel experience

Exploration of novel objects facilitates CA1 LTD in awake rats (Dong et al., 2012; Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999; Popkirov and Manahan-Vaughan, 2010) suggesting that LTD may contribute to learning or familiarity of a novel experience. In support, inhibition of mGluR5 or LTD mechanisms block novelty-related learning (Dong et al., 2012; Popkirov and Manahan-Vaughan, 2010). Importantly, Arc is implicated in late phase LTP (Bramham et al., 2010; Guzowski et al., 2000; Plath et al., 2006). Exploration of a novel open field (without objects) facilitates LTP induction (Kemp and Manahan-Vaughan, 2004), suggesting that Arc induction *in vivo* could also prime neurons for LTP. An interesting possibility is that the pattern and frequency of synaptic activation onto a neuron with recent Arc induction determines whether LTP or LTD is primed. Priming of mGluR-LTD by recent salient experience may contribute to the plasticity of active neural networks that encode that experience. Abnormal encoding and habituation of novel experiences occurs in Fragile X syndrome (Hagerman et al., 1985; Restivo et al., 2005), a cognitive disorder associated with altered mGluR-LTD (Huber et al., 2002). Understanding the contribution of mGluR-LTD to normal cognition will help understand how altered mGluR-LTD mechanisms lead to cognitive disorders (Auerbach et al., 2011; Bozdagi et al., 2012; Huber et al., 2002; Lee et al., 2005; Li et al., 2009).

EXPERIMENTAL PROCEDURES

Mice

The two mouse lines that were used, *ArcGFP-BAC* and *ArcGFP-KI* have been previously described (Grinevich et al., 2009; Wang et al., 2006) (Supplementary methods).

Novel Experience

Novelty-exposed and cage-anesthetized mice were habituated to human handling (4–5 days). After brief novelty (5 min), mice were left undisturbed in a standard empty cage for 3 hours prior to anesthesia. Cage-anesthetized mice were removed from their home cage and anesthetized within 10 sec with isoflurane. Repeated exposure to the same environment involved 5-min exposures, every 20 min for 4 hours. Mice in the standard-handling group were not habituated to human handling, and on experiment day were removed from their home cage, transported to the lab and anesthetized in the lab. (see Supplementary Methods).

Electrophysiology

Whole cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices was performed as described (Huber et al., 2000) (Supplementary methods).

Immunocytochemistry

Acute hippocampal slices (400 μm) were prepared as for electrophysiology and treated with Vehicle or DHPG. Slices were then fixed in 4% paraformaldehyde and resectioned in 3% agarose to yield 50 μm thick sections. Sections were blocked with 3% goat serum and 0.5% Triton X prior to treatment with primary and secondary antibodies (see Supplementary methods).

Fluorescent in situ hybridization (FISH) with co-immunostaining

ArcGFP-BAC mice were exposed to brief novelty as for electrophysiology experiments but perfused with 4% paraformaldehyde. 20 μm sections were prepared and processed for Arc/Arg3.1-specific FISH using Cy3-coupled tyramide amplification. (see Supplementary Methods)

Synaptoneurosome preparation, ^{35}S -Met/Cys incorporation and immunoprecipitation of Arc was conducted as previously described (Waung et al., 2008) with a minor modification (Supplementary Methods).

Statistical Analysis

Statistical analyses were performed using Prism 5.0 (GraphPad Software). Two-way or one-way ANOVA, independent Student's t-test, Mann-Whitney U test or Two-sample Kolmogorov-Smirnoff test were applied where appropriate unless specified otherwise. All data are expressed as the mean \pm SEM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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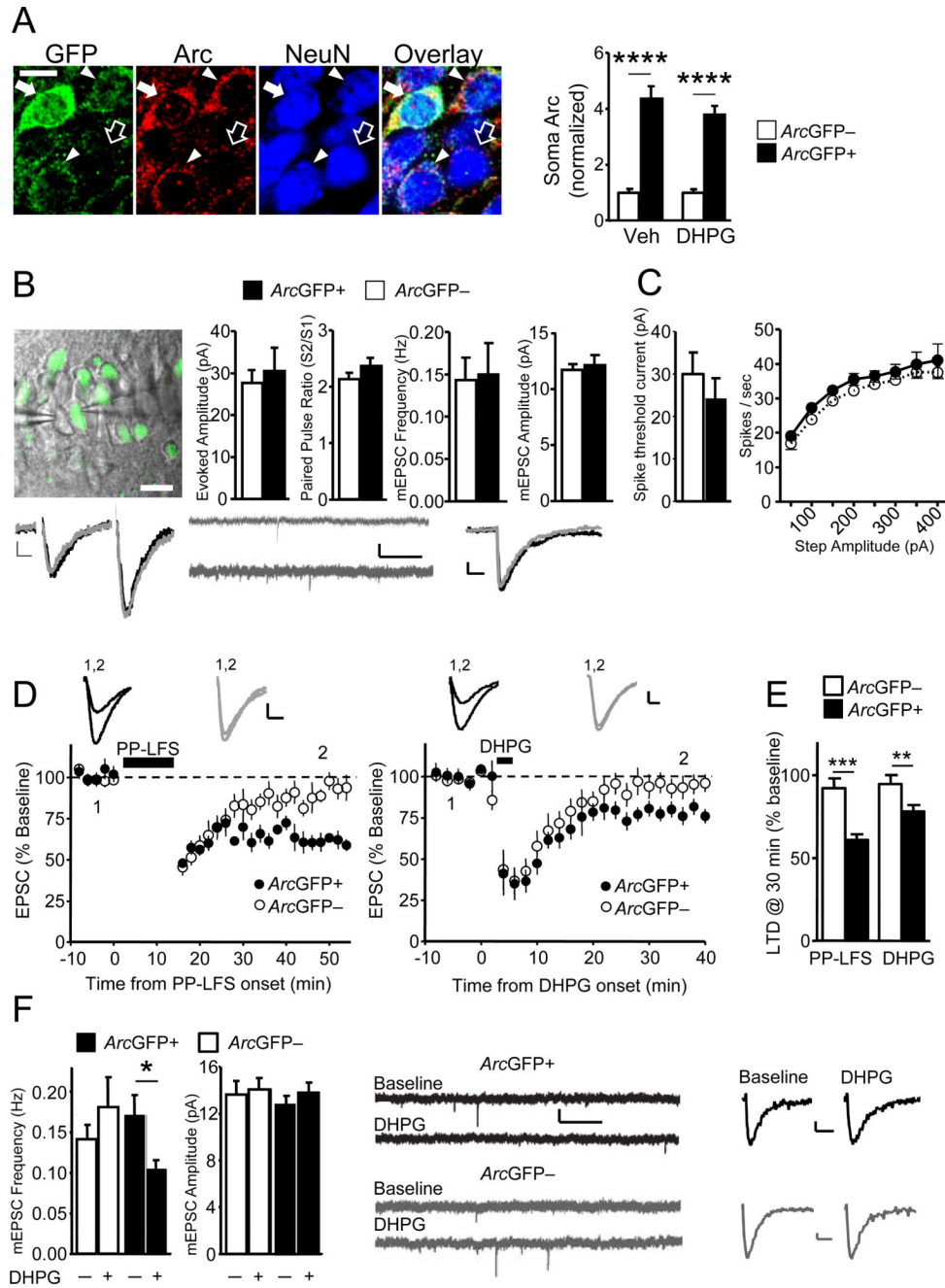


Figure 1. Group 1 mGluR activation induces LTD selectively in CA1 neurons with recent *in vivo* Arc induction

A). Left: Immunohistochemistry for NeuN, GFP and Arc in hippocampal CA1 from an *ArcGFP-BAC* mouse exposed to novelty. Empty and filled arrows denote *ArcGFP-* and *ArcGFP+* neurons, respectively. GFP and Arc levels in neuron soma are correlated (filled arrowheads and arrow). Scale-bar=5 μ m. Right: Group averages (\pm SEM) of soma Arc protein in *ArcGFP+* and *ArcGFP-* neurons (normalized to average soma Arc value in *ArcGFP-* neurons in the same image). The relative difference in soma Arc levels between *ArcGFP+* (n=108) and *ArcGFP-* neurons (n=32) is similar in ACSF and DHPG treated slices (*ArcGFP+*;n=97, *ArcGFP-*;n=28). **B)** Upper Left: Overlay of confocal fluorescence

and infrared DIC images from an acute hippocampal slice during simultaneous recordings from *ArcGFP+* and *ArcGFP-* CA1 neurons. **Upper Right:** Group averages (\pm SEM) of evoked EPSC ($n=10$ cell pairs) amplitudes, paired-pulse ratios, mEPSC frequency and amplitudes ($n=11$ cell pairs) from *ArcGFP+* and neighboring *ArcGFP-* neurons. **Lower:** Example evoked EPSCs (average of 4), raw and averaged mEPSCs (Scale bars: 10 ms/5 pA, 1 s/10 pA and 10 ms/2 pA respectively). **C) Left:** The minimal current required to elicit action potentials is similar *ArcGFP+* ($n=15$) and *ArcGFP-* ($n=10$) neurons. **Right:** Firing frequency versus current injection (F-I curve) is similar between *ArcGFP+* ($n=19$) and *ArcGFP-* ($n=15$) neurons. **D) Time course of average evoked EPSC amplitude (Avg \pm SEM) normalized to pre-PP-LFS (left panel) or pre-DHPG (right panel) baseline. mGluR activation by PP-LFS (15-min; black bar; $n=6$ per group) or 100 μ M DHPG (5-min; black bar; $n=9$ per group) induces LTD in *ArcGFP+* but not *ArcGFP-* neurons. Representative EPSCs taken at times indicated are given on the graph. Scale-bar=10 ms/25 pA. **E) LTD of EPSC amplitude at 30 min after cessation of PP-LFS or DHPG in *ArcGFP+* and *ArcGFP-* neurons. **F) Left:** DHPG application induced a persistent (30-min) decrease in mEPSC frequency in *ArcGFP+* ($n=13$) but not in *ArcGFP-* CA1 neurons ($n=10$). **Right:** Raw and averaged mEPSCs (Scale bars: 1 s/10 pA and 10 ms/2 pA) before and 30 min after DHPG (50 μ M; 5-min) for *ArcGFP+* (black) and *ArcGFP-* (grey) neurons. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. See also Figure S1.****

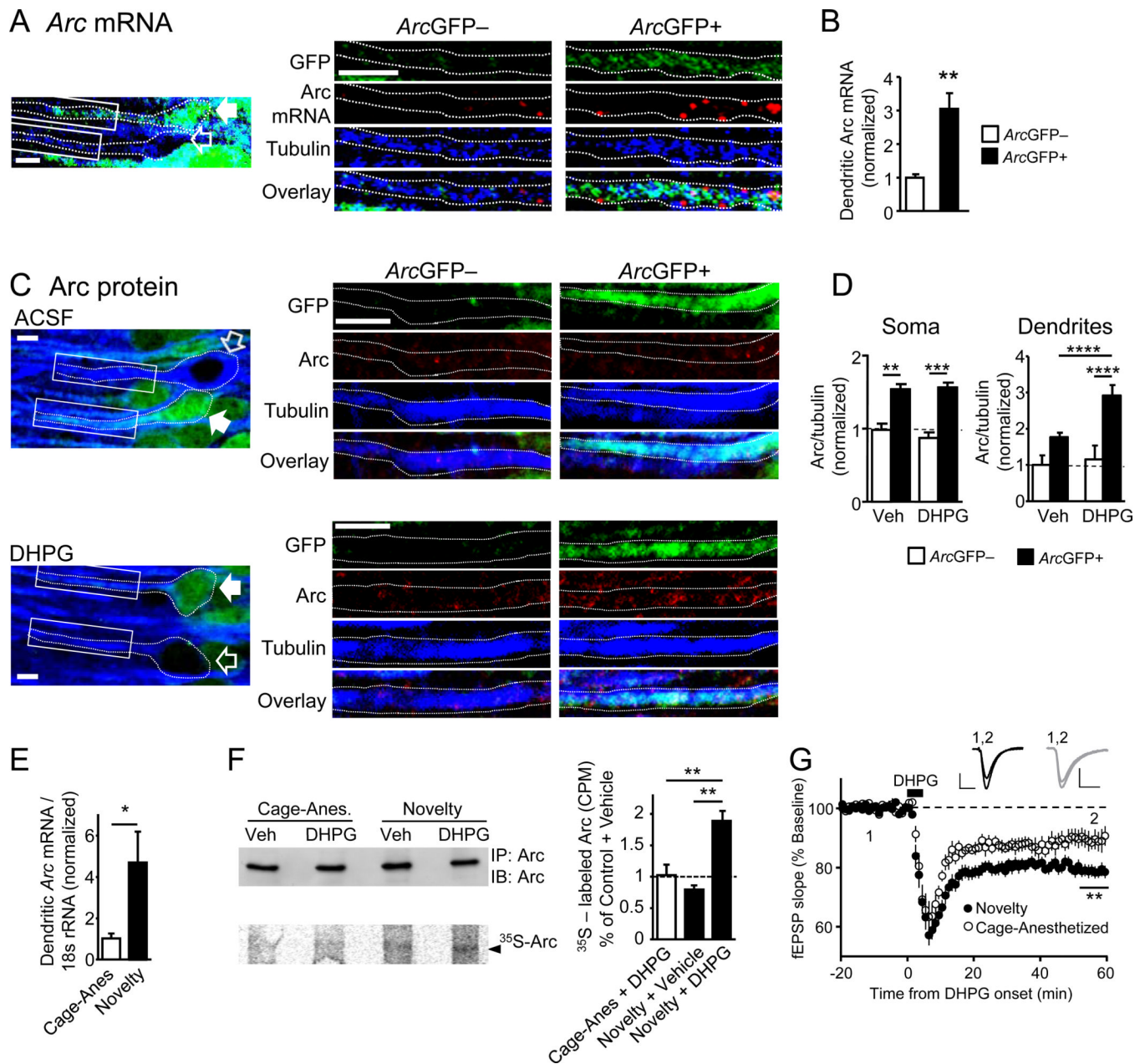


Figure 2. Novelty induction of Arc *in vivo* enhances group 1 mGluR stimulated dendritic Arc translation

A) Representative image of CA1 neurons from *ArcGFP-BAC* mice exposed to brief novelty and processed for FISH of *Arc* mRNA (red) and immunohistochemistry for GFP (green) and 3-tubulin (blue). Dotted outline of *ArcGFP+* neuron (filled white arrow) and *ArcGFP-* neuron (empty arrow) indicates source of dendrites at right. Scale-bar=5 μ m. **B)** Quantification of dendritic *Arc* mRNA levels in *ArcGFP+* neurons (n=48) normalized to that in neighbor *ArcGFP-* neurons (n=14). **C)** Representative images of CA1 neurons from *ArcGFP-BAC* mice exposed to brief novelty and processed for immunohistochemistry of 3-Tubulin (blue), GFP (green) and Arc protein (red). Dotted outline of neurons in left panel indicates the source of dendrites at right. Scale-bar=10 μ m **D)** Quantified group data of Arc/tubulin immunofluorescence in soma or dendrites of *ArcGFP+* (n for soma, dendrites-

Vehicle; =155,135; DHPG; n=87,77) and *ArcGFP-* (Vehicle; n=29,19; DHPG; n=22,19) CA1 neurons from vehicle or DHPG-treated acute slices in novelty exposed mice. **E**) qPCR of *Arc* mRNA in microdissected CA1 dendritic regions from mice with brief novelty exposure or cage-anesthetized (n = 6 mice/condition) **F**) Western blot of total Arc immunoprecipitated (upper) and phosphorImager gel scan (lower) of immunoprecipitated, ³⁵S-Met-labeled Arc from Vehicle (Veh) and DHPG-treated hippocampal synaptoneurosomes from cage-anesthetized or novelty-exposed mice. Right: Quantified group data of ³⁵S Met incorporation into Arc immunoprecipitates expressed as a fraction of total immunoprecipitated Arc and normalized to cage-anesthetized+vehicle condition. **G**) Group average of DHPG (100 μM; 5 min)-induced LTD of extracellularly recorded, evoked field (f)EPSPs (Avg±SEM) in cage-anesthetized (89±3% of baseline; n=10 slices) and novelty-exposed mice (79±2%; n=20), normalized to pre-DHPG baseline. *p<0.05;**p< 0.01; ***p< 0.001; ****p < 0.0001. See also Figure S2.

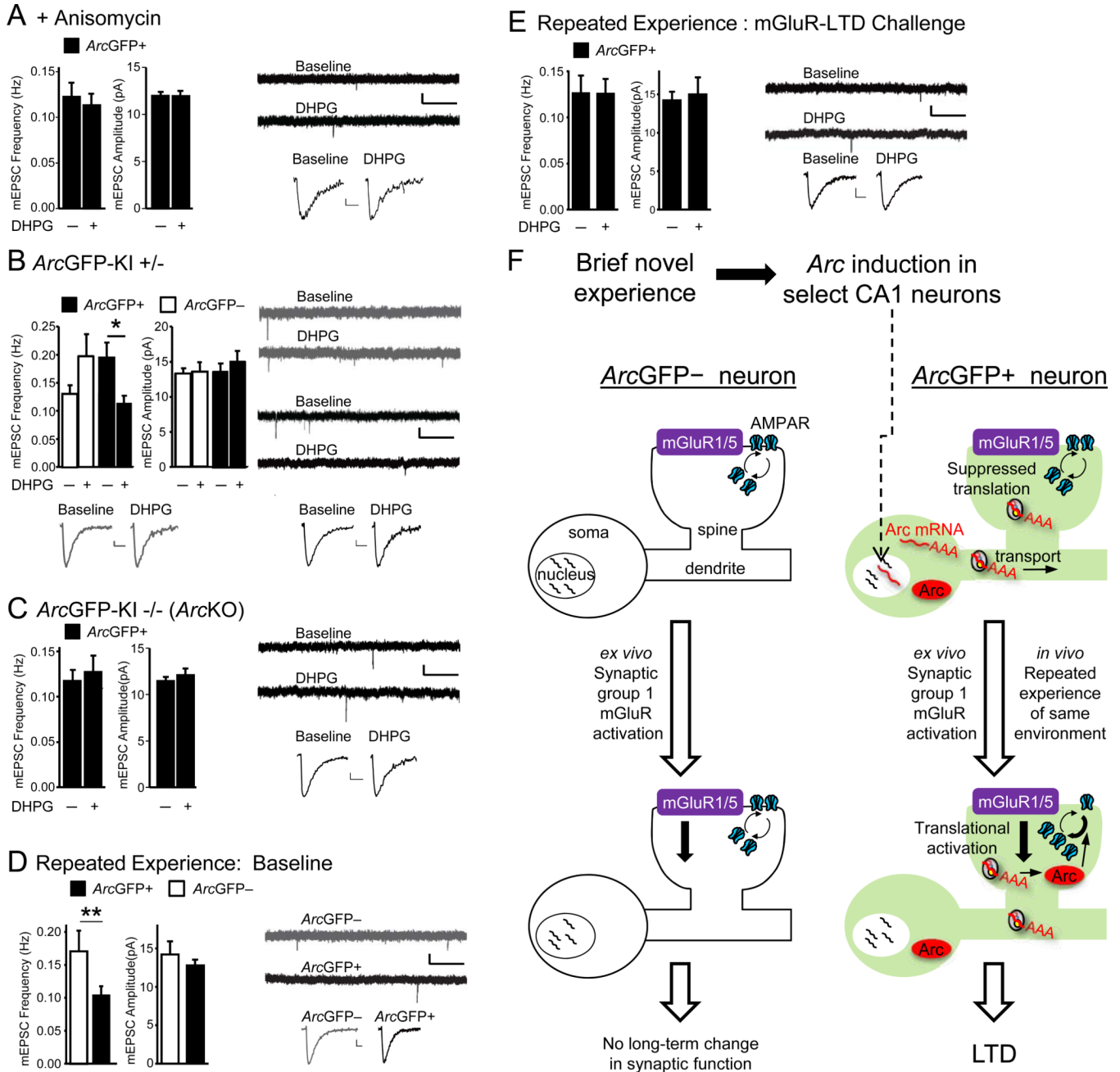


Figure 3. Group 1 mGluR triggered LTD in ArcGFP+ neurons requires *de novo* protein synthesis and Arc and occurs *in vivo* with repeated novelty experience
A) Anisomycin (25 μ M) blocks DHPG-induced decreases in mEPSC frequency (Avg \pm SEM) in ArcGFP+ neurons (n=13) from novelty exposed ArcGFP-BAC mice. **B)** Novelty-exposed heterozygous ArcGFP-KI^{+/-} mice exhibit DHPG-induced LTD of mEPSC frequency in ArcGFP+, but not ArcGFP- neurons (n=9,12). **C)** DHPG-induced LTD of mEPSCs is absent in ArcGFP+ neurons of homozygous ArcGFP-KI^{-/-} (ArcKO) mice (n=11). **D)** ArcGFP+ neurons from ArcGFP-BAC mice exposed to repeated experience of the same environment display reduced mEPSC frequency in comparison to neighboring ArcGFP- neurons (n=10 cell-pairs) **E)** DHPG has no effect on mEPSCs in ArcGFP+ neurons from ArcGFP-BAC mice with repeated experience (n=9). For Figures 3A–3E: left panels depict

group averages (\pm SEM) of mEPSC frequency and amplitude before and 30 min after DHPG and right panels depict representative raw and average mEPSCs at baseline and 30 min after DHPG for *Arc*GFP+ (black) and *Arc*GFP- (grey) neurons. Scale-bars: 1s/10 pA and 10 ms/2 pA. * $p < 0.05$, ** $p < 0.01$. **F**) Proposed model of *in vivo Arc* induction by novelty experience and LTD priming. See text and Figure S3.