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# LTP- and LTD-inducing stimulations cause opposite changes in *Arc/Arg3.1* mRNA level in hippocampal area CA1 *in vivo*

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## Abstract

Immediate early genes (IEGs) typically are the first genetic responders to a variety of cellular activations. The IEG that encodes activity-regulated cytoskeleton-associated protein (*arc/arg3.1*) has attracted much interest because its mRNA is transported to and translated near activated synapses. Moreover, *arc* has been implicated in both long-term potentiation (LTP) and long-term depression (LTD). However, little is known about the time course of altered *arc* expression during LTP and LTD. Here we characterized *arc* mRNA levels in area CA1 of the adult rat hippocampus *in vivo* after LTP-and LTD-inducing stimulations that were identical except for the temporal patterning of the stimulation pulses. We observed a persistent increase in *arc* mRNA level during LTP. In contrast, during LTD, *arc* mRNA level first was decreased and then transiently increased relative to control level. These findings demonstrate that *arc* mRNA is regulated differently during LTP and LTD, and they provide evidence for stimulation-induced down-regulation of mRNA availability during LTD. Findings of abbreviated LTD when transcription was inhibited indicate that the prolonged maintenance of the type of NMDA receptor-dependent LTD studied here requires *de novo* transcription. Furthermore, lack of evidence for a LTD-associated change in the mRNA level of the IEG *zif268* demonstrates that the decrease in *arc* mRNA during LTD is not a general genetic response. Thus, the regulation of *arc* expression not only differs between LTP and LTD but also diverges from that of other IEGs implicated in activity-dependent synaptic plasticity.

### Keywords

zif268; immediate early gene; synaptic plasticity; NMDA receptor; RNA stability

## Introduction

Alteration in the expression of immediate early genes (IEGs) is the first genetic response to many kinds of cellular activations (Dragunow, 1996; Lanahan and Worley, 1998; Miyashita et al., 2008). Many IEGs regulated by neural activity encode transcription factors; however, some encode effector proteins. One effector protein-encoding gene that has received much attention in the context of activity-dependent synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), is the gene that encodes activity-regulated cytoskeleton-associated protein (*arc*, also known as *arg3.1*) (Bramham et al., 2010; Bramham et al., 2008; Tzingounis and Nicoll, 2006). *Arc* mRNA levels were found to be increased and shown to be transported to activated synapses after LTP-inducing

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stimulation in hippocampus (Lyford et al., 1995; Steward et al., 1998; Steward and Worley, 2001a; Steward and Worley, 2001b); genetic deletion of *arc* was shown to be associated with impaired LTP and LTD in hippocampal slice preparations (Plath et al., 2006); and acute knock-down of *arc* was shown to disrupt LTP in area CA1 as well as the dentate gyrus of the hippocampus (Guzowski et al., 2000; Messaoudi et al., 2007). Furthermore, consistent with the idea of a link between activity-dependent synaptic plasticity and the establishment of long-term memories (Bliss and Collingridge, 1993; Braunewell and Manahan-Vaughan, 2001; Malenka and Bear, 2004), mice in which *arc* was either knocked out or acutely knocked down were found to exhibit consolidation deficits (Guzowski et al., 2000; Plath et al., 2006). However, an increase in *arc* expression after LTP-inducing stimulation has not been observed consistently (French et al., 2001; Miyashita et al., 2009), and overexpression of *arc* was found to be associated with a reduction in AMPA receptor-mediated currents and occlusion of N-methyl-D-aspartate (NMDA) receptor-dependent LTD (Rial Verde et al., 2006).

Despite much interest in *arc* as an early responder to plasticity-inducing synaptic input and a critical player in the establishment of long-term synaptic modification, relatively little is known about the time course of altered *arc* expression after LTP- or LTD-inducing stimulation. Miyashita et al. (2009) observed an increase in *arc* expression within minutes after LTP induction in area CA1; others reported an increase in *arc* mRNA levels 30 min to 4 hours after LTP induction in the dentate gyrus (Lyford et al., 1995; Steward et al., 1998). These findings suggest that *arc* induction in response to LTP-inducing stimulation is very rapid and quite prolonged. Such comparisons across hippocampal subregions, however, may not be warranted, especially in light of differential rates of *arc* induction in areas CA3 versus CA1 in response to behavioral manipulations (Gusev et al., 2005; Miyashita et al., 2009). The profile of *arc* expression during LTP in a given brain region, specifically one known to undergo experience-dependent plasticity, therefore remains to be determined.

There currently is no information about changes in *arc* expression during LTD. Based on findings of disrupted NMDA receptor-dependent LTD in the presence of transcription inhibitors (Kauderer and Kandel, 2000), together with the aforementioned observations that *arc* overexpression leads to LTD occlusion (Rial Verde et al., 2006), one might predict that LTD-inducing stimulation triggers an increase in *arc* expression. On the other hand, results showing no effect of transcription inhibitors on NMDA receptor-dependent LTD (Manahan-Vaughan et al., 2000) suggest that *arc* expression is not increased during LTD or that any increase is non-consequential for the establishment of this form of LTD.

In this study we aimed to characterize *arc* expression during NMDA receptor-dependent LTP and LTD in area CA1 of the adult rat hippocampus *in vivo*. To avoid that any differences in expression profiles between LTP and LTD that could be attributed to procedural differences, such as amount or duration of stimulation, we kept all of the stimulation parameters identical but varied only the temporal pattern of the plasticity-inducing stimulation between LTP- and LTD-experiments. Our results show that *arc* mRNA levels are regulated very differently during the two forms of synaptic plasticity. Whereas LTP is associated with a persistent increase in *arc* expression, LTD is associated a rapid decrease followed by a transient increase in *arc* mRNA level. These findings provide evidence for stimulation-induced down-regulation of transcriptional product during LTD.

## Materials and Methods

### In Vivo Electrophysiology

Electrophysiological methods were used as previously described (Thiels et al., 2002; Thiels et al., 1992). All procedures were in compliance with and approved by the Institutional

Animal Care and Use Committee, University of Pittsburgh. Briefly, male Sprague Dawley rats (Hilltop, Scottdale, PA; 250–350 g) were anesthetized first with an intraperitoneal (i.p.) injection of 8% chloral hydrate in 150 mM NaCl (0.4 g/kg) and then maintained under constant anesthesia throughout the remainder of the recording session with intravenous (i.v.) injection of the same anesthetic (0.15 g/kg/h). Rats were placed in a stereotaxic apparatus, and an incision was made on the scalp. After the skin was retracted, one small hole was drilled into the skull on the left side (relative to bregma: AP, −1.7 mm; ML, −1.1 mm) and one on the right side (relative to bregma: AP, −3.6 mm; ML, +2.3 mm). After removal of dura mater, a pair of bipolar metal electrodes, insulated except for the 100 μm to 150 μm at the tip, was lowered into area CA3 of the left dorsal hippocampus (final DV placements relative to the surface of the brain: about −3.5 mm) and a glass electrode, filled with 2 M saline (impedance of 0.9–1.4 MΩ), was lowered to either str. pyramidale or, in separate groups of animals, str. radiatum of area CA1 of the right dorsal hippocampus (final DV placements relative to the surface of the brain: about −1.8 mm and −2.1 mm, respectively). An input-output (I/O) function that relates the intensity of commissural stimulation (20–200 μA; 100-μs duration) to the amplitude of the evoked CA1 pyramidal cell population spike was determined at the beginning of each experiment. An additional I/O function that relates the intensity of commissural stimulation to the initial slope of the evoked CA1 population EPSP was determined for experiments involving recordings in str. radiatum. A stimulation intensity that produced a response with an amplitude that was 30% to 40% of the maximum amplitude of the evoked population spike for str. pyramidale recordings or an initial slope that was 30% to 40% of the maximum slope of the evoked population EPSP for str. radiatum recordings before delivery of high-frequency or paired-pulse stimulation (HFS and PPS, respectively) was used for test pulses (a series of 10 pulses at 0.1 Hz, delivered at 5-min intervals before and after HFS or PPS). Baseline response level was determined by delivery of successive series of test pulses for a total of 15 min to 20 min before HFS or PPS, i.e., until stable baseline responding had been established. LTP was induced by applying HFS (4 trains of 100 pulses at 100 Hz delivered at 130-sec intervals), and LTD was induced by applying PPS (200 pairs of pulses with a 25-ms interval delivered at 0.5 Hz) to the commissural fibers using a stimulation intensity that was 60% to 75% of the maximum amplitude of the population spike, as determined with the first I/O function. Thus, the total number of stimulation pulses (400 pulses), total duration of patterned stimulation (400 sec), and stimulation intensity were essentially identical between the two protocols. After termination of HFS or PPS, additional series of test pulses were delivered for a total of either 10 min, 30 min, 60 min, or 120 min using the same stimulation intensity as was used for test pulses before HFS or PPS delivery. Recorded data were amplified, filtered (0.1 Hz – 10 kHz), digitized, and stored on computer disk for later analysis. In experiments requiring local drug infusion, a glass pipette (tip inner diameter = 35–50 μm) connected to a positive-pressure syringe pump (Harvard Apparatus, Holliston, MA) was lowered into area CA1 within 200 μm to 300 μm of the tip of the recording electrode. D-aminophosphonovaleric acid (D-APV; 0.5 mM in the drug pipette; dissolved in 150 mM NaCl; Tocris, Ellisville, MO) was infused continuously (6–8 nl/min) from 60 min before HFS or PPS until the end of recording. Actinomycin D (ActD; 0.8 mM in the drug pipette; dissolved in 0.5% methanol/95% 150 mM NaCl; Calbiochem, La Jolla, CA) was infused over the course of 15 min to 20 min (50–60 nl/min) beginning 90 min before HFS or PPS. At the end of electrophysiological recording, rats were decapitated, the brains removed rapidly, and the right hippocampus excised in the presence of ice-cold artificial cerebrospinal fluid. Within one min of brain removal, a 1 $mm^3$-piece of tissue from dorsal area CA1 (recording site) and an equal-sized piece of tissue from ventral area CA1 (within-subject control) of the right hippocampus were dissected out and transferred to separate, color-coded 1.5-ml tubes maintained on dry ice for instantaneous freezing of the tissue samples. The samples were stored at −80°C until molecular analysis.

## Quantitative PCR (qPCR)

The experimenter conducting the PCR analyses was blind to the color code until completion of the experiments. From each tissue sample, total RNA was isolated using Trizol® reagent (Invitrogen, Carlsbad, CA) according to manufacturer instructions. Isolated RNA was treated with DNAse I (Invitrogen) prior to reverse transcription in order to remove genomic DNA. The integrity of the RNA was confirmed by agarose gel electrophoresis, and the yield was determined by measurement of the absorbance at 260–280 nm. Using the Superscript III kit (Invitrogen) 0.5 μg total RNA was reverse-transcribed with poly-dT primers, nucleotides, and buffers in a total reaction volume of 20 μl according to manufacturer instructions. In each well, 2 μl of the appropriate cDNA was added to the qPCR reaction mixture, which consisted of 12.5 μl SYBR-green master mix (SA Biosciences, Frederick, MD), 6 μl of primer mixture containing 400 nM of each forward and reverse primers (final concentration of each ~100 nM), and 4.5 μl nuclease-free water, to bring the total volume in each well to 25 μl. A minus reverse transcriptase negative control was included in all cases. Reactions were performed in triplicates using a Biorad IQ real-time thermocycler (Hercules, CA) with an initial *Taq* polymerase activation at 95°C for 10 min, followed by 50 cycles each of which involved 30 sec at 95°C, 30 sec at 60°C, and 30 sec at 72°C. The efficiency (E) of each primer set was determined using a serial dilution of standard cDNA, which was generated by reverse transcription of 2 μg of total RNA extracted from whole hippocampus using polydT primers in a reaction volume of 20 μl. The threshold cycle (Ct) for each sample was chosen to lie within the early exponential rise phase, and any wells with multiple peaks during the melting-point analysis were excluded from analysis. Data were normalized to glyceraldehyde 3-phosphate dehydrogenase (*gapdh)* run in the same qPCR experiment. Gene expression in dorsal area CA1 (experimental tissue), relative to expression in ventral area CA1 (within-subject control tissue), was determined by the efficiency-corrected delta cycle threshold (ΔCt) method using the following formula: relative quantity $(RQ)_{arc,zif268} = E_{arc,zif268}^{Ct(ventral)-Ct(dorsal)}/E_{gapdh}^{Ct(ventral)-Ct(dorsal)}$.

All primers were selected to have the same melting temperature of 60 °C to enable running them within the same plate. In addition, all primers were designed to be on two exons to allow us to distinguish between mature versus newly synthesized mRNA by the melting curves. In all cases a single peak was observed. *Arc*, *zif268*, and *gapdh* PCR products were cloned using the TA cloning kit (Invitrogen) according to instructions of the manufacturer. The sequence-specific amplification was confirmed by sequencing and subsequent NCBI blast. The primers and their product sizes were as follows:

Arc (fwd): 5′-AGTCTTGGGCAGCATAGCTC-3′, Arc (rev): 5′-GCCGAAGTCTGCTTTTCTTC-3′ (115 bp); Zif268 (fwd): 5′-CAGCGCTTTCAATCCTCAA-3′, Zif268 (rev): 5′-TGGGATAACTTGTCTCCACCA-3′ (119 bp); Gapdh (fwd): 5′-GAAGGGCTCATGACCACAGT-3′, Gapdh (rev): 5′-GGATGCAGGGATGATGTTCT-3′ (117 bp).

Calibration curves for estimating the PCR amplification efficiency of each of the three targets are shown in Supplementary Figure 1.

## Statistical analyses

To validate significant levels of synaptic potentiation or depression after HFS or PPS, respectively, two-tailed paired Student's *t*-tests were carried out on data collected during the last 5 min before HFS or PPS and during the last 5 min of electrophysiological recording. Differences in level of synaptic change between groups were compared with two-tailed Student's *t*-tests for independent groups. To determine whether delivery of baseline stimulation, HFS, or PPS is associated with a significant change in *arc* or *zif268* mRNA

level, we first transformed the RQ values calculated as described above on a $\log_2$ scale to achieve normal distribution of the data (Bland and Altman, 1996; Kubista et al., 2007) and then tested the resulting distributions against the null-hypothesis of equal mRNA level in dorsal and ventral samples (i.e., a population mean of 0.0) using two-tailed one-sample Student's *t*-tests. An α-level of ≤ 0.05 was applied for all comparisons to determine statistical significance.

## Results

### LTP in area CA1 *in vivo* is accompanied by a rapid and persistent increase in *arc* mRNA level

Previous studies on changes in *arc* expression after induction of LTP in area CA1 *in vivo* used *in situ* hybridization-based techniques and have led to conflicting findings, possibly due to procedural differences (French et al., 2001; Miyashita et al., 2009). Both groups of investigators induced LTP in anesthetized rats with two trains of HFS presented 30 sec apart. French and colleagues harvested tissue 30 min after LTP induction and examined IEG induction using autoradiographic *in situ* hybridization techniques with end-labeled oligodeoxynucleotide probes, whereas Miyashita and colleagues harvested tissue 5 min after LTP induction and used fluorescent *in situ* hybridization with digoxigenin-labeled intron-enriched riboprobes. French and colleagues did not find evidence for LTP-associated *arc* induction, whereas Miyashita and colleagues observed a robust increase in *arc* expression in a large proportion of the cases. Here, we investigated *arc* expression by applying qPCR on small samples of CA1 tissue that included the recording site and were harvested at different times after LTP-inducing stimulation. To induce LTP, we delivered 4 trains of HFS 130 sec apart to the dorsal CA3 commissural projections to contralateral area CA1; to map out the time course of *arc* expression, we collected tissue samples either 20 min, 40 min, 70 min, or 130 min after the start of HFS and conducted qPCR on those samples. Our stimulation protocol produced a persistent potentiation of the amplitude of the CA1 population spike evoked by test pulses delivered before and after HFS (Fig 1. A1). The potentiation of the evoked population spike lasted for at least 2 hr (20-min group: $t(6) = 6.36$; 40-min group: $t(6) = 4.64$; 70-min group: $t(5) = 6.34$; and 130-min group: $t(4) = 6.15$; all $p$'s < 0.01). Similarly, our HFS protocol produced a persistent potentiation of the initial slope of the CA1 population EPSP, and this potentiation lasted at least 2 hr (Fig 1. A2) (70-min group: $t(3) = 4.66$; and 130-min group: $t(4) = 5.16$; both $p$'s < 0.05). Baseline stimulation only (series of 10 test pulses delivered every 5 min), on the other hand, was not associated with a systematic change in either the amplitude of the evoked population spike (Fig. 1. A1) or the initial slope of the evoked population EPSP (Fig. 1. A2) across the 2.5-hr recording period (population spike: $t(2) = 1.15$; population EPSP: $t(2) = 1.29$, both $p$'s > 0.1). To determine whether *arc* level is altered after HFS, we compared mRNA levels in dorsal area CA1 near the recording site (experimental tissue) to that in ventral area CA1 (within-subject control). Because the CA3 commissural fibers stimulated in our experiments do not innervate ventral area CA1 (Ishizuka et al., 1990), ventral CA1 constitutes a useful within-subject control. Figure 1B depicts that before delivery of HFS, i.e., under baseline condition, *arc* mRNA level in dorsal area CA1 was comparable to that in ventral area CA1 ($t(8) < 1$). In contrast, after 4 trains of HFS, *arc* mRNA level was significantly higher in experimental relative to control tissue at each of the time points investigated (20 min: $t(6) = 3.55$, $p < 0.02$; 40 min: $t(6) = 2.52$, $p < 0.05$; 70 min: $t(9) = 3.52$, $p < 0.01$; and 130 min: $t(9) = 5.03$, $p < 0.01$). Our results confirm the findings by Miyashita et al. (2009) that *arc* mRNA levels increase rapidly after LTP-inducing stimulation of area CA1 *in vivo*. Furthermore, our results extend these earlier observations by showing that *arc* mRNA levels are increased for at least 2 hr after LTP induction in area CA1.

To rule out the possibility that the observed change in *arc* mRNA level was a non-specific consequence of patterned stimulation, we took advantage of the fact that HFS-induced LTP in area CA1 is dependent on NMDA receptor activation (Thiels et al., 1992). Thus, we infused the NMDA receptor antagonist D-APV (0.5 mM in the drug pipette) into area CA1 near the recording site throughout electrophysiological recording and assessed the effect on *arc* expression in CA1 tissue samples collected 20 min after HFS. Figure 2. A1 shows that in the presence of D-APV, HFS failed to produce a significant potentiation of the amplitude of the evoked CA1 population spike ($t(6) = 2.21$, $p > 0.07$.). Figure 2. A2 shows that the increase in *arc* mRNA level detected 20 min after HFS in the absence of drug was abolished completely in the presence of D-APV ($t(6) < 1$). These results confirm that the increase in *arc* mRNA level after HFS we observed above (Figure 1B) depends on prior induction of LTP. Furthermore, the results suggest that the LTP-associated increase in *arc* mRNA level is mediated via an NMDA receptor-dependent mechanism.

Our observations of an HFS-associated increase in *arc* mRNA level do not distinguish between the possibility that LTP is associated with an increase in *de novo arc* transcription versus an increase in *arc* mRNA stability. To address this issue, we assessed the effect of the general transcription inhibitor ActinomycinD (ActD) on the increase in *arc* mRNA level observed immediately after HFS. Specifically, we infused ActD (0.8 mM in the drug pipette) into area CA1 near the recording site over the course of about 15 min beginning 90 min before HFS. Dorsal and ventral area CA1 pieces were collected 20 min after HFS. Figure 2. B1 shows that the presence of ActD did not affect the ability of 4 trains of HFS to cause a significant increase in the amplitude of the evoked population spike ($t(8) = 6.71$, $p < 0.01$). No effect of the transcriptional inhibitor on the increase in the evoked response immediately after HFS was expected because only late but not early phases of LTP depend on transcription (Abraham and Williams, 2003; Nguyen et al., 1994). Figure 2. B2 shows that the marked increase in *arc* mRNA level 20 min after HFS observed in the absence of drug failed to emerge in the presence of ActD ($t(8) = 1.15$, $p > 0.1$). These results indicate that the observed LTP-associated elevation in *arc* mRNA level stems from a stimulation-induced increase in *arc* mRNA synthesis, rather than a decrease in *arc* mRNA degradation. Taken together, our findings demonstrate that the induction of NMDA receptor-dependent LTP in area CA1 *in vivo* is associated with a robust, rapid increase in *arc* transcription and that *arc* mRNA levels remain elevated above control levels for at least 2 hr after LTP induction.

## LTD in area CA1 *in vivo* is accompanied by a rapid decrease followed by a transient increase in *arc* mRNA level

To determine the time course of any changes in *arc* mRNA level in association with LTD area CA1 *in vivo*, we followed essentially the same approach as described above. We induced LTD in area CA1 by delivering one train of PPS, which consisted of 200 pairs of pulses with a 25-ms interval presented at 0.5 Hz, to the dorsal CA3 commissural fibers, and we harvested CA1 tissue samples for qPCR analysis either 20 min, 40 min, 70 min, or 130 min after the onset of the PPS. Similar to our previous observations (Thiels et al., 1994), PPS produced a persistent depression of the amplitude of the CA1 population spike (Fig. 3. A1) as well as the initial slope of the CA1 population EPSP (Fig. 3. A2) evoked by test pulses delivered before and after PPS, and these effects lasted for at least 2 hr after PPS (population spike: 20 min: $t(6) = 5.15$, $p < 0.01$; 40 min: $t(1) = 16.31$, $p < 0.05$; 70 min: $t(3) = 5.71$, $p < 0.02$; and 130 min: $t(4) = 7.63$, $p < 0.01$; population EPSP: 40 min: $t(4) = 10.44$; 70 min: $t(4) = 6.93$; 130 min: $t(4) = 12.60$, all $p$'s $< 0.01$). As in the above experiments, baseline stimulation was not associated with a systematic change in either the amplitude of the evoked population spike (Fig. 3. A1) or the initial slope of the evoked population EPSP (Fig. 3. A2) across the 2.5-hr recording period (population spike: $t(2) < 1$; population EPSP:

$t(2) = 1.13$, $p > 0.1$). The results from the qPCR analysis are shown in Figure 3B. Before delivery of PPS, *arc* mRNA level in dorsal area CA1 did not differ from that in ventral area CA1 ($t(6) < 1$). However, immediately after PPS, *arc* mRNA level was significantly decreased relative to the level detected in control tissue (20 min: $t(6) = 5.22$, $p < 0.01$). This significant decrease was short-lasting, because 40 min after PPS, a systematic difference in *arc* mRNA level between experimental and control tissue samples was no longer detectable ($t(6) < 1$). However, as time since PPS passed, *arc* mRNA levels began to increase such that 70 min after PPS, mRNA level in experimental CA1 samples was significantly higher relative to that in control samples ($t(8) = 4.34$, $p < 0.01$). This increase in *arc* mRNA level 1 hr after LTD induction, however, also did not persist; mRNA level in experimental CA1 samples no longer differed significantly from control level 130 min after PPS ($t(8) < 1$). Different from what one might have expected based on previous work showing that genetic reduction of *arc* interferes with LTD maintenance (Plath et al., 2006) whereas overexpression of *arc* occludes LTD (Rial Verde et al., 2006), the present results indicate that *arc* mRNA level undergoes a rapid decrease after LTD-inducing stimulation. Furthermore, our results suggest a remarkable bidirectional regulation of *arc* mRNA level after LTD induction in area CA1 *in vivo*, with levels decreasing below baseline during the early phase of LTD, increasing above baseline about 1 hr after LTD induction, and returning to baseline by 2 hr after LTD induction. This profile is distinctly different from the one we observed after induction of LTP.

We previously showed that induction of LTD by PPS in area CA1 *in vivo* is dependent on NMDA receptor activation (Thiels et al., 1994; Thiels et al., 2000). Therefore, to rule out that the observed decrease in *arc* mRNA level was some non-specific effect of patterned stimulation, we infused D-APV (0.5 mM in the drug pipette) into area CA1 near the recording site throughout the electrophysiological recording session and assessed the effect on *arc* mRNA level in CA1 tissue samples collected 20 min after PPS. Figure 4. A1 shows that in the presence of D-APV, PPS failed to produce a depression of the amplitude of the evoked CA1 population spike ($t(5) < 1$). Figure 4. A2 shows that the decrease in *arc* mRNA level detected 20 min after PPS in the absence of drug failed to emerge in the presence of D-APV ($t(5) < 1$). These results confirm that the decrease in *arc* mRNA level after PPS observed above (Figure 3B) depends on prior induction of LTD and is not a non-specific consequence of prior synaptic activation.

The increase in *arc* mRNA 70 min after PPS raises the question whether the observed change stems from *de novo* transcription or a dramatic reduction in *arc* mRNA degradation. To address this issue, we assessed the effect of ActD on the increase in *arc* mRNA level observed 70 min after PPS. Specifically, we infused ActD (0.8 mM in the drug pipette) into area CA1 near the recording site over the course of about 15 min beginning 90 min before PPS. Dorsal and ventral area CA1 pieces were collected 70 min after HFS. Figure 4. B1 shows that pre-infusion of ActD did not affect the early phase of LTD, as the amplitude of the evoked population spike immediately after PPS was not distinguishable from the depressed level observed in the vehicle group (for the first 4 test-pulse series after PPS, vehicle vs. ActD, $t(10) > 1$). However, the initial depression of the evoked response did not last in the group that received ActD but returned to baseline level by the end of the recording period ($t(6) < 1$). In contrast, the response depression lasted at least until the end of the recording period in vehicle-treated animals ($t(6) = 8.40$, $p < 0.01$) (Figure 4. B1). These findings demonstrate that the prolonged maintenance of PPS-induced LTD in area CA1 requires mRNA synthesis. Figure 4. B2 shows that the robust increase in *arc* mRNA level in dorsal hippocampus 70 min after PPS observed after infusion of vehicle solution ($t(6) = 4.83$, $p < 0.01$) was abolished completely after infusion of ActD; in fact, *arc* mRNA level detected in dorsal CA1 tissue 70 min after PPS in the presence of ActD was slightly but significantly below control level ($t(6) = 2.3$, $p < 0.05$). These results indicate that the LTD-

associated increase in *arc* mRNA level 70 min after PPS stems from a stimulation-induced increase in *arc* mRNA synthesis, rather than a decrease in *arc* mRNA degradation.

Taken together, these findings demonstrate that the induction of NMDA receptor-dependent LTD in area CA1 *in vivo* is associated with an immediate, pronounced decrease and a protracted, transient increase in *arc* mRNA level. The transient increase in *arc* mRNA level is the result of an increase in *arc* transcription. Importantly, this increase in arc transcription is necessary for the prolonged maintenance of NMDA receptor-dependent PPS-induced LTD in area CA1 *in vivo*. Relating these findings to those after LTP induction, our results show that *arc* mRNA levels are altered during both LTP and LTD in area CA1 *in vivo*. The pattern of *arc* mRNA regulation, however, differs markedly between these two forms of synaptic plasticity.

### The patterns of change in *arc* and *zif268* mRNA level differ from one another during LTD but not during LTP in area CA1 *in vivo*

Similar to *arc*, the IEG *zif268* was reported to be regulated in a synaptic activation-dependent manner (Jones et al., 2001; Lindecke et al., 2006). In fact, *zif268* was shown to be required for the maintenance of LTP (Davis et al., 2003; Renaudineau et al., 2009). Using the same tissue samples as we used for the analyses of *arc* mRNA level after induction of LTP, we found that *zif268* mRNA level also is increased significantly immediately after LTP induction (Fig. 5A). As was the case with *arc*, this increase in *zif268* mRNA level persisted for at least 2 hr (Base: $t(7) < 1$; 20 min: $t(6) = 2.91$, $p < 0.05$; 40 min: $t(6) = 3.53$, $p < 0.02$; 70 min: $t(9) = 3.79$, $p < 0.01$; and 130 min: $t(9) = 3.86$, $p < 0.01$). In light of an overall similarity in the expression patterns of *arc* and *zif268* during LTP, it became of interest to determine whether these two IEGs also are regulated similarly during LTD. Accordingly, we probed the same LTD- tissue samples as used for the *arc* analyses for *zif268* mRNA. Figure 5B shows that *zif268* mRNA level in dorsal area CA1 was comparable to that detected in ventral area CA1 before PPS. Although *zif268* mRNA level tended to increase, relative to control level, over the course of the first hour after PPS and then decrease, relative to control level, over the course of the second hour after PPS, none of these trends was statistically significant (Base: $t(7) < 1$; 20 min: $t(6) = 1.56$; 40 min: $t(6) = 1.92$; 70 min: $t(7) = 1.74$; and 130 min: $t(7) = 1.45$, all $p$'s $> 0.1$). These results indicate that, in contrast to *arc* mRNA, *zif268* mRNA level does not deviate significantly from control level at any time during the first 2 hr after LTD induction. Thus, taken together, these results show that *arc* and *zif268* are regulated differently from one another during LTD, whereas they appear to be regulated in overall similar fashion during LTP in area CA1 *in vivo*.

## Discussion

Previous work indicates that *arc* plays a critical role in LTP, LTD, and homeostatic plasticity (Bramham et al., 2008; Tzingounis and Nicoll, 2006). In this study we characterized the time course of *arc* expression during LTP and LTD in area CA1 of the adult rat hippocampus *in vivo*. Based on previous reports of changes in *arc* mRNA level after LTP induction (Link et al., 1995; Lyford et al., 1995; Messaoudi et al., 2007; Miyashita et al., 2009; Steward et al., 1998; Waltereit et al., 2001) and findings that *arc* overexpression occludes whereas *arc* deletion interferes with LTD induction (Plath et al., 2006; Rial Verde et al., 2006), we expected that *arc* mRNA expression would be increased after LTP-as well as LTD-inducing stimulation. To avoid that any differences in expression profiles between LTP and LTD could be attributed to procedural differences, such as amount of stimulation, we used the same number of stimulation pulses, stimulation intensity, and total duration of stimulation for the induction of LTP and LTD, but varied only the temporal pattern of the stimulation pulses between LTP and LTD experiments. Our results show that the regulation of *arc* expression differs markedly between LTP and LTD in area CA1 *in vivo* despite the

similar requirement of this gene's product for both forms of plasticity. Whereas there is a persistent up-regulation of *arc* during LTP, *arc* exhibits a biphasic regulation pattern during LTD.

The rapid induction of *arc* by a wide variety of cellular activations, and the transport to and local translation of *arc* mRNA near activated synapses (Lyford et al., 1995; Park et al., 2008; Steward et al., 1998; Steward and Worley, 2001a; Steward and Worley, 2001b; Wallace et al., 1998; Waung et al., 2008) have attracted much interest in the fields of molecular, synaptic, and behavioral neuroscience. *Arc* is a single copy gene that is highly conserved among vertebrates (Bramham et al., 2008; Miyashita et al., 2008), which suggests that *arc* serves a critical function. *Arc* is expressed in principal neurons in the brain (Vazdarjanova et al., 2006), and rapid increases in *arc* mRNA level have been observed after spatial exploration (Chawla et al., 2005; Guzowski et al., 1999; Miyashita et al., 2009; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006), implicit and explicit memory tasks (Guzowski et al., 2000; McIntyre et al., 2005; Montag-Sallaz and Montag, 2003; Plath et al., 2006; Soule et al., 2008), LTP-inducing stimulation (Guzowski et al., 2000; Link et al., 1995; Lyford et al., 1995; Waltereit et al., 2001), seizures (Link et al., 1995; Lyford et al., 1995), and stress (Ons et al., 2004). Our findings of a *reduction* in *arc* mRNA level after LTD-inducing stimulation demonstrate that not all types of cellular activation lead to *arc* induction and, hence, that *arc* mRNA is not a general marker of cellular activity but regulated in differential fashion.

The basis of the decrease in *arc* mRNA level after LTD induction remains to be determined. One possibility is that the transcription of *arc* is reduced immediately after PPS. We showed previously that PPS-induced LTD in area CA1 *in vivo* is associated with an increase in the activity of the serine/threonine protein phosphatases PP1 and PP2A (Thiels et al., 1998). Both PP1 and PP2A can affect the phosphorylation state of transcription factors, such as CREB (Genoux et al., 2002; Hagiwara et al., 1992), including after PPS in area CA1 (Mauna et al., submitted), and they thereby reduce the DNA binding ability of these transcriptional regulators. The promoter sequence of *arc* contains binding consensus sequences for several transcription factors, including CREB, MEF2, SRF, and Elk-1 (Flavell et al., 2006; Kawashima et al., 2009; Pintchovski et al., 2009; Waltereit et al., 2001). In light of findings that CREB phosphorylation is decreased below basal level during LTD (Thiels et al., 2002; Mauna et al., submitted), it is tempting to speculate that the initial decrease in *arc* mRNA level observed here is related to the LTD-associated decrease in CREB phosphorylation and, hence, that our results reflect a reduction in transcription. This scenario is consistent with previous work attributing to CREB a possible role in the regulation of *arc* expression (Kawashima et al., 2009; Waltereit et al., 2001). A prominent regulatory role by CREB does not preclude a contribution by other transcription factors; however, we consider a significant role by either SRF/Elk-1 or MEF2 in the LTD-associated decrease of *arc* mRNA level less likely because (1) we previously found Elk-1 phosphorylation to be increased immediately after the induction of LTD in area CA1 *in vivo* (Thiels et al., 2002), and (2) calcineurin, a protein phosphatase that is activated during LTD (Mulkey et al., 1994), was shown to promote, rather than attenuate, MEF2-mediated *arc* expression (Flavell et al., 2006).

An alternative explanation for the decrease in *arc* mRNA level immediately after LTD-inducing stimulation is that *arc* mRNA undergoes rapid degradation in response to the synaptic input pattern. Recently, *arc* mRNA was shown to be degraded by the nonsense-mediated mRNA decay (NMD) pathway (Giorgi et al., 2007). The NMD pathway targets newly transcribed mRNAs to degradation and thereby limits the total translation time and amount of these mRNAs (Ishigaki et al., 2001; Maquat, 2004). A key RNA-binding protein required for NMD is eIF4AIII (Palacios et al., 2004), and *arc* was shown to be co-localized

with eIF4AIII (Giorgi et al., 2007). When NMD was inhibited in neurons by knockdown of eIF4AIII, the level of both *arc* mRNA and Arc protein were found to be elevated (Giorgi et al., 2007). Thus, it is possible that PPS triggers increased targeting of *arc* mRNA by the NMD pathway and consequently initiates degradation of *arc* mRNA that has rapidly been translated immediately after LTD-inducing stimulation. Future studies focused on targeting of *arc* mRNA by the NMD pathway and on determining pre-*arc* mRNA levels may help resolve the question of whether the observed decrease in *arc* mRNA level stems from a decrease in *de novo* transcription or an increase in rapid mRNA degradation.

The increase in *arc* mRNA level about 1 hr after LTD induction revealed to be the result of *de novo* transcription of *arc.* Interference with *de novo* transcription, in turn, prevented the prolonged maintenance of LTD. This effect of transcription inhibition on LTD in area CA1 *in vivo* is in agreement with a previous report of transcription-dependent NMDA receptor-mediated LTD in area CA1 *in vitro* (Kauderer and Kandel, 2000; but see Manahan-Vaughan et al., 2000). It remains to be determined whether the increase in *arc* transcription is critical for LTD maintenance. This intriguing possibility is suggested by findings of impaired LTD in area CA1 of *arc* knockout mice (Plath et al., 2006).

The increase in *arc* mRNA level during LTD did not emerge until approximately 1 hr after PPS. It is possible that upregulation of *arc* transcription is triggered by the initial synaptic input, but that its manifestation at the mRNA level is masked by the mRNA degradation pathway that rapidly degrades translated *arc* mRNA (Giorgi et al., 2007). Alternatively, the late increase in *arc* mRNA level may be caused by a latent transcriptional mechanism that operates in parallel with the mechanisms that underlie the initial decrease in *arc* mRNA level. Future studies assessing pre-*arc* mRNA levels at various time points after LTD induction may inform whether a prolonged upregulation of *arc* transcription is masked by rapid mRNA degradation or whether *de novo* transcription is increased only for a short period of time approximately 1 hr after PPS. Regardless of which specific mechanisms underlie the respective changes in *arc* mRNA level during LTD, our findings of a distinct bidirectional pattern of change highlight the dynamic nature of *arc* mRNA regulation during LTD. Furthermore, the findings illustrate that the time point at which mRNA levels are assessed affects greatly the direction of change that is observed. Accordingly, models of *arc* regulation and its role in synaptic plasticity based on singular temporal measurements may be incomplete.

Similar to observations by others using a range of cellular activation paradigms (Guzowski et al., 2000; Guzowski et al., 1999; Link et al., 1995; Lyford et al., 1995; Steward and Worley, 2001a; Steward and Worley, 2001b), we found that our LTP-inducing stimulation protocol resulted in a persistent increase in *arc* mRNA level that lasted at least 2 hr. Our findings that the initial increase in *arc* mRNA level is abolished when transcription was blocked indicates that the change in mRNA level was due to an increase in *arc* transcription. The prolonged increase in *arc* mRNA level might be considered surprising given that *arc* mRNA has a half-life of ~47 min (Vazdarjanova et al., 2006). One possible mechanism underlying the prolonged increase in mRNA level may be extended activation of *arc* transcription. In addition to CREB, which is phosphorylated rapidly after LTP-inducing stimulation (Impey et al., 1998; Kasahara et al., 2001; Racaniello et al., 2009; Schulz et al., 1999), other transcriptional regulators, including MEF2, SRF/Elk-1, Zeste-like response element binding proteins, or Zif268, may mediate *arc* transcription and may do so at later time points (Flavell et al., 2006; Kawashima et al., 2009; Li et al., 2005; Pintchovski et al., 2009). Thus, the prolonged elevation in *arc* mRNA may result from enhanced transcription via a number of different transcription factors operating in sequence. Alternatively, the prolonged increase in *arc* mRNA may stem from an enhancement of *arc* mRNA stability after the initial increase in *arc* transcription. In fact, control of mRNA stability has been

implicated to play a critical role in neuronal function and synaptic plasticity (Deschenes-Furry et al., 2006; Perrone-Bizzozero and Bolognani, 2002). Thus, it would be interesting to determine in future work whether LTP-inducing stimulation triggers a reduction in *arc* mRNA degradation, thereby enabling an increase in *arc* mRNA availability through an epigenetic mechanism.

In conclusion, our observations reveal that LTP and LTD are associated with different profiles of *arc* mRNA changes that may result from different combinations of alterations in *arc* transcription and *arc* mRNA stability within as well as between LTP and LTD. Taken together with our findings of *zif268* mRNA levels during these two forms of synaptic plasticity, it appears that the regulatory mechanisms underlying *arc* expression differ not only between and within LTP and LTD but also from those of other IEGs implicated in synaptic plasticity. We believe our study presents a useful starting point for future dissection of the varied mechanisms that regulate *arc* expression during bidirectional plasticity at glutamatergic synapses in hippocampal area CA1.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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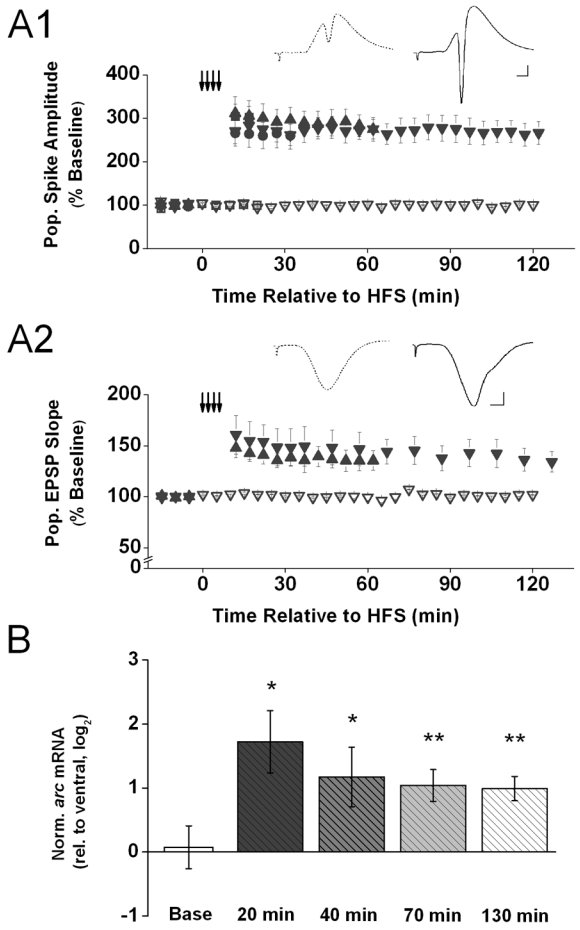


**Figure 1. Rapid and persistent increase of *arc* mRNA level after induction of LTP in area CA1 *in vivo***

**A1)** Means ± s.e.m.s of the amplitude of the population spike, expressed as a percent of baseline, evoked by commissural stimulation before and after 4 trains of high-frequency stimulation (HFS; small down-ward arrows) recorded from animals decapitated either 20 min (filled squares; n = 7), 40 min (filled circles; n = 7), 70 min (filled upward triangles; n = 6), or 130 min (filled down-ward triangles; n = 5) after the onset of HFS, or after either 20 min (open squares; n = 2) or 120 min (open down-ward triangles; n = 3) of only test pulse stimulation after the initial baseline stimulation period. Inserts above show representative waveforms (average of 10 recordings) recorded from an animal 5 min before (stippled line) and 127 min after HFS (solid line). Scale: 2 mV, 2 msec. **A2)** Similar data as shown above for the initial slope of the population EPSP evoked by commissural stimulation before and after HFS. (n = 3 for baseline, n = 4 for 70 min, n = 5 for 130 min). Scale for the insert is identical. **B)** Means ± s.e.m.s of *arc* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples harvested after either baseline stimulation (Base) or either 20 min, 40 min, 70 min, or 130 min after HFS. *Arc* expression levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ -transformed. Fold differences (FD) can be calculated from $\log_2$(RQ) by the formula FD= $2^{\log2(RQ)}$. (n = 8 for baseline, n = 7 for 20 min, n = 7 for 40 min, n = 10 for 70 min, n = 10 for 130 min). Determined Ct values for each of these groups are shown in Table 1 in the Supplementary Information. Two-tailed *t*-tests indicate a significant increase in *arc* mRNA above control level at all time points after HFS (* $p < 0.05$, ** $p < 0.01$).

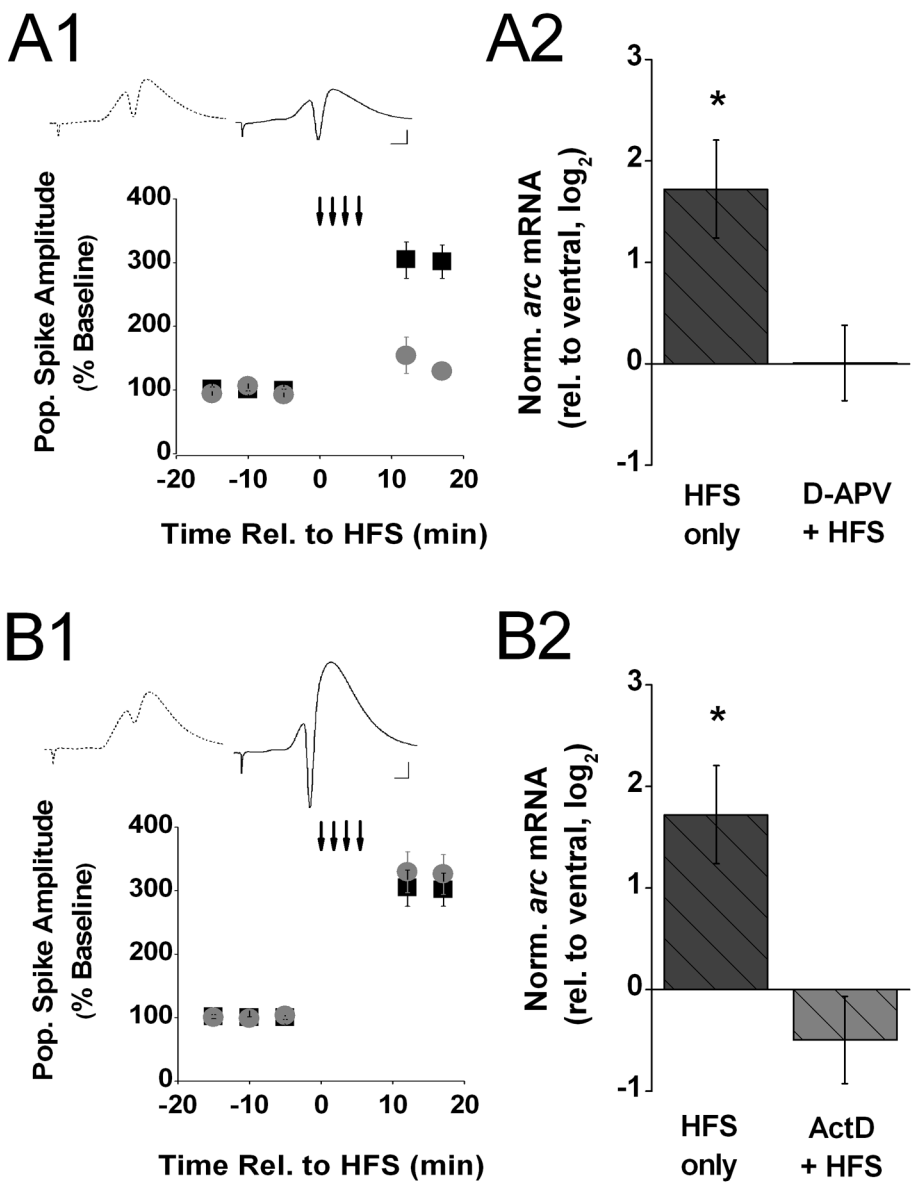


**Figure 2. Increase of arc mRNA level after HFS fails to occur when either NMDA receptors are blocked or new RNA synthesis is inhibited**

**A1)** Means ± s.e.m.s of the amplitude of the population spike, expressed as a percent of baseline, evoked by commissural stimulation before and after 4 trains of high-frequency stimulation (HFS; small down-ward arrows) recorded from animals decapitated 20 min after HFS delivered in either the presence of D-APV (gray circles, n=6) or the absence of drug (black squares, n=7). The data from the latter group were shown in Fig. 1. A1 and are included here for purposes of comparison. Insert above shows representative waveforms (average of 10 recordings) recorded 5 min before (stippled line) and 17 min after the onset of HFS (solid line) from an animal that received HFS in the presence of D-APV. Scale: 2 mV, 2 ms. **A2)** Means ± s.e.m.s of *arc* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples harvested 20 min after HFS delivered in either the presence of D-APV (D-APV + HFS, gray bar) or the absence of drug (HFS only, black bar). The data from the latter group were shown in Fig. 1. B and are

included here for purposes of comparison. *Arc* expression levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ -transformed. Fold differences (FD) can be calculated from $\log_2$(RQ) by the formula FD= $2^{\log2(RQ)}$. Determined Ct values for the two groups are shown in Table 1 in the Supplementary Information. Two-tailed *t*-tests indicate that the significant increase in *arc* mRNA level 20 min after HFS in the absence of drug was abolished in the presence of D-APV. **B1)** Means ± s.e.m.s of the amplitude of the population spike, expressed as a percent of baseline, evoked by commissural stimulation before and after 4 trains of HFS (small down-ward arrows) recorded from animals decapitated 20 min after HFS delivered in either the presence of ActD (gray circles, n=9) or the absence of drug (black squares, n=7). The data from the latter group were shown in Fig. 1. A1 and are included here for purposes of comparison. Insert above shows representative waveforms (average of 10 recordings) recorded 5 min before (stippled line) and 17 min after HFS (solid line) from an animal that received HFS in the presence of ActD. Scale: 2 mV, 2 ms. **B2)** Means ± s.e.m.s of *arc* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples harvested 20 min after HFS delivered in either the presence of ActD (ActD + HFS, gray bar) or the absence of drug (HFS only, black bar). The data from the latter group were shown in Fig. 1. B and are included here for purposes of comparison. *Arc* expression levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ -transformed. Fold differences (FD) can be calculated from $\log_2$(RQ) by the formula FD= $2^{\log2(RQ)}$. Determined Ct values for the two groups are shown in Table 1 in the Supplementary Information. Two-tailed *t*-tests indicate that the significant increase in *arc* mRNA level 20 min after HFS in the absence of drug was abolished in the presence of ActD.

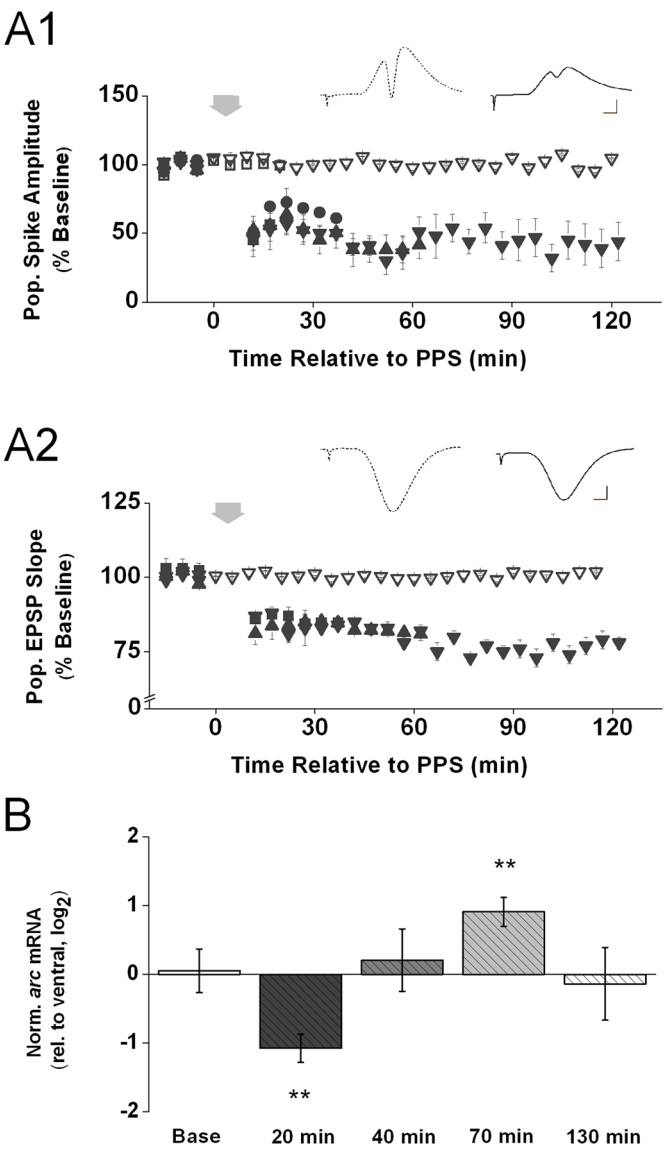


**Figure 3. Rapid decrease followed by transient increase of *arc* mRNA level after induction of LTD in area CA1 *in vivo***

**A1)** Means ± s.e.m.s of the amplitude of the population spike, expressed as a percent of baseline, evoked by commissural stimulation before and after paired-pulse stimulation (PPS; wide down-ward arrow) recorded from animals decapitated either 20 min (filled squares; n = 7), 40 min (filled circles; n = 2), 70 min (filled upward triangles; n = 5), or 130 min (filled down-ward triangles; n = 5) after the onset of PPS, or after either 20 min (open squares; n = 3) or 120 min (open down-ward triangles; n = 2) of only test pulse stimulation after the initial baseline stimulation period. Inserts above show representative waveforms (average of 10 recordings) recorded from an animal 5 min before (stippled line) and 127 min after PPS (solid line). Scale: 2 mV, 2 msec. **A2)** Similar data as shown above for the initial slope of the population EPSP evoked by commissural stimulation before and after PPS. (n = 3 for baseline, n = 5 for 40 min, n = 5 for 70 min, n = 5 for 130 min). Scale for the insert is identical. **B)** Means ± s.e.m.s of *arc* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples harvested after either baseline stimulation (Base) or either 20 min, 40 min, 70 min, or 130 min after PPS. *Arc* expression

levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ –transformed. Fold differences (FD) can be calculated from $\log_2$(RQ) by the formula FD= $2^{\log2(RQ)}$. (n = 8 for baseline, n = 7 for 20 min, n = 7 for 40 min, n = 10 for 70 min, n = 10 for 130 min). Determined Ct values for each of these groups are shown in Table 1 in the Supplementary Information. Two-tailed *t*-tests indicate a significant decrease in *arc* mRNA level 20 min after PPS followed by a significant increase in *arc* mRNA level 70 min after PPS (** $p < 0.01$).

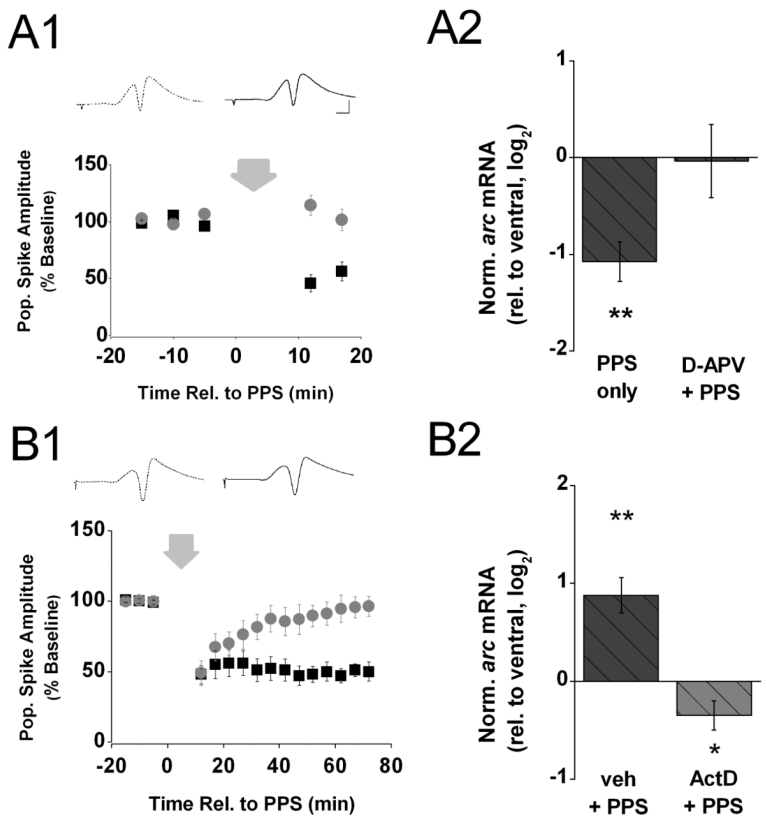


**Figure 4. Decrease of *arc* mRNA level after PPS fails to occur when NMDA receptors are blocked**

**A1)** Means ± s.e.m.s of the amplitude of the population spike, expressed as a percent of baseline, evoked by commissural stimulation before and after PPS (wide down-ward arrow) recorded from animals decapitated 20 min after PPS delivered in either the presence of D-APV (gray circles, n=9) or the absence of drug (black squares, n=7). The data from the latter group were shown in Fig. 3. A1 and are included here for purposes of comparison. Inserts above show representative waveforms (average of 10 recordings) recorded 5 min before (stippled line) and 17 min after PPS (solid line) from an animal that received PPS in the presence of D-APV. Scale: 2 mV, 2 ms. **A2)** Means ± s.e.m.s of *arc* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples harvested 20 min after PPS delivered in either the presence of D-APV (D-APV + PPS, gray bar) or the absence of drug (PPS only, black bar). The data from the latter group were shown in Fig. 3. B and are included here for purposes of comparison. *Arc* expression levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ -transformed. Fold differences (FD) can be calculated from $\log_2$(RQ) by the formula FD= $2^{\log2(RQ)}$. Determined Ct values for the two groups are shown in Table 1 in the Supplementary Information. Two-tailed *t*-tests indicate that the significant decrease of *arc* mRNA level 20 min after PPS in the absence of drug is abolished in the presence of D-APV. **B1)** Means ± s.e.m.s of the amplitude of the population spike, expressed as a percent of baseline, evoked by commissural stimulation before and after PPS (wide down-ward arrow) recorded from animals decapitated 70 min after PPS delivered in the presence of either ActD (gray circles, n=6) or vehicle solution (black squares, n=6). Insert above shows representative waveforms (average of 10 recordings) recorded 5 min before (stippled line) and 70 min after PPS (solid line) from an animal that received PPS in the presence of ActD. Scale: 2 mV, 2 ms. **B2)** Means ± s.e.m.s of *arc* mRNA level in dorsal

area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples harvested 70 min after PPS delivered in the presence of either ActD (ActD + PPS, gray bar) or vehicle solution (veh + PPS, black bar). *Arc* expression levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ -transformed. Fold differences (FD) can be calculated from $\log_2(RQ)$ by the formula $FD= 2^{\log2(RQ)}$. Determined Ct values for the two groups are shown in Table 1 in the Supplementary Information. A two-tailed *t*-test indicates a significant increase in *arc* mRNA level 70 min after PPS in the presence of vehicle solution (** $p < 0.01$). This effect was abolished completely in the presence of ActD; in fact, a two-tailed *t*-test indicates a small but significant decrease in *arc* mRNA level 70 min after PPS in the presence of ActD (* $p < 0.05$).

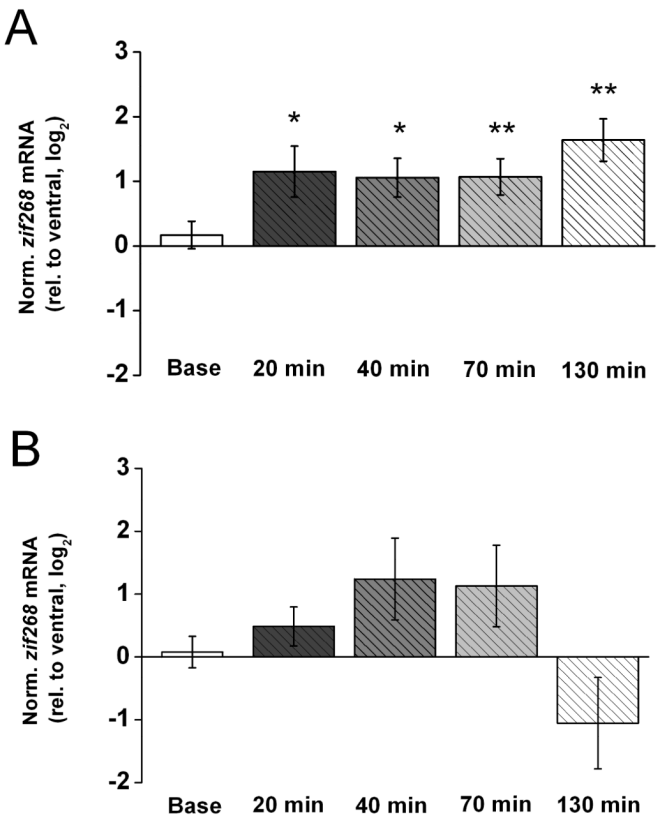


**Figure 5. *Zif268* mRNA levels also increase during LTP but do not change from control levels during LTD in area CA1 *in vivo.***

**A)** Means ± s.e.m.s of *zif268* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in the same tissue samples used in Fig. 1. B. (n = 8 for baseline (Base), n = 7 for 20 min, n = 7 for 40 min, n = 10 for 70 min, n = 10 for 130 min). *Zif268* expression levels were normalized by *gapdh* expression in the respective tissue samples and, to approximate a normal distribution of the data, $\log_2$ -transformed. Fold differences (FD) can be calculated from $\log_2$(RQ) by the formula FD= $2^{\log 2(RQ)}$. Two-tailed *t*-tests indicate a significant increase in *zif268* mRNA above control level at all time points after HFS (* $p < 0.05$, ** $p < 0.01$). **B)** Means ± s.e.m.s of *zif268* mRNA level in dorsal area CA1 (experimental), relative to ventral area CA1 (control), detected in tissue samples used in Fig. 3. B. (n = 8 for baseline (Base), n = 7 for 20 min, n = 7 for 40 min, n = 10 for 70 min, n = 10 for 130 min). *Zif268* expression levels were normalized and transformed as described above. Determined Ct values for each of these groups are shown in Table 2 in the Supplementary Information. Two-tailed *t*-tests indicate that the variations in *zif268* mRNA level after PPS do not differ significantly from control level for any of the time points.