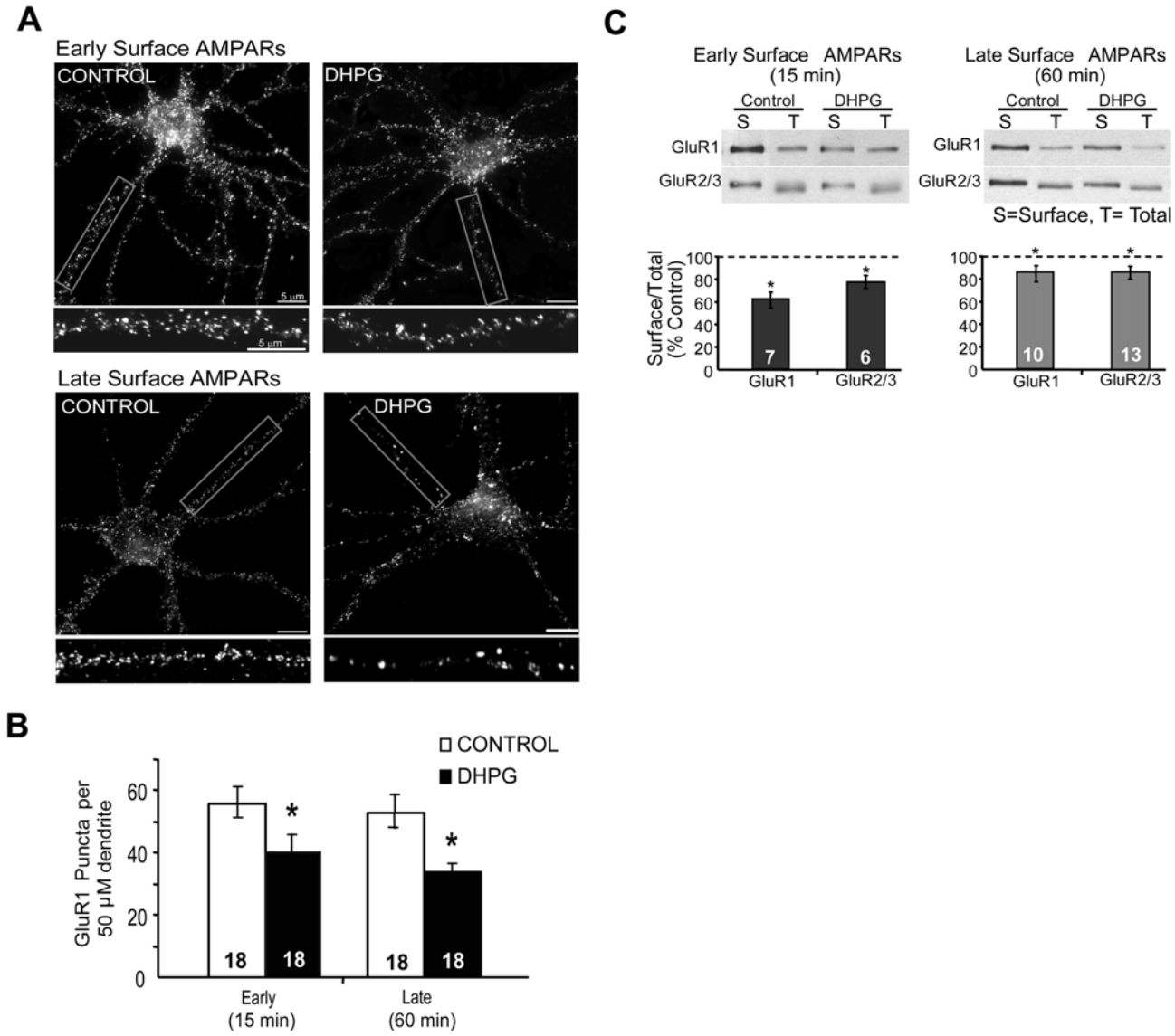
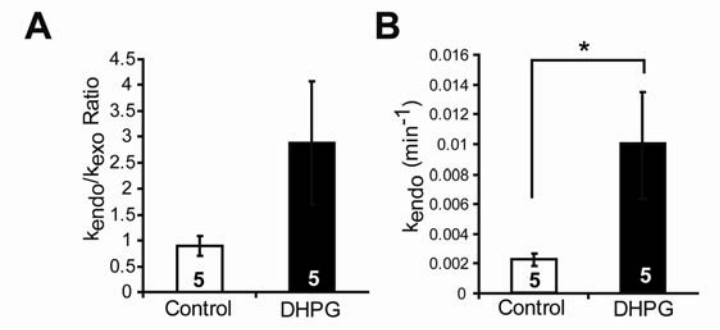
Supplemental Figures:



Supplemental Figure 1. Brief mGluR activation induces an early and persistent decrease in surface GluR1 and GluR2/3 expression. A, Representative images of surface GluR1 staining in low-density dissociated hippocampal neurons 15 min (early) or 1 hour (late) after treatment with media or DHPG (100 μM; 5 min). Scale bars = 10 μm. **B,** Quantification of surface GluR1 puncta number from group data. N = # cells per condition is on each bar. Data pooled from 3 cultures each. **C,** Representative blots of total (T) and biotinylated surface (S) GluR1 and GluR2/3 subunits from high-density dissociated hippocampal neuron cultures 15 min or one hour after DHPG treatment in media. **D,** Brief DHPG treatment decreases surface GluR1 and GluR2/3 subunits relative to untreated cultures, as measured by western blot following biotinylation of surface receptors. N = # cultures per condition is on each bar. * $p < 0.05$.



Supplemental Figure 2. DHPG results in a persistent increase in endocytosis rate constant (k_{endo}) A, The ratio of endocytosis/exocytosis rate constants (k_{endo} / k_{exo}) for GluR1 was calculated from steady-state measurements of surface and total GluR1 in high-density cultures one hour after brief DHPG treatment (shown in Figure 1A-D) based on the following simplified model. The AMPA receptor pools can be described as follows:

$$R_T = R_S + R_I \qquad \text{Eq. 1,}$$

where R_T is the total number of receptors, R_s is the number of receptors at the surface, and R_I is the number of internal receptors. In a simplified model, the entire pool of R_s is available for endocytosis, and the entire pool of R_I is available for exocytosis and R_T does not change. The latter is supported by empirical evidence that total GluR1 levels are not different between control and DHPG treated cultures (DHPG total GluR1 levels = 107 ± 7% of control; n = 17 cultures; p = 0.5)

At steady state, the rate of endocytosis equals the rate of exocytosis:

Eq. 2

$$k_{endo} \cdot R_S = k_{exo} \cdot R_I$$
$$k_{endo} \cdot R_S = k_{exo} \cdot (R_T - R_S)$$
$$\frac{k_{endo}}{k_{exo}} = \frac{R_T - R_S}{R_S}$$
$$\frac{k_{endo}}{k_{exo}} = \frac{R_T}{R_S} - 1$$
$$1 + \frac{k_{endo}}{k_{exo}} = \frac{R_T}{R_S}$$
$$\frac{R_S}{R_T} = \frac{1}{1 + \frac{k_{endo}}{k_{exo}}}$$

Based upon steady state concentrations of receptors at the surface and total receptors, a ratio of the rate constant of endocytosis (k_{endo}) to the rate constant of exocytosis (k_{exo}) can be determined from Eq. 3.

$$\frac{k_{endo}}{k_{exo}} = \frac{R_T}{R_S} - 1 \qquad \text{Eq. 3}$$

B, Empirically, the rate constant of endocytosis (k_{endo}) can also be determined from pulse chase experiments, where surface receptors are pulsed with a biotin label and chased with media to allow for endocytosis (from Figure 1A,B,D). After stripping of remaining surface biotinylated proteins, internalized receptors (or the change in surface receptors, ΔR_S) can be measured, as long as the change in time (Δt) is small relative to the percentage of internalized receptors (ΔR_S) to surface receptors (R_S).

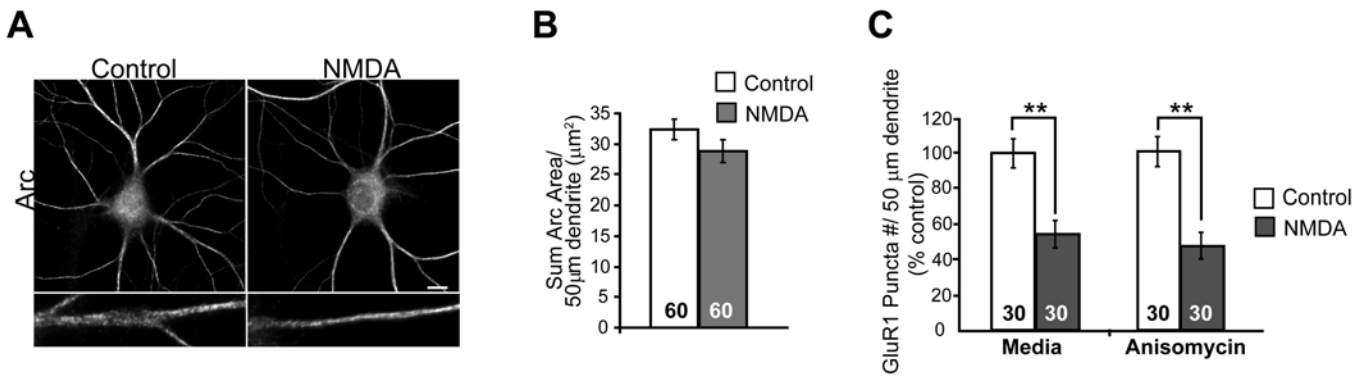
$$\Delta R_S = R_S \cdot k_{endo} \cdot \Delta t$$

$$k_{endo} = \frac{\Delta R_S}{R_S \cdot \Delta t} \qquad \text{Eq. 4}$$

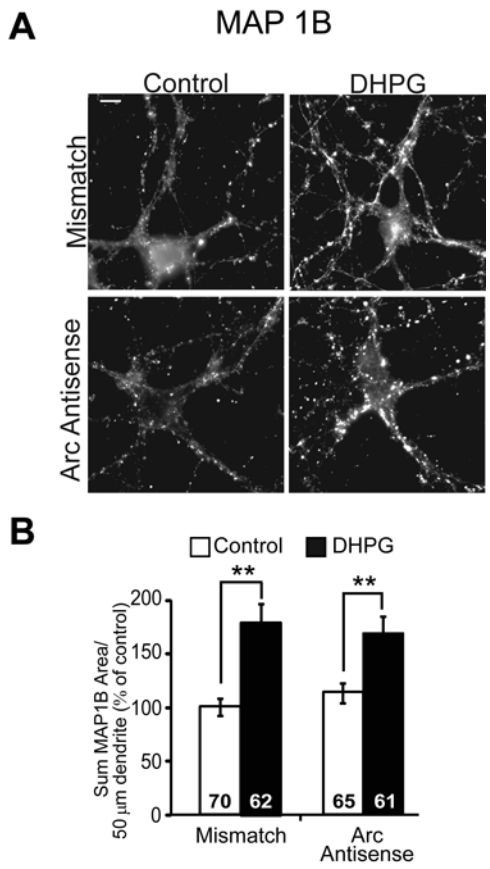
Plotted are calculated values for k_{endo} based upon measurements of internal accumulation of GluR1 in 5 min, one hour after brief DHPG treatment (from Figure 1A,B,D). N = # cultures per condition is on each bar. * p <0.05.

Using the rate constant of endocytosis (k_{endo}) obtained from Eq. 4, one can also determine the rate constant of exocytosis (k_{exo}) using the ratio obtained from Eq. 3.

$$k_{exo} = \frac{\Delta R_S}{(R_T - R_S) \cdot \Delta t} \qquad \text{Eq. 5}$$



Supplemental Figure 3. Chemical induction of NMDAR-LTD does not increase Arc protein levels or require protein synthesis. A, Representative Arc immunofluorescence in low-density dissociated hippocampal cultures exposed to control media or NMDA treatment (20 µM; 3 min). Neurons were fixed 10 min after treatment onset. Scale bar = 10 µm. **B,** Quantification of Arc immunofluorescence from one culture reveal that brief NMDA treatment does not alter Arc-positive staining 10 min after onset of treatment. N = # cells per condition is on each bar. **C,** NMDAR-induced decreases in surface GluR1 do not require new protein synthesis. Dissociated cultured neurons were treated with NMDA (20 µM; 3 min) in the presence or absence of the protein synthesis inhibitor anisomycin and surface GluR1 puncta number were quantified one hour after treatment. N = # cells on each bar. Data from 2 cultures. ** $p < 0.01$.



Supplemental Figure 4. Arc antisense oligonucleotide does not block mGluR-induced increases in dendritic MAP1b protein levels. A, Representative MAP1b immunofluorescence in low-density dissociated hippocampal cultures exposed to control media or DHPG treatment (100 µM; 5 min) in the presence of Mismatch control oligo or Arc antisense oligo. Neurons were fixed 10 min after treatment onset. Scale bar = 10 µm. **B,** Quantification of MAP1b immunofluorescence reveals that DHPG induces increases in MAP1b protein levels which are unaffected by oligo pretreatment. N = # cells on each bar. Data from 4 cultures. ** $p < 0.01$.

Treatment	Media		+ Anisomycin		Media	
	Control	**DHPG**	**Control**	**DHPG**	**Control**	**NMDA**
Surface/Total GluR1	6.51	4.05	5.87	6.17	2.25	1.62
SEM	0.69	0.53	1.18	1.17	0.47	0.55
N	5	5	5	5	6	6
p value		**0.02***		0.86		**0.01***
Internal/Total GluR1	0.06	0.17	0.07	0.06	0.26	0.18
SEM	0.01	0.03	0.01	0.01	0.06	0.04
N	5	5	5	5	6	6
P value		**0.04***		0.39		0.10

Supplemental Table 1: Raw ratios of surface/total GluR1 and internal/total GluR1 from biotinylation experiments presented in Figure 1B-D. n = number of cultures. P values from a paired t-test comparing ratio values in treated (DHPG or NMDA) cultures to their respective, sister control (untreated) cultures.