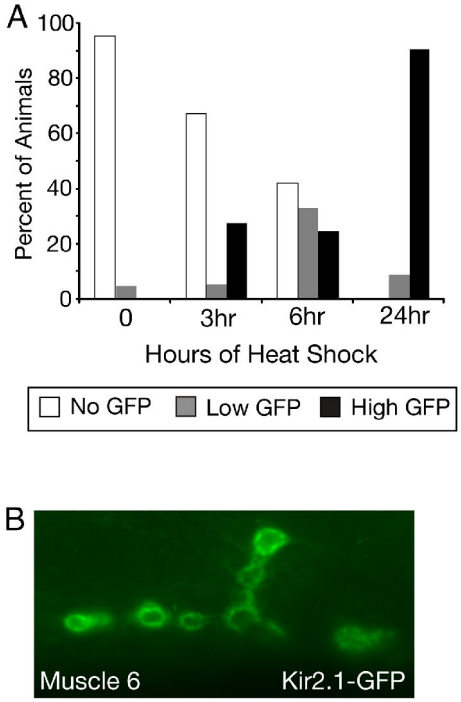
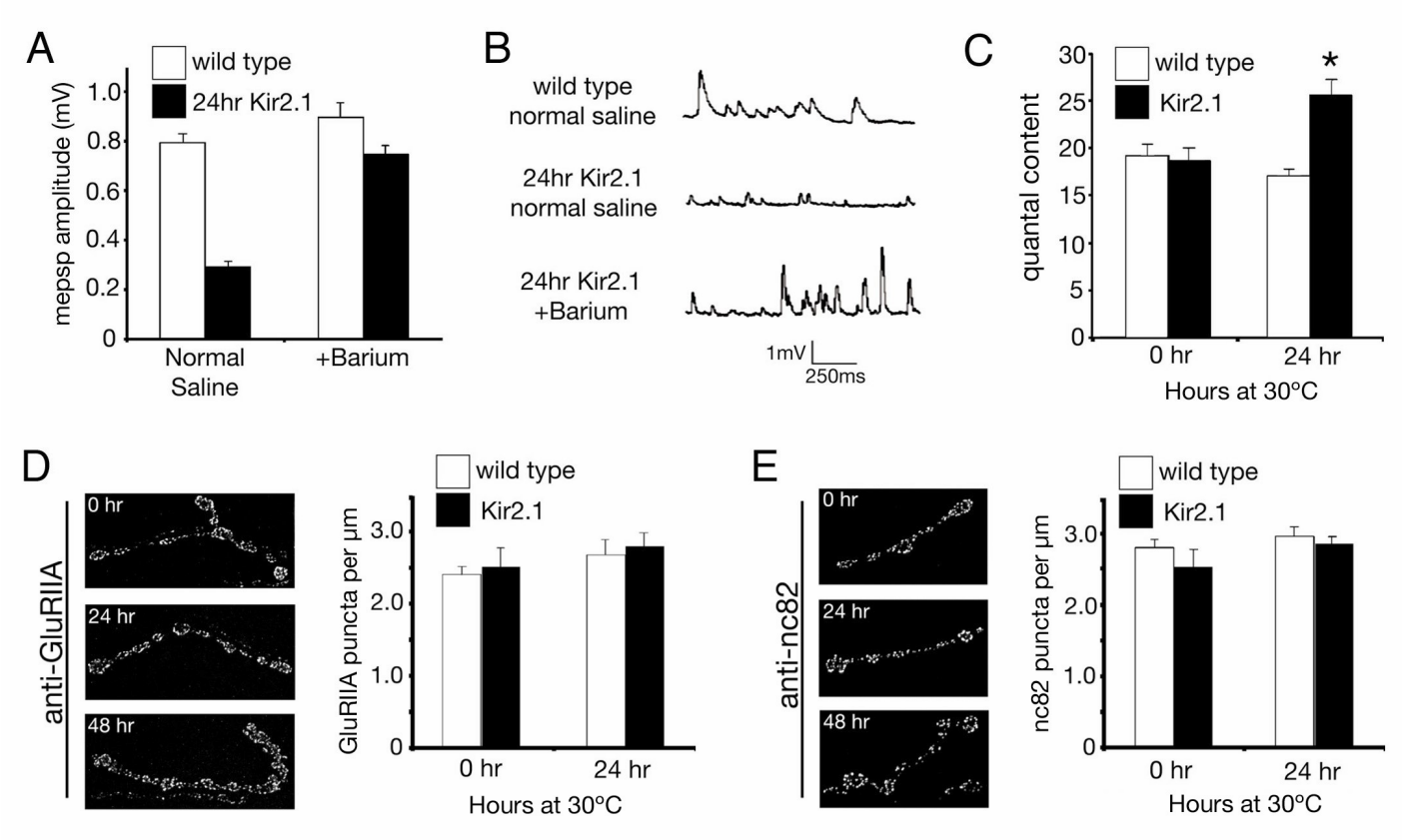
Supplemental Figure 1



Supplemental Figure 1. Temporal control of UAS-Kir2.1-GFP expression in Drosophila muscle. At 20ºC, *GAL80ts* is active and inhibits GAL4-dependent expression of *UAS*-driven transgenes (McGuire, 2003). A temperature shift to 30ºC is sufficient to inactivate *GAL80ts* and thereby enable GAL4-dependent transgene expression to proceed. We used *GAL80ts* in combination with a muscle-specific GAL4 driver (*MHC-GAL4*) and *UAS-Kir2.1-GFP* to temporally restrict *UAS-Kir2.1-GFP* expression to the final 24 hours of larval development. **A)** To determine optimal conditions and to define the time course of *UAS-Kir2.1-GFP* expression we assayed the emergence of Kir2.1-GFP fluorescence at muscles 6 and 7 of third instar larvae. In each experiment we raised larvae for a total of 8 days, varying the amount of time at 18ºC, 30ºC, and room temperature (RT). When larvae of the experimental genotype (*MHC-GAL4*, *UAS-Kir2.1-GFP*/*Tub-Gal80ts*) are raised at 18ºC for 8 days without heat shock, greater than 95% of animals completely lack GFP expression (0 hours heat shock). Thus, in the absence of heat shock, Kir2.1-GFP expression is prevented. When larvae are raised for 7 days at 18ºC and then shifted to 30ºC for 24 hours, greater than 90% of animals show high levels of Kir2.1-GFP expression at muscle 6 and 7 in every segment of the animal (24hr heat shock). Larvae shifted to 30ºC for less than 24 hours showed less robust expression of Kir2.1-GFP and expression was not consistently induced in all segments of each animal (3hr and 6hr heat shock). Thus, we can achieve robust expression of the Kir2.1 potassium channel in muscle within 24 hours. To understand the timeframe of altered muscle excitation in these experiments we assayed muscle physiology. We have documented the change in muscle excitation following 24 hour expression of *UAS-Kir2.1-GFP*. Muscle excitation is severely perturbed, as evidenced by a hyperpolarized resting potential (–84.4 ± 0.3 mV compared to –67.1 ± 1.0 mV for wild type and –66.5 ± 1.4 mV for the genetic control *UAS-Kir2.1-GFP, MCH-GAL4/TubGal80ts* raised at 18ºC throughout development; $p<0.01$), decreased muscle input resistance (wt = 9.4±0.9 MΩ, the genetic control = 6.5 ± 0.4 MΩ and the experimental condition *UAS-Kir2.1-GFP; MHC-GAL4* = 0.2±0.05 MΩ; $p<0.01$) and decreased quantal size (Supplemental Figures 2a, b).

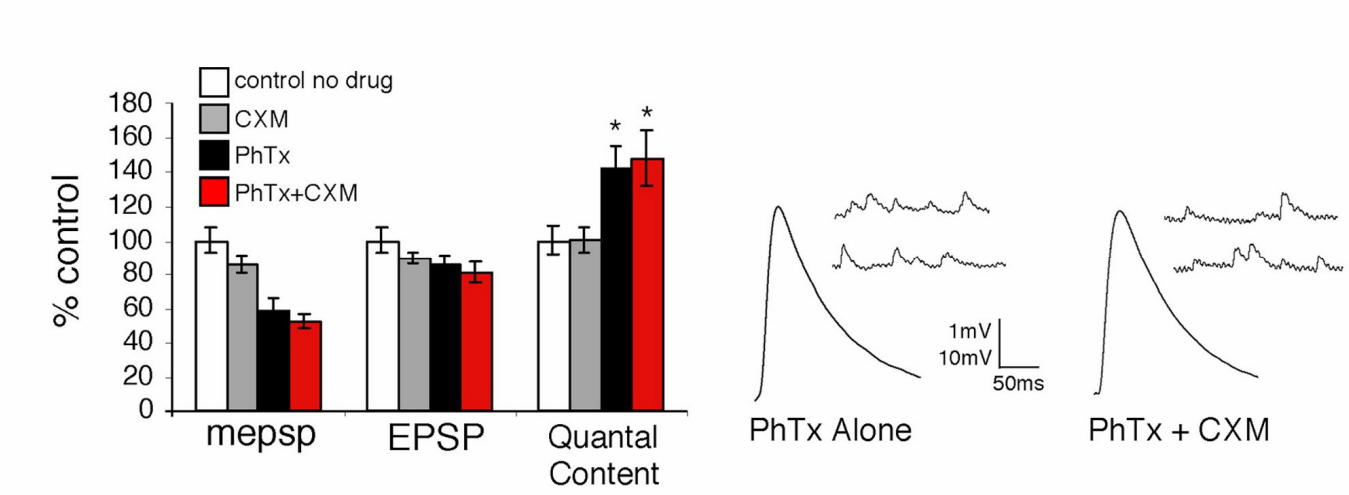
As shown previously (Paradis et al., 2001), altered excitability is rescued by including Ba^{2+} (0.3 mM) in the recording saline, thereby blocking the Kir2.1 channel (Supplemental Figure 2b, and data not shown). **B)** An image of *UAS-Kir2.1-GFP* expression following the 24hr heat shock protocol. Kir2.1-GFP localizes to the synaptic membranes, but this localization is not necessary for altered muscle excitation as shown previously (Paradis et al., 2001).

Supplemental Figure 2



Supplemental Figure 2. Rapid induction of synaptic homeostasis at late stages of development in a behaving animal. **A)** Quantification of mepsp amplitude in wild type (open bars; n=10) versus animals expressing *UAS-Kir2.1-GFP* for 24 hours (filled bars; n=12). Recording in the presence of 0.3mM barium blocks Kir2.1 and restores mepsp amplitudes toward wild-type average amplitudes. **B)** Sample traces for data presented in (A). **C)** A significant, homeostatic increase in quantal content is observed in animals expressing Kir2.1-GFP in muscle for 24 hours ($p<0.01$; Student's T-test; $n\geq10$). **D-E)** Quantification of active zone density at wild-type and homeostatically compensated NMJs (as in A and B) using antibodies to postsynaptic GluRIIA (D) or the presynaptic active zone marker nc82 (E). No significant changes were found in active zone density using either antibody, comparing experimental animals and controls ($p>0.3$).

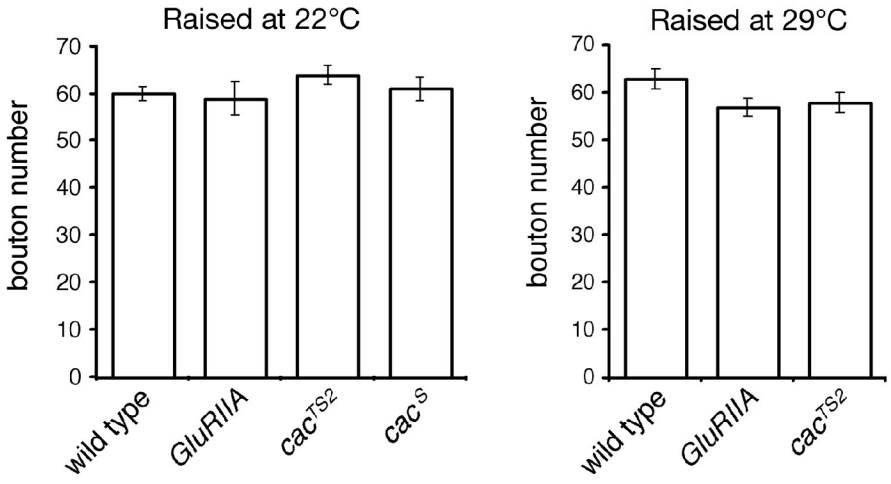
Supplemental Figure 3



Supplemental Figure 3. Synaptic homeostasis is independent of new protein synthesis.

It remains unknown whether homeostatic compensation at the NMJ requires new protein synthesis. Prior manipulation of genes involved in local protein synthesis have suggested a role for local protein synthesis in the regulation of synaptic efficacy at the Drosophila NMJ (Sigrist et al., 2000). However, since these genetic manipulations persist throughout development, it is difficult to dissociate a requirement for synapse development versus homeostatic signaling. Our demonstration that synaptic homeostasis at the NMJ can occur in 3-10 minutes argues against, but does not rule out, the involvement of new protein synthesis. Here we take advantage of our ability to apply pharmacological tools to a semi-intact preparation and provide evidence that the rapid induction of synaptic homeostasis is independent of new protein synthesis. **A)** We pre-incubated semi-intact preparations in 50μg/ml cyclohexamide (CXM) for 20 minutes, a concentration previously shown to block protein synthesis in Drosophila (Tully et al., 1994). Cyclohexamide pre-incubation has no effect on the expression of synaptic homeostasis compared to preparations pre-incubated in saline without cyclohexamide. Average values for mepsp, EPSP and quantal content are shown, normalized to wild-type controls in the absence of PhTox. Conditions include pre-incubation (20 min) in cyclohexamide (CXM), pre-incubation in cyclohexamide (20 min) followed by a 10 min incubation in PhTox (PhTx + CXM), saline pre-incubation (20 min) followed by a 10 min PhTox incubation (PhTox), and wild-type animals (20 min; control no drug). In conditions using CXM, the CXM was included in the recording saline. A homeostatic increase in quantal content is observed in the presence of CXM ($p<0.01$). Sample traces at right for indicated conditions 10 min following PhTox incubation. Similar results were obtained by pre-incubation with anisomycin (40μM) (data not shown).

Supplemental Figure 4



Supplemental Figure 4. The *cac*^{TS2} and *cac*^{S} mutations do not substantially alter morphological synaptic growth. Bouton number was quantified at the NMJ formed at muscles 6/7 in abdominal segment 3. Average bouton number is unchanged when animals are raised at room temperature (22ºC) (left). When animals are raised at 29ºC (right) there is a slight increase in the number of boutons in wild type controls that is not observed in any of the mutant backgrounds used in this study. However, the failure to increase bouton number at elevated temperature is not correlated with any defect the homeostatic regulation of synaptic function since bouton numbers are identical in *cac*^{TS2} and *GluRIIA* while the homeostatic control of synaptic function is perturbed only in the *cac*^{TS2} animals. Thus, under the conditions used in this study, any adverse effects on morphological synapse development are minimized, allowing us to specifically address the role of this channel in the homeostatic control of synaptic function (Xing et al., 2005; Rieckhof et al., 2003).