

1 **Title:** Synaptic homeostasis at the Drosophila neuromuscular junction is a reversible signaling
2 process that is sensitive to high temperature

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4 **Abbreviated Title:** Reversibility and limits of synaptic homeostasis

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23 **ABSTRACT**

24 Homeostasis is a vital mode of biological self-regulation. The hallmarks of homeostasis
25 for any biological system are a baseline set point of physiological activity, detection of unac-
26 ceptable deviations from the set point, and effective corrective measures to counteract devia-
27 tions. Homeostatic synaptic plasticity (HSP) is a form of neuroplasticity in which neurons and
28 circuits resist environmental perturbations in order to maintain appropriate levels of activity. One
29 assumption is that if an environmental perturbation triggers homeostatic corrective changes in
30 neuronal properties, those corrective measures should be reversed upon removal of the pertur-
31 bation. We test the reversibility and limits of HSP at a well-studied model synapse, the *Dro-*
32 *sophila melanogaster* neuromuscular junction (NMJ). At the *Drosophila* NMJ, impairment of glu-
33 tamate receptors causes a decrease in quantal size, which is offset by a corrective, homeostatic
34 increase in the number of vesicles released per evoked presynaptic stimulus, or quantal con-
35 tent. This process has been termed presynaptic homeostatic potentiation (PHP). Taking ad-
36 vantage of a GAL4/GAL80^{TS}/UAS expression system, we triggered PHP by expressing a domi-
37 nant-negative glutamate receptor subunit at the NMJ. We then reversed PHP by halting expres-
38 sion of the dominant-negative receptor. Our data show that PHP is fully reversible over a time
39 course of 48-72 hours after the dominant-negative glutamate receptor stops being genetically
40 expressed. Additionally, we found that the PHP response triggered by the dominant-negative
41 subunit was ablated at high temperatures. Our data show that the long-term maintenance of
42 PHP at the *Drosophila* NMJ is a reversible regulatory process that is sensitive to temperature.

43

44 **SIGNIFICANCE STATEMENT**

45 Biological homeostatic systems must upregulate or downregulate cellular parameters in
46 order to maintain appropriate set points of physiological activity. Homeostasis is a well-
47 documented mode of regulation in metazoan nervous systems. True homeostatic control should
48 be a reversible process – but due to technical difficulties of presenting and removing functional

49 challenges to living synapses, the reversibility of homeostatic forms of synapse regulation has
50 not been rigorously examined *in vivo* over extended periods of developmental time. Here we
51 formally demonstrate that homeostatic regulation of *Drosophila melanogaster* neuromuscular
52 synapse function is reversible and temperature-labile. This is significant because developing
53 methods to study how homeostatic regulatory systems are turned on and off could lead to fun-
54 damental new insights about control of synaptic output.

55

56 INTRODUCTION

57 Homeostasis is a strong form of biological regulation. It permits individual cells or entire
58 systems of cells to maintain core physiological properties that are compatible with life. In the
59 nervous system, decades of study have shown that while synapses and circuits are inherently
60 plastic, they also possess robust homeostatic regulatory systems in order to maintain physiolog-
61 ical stability. Homeostatic plasticity in the nervous system is a non-Hebbian strategy to counter-
62 act challenges to neuronal function that may threaten to disrupt essential neuronal and circuit
63 activities (Turrigiano, 2017). Depending upon the synaptic preparation examined and the envi-
64 ronmental challenge presented to the synapse, homeostatic responses may be executed via
65 compensatory adjustments to presynaptic neurotransmitter release (Cull-Candy et al., 1980;
66 Davis and Müller, 2015; Frank et al., 2006; Murthy et al., 2001; Petersen et al., 1997;
67 Thiagarajan et al., 2005), postsynaptic neurotransmitter receptor composition (O'Brien et al.,
68 1998; Rongo and Kaplan, 1999; Turrigiano, 2008; Turrigiano et al., 1998), neuronal excitability
69 (Bergquist et al., 2010; Marder and Bucher, 2007; Marder and Goaillard, 2006; Marder and
70 Prinz, 2002; Parrish et al., 2014) – or even developmentally, via changes in synaptic contact
71 formation and maintenance (Burrone et al., 2002; Davis and Goodman, 1998; Wefelmeyer et
72 al., 2016).

73 Bi-directionality has been documented in several homeostatic systems, perhaps most
74 prominently in the case of synaptic scaling of neurotransmitter receptors. For vertebrate neu-

75 ronal culture preparations – such as cortical neurons or spinal neurons – global silencing of
76 network firing can induce increases in excitatory properties, such as increased AMPA-type glu-
77 tamate receptor accumulation; by contrast, global enhancement of activity can induce the oppo-
78 site type of response (O'Brien et al., 1998; Turrigiano, 2008; Turrigiano et al., 1998; Wierenga et
79 al., 2005).

80 Bi-directionality is also a key feature underlying homeostatic alterations of neurotransmit-
81 ter release at peripheral synapses like the neuromuscular junction (NMJ). At the *Drosophila*
82 *melanogaster* and mammalian NMJs, impairing neurotransmitter receptor function
83 postsynaptically results in diminished sensitivity to single vesicles of transmitter.
84 Electrophysiologically, this manifests as decreased quantal size. NMJs respond to this chal-
85 lenge by enhancing neurotransmitter vesicle release (Cull-Candy et al., 1980; Davis et al., 1998;
86 Frank et al., 2009; Petersen et al., 1997; Plomp et al., 1995; Plomp et al., 1992). By contrast,
87 perturbations that enhance quantal size – for example, overexpression of the vesicular neuro-
88 transmitter transporter – can result in decreased quantal content (Daniels et al., 2004; Gaviño et
89 al., 2015).

90 Synapses and circuits possess myriad solutions to assume appropriate functional out-
91 puts in the face of perturbations (Marder and Goaillard, 2006; Marder and Prinz, 2002). There-
92 fore, a corollary to bi-directional regulation is that homeostatic forms of regulation should also be
93 reversible. There are experimental difficulties of presenting and removing a synaptic challenge
94 in the context of a living synapse, so homeostatic reversibility has not been rigorously studied in
95 an *in vivo* system or over extended periods of developmental time. However, understanding
96 how homeostatic regulatory systems are reversibly turned on and off could have profound impli-
97 cations for elucidating fundamental properties of circuit regulation.

98 Here we focus on the aforementioned *Drosophila melanogaster* NMJ as a living synapse
99 to test homeostatic reversibility. At the *Drosophila* NMJ, a canonical way to challenge synapse
100 function is through glutamate receptor impairment (Frank, 2014), either genetically (Petersen et

101 al., 1997) or pharmacologically (Frank et al., 2006). Impairments of muscle glutamate receptor
102 function decrease quantal size. Decreased quantal size spurs muscle-to-nerve signaling that
103 ultimately results in a homeostatic increase in presynaptic vesicle release – a process that has
104 been termed presynaptic homeostatic potentiation (PHP). The most widely used experimental
105 homeostatic challenges to *Drosophila* NMJ function are not easily reversed – including pharma-
106 cological inhibition with PhTox, due to an irreversible impairment of glutamate receptor function
107 (Frank et al., 2006).

108 For this study, we engineered a way to challenge NMJ function *in vivo* for significant pe-
109 riods of time, verify the effectiveness of the challenge at a defined time developmental time
110 point, remove the challenge, and then assess the homeostatic capacity of the NMJ at a later
111 developmental time point. To do this, we utilized the TARGET (temporal and regional gene ex-
112 pression targeting) GAL4/GAL80^{TS}/UAS expression system (McGuire et al., 2003). By using this
113 expression system to temporally control the expression of a dominant-negative GluRIIA receptor
114 subunit (DiAntonio et al., 1999), we found that homeostatic potentiation of neurotransmitter re-
115 lease is fully reversible. In the course of conducting our studies, we also uncovered a high tem-
116 perature limitation of homeostatic potentiation at the NMJ.

117

118 MATERIALS AND METHODS

119

120 *Drosophila* Stocks and Husbandry

121 Fruit fly stocks were either obtained from the Bloomington *Drosophila* Stock Center
122 (BDSC, Bloomington, Indiana) or from the labs that generated them. *w*¹¹¹⁸ was used as a wild-
123 type (WT) control (Hazelrigg et al., 1984). The *GluRIIA*^{SP16} deletion was used as a genetic loss-
124 of-function (Petersen et al., 1997). Transgenes included the UAS-driven dominant-negative glu-
125 tamate receptor subunit, *UAS-GluRIIA*^{M614R} (DiAntonio et al., 1999) and a ubiquitous
126 *Tubulin*_{Promoter}-*Gal80*^{TS} (*TubP-Gal80*^{TS}) (McGuire et al., 2003). Muscle-specific GAL4 drivers in-

127 cluded *MHC-Gal4* (Schuster et al., 1996a, b) and *BG57-Gal4* (also known as *C57-Gal4*) (Budnik
128 et al., 1996). For reversibility experiments, the full genotypes for the crosses were $w^{1118}; CyO-$
129 $GFP/UAS-GluRIIA^{M614R}; TM6b(Tb)/Tub_P-Gal80^{TS} \times w^{1118}; ; TM6b(Tb)/MHC-Gal4$ or $BG57-Gal4$.
130 Non-tubby, non-GFP larvae were selected for recording. In control recordings, we found no dis-
131 cernable differences between male and female third-instar electrophysiology, but for reversibility
132 experiments (Figs. 3-5), single sexes of larvae were chosen to eliminate sex as a possible con-
133 founding variable.

134 Fruit flies were raised on cornmeal, molasses, and yeast medium (see BDSC website for
135 standard recipe) in temperature-controlled conditions. For most experiments, animals were
136 reared at the temperatures noted (including temperature shifts) until they reached the wandering
137 third instar larval stage, at which point they were chosen for electrophysiological recording. For
138 experiments in Figs. 2 and 3, mated animals were placed at either 25°C or 29°C and allowed to
139 lay eggs for 6-8 hours. Stage-matched and size-matched early third instar larvae (approximately
140 48-54 hours after the egg laying period) were subjected to electrophysiological recording (Fig. 2)
141 or temperature swaps (Fig. 3), as indicated.

142

143 **Electrophysiology and Analysis**

144 For Figs. 1 and 3-6, wandering third instar larvae were used for electrophysiological re-
145 cordings. For Fig. 2, early third instar larvae were used. In both cases, sharp electrode electro-
146 physiological recordings were taken from muscle 6 of abdominal segments 2 and 3. Briefly, lar-
147 vae were dissected in a modified HL3 saline comprised of: NaCl (70 mM), KCl (5 mM), MgCl₂
148 (10 mM), NaHCO₃ (10 mM), sucrose (115 mM = 3.9%), trehalose (4.2 mM = 0.16%), HEPES
149 (5.0 mM = 0.12%), and CaCl₂ (0.5 mM). Electrophysiological data were collected using an
150 Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in bridge mode, digitized using a
151 Digidata 1440A data acquisition system (Molecular Devices), and recorded with pCLAMP 10
152 acquisition software (Molecular Devices). A Master-8 pulse stimulator (A.M.P. Instruments, Je-

153 rusalem, Israel) and an ISO-Flex isolation unit (A.M.P. Instruments) were utilized to deliver 1 ms
154 suprathreshold stimuli to the appropriate segmental nerve. The average spontaneous miniature
155 excitatory postsynaptic potential (mEPSP) amplitude per NMJ was quantified by hand, approxi-
156 mately 100-200 individual spontaneous release events per NMJ (MiniAnalysis, Synaptosoft, Fort
157 Lee, NJ). In the case that the mEPSP frequency was extremely low (usually for expression of
158 the dominant-negative glutamate receptor subunit), several minutes of spontaneous recording
159 were done and all events measured. Measurements from all NMJs of a given condition were
160 then averaged. For evoked neurotransmission, 30 excitatory postsynaptic potentials (EPSPs)
161 were averaged to find a value for each NMJ. These were then averaged to calculate a value for
162 each condition. Quantal content (QC) was calculated by the ratio of average EPSP and average
163 mEPSP amplitudes for each individual NMJ. An average quantal content was then calculated
164 for each condition.

165

166 **Statistical Analyses**

167 Statistical analyses were conducted using GraphPad Prism Software. Statistical signifi-
168 cance was assessed either by Student's T-Test when one experimental data set was being di-
169 rectly compared to a control data set, or one-way ANOVA with Tukey's post-hoc test when mul-
170 tiple data sets were being compared. To assess potential correlations between incubation tem-
171 perature recovery times and electrophysiological parameters (Fig. 4), Pearson correlation coef-
172 ficients were calculated (r) and reported on the graphs, and two-tailed statistical analyses per-
173 formed to check correlation significance.

174 Specific p value ranges are noted in the Fig. legends and shown in graphs as follows: * p
175 < 0.05 , ** $p < 0.01$, and *** $p < 0.001$. For some p values that potentially trend toward statistical
176 significance ($0.05 < p < 0.1$), specific values are given. Most values reported in Table 1 or plot-
177 ted on bar graphs are mean \pm SEM.

178

179 **RESULTS**

180

181 **Homeostatic Potentiation Utilizing a Dominant-Negative Glutamate Receptor Subunit**

182 A prior study described a transgene encoding a dominant-negative GluRIIA subunit,
183 *UAS-GluRIIA^{M614R}* (herein termed *UAS-GluRIIA^{M/R}* or “dominant-negative”) (DiAntonio et al.,
184 1999). The GluRIIA M614R amino-acid substitution resides in the ion conduction pore of the
185 GluRIIA subunit, and it cripples channel function (DiAntonio et al., 1999). Transgenic expression
186 of *UAS-GluRIIA^{M/R}* in muscles renders a strong homeostatic challenge (markedly diminished
187 quantal size) and an equally strong compensatory response (increase in quantal content). The
188 prior report demonstrated that evoked amplitudes remain normal as a result of this compensa-
189 tion (DiAntonio et al., 1999).

190 We acquired this dominant-negative transgenic line to test homeostatic reversibility.
191 First, we replicated the published experiments – this time raising fruit fly larvae at temperatures
192 compatible with the TARGET system that we planned to use to test reversibility (McGuire et al.,
193 2003). We tested 25°C (a common culturing temperature) and 29°C (a temperature at which the
194 GAL80^{TS} protein ceases to inhibit GAL4). We drove *UAS-GluRIIA^{M/R}* expression with *MHC-Gal4*,
195 which turns on in first-instar larval muscles (Schuster et al., 1996a, b). We recorded from NMJs
196 of control and dominant-negative wandering third instar larvae.

197 For animals reared at 25°C, wild-type (WT) electrophysiology was robust (Figs. 1A, C, D,
198 Table 1 – see Table 1 for all raw electrophysiological data). By contrast, muscle-specific *UAS-*
199 *GluRIIA^{M/R}* expression at 25°C caused a large diminishment of quantal size compared to WT,
200 evident from a small average miniature excitatory postsynaptic potential (mEPSP) amplitude
201 (Figs. 1A, C, Table 1). Despite this decrease in quantal size, there was no diminishment in the
202 average evoked excitatory postsynaptic potential (EPSP) amplitude for dominant-negative
203 NMJs (Figs. 1A, C, D, Table 1) because of an offsetting homeostatic increase in quantal content
204 (QC) (Figs. 1A, C, Table 1). In sum, recordings at 25°C agreed with the prior finding for the

205 dominant-negative transgene (DiAntonio et al., 1999): perfect presynaptic homeostatic potentia-
206 tion (PHP). We did note one previously unreported phenotype at 25°C: starkly diminished
207 quantal frequency (Fig. 1C). This diminished mEPSP frequency phenotype mirrored numerous
208 cases in which there was greatly diminished glutamate receptor subunit expression or function
209 (Brusich et al., 2015; Daniels et al., 2006; Featherstone et al., 2005; Kim et al., 2012; Kim et al.,
210 2015; Ramos et al., 2015).

211 For animals raised at 29°C, we garnered similar results as 25°C, noting a few differ-
212 ences. First, at 29°C, WT NMJs had slightly smaller mEPSP quantal amplitude and frequency
213 than 25°C WT NMJs (Figs. 1A-D, Table 1). This finding was consistent with a prior report meas-
214 uring NMJ physiology at elevated temperatures (Ueda and Wu, 2015). Nevertheless, WT
215 evoked amplitudes and QC at 29°C were robust, similar to the values garnered at 25°C (Figs.
216 1A-D, Table 1). *UAS-GluRIIA^{M/R}*-expressing NMJs from 29°C showed a profound decrease in
217 mEPSP size, a strong compensatory increase in QC, and markedly reduced quantal frequency
218 compared to WT controls (Fig. 1B). However, unlike at 25°C, homeostatic compensation was
219 not perfect at 29°C for dominant-negative NMJs. Even though QC was significantly increased in
220 dominant-negative NMJs compared to WT, it did not increase enough to bring average *MHC-*
221 *Gal4 >> UAS-GluRIIA^{M/R}* EPSP amplitudes at 29°C fully back to control levels (Figs. 1B, C, D).

222

223 **Early Third Instar Larvae Express Homeostatic Plasticity**

224 Our initial experiments showed that it is possible to observe compensatory increases in
225 quantal content for *UAS-GluRIIA^{M/R}*-expressing animals raised throughout life, at either 25°C or
226 29°C. We sought to test homeostatic reversibility using the TARGET system. For this study,
227 TARGET augments GAL4/UAS expression adding a ubiquitously expressed *Tubulin_P-Gal80^{TS}*
228 GAL4 inhibitor transgene (McGuire et al., 2003) to the *MHC-Gal4 >> UAS-GluRIIA^{M/R}* genetic
229 background. The temperature sensitivity of the GAL80^{TS} protein permits tight control over when
230 GAL4-responsive transgenes are expressed (McGuire et al., 2003). We predicted that the dom-

231 inant-negative transgene should be repressed by GAL80^{TS} at low, permissive temperatures; and
232 conversely, *UAS-GluRIIA^{M/R}* should be actively expressed when GAL80^{TS} is inactive (~29°C or
233 higher) (McGuire et al., 2003). In order to study the reversibility of PHP, animals could be reared
234 at high temperature and then swapped to a lower temperature at an appropriate developmental
235 time point.

236 We needed to identify a suitable developmental stage for temperature swaps. An ideal
237 swap point would be late enough to detect PHP, but early enough to allow recovery time after
238 cessation of the dominant-negative *UAS-GluRIIA^{M/R}* expression. We crossed *UAS-GluRIIA^{M/R}*;
239 *Tub_P-Gal80^{TS}* x *MHC-Gal4* stocks. Mated animals were transferred to 25°C or 29°C for egg lay-
240 ing and subsequent larval development. We selected early third instar progeny for electrophysi-
241 ological recording. At temperature ranges of 25-29°C, early third instar larvae emerge roughly
242 48-60 hours after an egg-laying period. We staged small animals unambiguously by examining
243 their posterior spiracles for an orange-colored tip.

244 For animals raised entirely at 25°C, early third instar NMJ mEPSP size was large – sig-
245 nificantly larger than one would observe for third instar larvae (Figs 2A, B, Table 1). This was
246 expected because for developing NMJs, small muscles have a significantly greater input re-
247 sistance and enhanced quantal size (Davis and Bezprozvanny, 2001; Lnenicka and Mellon,
248 1983a, b) (Table 1). Early third instar larval NMJs showed normal evoked amplitudes, consistent
249 with a stable level of evoked muscle excitation throughout development (Figs 2A, B, Table 1).

250 We predicted genotypically equivalent early third instars raised entirely at 29°C would
251 express the dominant-negative transgene. As expected, NMJs from these animals showed
252 sharply reduced mEPSP amplitude and frequency compared to their stage- and size-matched
253 counterparts raised and 25°C (Figs. 2A, B, Table 1). There was a robust increase in quantal
254 content at 29°C, resulting in EPSP amplitudes that were nearly normal, but not quite at the
255 same level as at 25°C. As with the earlier experiments, presynaptic homeostatic potentiation

256 (PHP) for early third instar NMJs raised exclusively at 29°C was present, but not perfect (Figs.
257 2A, B, Table 1).

258

259 **Imperfect Homeostatic Potentiation is Reversible**

260 At 29°C, PHP was not perfect, but QC increases versus controls were robust, making it
261 possible to test reversibility. We generated additional *MHC-Gal4 >> UAS-GluRIIA^{M/R}* larvae with
262 the *TubP-GAL80^{TS}* transgene. This time we chose 21°C as a permissive GAL80^{TS} shift tempera-
263 ture to examine because 21°C permitted multiple electrophysiological time point measurements
264 over a long recovery window (Fig. 3A).

265 Control (no PHP) animals raised entirely at 21°C took ~120 hours after the egg laying
266 period to reach the wandering third instar stage, while control (PHP) animals raised entirely at
267 29°C took ~96 hours to reach the same stage (Fig. 3A). A 1-day recovery condition was utilized
268 – exposing animals to the *UAS-GluRIIA^{M/R}* challenge for ~72 hours (29°C) and allowing them to
269 recover at 21°C for 1 day prior to recording. We also tested 2- and 3-day recovery conditions,
270 rearing larvae at 29°C for approximately ~49-50 hours and then swapping them to 21°C until
271 they reached wandering third instar stage. Due to the length of the egg lays and small variations
272 in developmental time, some animals reached wandering third instar stage after about 2 days at
273 21°C, while others took closer to 3 days (Fig. 3A).

274 Control *MHC-Gal4 >> UAS-GluRIIA^{M/R}* larvae with ubiquitously expressed *TubP-Gal80^{TS}*
275 and raised at 21°C showed physiology indistinguishable from WT control animals reared at
276 25°C. This was true for all electrophysiological parameters (Compare Figs. 1, 3, Table 1). By
277 contrast, genetically identical control animals raised at 29°C throughout life showed electrophys-
278 iological phenotypes similar to dominant-negative *MHC-Gal4 >> UAS-GluRIIA^{M/R}* animals raised
279 at 29°C (Compare Figs. 1, 3, Table 1). Compared to counterparts raised at 21°C, animals raised
280 at 29°C showed reduced mEPSP frequency, reduced mEPSP size, slightly below-normal EPSP
281 amplitudes, and increased QC, indicating robust PHP (Fig. 3, Table 1).

282 Recovery conditions showed physiological signatures that corresponded with how much
283 time was spent at 21°C. Compared to the 29°C condition, the 1-day 21°C recovery condition
284 showed no significant changes in physiological properties and no reversal of PHP (Figs 3B-D).
285 By contrast, the 2-day recovery condition showed intermediate phenotypes. Compared to con-
286 stant exposure to 21°C, the 2-day recovery condition still had diminished mEPSP amplitude and
287 frequency – but not nearly as diminished as constant exposure to 29°C (Figs. 3B-D). Interest-
288 ingly, the 2-day recovery EPSP amplitudes revealed restored levels of excitation, due to an in-
289 crease in QC (Figs. 3C, D). The 3-day recovery condition showed electrophysiology that was
290 not significantly different from the constant 21°C condition (Figs 3B-D), indicating a full reversal
291 of PHP.

292 We further analyzed the aggregate data from the reversibility experiment. We wished to
293 test for hallmarks of PHP and reversal related to recovery time. Prior studies of homeostatic
294 plasticity at the *Drosophila* NMJ have shown that by plotting hundreds individual recording val-
295 ues, quantal content inversely scales with quantal size across genotypes – and as a result,
296 evoked excitation levels remain stable (Davis and Müller, 2015; Frank et al., 2006; Gaviño et al.,
297 2015). For our temperature swap experiments – this time conducting a comparison within a sin-
298 gle genotype – this also proved to be the case (Fig. 4A).

299 Next, we plotted mEPSP and QC values versus the number of animals spent at the re-
300 covery temperature, with a specific recovery time value for each NMJ, time locked to the egg
301 laying period and the recording time after recovery/development at 21°C. Both plots showed
302 hallmarks of PHP reversal: mEPSP amplitudes significantly positively correlated with time at
303 21°C (Fig. 4B), and QC values significantly inversely correlated with time at 21°C (Fig. 4C).
304 Consistent with the observation that PHP was present – but not perfect – at 29°C (Figs. 1-3),
305 individual data points from NMJs of animals raised entirely at 29°C showed a wide variability of
306 QC values (Fig. 4C).

307

308 **Perfect Homeostatic Potentiation is Also Reversible**

309 Homeostatic potentiation was robust, yet imperfect, at the 29°C condition. We hoped to
310 test a condition that was both compatible with perfect PHP and the reversibility assay. We at-
311 tempted a new temperature swap, changing a few parameters. For one change, we lowered the
312 restrictive Gal80^{TS} temperature from 29°C to 28.5°C. Other studies using the TARGET system
313 in *Drosophila melanogaster* have reported that 28.5°C is somewhat effective at impairing
314 Gal80^{TS} function (Corrigan et al., 2014; Redhai et al., 2016; Staley and Irvine, 2010). Second,
315 there was a formal possibility that imperfect compensation at 29°C reflected a GAL4 driver-
316 specific phenomenon, rather than a temperature-specific phenomenon. Therefore, we replaced
317 the *MHC-Gal4* muscle driver with the *BG57-Gal4* muscle driver (Budnik et al., 1996). Finally, we
318 returned to 25°C as the permissive condition.

319 We generated new sets of larvae for this swap experiment – *BG57-Gal4* >> *UAS-*
320 *GluRIIA^{MR}* larvae with the *TubP-Gal80^{TS}* transgene. As expected, animals raised at 25°C
321 throughout life (GAL80^{TS} on) developed NMJs with electrophysiological properties similar to
322 other control conditions already reported (Figs. 5A, C, Table 1). Also, as expected, animals
323 raised at 28.5°C throughout life (GAL80^{TS} impaired) had significantly diminished NMJ mEPSP
324 amplitudes and mEPSP frequency (Figs. 5A, B, Table 1). NMJs from those 28.5°C animals also
325 showed completely normal NMJ EPSP amplitudes because of a perfect, offsetting homeostatic
326 increase in QC (Figs. 5A, C, Table 1). Of note, the 28.5°C NMJs had markedly diminished
327 mEPSP frequency, an indication of successful expression of the dominant-negative *GluRIIA^{MR}*
328 transgene (Fig. 1). Animals raised at 28.5°C until early third instar and then swapped to 25°C for
329 the final two days of larval development showed NMJ electrophysiology indistinguishable from
330 that of animals raised at 25°C throughout life, indicating a complete reversal of PHP (Fig. 5).

331

332 **Homeostatic potentiation induced by *GluRIIA^{MR}* is impaired at high temperatures**

333 *GluRIIA^{M/R}* transgene-induced homeostatic potentiation was robust, yet imperfect at the
334 29°C condition. We wondered if PHP might specifically be impaired at the NMJ when flies are
335 raised at high temperatures. To test this idea further, we drove the dominant-negative transgene
336 in the muscle, setting up crosses to generate both *MHC-Gal4 >> UAS-GluRIIA^{M/R}* and *BG57-
337 Gal4 >> UAS-GluRIIA^{M/R}* animals, as well as driver-specific controls. For the driver controls,
338 quantal size was somewhat diminished at 30°C (Figs. 6A-D, Table 1). This was consistent with
339 the idea that quantal size is generally diminished at very high temperatures (Ueda and Wu,
340 2015) – though it could also be the case that high levels of muscle-driven GAL4 protein at high
341 temperatures contributes to this phenotype. Evoked EPSP amplitudes were still robust for driver
342 controls (Figs. 6A-D, Table 1). For the dominant-negative NMJs, there was a significant reduc-
343 tion in mEPSP size – beyond what was measured for the driver controls. Moreover, evoked am-
344 plitudes were weak, diminished significantly versus driver controls because of no significant in-
345 crease in QC – in fact, there was a significant decrease in QC (Figs. 6A-D, Table 1). These data
346 indicated that signaling processes that maintain PHP in response to the dominant-negative
347 transgene may break down at high temperatures.

348 To extend this line of inquiry, we examined a second homeostatic challenge to NMJ
349 function. We raised WT and *GluRIIA^{SP16}* deletion flies (Petersen et al., 1997) at 30°C. WT NMJs
350 showed relatively normal physiology at 30°C (Figs., 6E, F, Table 1). NMJs from *GluRIIA^{SP16}* de-
351 letion animals raised at 30°C showed significant PHP, with reduced mEPSP amplitudes and ro-
352 bust increase in QC compared to WT (Figs. 6A, B, Table 1). We noted that EPSP amplitudes
353 were somewhat reduced compared to WT (Table 1). Collectively, our data show that normal
354 baseline neurotransmission is not disrupted at 30°C. Moreover, PHP is possible and robust (yet
355 imperfect) at 30°C for *GluRIIA^{SP16}* mutants, but it is abolished in the case of the dominant-
356 negative *UAS-GluRIIA^{M/R}* transgene.

357

358 **DISCUSSION**

359 We present evidence that presynaptic homeostatic potentiation (PHP) at the *Drosophila*
360 *melanogaster* neuromuscular synapse is a reversible process. In doing so, we confirm prior find-
361 ings showing that there is a tight inverse relationship between quantal amplitude and quantal
362 content at the NMJ (Fig. 4). We complement those findings by conducting temperature shift ex-
363 periments. We find that PHP is measurable at an early stage of larval development (Fig. 2) and
364 can be erased over a matter of days (Figs. 3, 5). Interestingly, PHP fails or falls short of perfect
365 compensation at high temperatures (Figs. 1, 2, 3, 6).

366 For the *Drosophila* NMJ, homeostatic potentiation is a robust and sensitive process. One
367 assumption supported by all available data is that the larval NMJ is capable of modulating its
368 vesicle release at any time point during development – in accordance with the presence or ab-
369 sence of a homeostatic challenge to synapse function. Rapid, acute induction of homeostatic
370 signaling has previously been demonstrated at the *Drosophila* NMJ by application of
371 Philanthotoxin-433 (PhTox) to impair the glutamate receptors (Frank et al., 2006), but the re-
372 versibility of this particular modulation (or any other modulation at the *Drosophila* NMJ) had not
373 been studied. More generally, it is not clear what happens in metazoan nervous systems when
374 harsh perturbations that induce homeostatic signaling are introduced for long periods of devel-
375 opmental time and then later removed.

376

377 **Why is reversibility slow after dominant-negative *GluRIIA*^{M/R} removal?**

378 There was a robust expression of presynaptic homeostatic potentiation (PHP) for NMJs
379 of *MHC-Gal4 >> UAS-GluRIIA*^{M/R} larvae with the *Tub ρ -Gal80^{TS}* transgene raised at 29°C for 48
380 hours post egg-laying (Fig. 2). Once the expression of the dominant-negative *UAS-GluRIIA*^{M/R}
381 transgene was halted, this expression of PHP was erased over a slow 48- to 72-hour period
382 (Fig. 3). 24 hours of halted dominant-negative expression provided no relief (Fig. 3).

383 If PHP is a readily reversible homeostatic process, why is there a days-long time lag in
384 order to reverse it? The answer is likely a constraint of the dominant-negative *GluRIIA*^{M/R} exper-

385 imental perturbation, rather than a reflection of the NMJ's capacity to respond quickly to the
386 changed environment. In a prior study, researchers expressed functional, tagged GluRIIA trans-
387 genic subunits at the NMJ and performed fluorescence recovery after photobleaching (FRAP)
388 experiments (Rasse et al., 2005). Those experiments demonstrated that receptor turnover rates
389 at the *Drosophila* NMJ are extremely slow: it appears that once postsynaptic densities (PSDs)
390 reach a critical size, GluRIIA subunits are stably incorporated (Rasse et al., 2005). For our
391 study, this likely means that the temperature downshift represented an opportunity for the NMJ
392 to incorporate endogenous wild-type GluRIIA into a significant number of new PSDs while it
393 continued to grow (Rasse et al., 2005; Schmid et al., 2008; Schmid et al., 2006). Given sufficient
394 growth, the endogenously expressed GluRIIA would gradually overcome the previously incorpo-
395 rated dominant-negative GluRIIA^{M/R} subunits. As a result, this gradually restored neurotransmis-
396 sion to normal levels over a time course of 24-48 hours (Fig. 3), during which time many of the
397 dominant-negative GluRIIA^{M/R} subunits that had previously been stably incorporated at the NMJ
398 likely remained at the synapse.

399

400 **Reversibility of rapid and sustained forms of homeostatic plasticity**

401 The majority of recent studies about synaptic homeostasis at the *Drosophila* NMJ have
402 emphasized that presynaptic adjustments to neurotransmitter release properties must occur
403 within minutes of drug-induced (PhTox) postsynaptic receptor inhibition in order to induce a rap-
404 id and offsetting response to PhTox challenge. Important presynaptic parameters uncovered
405 through these studies include regulation of presynaptic Ca²⁺ influx (Frank et al., 2006; Frank et
406 al., 2009; Müller and Davis, 2012; Wang et al., 2014; Wang et al., 2016a; Younger et al., 2013);
407 regulation of the size of the readily releasable pool (RRP) of presynaptic vesicles (Harris et al.,
408 2015; Müller et al., 2015; Müller et al., 2012; Wang et al., 2016a; Weyhersmüller et al., 2011);
409 control of SNARE-mediated fusion events (Dickman and Davis, 2009; Dickman et al., 2012;
410 Müller et al., 2011); control of neuronal excitability (Bergquist et al., 2010; Parrish et al., 2014;

411 Younger et al., 2013); and recently, ER calcium-sensing downstream of presynaptic calcium in-
412 flux (Genç et al., 2017). For almost all of the cases in which a mutation or an experimental con-
413 dition blocks the short-term induction of homeostatic signaling, the same perturbation has also
414 proven to block its long-term maintenance. Interestingly, the converse is not true. Additional
415 studies have amassed evidence that the long-term consolidation of homeostatic signaling at the
416 NMJ can be genetically uncoupled from its induction, and select molecules seem to be dedicat-
417 ed to a maintenance program that involves protein translation and signaling processes in both
418 the neuron and the muscle (Brusich et al., 2015; Frank et al., 2009; Kauwe et al., 2016; Marie et
419 al., 2010; Penney et al., 2012; Spring et al., 2016). These long-term processes seem to take six
420 hours or more to take full effect (Kauwe et al., 2016).

421 As more molecular detail about HSP is elucidated, it will be interesting to test if the rapid
422 induction and sustained consolidation of PHP can be reversed by similar or separate mecha-
423 nisms – and what the time courses of those reversal mechanisms are. At the mouse NMJ, re-
424 versibility was recently demonstrated pharmacologically. D-Tubocurarine was applied to at a
425 sub-blocking concentration in order to impair postsynaptic acetylcholine receptors. Within se-
426 conds of drug application, QC increased – and then within seconds of drug washout, it de-
427 creased again (Wang et al., 2016b). Follow-up experiments suggested that those rapid, dynam-
428 ic changes in PHP dynamics at the mouse NMJ were mediated by a calcium-dependent in-
429 crease in the size of the readily-releasable pool (RRP) of presynaptic vesicles (Wang et al.,
430 2016b). Since there seem to be several similarities between the mouse NMJ and the *Drosophila*
431 NMJ (Davis and Müller, 2015; Frank, 2014), it is possible that PHP at the insect NMJ can also
432 be rapidly reversed.

433 Finally, it is instructive to examine mammalian synaptic preparations to study how ho-
434 meostatic forms of synaptic plasticity are turned on and off. Groundbreaking work on cultured
435 excitatory vertebrate synapses showed that in response to activity deprivation (or promotion),
436 synapses employ scaling mechanisms by adding (or subtracting) AMPA-type glutamate recep-

437 tors in order to counteract the perturbation (O'Brien et al., 1998; Turrigiano et al., 1998). Bi-
438 directional scaling suggested that reversible mechanisms likely dictate homeostatic scaling pro-
439 cesses. Complementary studies testing scaling reversibility have borne out this prediction
440 (Rutherford et al., 1997; Swanwick et al., 2006; Wang et al., 2011). Additionally, evidence for
441 reversible forms of homeostatic scaling have also been found in rodent sensory systems, such
442 as auditory synapses after hearing deprivation (and restoration to reverse) (Whiting et al., 2009)
443 and in the visual cortex after light deprivation (and restoration to reverse) (Goel and Lee, 2007).
444 Collectively, the vertebrate and invertebrate studies support the notion that reversible fine-tuning
445 is an efficient strategy used to stabilize activities in metazoan nervous systems. One advantage
446 offered by the *Drosophila* system is a toolkit to uncover possible reversibility factors.

447

448 **Homeostatic signaling can crash at extreme temperatures**

449 Are there environmental limitations for homeostatic potentiation at the *Drosophila* NMJ?
450 Our data suggest that high temperatures represent a potential limitation on the system. It is not
451 clear what the molecular or anatomical basis of this limit is. We do know that it is not an issue of
452 NMJ excitation at high temperatures. This is because evoked neurotransmission for WT (or
453 driver control) NMJs remains remarkably robust over a range of temperatures, including 30°C
454 (Table 1). Nor does it seem to be an elimination of PHP in general because PHP was still pre-
455 sent in the case of *GluRIIA*^{SP16} animals raised at 30°C (Fig. 6, Table 1). Rather, the limitation
456 seems to be on homeostatic signaling that supports PHP at high temperatures in the face of the
457 dominant-negative transgene expression.

458 Temperature effects on neurophysiology are well documented. Recent work in crusta-
459 ceans demonstrates that robust and reliable circuits like the neurons driving the rhythmicity
460 stomatogastric nervous system can “crash” under extreme temperature challenges (Marder et
461 al., 2015; Rinberg et al., 2013; Tang et al., 2012). For the *Drosophila* NMJ, prior studies of larval
462 development documented a significant enhancement of synaptic arborization when larvae were

463 raised at high temperatures (Sigrist et al., 2003; Zhong and Wu, 2004). Additional studies have
464 shown that NMJ growth plasticity can be additionally affected by mutations that affect neuronal
465 excitability (Budnik et al., 1990; Lee and Wu, 2010; Zhong et al., 1992). Given the backdrop of
466 these data, it is not unreasonable to hypothesize that the tolerable limits of synaptic activity
467 challenge could be different at different temperatures.

468 For our experiments, 29-30°C represents a potential “crash” point for homeostatic poten-
469 tiation at the *Drosophila* NMJ. We must note, however, that our data suggest that the coping
470 capacity of the NMJ is dependent on genotype. WT NMJs cope at all temperatures. By contrast,
471 for dominant-negative *GluRIIA*-expressing NMJs, 29°C is a point at which PHP becomes imper-
472 fect (Figs. 1-2), and 30°C is a point at which it crashes (Fig. 6). For *GluRIIA*^{SP16} subunit deletion
473 NMJs, there is robust, but imperfect PHP at 30°C (Fig. 6) – not unlike the compensation seen
474 for the dominant-negatives at 29°C. Why do these differences persist? The answer could relate
475 to the well-documented temperature-induced alterations in NMJ growth – or alternatively, a lim-
476 ited availability of synaptic factors that are needed in order to cope with a double challenge of
477 high temperature and particular impairment glutamate receptor function. Future molecular and
478 physiological work will be needed to unravel those possibilities in the contexts of different genet-
479 ic backgrounds and culturing conditions.

480

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488

489

490 REFERENCES

- 491 Bergquist, S., Dickman, D.K., and Davis, G.W. (2010). A hierarchy of cell intrinsic and target-
492 derived homeostatic signaling. *Neuron* 66, 220-234.
- 493 Brusich, D.J., Spring, A.M., and Frank, C.A. (2015). A single-cross, RNA interference-based
494 genetic tool for examining the long-term maintenance of homeostatic plasticity. *Frontiers in*
495 *cellular neuroscience* 9, 107.
- 496 Budnik, V., Koh, Y.H., Guan, B., Hartmann, B., Hough, C., Woods, D., and Gorczyca, M. (1996).
497 Regulation of synapse structure and function by the *Drosophila* tumor suppressor gene *dlg*.
498 *Neuron* 17, 627-640.
- 499 Budnik, V., Zhong, Y., and Wu, C.F. (1990). Morphological plasticity of motor axons in
500 *Drosophila* mutants with altered excitability. *J Neurosci* 10, 3754-3768.
- 501 Burrone, J., O'Byrne, M., and Murthy, V.N. (2002). Multiple forms of synaptic plasticity triggered
502 by selective suppression of activity in individual neurons. *Nature* 420, 414-418.
- 503 Corrigan, L., Redhai, S., Leiblich, A., Fan, S.J., Perera, S.M., Patel, R., Gandy, C., Wainwright,
504 S.M., Morris, J.F., Hamdy, F., *et al.* (2014). BMP-regulated exosomes from *Drosophila* male
505 reproductive glands reprogram female behavior. *J Cell Biol* 206, 671-688.
- 506 Cull-Candy, S.G., Miledi, R., Trautmann, A., and Uchitel, O.D. (1980). On the release of
507 transmitter at normal, myasthenia gravis and myasthenic syndrome affected human end-plates.
508 *J Physiol* 299, 621-638.
- 509 Daniels, R.W., Collins, C.A., Chen, K., Gelfand, M.V., Featherstone, D.E., and DiAntonio, A.
510 (2006). A single vesicular glutamate transporter is sufficient to fill a synaptic vesicle. *Neuron* 49,
511 11-16.
- 512 Daniels, R.W., Collins, C.A., Gelfand, M.V., Dant, J., Brooks, E.S., Krantz, D.E., and DiAntonio,
513 A. (2004). Increased expression of the *Drosophila* vesicular glutamate transporter leads to
514 excess glutamate release and a compensatory decrease in quantal content. *J Neurosci* 24,
515 10466-10474.
- 516 Davis, G.W., and Bezprozvanny, I. (2001). Maintaining the stability of neural function: a
517 homeostatic hypothesis. *Annu Rev Physiol* 63, 847-869.
- 518 Davis, G.W., DiAntonio, A., Petersen, S.A., and Goodman, C.S. (1998). Postsynaptic PKA
519 controls quantal size and reveals a retrograde signal that regulates presynaptic transmitter
520 release in *Drosophila*. *Neuron* 20, 305-315.
- 521 Davis, G.W., and Goodman, C.S. (1998). Synapse-specific control of synaptic efficacy at the
522 terminals of a single neuron. *Nature* 392, 82-86.
- 523 Davis, G.W., and Müller, M. (2015). Homeostatic control of presynaptic neurotransmitter
524 release. *Annu Rev Physiol* 77, 251-270.

- 525 DiAntonio, A., Petersen, S.A., Heckmann, M., and Goodman, C.S. (1999). Glutamate receptor
526 expression regulates quantal size and quantal content at the *Drosophila* neuromuscular
527 junction. *J Neurosci* 19, 3023-3032.
- 528 Dickman, D.K., and Davis, G.W. (2009). The schizophrenia susceptibility gene dysbindin
529 controls synaptic homeostasis. *Science* 326, 1127-1130.
- 530 Dickman, D.K., Tong, A., and Davis, G.W. (2012). Snapin is critical for presynaptic homeostatic
531 plasticity. *J Neurosci* 32, 8716-8724.
- 532 Featherstone, D.E., Rushton, E., Rohrbough, J., Liebl, F., Karr, J., Sheng, Q., Rodesch, C.K.,
533 and Broadie, K. (2005). An essential *Drosophila* glutamate receptor subunit that functions in
534 both central neuropil and neuromuscular junction. *J Neurosci* 25, 3199-3208.
- 535 Frank, C.A. (2014). Homeostatic plasticity at the *Drosophila* neuromuscular junction.
536 *Neuropharmacology* 78, 63-74.
- 537 Frank, C.A., Kennedy, M.J., Goold, C.P., Marek, K.W., and Davis, G.W. (2006). Mechanisms
538 underlying the rapid induction and sustained expression of synaptic homeostasis. *Neuron* 52,
539 663-677.
- 540 Frank, C.A., Pielage, J., and Davis, G.W. (2009). A presynaptic homeostatic signaling system
541 composed of the Eph receptor, ephexin, Cdc42, and CaV2.1 calcium channels. *Neuron* 61, 556-
542 569.
- 543 Gaviño, M.A., Ford, K.J., Archila, S., and Davis, G.W. (2015). Homeostatic synaptic depression
544 is achieved through a regulated decrease in presynaptic calcium channel abundance. *eLife* 4.
- 545 Genç, Ö., Dickman, D.K., Ma, W., Tong, A., Fetter, R.D., and Davis, G.W. (2017). MCTP is an
546 ER-resident calcium sensor that stabilizes synaptic transmission and homeostatic plasticity.
547 *eLife* 6.
- 548 Goel, A., and Lee, H.K. (2007). Persistence of experience-induced homeostatic synaptic
549 plasticity through adulthood in superficial layers of mouse visual cortex. *J Neurosci* 27, 6692-
550 6700.
- 551 Harris, N., Braiser, D.J., Dickman, D.K., Fetter, R.D., Tong, A., and Davis, G.W. (2015). The
552 Innate Immune Receptor PGRP-LC Controls Presynaptic Homeostatic Plasticity. *Neuron* 88,
553 1157-1164.
- 554 Hazelrigg, T., Levis, R., and Rubin, G.M. (1984). Transformation of white locus DNA in
555 *drosophila*: dosage compensation, zeste interaction, and position effects. *Cell* 36, 469-481.
- 556 Kauwe, G., Tsurudome, K., Penney, J., Mori, M., Gray, L., Calderon, M.R., Elazouzzi, F.,
557 Chicoine, N., Sonenberg, N., and Haghghi, A.P. (2016). Acute Fasting Regulates Retrograde
558 Synaptic Enhancement through a 4E-BP-Dependent Mechanism. *Neuron* 92, 1204-1212.
- 559 Kim, Y.J., Bao, H., Bonanno, L., Zhang, B., and Serpe, M. (2012). *Drosophila* Neto is essential
560 for clustering glutamate receptors at the neuromuscular junction. *Genes Dev* 26, 974-987.

- 561 Kim, Y.J., Igiesuorobo, O., Ramos, C.I., Bao, H., Zhang, B., and Serpe, M. (2015). Prodomain
562 removal enables neto to stabilize glutamate receptors at the *Drosophila* neuromuscular junction.
563 *PLoS genetics* 11, e1004988.
- 564 Lee, J., and Wu, C.F. (2010). Orchestration of stepwise synaptic growth by K⁺ and Ca²⁺
565 channels in *Drosophila*. *J Neurosci* 30, 15821-15833.
- 566 Lnenicka, G.A., and Mellon, D., Jr. (1983a). Changes in electrical properties and quantal current
567 during growth of identified muscle fibres in the crayfish. *J Physiol* 345, 261-284.
- 568 Lnenicka, G.A., and Mellon, D., Jr. (1983b). Transmitter release during normal and altered
569 growth of identified muscle fibres in the crayfish. *J Physiol* 345, 285-296.
- 570 Marder, E., and Bucher, D. (2007). Understanding circuit dynamics using the stomatogastric
571 nervous system of lobsters and crabs. *Annu Rev Physiol* 69, 291-316.
- 572 Marder, E., and Goaillard, J.M. (2006). Variability, compensation and homeostasis in neuron
573 and network function. *Nat Rev Neurosci* 7, 563-574.
- 574 Marder, E., Haddad, S.A., Goeritz, M.L., Rosenbaum, P., and Kispersky, T. (2015). How can
575 motor systems retain performance over a wide temperature range? Lessons from the
576 crustacean stomatogastric nervous system. *J Comp Physiol A Neuroethol Sens Neural Behav*
577 *Physiol* 201, 851-856.
- 578 Marder, E., and Prinz, A.A. (2002). Modeling stability in neuron and network function: the role of
579 activity in homeostasis. *Bioessays* 24, 1145-1154.
- 580 Marie, B., Pym, E., Bergquist, S., and Davis, G.W. (2010). Synaptic homeostasis is consolidated
581 by the cell fate gene gooseberry, a *Drosophila* pax3/7 homolog. *J Neurosci* 30, 8071-8082.
- 582 McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., and Davis, R.L. (2003). Spatiotemporal
583 rescue of memory dysfunction in *Drosophila*. *Science* 302, 1765-1768.
- 584 Müller, M., and Davis, G.W. (2012). Transsynaptic control of presynaptic Ca²⁺(+) influx
585 achieves homeostatic potentiation of neurotransmitter release. *Curr Biol* 22, 1102-1108.
- 586 Müller, M., Genç, Ö., and Davis, G.W. (2015). RIM-Binding Protein Links Synaptic Homeostasis
587 to the Stabilization and Replenishment of High Release Probability Vesicles. *Neuron* 85, 1056-
588 1069.
- 589 Müller, M., Liu, K.S., Sigrist, S.J., and Davis, G.W. (2012). RIM Controls Homeostatic Plasticity
590 through Modulation of the Readily-Releasable Vesicle Pool. *J Neurosci* 32, 16574-16585.
- 591 Müller, M., Pym, E.C., Tong, A., and Davis, G.W. (2011). Rab3-GAP controls the progression of
592 synaptic homeostasis at a late stage of vesicle release. *Neuron* 69, 749-762.
- 593 Murthy, V.N., Schikorski, T., Stevens, C.F., and Zhu, Y. (2001). Inactivity produces increases in
594 neurotransmitter release and synapse size. *Neuron* 32, 673-682.

- 595 O'Brien, R.J., Kamboj, S., Ehlers, M.D., Rosen, K.R., Fischbach, G.D., and Huganir, R.L.
596 (1998). Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* 21,
597 1067-1078.
- 598 Parrish, J.Z., Kim, C.C., Tang, L., Bergquist, S., Wang, T., Derisi, J.L., Jan, L.Y., Jan, Y.N., and
599 Davis, G.W. (2014). Kruppel mediates the selective rebalancing of ion channel expression.
600 *Neuron* 82, 537-544.
- 601 Penney, J., Tsurudome, K., Liao, E.H., Elazzouzi, F., Livingstone, M., Gonzalez, M., Sonenberg,
602 N., and Haghghi, A.P. (2012). TOR is required for the retrograde regulation of synaptic
603 homeostasis at the *Drosophila* neuromuscular junction. *Neuron* 74, 166-178.
- 604 Petersen, S.A., Fetter, R.D., Noordermeer, J.N., Goodman, C.S., and DiAntonio, A. (1997).
605 Genetic analysis of glutamate receptors in *Drosophila* reveals a retrograde signal regulating
606 presynaptic transmitter release. *Neuron* 19, 1237-1248.
- 607 Plomp, J.J., Van Kempen, G.T., De Baets, M.B., Graus, Y.M., Kuks, J.B., and Molenaar, P.C.
608 (1995). Acetylcholine release in myasthenia gravis: regulation at single end-plate level. *Ann*
609 *Neurol* 37, 627-636.
- 610 Plomp, J.J., van Kempen, G.T., and Molenaar, P.C. (1992). Adaptation of quantal content to
611 decreased postsynaptic sensitivity at single endplates in alpha-bungarotoxin-treated rats. *J*
612 *Physiol* 458, 487-499.
- 613 Ramos, C.I., Igiesuorobo, O., Wang, Q., and Serpe, M. (2015). Neto-mediated intracellular
614 interactions shape postsynaptic composition at the *Drosophila* neuromuscular junction. *PLoS*
615 *genetics* 11, e1005191.
- 616 Rasse, T.M., Fouquet, W., Schmid, A., Kittel, R.J., Mertel, S., Sigrist, C.B., Schmidt, M.,
617 Guzman, A., Merino, C., Qin, G., *et al.* (2005). Glutamate receptor dynamics organizing
618 synapse formation in vivo. *Nat Neurosci* 8, 898-905.
- 619 Redhai, S., Hellberg, J.E., Wainwright, M., Perera, S.W., Castellanos, F., Kroeger, B., Gandy,
620 C., Leiblich, A., Corrigan, L., Hilton, T., *et al.* (2016). Regulation of Dense-Core Granule
621 Replenishment by Autocrine BMP Signalling in *Drosophila* Secondary Cells. *PLoS genetics* 12,
622 e1006366.
- 623 Rinberg, A., Taylor, A.L., and Marder, E. (2013). The effects of temperature on the stability of a
624 neuronal oscillator. *PLoS Comput Biol* 9, e1002857.
- 625 Rongo, C., and Kaplan, J.M. (1999). CaMKII regulates the density of central glutamatergic
626 synapses in vivo. *Nature* 402, 195-199.
- 627 Rutherford, L.C., DeWan, A., Lauer, H.M., and Turrigiano, G.G. (1997). Brain-derived
628 neurotrophic factor mediates the activity-dependent regulation of inhibition in neocortical
629 cultures. *J Neurosci* 17, 4527-4535.
- 630 Schmid, A., Hallermann, S., Kittel, R.J., Khorramshahi, O., Frolich, A.M., Quentin, C., Rasse,
631 T.M., Mertel, S., Heckmann, M., and Sigrist, S.J. (2008). Activity-dependent site-specific
632 changes of glutamate receptor composition in vivo. *Nat Neurosci* 11, 659-666.

- 633 Schmid, A., Qin, G., Wichmann, C., Kittel, R.J., Mertel, S., Fouquet, W., Schmidt, M.,
634 Heckmann, M., and Sigrist, S.J. (2006). Non-NMDA-type glutamate receptors are essential for
635 maturation but not for initial assembly of synapses at *Drosophila* neuromuscular junctions. *J*
636 *Neurosci* 26, 11267-11277.
- 637 Schuster, C.M., Davis, G.W., Fetter, R.D., and Goodman, C.S. (1996a). Genetic dissection of
638 structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic
639 stabilization and growth. *Neuron* 17, 641-654.
- 640 Schuster, C.M., Davis, G.W., Fetter, R.D., and Goodman, C.S. (1996b). Genetic dissection of
641 structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic
642 structural plasticity. *Neuron* 17, 655-667.
- 643 Sigrist, S.J., Reiff, D.F., Thiel, P.R., Steinert, J.R., and Schuster, C.M. (2003). Experience-
644 dependent strengthening of *Drosophila* neuromuscular junctions. *J Neurosci* 23, 6546-6556.
- 645 Spring, A.M., Brusich, D.J., and Frank, C.A. (2016). C-terminal Src Kinase Gates Homeostatic
646 Synaptic Plasticity and Regulates Fasciclin II Expression at the *Drosophila* Neuromuscular
647 Junction. *PLoS genetics* 12, e1005886.
- 648 Staley, B.K., and Irvine, K.D. (2010). Warts and Yorkie mediate intestinal regeneration by
649 influencing stem cell proliferation. *Curr Biol* 20, 1580-1587.
- 650 Swanwick, C.C., Murthy, N.R., and Kapur, J. (2006). Activity-dependent scaling of GABAergic
651 synapse strength is regulated by brain-derived neurotrophic factor. *Mol Cell Neurosci* 31, 481-
652 492.
- 653 Tang, L.S., Taylor, A.L., Rinberg, A., and Marder, E. (2012). Robustness of a rhythmic circuit to
654 short- and long-term temperature changes. *J Neurosci* 32, 10075-10085.
- 655 Thiagarajan, T.C., Lindskog, M., and Tsien, R.W. (2005). Adaptation to synaptic inactivity in
656 hippocampal neurons. *Neuron* 47, 725-737.
- 657 Turrigiano, G.G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell*
658 135, 422-435.
- 659 Turrigiano, G.G. (2017). The dialectic of Hebb and homeostasis. *Philos Trans R Soc Lond B*
660 *Biol Sci* 372.
- 661 Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C., and Nelson, S.B. (1998). Activity-
662 dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391, 892-896.
- 663 Ueda, A., and Wu, C.F. (2015). The role of cAMP in synaptic homeostasis in response to
664 environmental temperature challenges and hyperexcitability mutations. *Frontiers in cellular*
665 *neuroscience* 9, 10.
- 666 Wang, H.L., Zhang, Z., Hintze, M., and Chen, L. (2011). Decrease in calcium concentration
667 triggers neuronal retinoic acid synthesis during homeostatic synaptic plasticity. *J Neurosci* 31,
668 17764-17771.

- 669 Wang, T., Hauswirth, A.G., Tong, A., Dickman, D.K., and Davis, G.W. (2014). Endostatin Is a
670 Trans-Synaptic Signal for Homeostatic Synaptic Plasticity. *Neuron* 83, 616-629.
- 671 Wang, T., Jones, R.T., Whippen, J.M., and Davis, G.W. (2016a). α 2delta-3 Is Required for
672 Rapid Transsynaptic Homeostatic Signaling. *Cell reports* 16, 2875-2888.
- 673 Wang, X., Pinter, M.J., and Rich, M.M. (2016b). Reversible Recruitment of a Homeostatic
674 Reserve Pool of Synaptic Vesicles Underlies Rapid Homeostatic Plasticity of Quantal Content. *J*
675 *Neurosci* 36, 828-836.
- 676 Wefelmeyer, W., Puhl, C.J., and Burrone, J. (2016). Homeostatic Plasticity of Subcellular
677 Neuronal Structures: From Inputs to Outputs. *Trends Neurosci* 39, 656-667.
- 678 Weyhersmüller, A., Hallermann, S., Wagner, N., and Eilers, J. (2011). Rapid active zone
679 remodeling during synaptic plasticity. *J Neurosci* 31, 6041-6052.
- 680 Whiting, B., Moiseff, A., and Rubio, M.E. (2009). Cochlear nucleus neurons redistribute synaptic
681 AMPA and glycine receptors in response to monaural conductive hearing loss. *Neuroscience*
682 163, 1264-1276.
- 683 Wierenga, C.J., Ibata, K., and Turrigiano, G.G. (2005). Postsynaptic expression of homeostatic
684 plasticity at neocortical synapses. *J Neurosci* 25, 2895-2905.
- 685 Younger, M.A., Müller, M., Tong, A., Pym, E.C., and Davis, G.W. (2013). A presynaptic ENaC
686 channel drives homeostatic plasticity. *Neuron* 79, 1183-1196.
- 687 Zhong, Y., Budnik, V., and Wu, C.F. (1992). Synaptic plasticity in *Drosophila* memory and
688 hyperexcitable mutants: role of cAMP cascade. *J Neurosci* 12, 644-651.
- 689 Zhong, Y., and Wu, C.F. (2004). Neuronal activity and adenylyl cyclase in environment-
690 dependent plasticity of axonal outgrowth in *Drosophila*. *J Neurosci* 24, 1439-1445.
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693 **FIGURE AND TABLE LEGENDS**

694

695 **Figure 1. Postsynaptic expression of *GluRIIA^{M/R}* causes a decrease in mEPSP size and a**
696 **homeostatic increase in quantal content. (A)** Electrophysiological profiles comparing *w¹¹¹⁸*
697 (WT) control NMJs to NMJs with postsynaptic expression of *UAS-GluRIIA^{M/R}* (*w; UAS-*
698 *GluRIIA^{M/R}/+; MHC-Gal4/+*). Animals were reared at 25°C. mEPSP amplitude is markedly de-
699 creased compared to WT (***p* < 0.001). Quantal content (QC) is significantly increased for the
700 dominant-negative NMJs (***p* < 0.001), showing a homeostatic response that maintains EPSP
701 amplitude at control levels. **(B)** The same genotypes were raised at 29°C. mEPSP amplitude is
702 significantly decreased in the dominant-negative (***p* < 0.001). EPSP size is also decreased in
703 the dominant-negative compared to WT (***p* < 0.001), with QC significantly increased (***p* <
704 0.001) but not enough to completely offset the decrease in quantal size. **(C)** Raw value compar-
705 isons of the recordings normalized in (A) and (B). mEPSP amplitude, mEPSP frequency, EPSP
706 amplitude and QC were compared for these four conditions: WT 25°C, WT 29°C, *w;*
707 *GluRIIA^{M/R}/+; MHC-GAL4/+* 25°C, and *w; GluRIIA^{M/R}/+; MHC-GAL4/+* 29°C. In addition to the
708 observations above, WT at 29°C had a decreased mEPSP amplitude (* *p* < 0.05) and mEPSP
709 frequency (***p* < 0.001) compared to WT at 25°C. There was no difference in quantal content
710 between the two WT conditions (*p* = 0.32). At 25°C, the dominant-negative showed a decrease
711 in mEPSP frequency compared to WT at the same temperature (***p* < 0.001). The dominant-
712 negative at 29°C also showed a significant decrease in mEPSP frequency compared to WT at
713 29°C (***p* < 0.001). **(D)** Electrophysiological traces. Scale bars for EPSPs (and mEPSPs) are 5
714 mV (1 mV) and 50 ms (1000 ms). All statistical comparisons done by one-way ANOVA with
715 Tukey's post-hoc, collectively comparing the four total conditions.

716

717 **Figure 2. Animals expressing *GluRIIA^{M/R}* show homeostatic compensation early in devel-**
718 **opment. (A)** *w; GluRIIA^{M/R}/+; MHC-GAL4/GAL80^{TS}* animals were reared at 25°C or 29°C.

719 Compared to animals reared at 25°C, the animals reared at 29°C had a decrease in mEPSP
720 amplitude (** $p < 0.001$, Student's T-test) and frequency ($p < 0.001$, see Table 1). EPSP ampli-
721 tude was decreased (** $p < 0.01$), but there was a significant increase in QC (** $p < 0.001$) **(B)**
722 Representative electrophysiological traces. Scale bars for EPSPs (and mEPSPs) are 5 mV (1
723 mV) and 50 ms (1000 ms).

724

725 **Figure 3. Presynaptic homeostatic potentiation is reversible. (A)** Diagram of a temperature
726 swap paradigm. Mated animals were allowed to lay eggs for 6-8 hours. One set of animals was
727 reared entirely at 21° from egg lay to electrophysiological recording. A second condition was
728 raised entirely at 29°C. To test for reversibility of homeostatic potentiation, animals were reared
729 initially at 29°C and then swapped to 21°C. Animals were allowed to recover for either 1, 2, or 3
730 days before recording. **(B)** Expression of the dominant-negative transgene throughout life
731 (29°C) causes a dramatic decrease in mEPSP frequency (** $p < 0.001$, one-way ANOVA with
732 Tukey's post-hoc). Frequency remains low after 1 day of recovery at 21°C, compared 21°C rear-
733 ing controls. By 2 days recovery, the frequency is significantly increased compared to 29°C (**
734 $p < 0.001$), but still significantly decreased compared to 21°C controls (** $p < 0.01$). By 3 days
735 recovery, mEPSP frequency is no different from animals raised entirely at 21°C. **(C)** Normalized
736 electrophysiological data for mEPSP amplitude, EPSP amplitude, and QC for the NMJs of ani-
737 mals raised as described in (A). Longer recovery periods yield electrophysiology that more
738 closely approximates the control 21°C rearing condition (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
739 compared to 21°C control by one-way ANOVA with Tukey's post-hoc). **(D)** Representative elec-
740 trophysiological traces for 21°C and 29°C. Scale bars refer to 5 mV and 50 ms for EPSPs and 1
741 mV and 1000 ms for mEPSPs. **(E)** Traces for recovery conditions. Scale bars for EPSPs (and
742 mEPSPs) are 5 mV (1 mV) and 50 ms (1000 ms) for both (D) and (E).

743

744 **Figure 4. Strong correlations between recovery time and classical homeostatic parame-**
745 **ters (A)** There is a significant inverse correlation between mEPSP amplitude and QC for the
746 swap experiments described in Figure 3 (Pearson's $r = -0.68$, *** $p < 0.001$, $n = 59$). **(B)** There is
747 a significant positive correlation between the number of hours an animal has spent at 21°C and
748 mEPSP amplitude. ($r = 0.81$, $p < 0.001$, $n = 59$). Animals reared for “0 hours” at 21°C are the
749 29°C condition, with animals in the 21°C condition being reared at that temperature for around
750 120 hours before recording. **(C)** A significant inverse correlation between hours at 21°C and
751 quantal content was found ($r = -0.58$, *** $p < 0.001$, $n = 59$). Animals with the highest quantal
752 content were either at 21°C for 0 hours (29°C condition) or 24 hours (1-day recovery).

753
754 **Figure 5. Perfect homeostatic compensation is also reversible (A)** Dominant-negative ani-
755 mals raised at 28.5°C show a significant decrease in mEPSP amplitude (* $p < 0.05$, compared
756 to 25°C condition; one-way ANOVA with Tukey's post-hoc), accompanied by a perfectly offset-
757 ting increase in QC (** $p < 0.01$). Reversibility of homeostatic potentiation was demonstrated by
758 initially rearing *w; GluRIIA^{M/R}/+* ; *BG57-GAL4/GAL80^{TS}* animals at 28.5°C for 2 days and swap-
759 ping them to 25°C for 2 days. mEPSP amplitude, EPSP amplitude, and quantal content returned
760 to control (25°C throughout life) levels. EPSP amplitudes were virtually identical across all of the
761 conditions. **(B)** Dominant-negative animals raised at 28.5°C show a significant decrease in
762 mEPSP frequency (* $p < 0.05$, one-way ANOVA with Tukey's post-hoc). By contrast, the mEPSP
763 frequency in animals after two days of recovery was not significantly different from that of the
764 animals reared at 25°C. **(C)** Representative electrophysiological traces. Scale bars for EPSPs
765 (and mEPSPs) are 5 mV (1 mV) and 50 ms (1000 ms).

766
767 **Figure 6. Presynaptic homeostatic potentiation can be impaired at high temperatures (A)**
768 *MHC-Gal4* was used to drive expression of the dominant-negative GluRIIA^{M/R} subunit. Domi-
769 nant-negative animals were compared at 30°C to driver controls, *MHC-GAL4* crossed to WT.

770 mEPSP amplitude was significantly decreased in the dominant-negative animals ($*** p < 0.001$,
771 Student's T-test). A dramatic decrease in EPSP amplitude also occurred ($*** p < 0.001$) because
772 of a significant decrease in QC in the dominant-negative animals ($*** p < 0.001$). **(B)** Repre-
773 sentative electrophysiological traces. Scale bars for EPSPs (and mEPSPs) are 5 mV (1 mV)
774 and 50 ms (1000 ms). **(C)** *BG57-Gal4* was used to drive expression of the dominant-negative
775 *GluRIIA^{M/R}* subunit. Dominant-negative animals were compared at 30°C to driver controls,
776 *BG57-Gal4* crossed to WT. mEPSP amplitude was significantly decreased for the dominant-
777 negative NMJs ($** p < 0.01$, Student's T-test). A dramatic decrease in EPSP amplitude also oc-
778 curred ($*** p < 0.001$) because of a significant decrease in QC in the dominant-negative animals
779 ($*** p < 0.001$). **(D)** Representative electrophysiological traces. Scale bars as in (B). **(E)**
780 *GluRIIA^{SP16}* animals and WT controls were raised at 30°C. mEPSPs were significantly de-
781 creased for *GluRIIA* mutants (Student's T-test, $*** p < 0.001$, Student's T-test) and QC was sig-
782 nificantly increased ($** p < 0.01$). EPSP values were also decreased ($*** p < 0.001$) but not to
783 the same degree as mEPSP amplitudes. **(F)** Representative electrophysiological traces. Scale
784 bars as in (B).

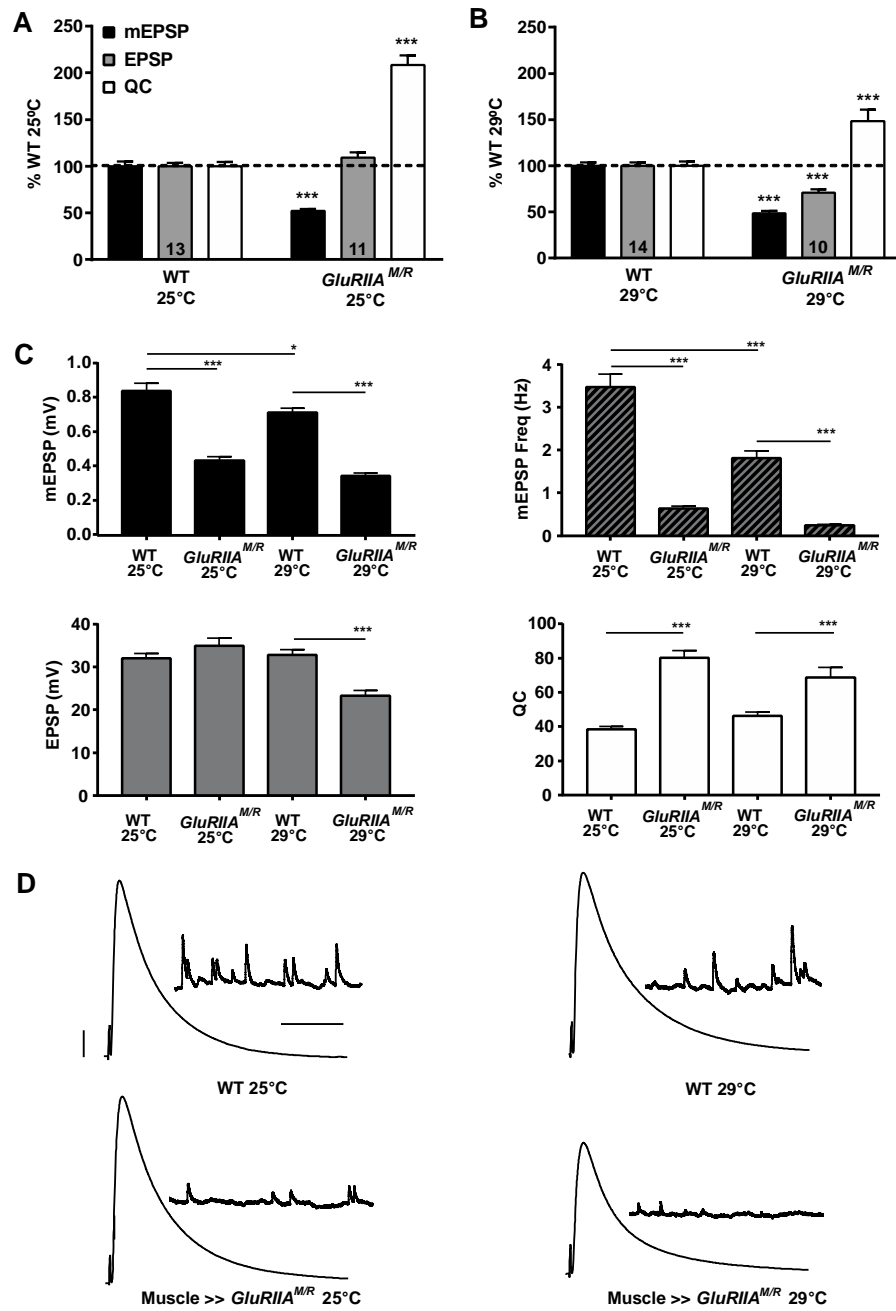
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786 **Table 1. Raw Electrophysiological Data.** Full genotypes and rearing conditions for electro-
787 physiological data presented in the study. Average values \pm SEM are presented for each pa-
788 rameter, with n = number of NMJs recorded. Values include miniature excitatory postsynaptic
789 potential (mEPSP) amplitude, mEPSP frequency, excitatory postsynaptic potential (EPSP) am-
790 plitude, quantal content (QC), muscle input resistance, and resting membrane potential.

791

792 **Figure 1**

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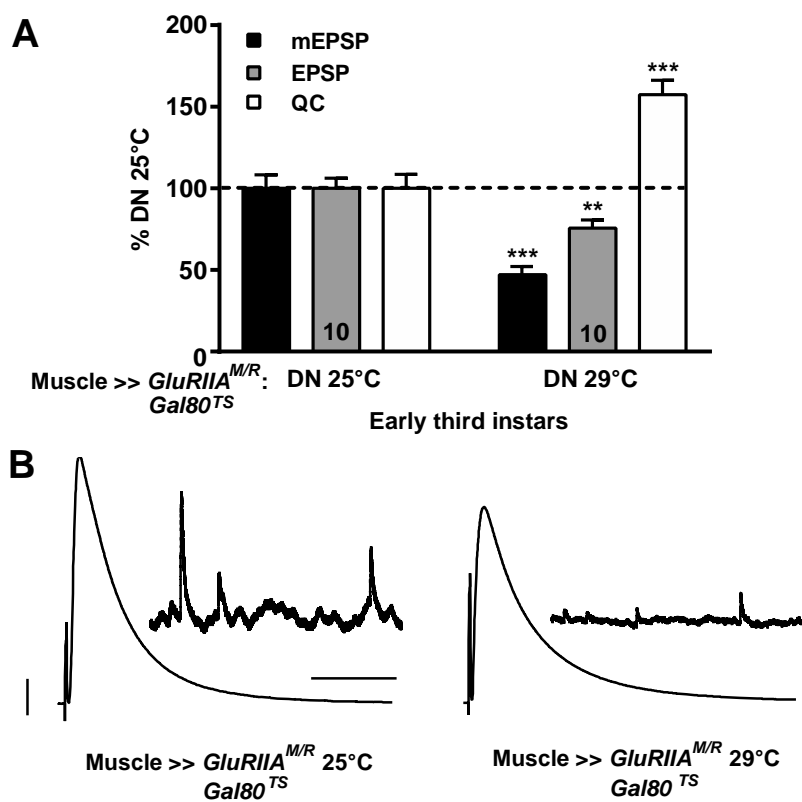
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797 **Figure 2**

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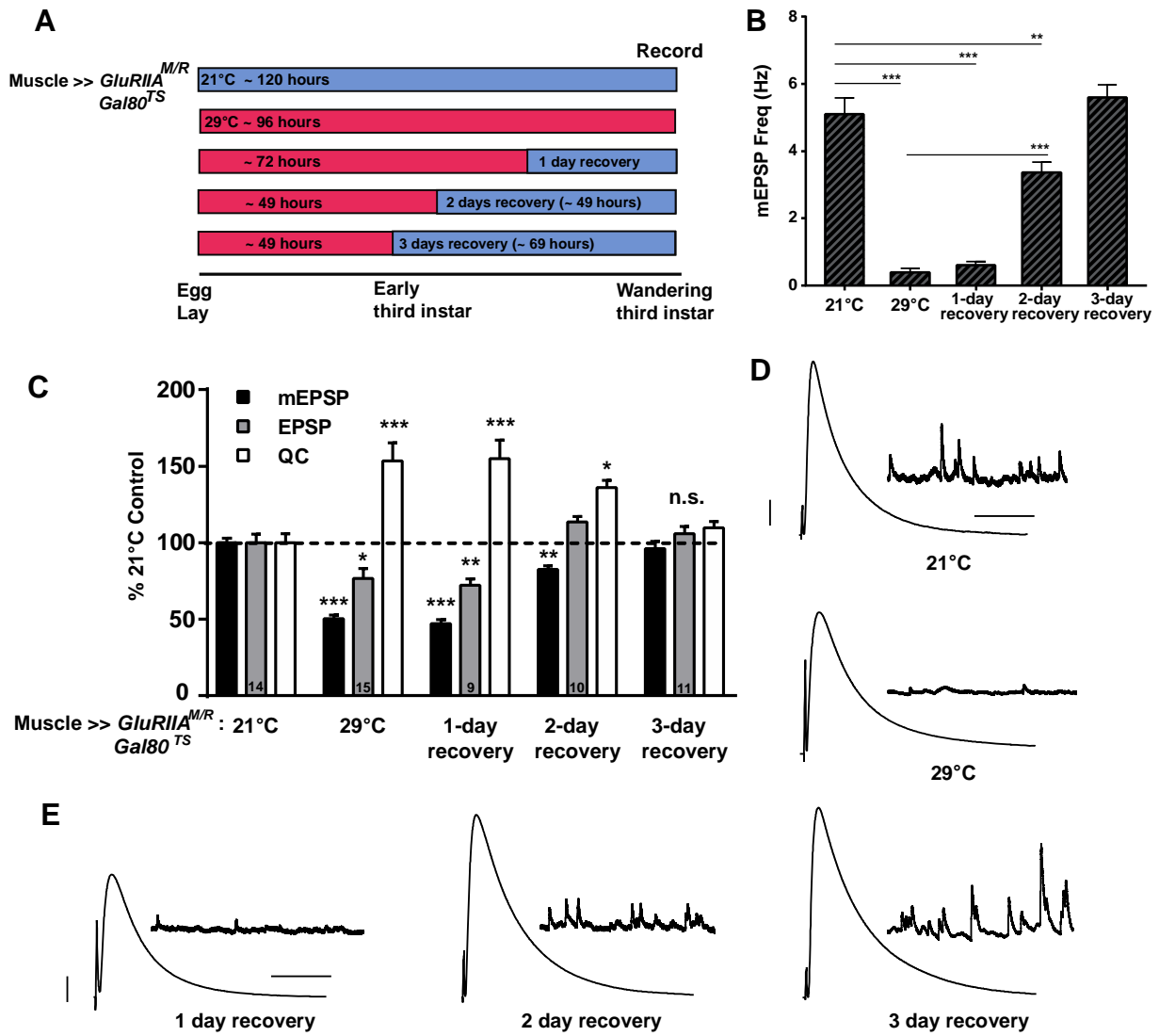


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801 **Figure 3**

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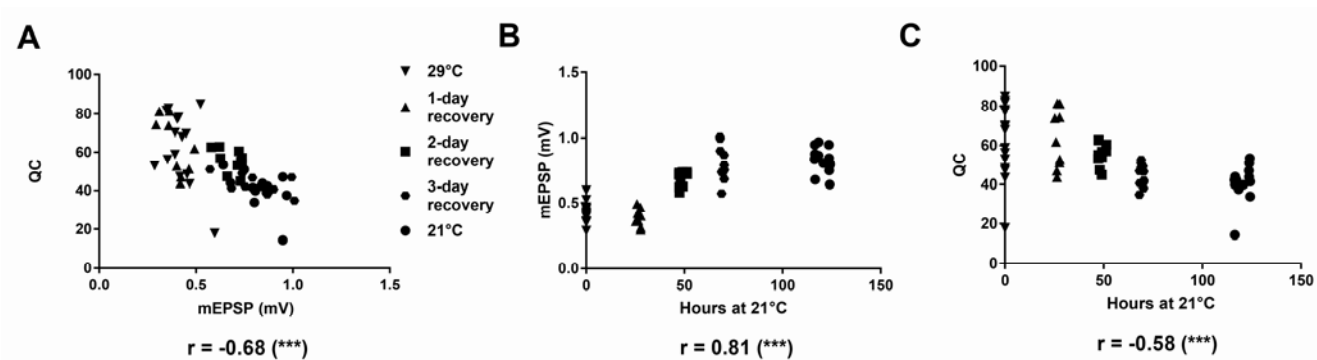
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807 **Figure 4**

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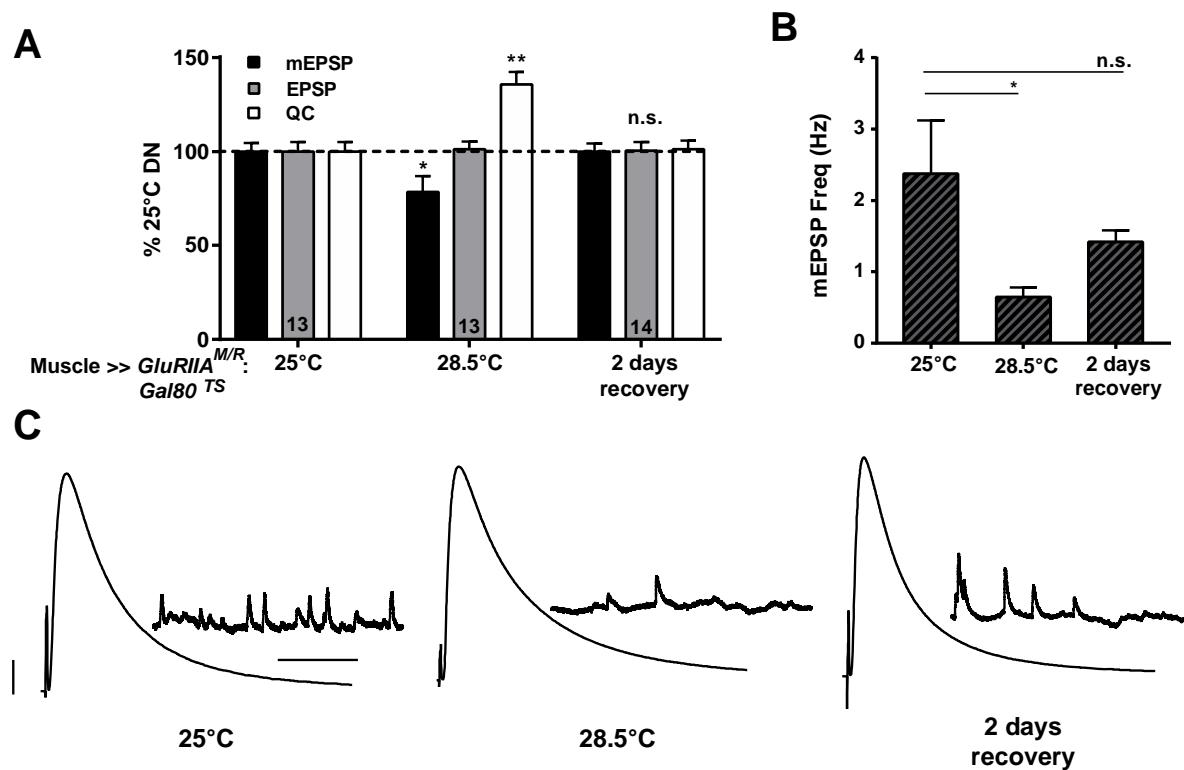
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812 **Figure 5**

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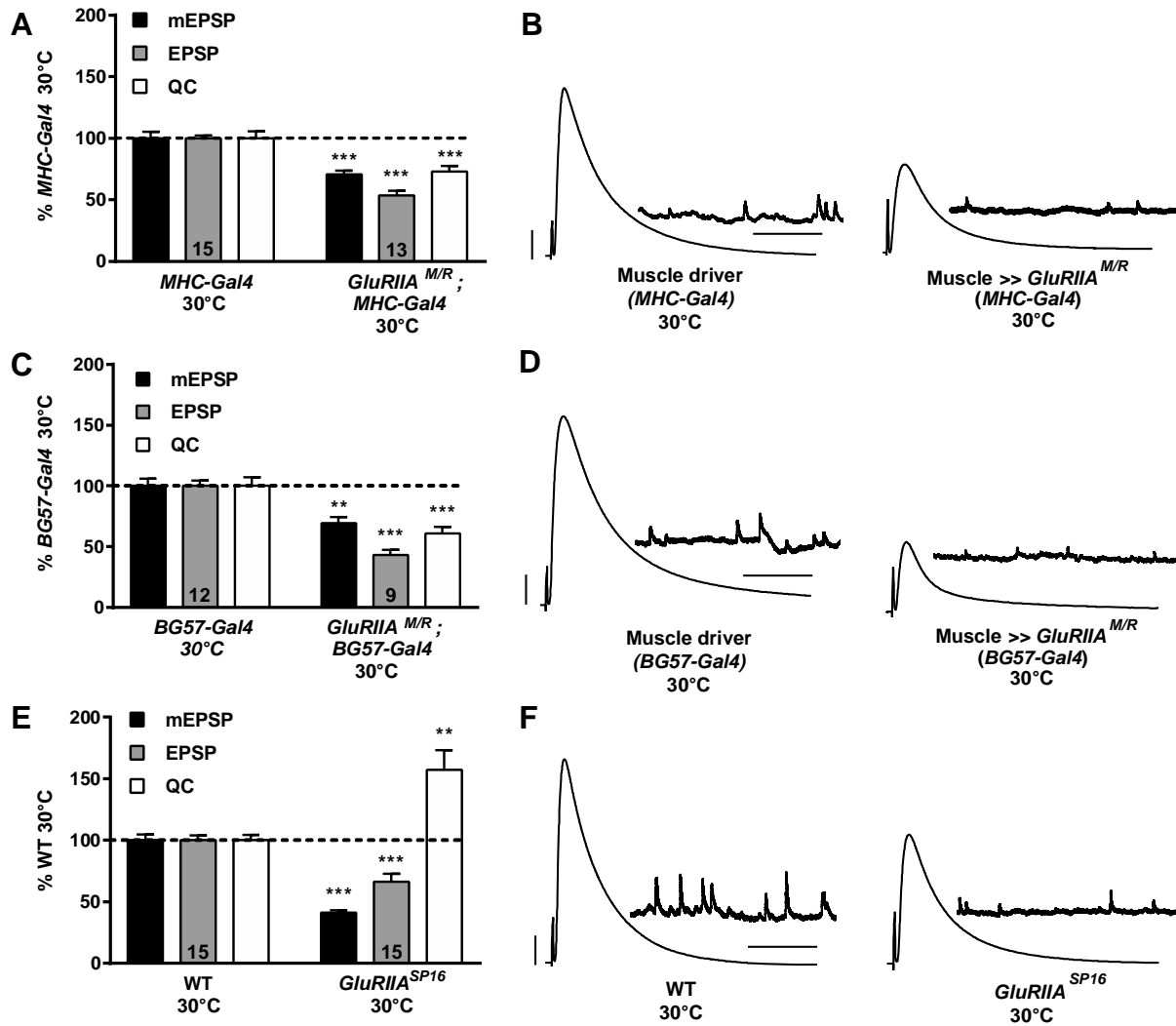
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817 **Figure 6**

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822 **Table 1**

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824

Genotype	Condition	mEPSP (mV)	mEPSP Freq (Hz)	EPSP (mV)	QC	I _h (mV)	RMP (mV)	n	Figure
<i>W¹¹¹⁸</i> (wild-type)	25°C	0.85 ± 0.04	3.5 ± 0.3	32.0 ± 1.2	38.5 ± 1.7	6.4 ± 0.6	-64.6 ± 1.1	13	1
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/+</i>	25°C	0.44 ± 0.02	0.6 ± 0.1	35.0 ± 1.8	80.3 ± 4.1	5.8 ± 0.5	-69.1 ± 1.2	11	1
<i>W¹¹¹⁸</i>	29°C	0.72 ± 0.03	1.8 ± 0.2	32.9 ± 1.2	46.4 ± 2.2	6.2 ± 0.5	-64.4 ± 0.9	14	1
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/+</i>	29°C	0.35 ± 0.02	0.2 ± 0.0	23.3 ± 1.2	68.9 ± 5.7	5.2 ± 0.2	-65.0 ± 1.1	10	1
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	Early third instar 25°C	1.27 ± 0.1	2.0 ± 0.3	33.2 ± 2.1	27.4 ± 2.4	9.5 ± 0.8	-64.7 ± 2.0	10	2
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	Early third instar 29°C	0.60 ± 0.05	0.4 ± 0.1	25.1 ± 1.4	43.1 ± 2.6	13.6 ± 0.9	-63.0 ± 0.8	10	2
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	21°C	0.83 ± 0.03	5.1 ± 0.5	33.3 ± 1.9	40.7 ± 2.4	5.6 ± 0.4	-66.3 ± 0.8	14	3, 4
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	29°C	0.42 ± 0.02	0.4 ± 0.1	25.6 ± 2.1	62.5 ± 4.8	6.0 ± 0.3	-66.8 ± 1.1	15	3, 4
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	1-day recovery at 21°C	0.39 ± 0.02	0.6 ± 0.1	24.1 ± 1.4	63.1 ± 5.0	5.3 ± 0.3	-66.0 ± 1.1	9	3, 4
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	2-day recovery at 21°C	0.69 ± 0.02	3.4 ± 0.3	37.8 ± 1.2	55.4 ± 1.9	5.3 ± 0.3	-67.7 ± 0.7	10	3, 4
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/GAL80^{TS}</i>	3-day recovery at 21°C	0.80 ± 0.04	5.6 ± 0.4	35.3 ± 1.5	44.7 ± 1.7	5.5 ± 0.2	-68.4 ± 0.9	11	3, 4
<i>GU/RIIA^{W614R}/+</i> ; <i>BGST-GAL4/GAL80^{TS}</i>	25°C	0.79 ± 0.04	2.4 ± 0.7	31.1 ± 1.6	39.5 ± 1.9	5.3 ± 0.4	-68.4 ± 1.1	13	5
<i>GU/RIIA^{W614R}/+</i> ; <i>BGST-GAL4/GAL80^{TS}</i>	28.5°C	0.62 ± 0.05	0.6 ± 0.1	31.5 ± 1.3	53.6 ± 3.6	7.2 ± 0.4	-67.0 ± 1.3	13	5
<i>GU/RIIA^{W614R}/+</i> ; <i>BGST-GAL4/GAL80^{TS}</i>	2-day recovery at 25°C	0.79 ± 0.03	1.4 ± 0.2	31.3 ± 1.4	39.9 ± 1.8	6.3 ± 0.3	-66.7 ± 1.1	14	5
<i>W¹¹¹⁸</i>	30°C	0.78 ± 0.04	2.4 ± 0.2	33.1 ± 1.4	42.9 ± 1.9	6.8 ± 0.3	-66.2 ± 0.8	15	6
<i>GU/RIIA^{S⁷¹⁶}</i>	30°C	0.32 ± 0.02	0.8 ± 0.1	21.9 ± 2.2	67.4 ± 6.8	6.0 ± 0.4	-63.0 ± 0.5	15	6
<i>MHC-GAL4/+</i>	30°C	0.47 ± 0.02	1.1 ± 0.1	28.9 ± 0.6	64.3 ± 3.6	4.9 ± 0.2	-63.9 ± 0.9	15	6
<i>GU/RIIA^{W614R}/+</i> ; <i>MHC-GAL4/+</i>	30°C	0.33 ± 0.01	0.2 ± 0.0	15.4 ± 1.1	47.0 ± 2.8	5.3 ± 0.2	-64.4 ± 0.9	13	6
<i>BGST-GAL4/+</i>	30°C	0.51 ± 0.03	0.6 ± 0.1	31.3 ± 1.4	63.9 ± 4.5	6.6 ± 0.3	-70.2 ± 1.4	12	6
<i>GU/RIIA^{W614R}/+</i> ; <i>BGST-GAL4/+</i>	30°C	0.35 ± 0.03	0.8 ± 0.7	13.5 ± 1.3	39.1 ± 3.4	6.1 ± 0.5	-65.5 ± 1.6	9	6