The Innate Immune Receptor PGRP-LC Controls Presynaptic Homeostatic Plasticity

Highlights

- A conserved innate immune receptor, PGRP, is necessary for presynaptic homeostasis

- The PGRP-LCx isoform is necessary in motoneurons for presynaptic homeostasis

- PGRP-LC controls the homeostatic modulation of the release-ready vesicle pool

- PGRP-LC genetically interacts with RIM and Multiplexin

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In Brief

Harris et al. demonstrate that an evolutionarily conserved innate immune receptor, PGRP-LC, is required for homeostatic plasticity. A novel, neuronal function for PGRP-LC highlights a potential intersection of innate immune signaling with homeostatic mechanisms that stabilize neural function throughout life.

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The Innate Immune Receptor PGRP-LC Controls Presynaptic Homeostatic Plasticity

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SUMMARY

It is now appreciated that the brain is immunologically active. Highly conserved innate immune signaling responds to pathogen invasion and injury and promotes structural refinement of neural circuitry. However, it remains generally unknown whether innate immune signaling has a function during the day-to-day regulation of neural function in the absence of pathogens and irrespective of cellular damage or developmental change. Here we show that an innate immune receptor, a member of the peptidoglycan pattern recognition receptor family (PGRP-LC), is required for the induction and sustained expression of homeostatic synaptic plasticity. This receptor functions presynaptically, controlling the homeostatic modulation of the readily releasable pool of synaptic vesicles following inhibition of postsynaptic glutamate receptor function. Thus, PGRP-LC is a candidate receptor for retrograde, *trans*-synaptic signaling, a novel activity for innate immune signaling and the first known function of a PGRP-type receptor in the nervous system of any organism.

INTRODUCTION

The homeostatic modulation of presynaptic neurotransmitter release has been observed at mammalian central synapses and at neuromuscular synapses in species ranging from *Drosophila* to mouse and human (for review, see Davis and Müller, 2015). The homeostatic enhancement of presynaptic release following inhibition of postsynaptic glutamate receptors is achieved by an increase in presynaptic calcium influx through presynaptic CaV2.1 calcium channels and the simultaneous expansion of the readily releasable pool (RRP) of synaptic vesicles (for review, see Davis and Müller, 2015). To date, the retrograde, *trans*-synaptic signaling system that initiates these presynaptic changes following disruption of postsynaptic glutamate receptors remains largely unknown (but see Wang et al., 2014).

A large-scale forward genetic screen for homeostatic plasticity genes, using synaptic electrophysiology at the neuromuscular junction (NMJ) as the primary assay (Dickman and Davis, 2009; Younger et al., 2013), identified mutations in the *PGRP-LC* locus that block presynaptic homeostasis. In *Drosophila*, PGRP-LC is the primary receptor that initiates an innate immune response through the immune deficiency (IMD) pathway (Figure 1; Royet and Dziarski, 2007). In mammals, there are four PGRPs including a "long" isoform (PGRP-L or PGlyRP2) that appears to have both secreted and membrane-associated activity (Royet and Dziarski, 2007). In mice, the PGlyRP2 protein has two functions in the innate immune response: a well-documented extracellular enzymatic (amidase) activity (Royet and Dziarski, 2007) and a pro-inflammatory signaling function that is independent of its extracellular enzymatic activity (Saha et al., 2009). As yet, there are no known functions for PGRPs in the nervous system of any organism.

RESULTS

PGRP Is Required for the Robust Expression of Presynaptic Homeostatic Plasticity

Two major isoforms of *PGRP-LC* are transcribed, *PGRP-LCx* and *PGRP-LCa* (Figure 1B), both of which are known to participate in the innate immune response (Choe et al., 2002). Presynaptic homeostasis was quantified (0.4 mM $[Ca^{2+}]_e$) in two independent loss-of-function alleles, a deletion of the *PGRP-LC* gene locus (*PGRP^{del}*; Gottar et al., 2002) that removes both *PGRP* isoforms and a nonsense mutation in the PGRP domain of the *PGRP-LCx* isoform that results in an isoform-specific, loss of function allele (*PGRP^2*; Choe et al., 2002) (see Figure 1B). We demonstrate that application of the glutamate receptor antagonist PhTx (10–20 μM) decreases mEPSP amplitudes by ∼50% in all genotypes (Figures 1C–1F; compare light and dark bars for each genotype, representing the absence [−] and presence [+] of PhTx; $p < 0.01$). In wild-type, we observe a significant homeostatic increase in quantal content ($p < 0.01$) that brings EPSP amplitudes back toward the levels observed in the absence of PhTx (Figures 1D–1F). We then show, in both *PGRP-LC* mutant alleles as well as the trans-heterozygous allelic combination, that there is no homeostatic enhancement of quantal content following PhTx application (Figure 1F; $p > 0.5$ compare dark and light bars for each

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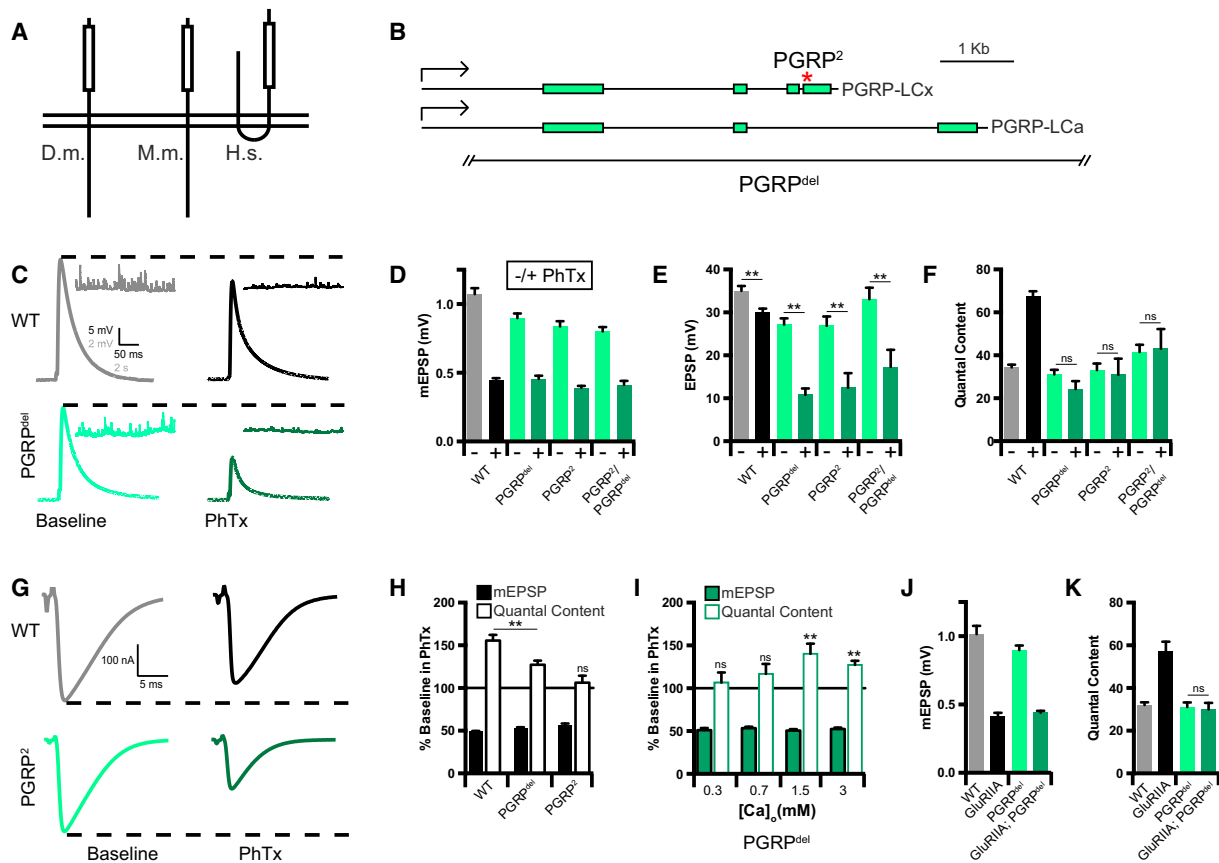
Figure 1. Identification of PGRP-LC as an Innate Immune Receptor Involved in the Rapid Induction and Sustained Expression of Presynaptic Homeostasis

(A) Diagram of PGRP-LC in different species (PGLYRP 2 in mouse, M.m.; and human, H.s.). Rectangles represent PGRP domains.

(B) Schematic of the *PGRP-LC* locus showing two alternative splice variants involved in innate immune signaling (*PGRP-LCa* and *PGRP-LCx*). The entire coding region is deleted in *PGRP*^{del}. Star denotes a nonsense mutation (*PGRP*^{2}) truncating the PGRP domain of the *PGRP-LCx* isoform.

(C) Sample data showing EPSP and mEPSP amplitudes in the absence and presence of PhTx for WT (gray/black respectively) and *PGRP*^{del} (light green/dark green).

(D) Average data for mEPSP amplitude for indicated genotypes in the absence (light gray/light green) and presence of PhTx (black/dark green). PhTx application reduces amplitudes in all genotypes ($p < 0.01$).

(E) Average data for EPSP amplitude as in (D) (**$p < 0.01$).

(F) Average data for quantal content as in (D) (ns; $p > 0.05$).

(G) Sample data showing EPSC amplitudes as in (C), 3 mM $[Ca^{2+}]_e$.

(H) Average data for mEPSP amplitudes and quantal content following application of PhTx expressed as percent change relative to baseline in the absence of PhTx for each genotype. Homeostasis is suppressed in *PGRP*^{del} compared to wild-type (**$p < 0.01$) and is blocked in *PGRP*^{2} at 3 mM extracellular calcium.

(I) Analysis of *PGRP*^{del}, extracellular calcium as indicated. Homeostasis is blocked at 0.3 mM $[Ca^{2+}]_e$ ($p > 0.3$) and 0.7 mM $[Ca^{2+}]_e$ ($p > 0.1$), and is suppressed at 1.5 (see H) and 3 mM (although statistically significant homeostasis is observed above *PGRP*^{del} baseline, $p < 0.01$).

(J–K) Average data for mEPSP amplitude and quantal contents for indicated genotypes. Data are mean ± SEM for all figures. Student's t test.

genotype) and EPSP amplitudes are dramatically decreased compared to each genotype in the absence of PhTx (Figure 1E; $p < 0.01$). Sample sizes for all experiments are presented in Table S1. Thus, *PGRP-LC* is essential for the rapid induction of presynaptic homeostasis.

Examination of baseline synaptic transmission reveals that loss of PGRP-LC has a small, but measurable, effect on baseline parameters, but completely blocks the rapid induction of presynaptic homeostasis measured at 0.4 mM $[Ca^{2+}]_e$. In both *PGRP*^{del} and *PGRP*^{2}, we observe small, but statistically significant, decreases in spontaneous miniature excitatory postsynaptic po-

tential (mEPSP) amplitude and excitatory postsynaptic potential (EPSP) amplitude, but no significant change in quantal content (Figures 1D–1F). When *PGRP*^{2} is placed in *trans* to *PGRP*^{del}, mEPSP amplitudes remain decreased ($p < 0.05$), EPSP amplitude is unchanged compared to wild-type ($p > 0.05$) and there is a small, but statistically significant, increase in quantal content compared to wild-type ($p < 0.05$). When mEPSP amplitudes are plotted as cumulative frequency distributions, the entire distribution of amplitudes is slightly shifted in the *PGRP* mutants compared to wild-type (Figure S1). Finally, we demonstrate that wild-type and *PGRP*^{2} NMJs show statistically similar

sensitivity to application of EGTA-AM (25 μM for 10 min) (p > 0.5; data not shown).

We then tested whether loss of PGRP-LC blocks presynaptic homeostasis induced by a mutation in the *GluRIIA* subunit of the postsynaptic glutamate receptor. This mutation is present throughout the life of the organism and, therefore, tests for the sustained expression of presynaptic homeostasis. The homeostatic potentiation of quantal content normally observed in the *GluRIIA* mutant background (p < 0.01) is completely blocked in the *GluRIIA; PGRP^del^* double mutant (Figures 1J and 1K; p > 0.5). Thus, *PGRP-LC* is also necessary for the sustained expression of presynaptic homeostasis.

We next repeated our assays of presynaptic homeostasis at $[Ca^{2+}]_e$ ranging from 0.3 to 3 mM, a 10-fold range of $[Ca^{2+}]_e$ that encompasses what is believed to be physiological $[Ca^{2+}]_e$ at this synapse. In *PGRP^2^*, we find that homeostatic plasticity is blocked at 0.4 mM (Figure 1F) as well as at 3.0 mM $[Ca^{2+}]_e$ (Figures 1G and 1H), demonstrating that homeostatic plasticity is blocked in the *PGRP^2^* mutant at all $[Ca^{2+}]_e$. However, we observe a statistically significant (p < 0.01), homeostatic response in the *PGRP-LC* deletion allele at 3 mM $[Ca^{2+}]_e$ (Figure 1H), although the magnitude of this homeostatic response is significantly less that that observed in wild-type (p < 0.05), indicating that homeostatic plasticity is not normal in this allele, even at this elevated extracellular calcium concentration. When we examined two intervening calcium concentrations, specifically testing the *PGRP^del^* allele, we find no homeostatic increase in quantal content at 0.7 mM extracellular calcium (Figure 1I; p > 0.1) and statistically significant (p < 0.01) homeostatic plasticity at 1.5 mM extracellular calcium. While it remains unclear why the *PGRP^2^* allele is more severe than *PGRP^del^*, we considered several issues. We tested the heterozygous *PGRP^2^/+* allele and find no evidence of a dominant interfering effect since both baseline release and homeostatic plasticity are normal (Figure S2). It remains possible that enhanced transcription of another, related innate immune receptor could partially restore signaling in the *PGRP^del^* gene deletion background, but not when a mutant receptor isoform is transcribed, as in *PGRP^2^*. There are several PGRP receptors including modulatory and inhibitory receptors that could mediate this effect (Dziarski, 2004). Regardless, two conclusions are possible based upon our existing data. First, deletion of the *PGRP* gene clearly impairs presynaptic homeostasis and renders it less robust to changes in extracellular calcium, with an unequivocal blockade of homeostatic plasticity in the range of 0.3–0.7 mM $[Ca^{2+}]_e$. Second, a mutation that specifically disrupts the *PGRP-LCx* isoform causes a complete failure of presynaptic homeostatic plasticity.

### PGRP-LCx Functions Neuronally During Presynaptic Homeostasis

To determine the cell type in which PGRP-LC functions during homeostatic plasticity, we pursued rescue experiments with a Venus-tagged *PGRP-LCx* isoform under *UAS*-control (Figures 2A and 2E–2G). Presynaptic expression of *UAS-PGRP-LCx-Venus* fully restores homeostatic potentiation to the *PGRP^del^* mutant (0.4 mM $[Ca^{2+}]_e$), whereas postsynaptic muscle expression does not (Figure 2G). We note that muscle-specific *PGRP-LCx* expression in the *PGRP* mutant background causes a substantial

reduction in both mEPSP and EPSP amplitudes. To control for the possibility that muscle-specific expression might dominantly interfere with presynaptic homeostasis, we overexpressed *PGRP-LCx-Venus* in wild-type muscle. We document a similar decrease in baseline mEPSP and EPSP amplitudes compared to wild-type (Figures 2H and 2I) that can be attributed, in part, to diminished input resistance and a depolarized resting potential (Figures 2K and 2L). Importantly, homeostatic plasticity is fully expressed (Figure 2J). Thus, we can conclude that muscle-specific expression of *PGRP-LCx* fails to rescue homeostatic plasticity. We also note that motoneuron-specific expression of *PGRP-LCx* induces a modest increase in EPSP amplitude (p < 0.01) and a trend toward increased mEPSP amplitude (p = 0.08). This activity could reasonably contribute to the rescue of baseline defects in mEPSP and EPSP amplitudes when *PGRP-LCx-Venus* is expressed neuronally in the *PGRP* mutant background (compare Figures 2E and 2F with Figures 1D and 1E). But, the observed rescue of homeostatic plasticity when *PGRP-LCx-Venus* is expressed in motoneurons (Figure 2G) clearly occurs over and above any minor effects on baseline transmission. Therefore, we conclude that *PGRP-LCx* isoform expression in motoneurons supports robust homeostatic plasticity. This complements the observed necessity of the PGRP-LCx isoform, demonstrated by the elimination of presynaptic homeostasis in the *PGRP^2^* mutant.

Next, we perform the converse experiment using RNAi to deplete PGRP expression specifically in neurons or muscle (Figure 2). We demonstrate that expression of *UAS-PGRP-LC* RNAi in neurons blocks homeostatic plasticity, while expression in muscle does not (Figures 2A–2D). Finally, we find that *PGRP-LC* mRNA is weakly, but endogenously expressed in motoneurons, determined by custom micro-array analysis of mRNA expression in FACS isolated, GFP-expressing motoneurons (Parrish et al., 2014; data not shown). Taken together, these data are consistent with the conclusion that PGRP-LC, and PGRP-LCx in particular, functions presynaptically to enable homeostatic plasticity. These experiments also demonstrate that failed presynaptic homeostasis following loss of PGRP-LC cannot be attributed to the systemic effects associated with impaired innate immunity. More specifically, neuronal expression of *PGRP-LC-RNAi* blocks homeostatic plasticity even though innate immune signaling throughout the rest of the organism is unaltered. In addition, conversely, in our rescue experiments, innate immune signaling should be compromised everywhere except the nervous system and homeostatic plasticity is fully functional.

PGRP-LC Traffics to the Nerve Terminal and Does Not Alter Synaptic Growth

We next addressed where PGRP-LC resides within motoneurons. Analysis of PGRP-LCx-Venus distribution in motoneurons demonstrates that the Venus-tagged receptor traffics to the presynaptic nerve terminal. We observe PGRP-LCx-Venus puncta distributed throughout the presynaptic terminal (Figure 3A). Co-staining with the active zone marker Bruchpilot (BRP) reveals that the receptors are not present at the active zone. These data suggest that PGRP-LCx is a presynaptic receptor, consistent with a required function during the rapid induction of presynaptic homeostasis, which occurs locally at the isolated NMJ.
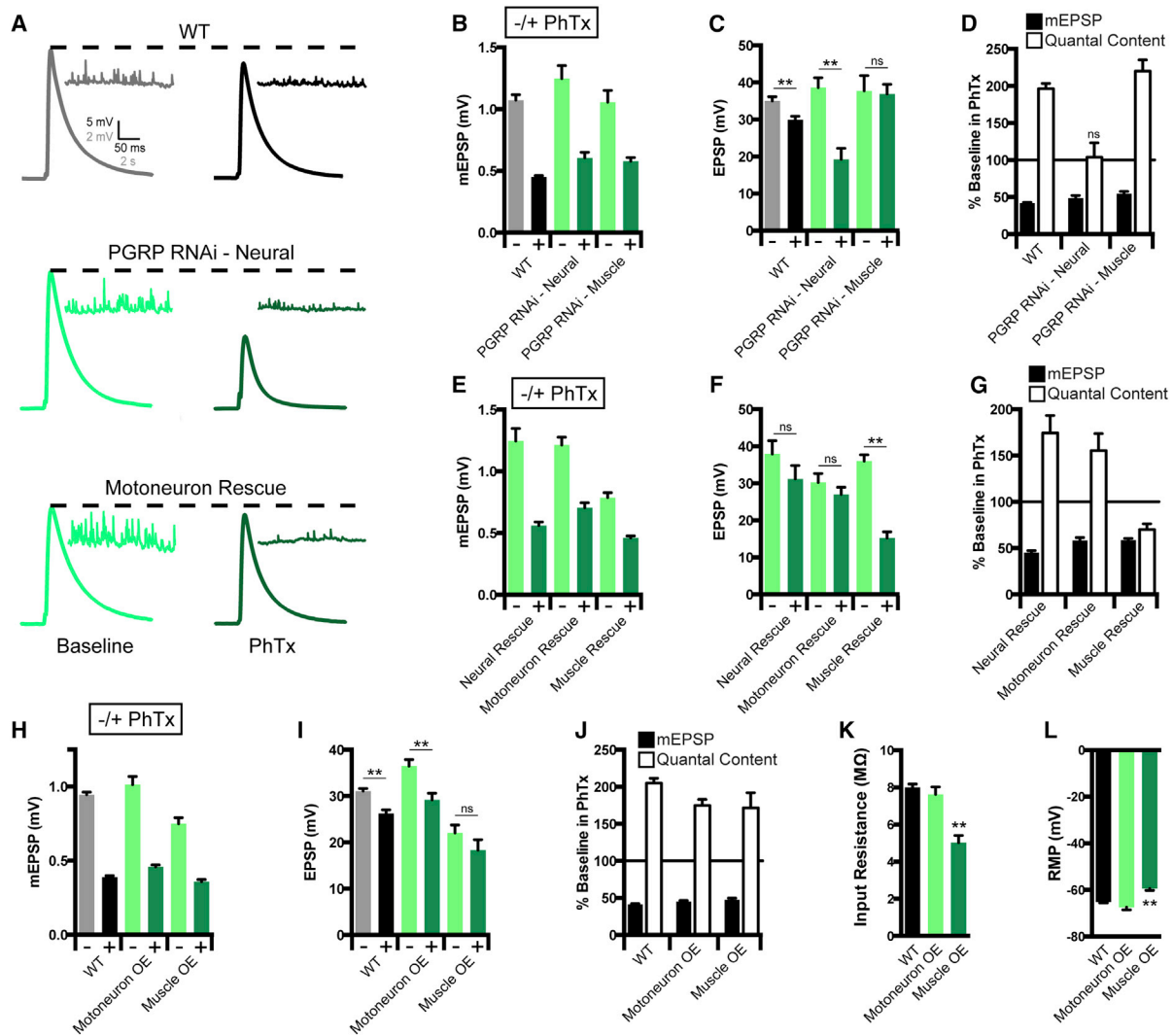
Figure 2. PGRP Is Required in Motoneurons for Presynaptic Homeostasis

(A) Sample data showing EPSP and mEPSP waveforms ± PhTx for the indicated genotypes.

(B) Average mEPSP amplitude when *PGRP-RNAi* is expressed neuronally (*elaV-GAL4*) or in muscle (*BG57-GAL4*), ± PhTx as indicated. PhTx application reduces amplitudes in all genotypes ($p < 0.01$).

(C) Average EPSP amplitude as in B. (**$p < 0.01$).

(D) Average percent change in mEPSP amplitude and quantal content in PhTx compared to baseline for the indicated conditions.

(E) Average mEPSP amplitude for indicated rescue experiments as follows: Neural rescue = *elaV-GAL4* expression of *UAS-PGRP-LCx* in the *PGRP^del* mutant; motoneuron rescue = *OK371-GAL4*; muscle rescue = *MHC-GAL4*. PhTx application reduces amplitudes in all genotypes ($p < 0.01$).

(F) Average EPSP amplitude as in (E).

(G) Average percent change in mEPSP amplitude and quantal content as in (D). When *UAS-PGRP-LCx* is expressed presynaptically, homeostasis is rescued ($p < 0.05$). Expression in muscle does not ($p > 0.05$).

(H) Average mEPSP amplitude for expression of *UAS-PGRP-LCx* in a wild-type background, drivers as indicated in (E).

(I) Average EPSP amplitudes for genotypes as in (H).

(J) Average percent change in mEPSP amplitude and quantal content for genotypes as in (H). Significant homeostasis is observed in all conditions ($p < 0.01$).

(K) Muscle input resistance for the indicated genotypes (**$p < 0.01$).

(L) Muscle resting membrane potential. Average RMP (**$p < 0.01$). Student's t test.

An analysis of synapse morphology reveals a grossly normal NMJ anatomy. There are no significant changes in synaptic bouton number or active zone number that could account for the change in homeostatic plasticity (Figure 3B). Active zone number is quantified as the number of Bruchpilot (Brp) puncta at the NMJ,

consistent with previous studies (Müller et al., 2012). Similarly, there are no major deficits in the abundance of essential synaptic vesicle proteins. Indeed, there is a slight increase in the abundance of Cysteine String Protein (CSP) and Synaptotagmin1 (Syt1) (see Figure S3). Thus, the growth and general organization
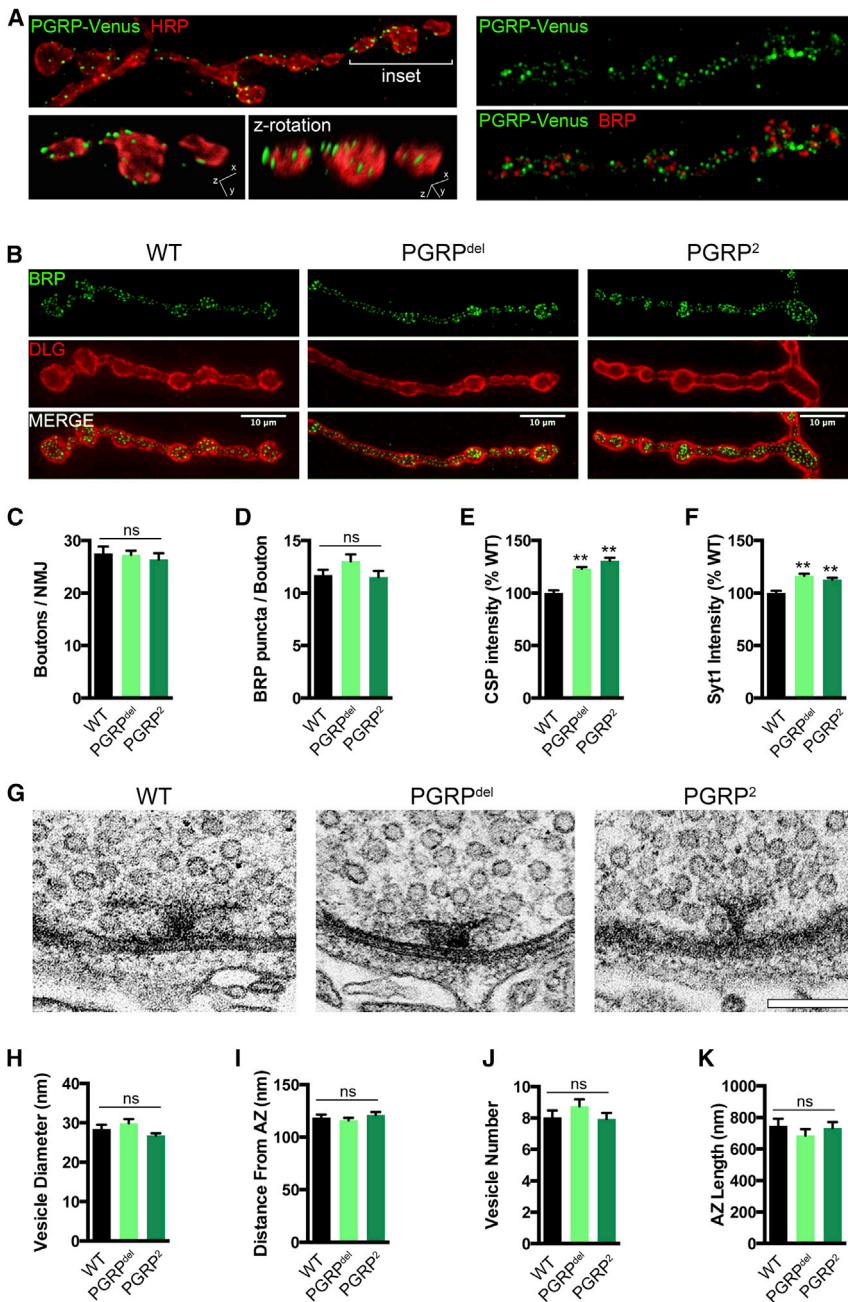
Figure 3. PGRP-LC-Venus Localizes to the Presynaptic Terminal

(A) Left: Image in of an NMJ in which *UAS-PGRP-LCx-Venus* was expressed using *Ok371-Gal4*. The NMJ was co-stained with HRP (red) and anti-GFP (green). Magnified and rotated images (below) indicate the membrane-proximal localization of PGRP-LCx-Venus. Right: NMJ expressing *UAS-PGRP-LCx-Venus* as in (A). The NMJ was co-stained with the presynaptic active zone marker Brp (red) and anti-Venus (green).

(B) WT NMJ at muscle 4 (left) co-stained with the presynaptic active zone marker Brp (top, green) and the postsynaptic marker Dlg (middle, red). Merge at bottom.

(C) Quantification of bouton number per NMJ at muscle 4.

(D) Brp puncta per bouton at muscle 4.

(E and F) Quantification of average staining intensities for anti-Syt1 and anti-CSP (**p < 0.01).

(G) Representative active zone images. Scale bar represents 150 nm.

(H–K) Quantification of vesicle diameter per active zone (AZ) (H), average vesicle distance from T-bar base (see text) (I), average vesicle number per active zone within a 150 nm radius of T-bar base (J), average AZ length (K). Student's t test with Bonferroni correction.

dimensional length of the active zone, defined as the continuous electron density on either side of the T-bar. There is no significant difference in any of these parameters comparing wild-type with the *PGRP^{del}* or the *PGRP^2* mutants. We conclude that loss of PGRP does not impair the integrity or organization of the active zone. By extension, the failure of presynaptic homeostatic plasticity is not a secondary consequence of impaired active zone organization, vesicle number, or vesicle distance from the T-bar (Figures 3G–3K).

PGRP Controls the Homeostatic Modulation of the Readily Releasable Vesicle Pool

The homeostatic modulation of presynaptic neurotransmitter release has been demonstrated to require potentiation of the readily releasable pool (RRP) of presynaptic vesicles (Davis and Müller, 2015). We define the RRP as the number of vesicles released during a brief (500 ms) high frequency stimulus train at elevated extracellular calcium concentrations (3 mM $[Ca^{2+}]_e$), according to published methods (Schneggenburger et al., 1999). For this analysis, we focused on the *PGRP^2* mutation because it blocks homeostatic plasticity at 3 mM $[Ca^{2+}]_e$. At this $[Ca^{2+}]_e$ *PGRP^2* mutants show a small decrease in mEPSP and cumulative EPSC amplitudes (Figures 4A–4C), but no significant difference in the baseline RRP (WT = 2,370 ± 227; *PGRP^2* = 2,236 ± 190; p > 0.3),

of the NMJ are not adversely affected by the absence of an essential innate immune receptor.

Finally, we examined synaptic ultrastructure. Active zones were identified by the presence of a presynaptic "T-bar" within a zone of pre- and postsynaptic electron dense membrane. For each active zone, we identified those synaptic vesicles that reside within 150 nm of the center of the pedestal of the T-bar (centroid), where presynaptic voltage gated calcium channels reside. For this population of vesicles, at each active zone, we quantified average vesicle diameter, average vesicle number, average vesicle distance from the T-bar centroid and, finally, the two-
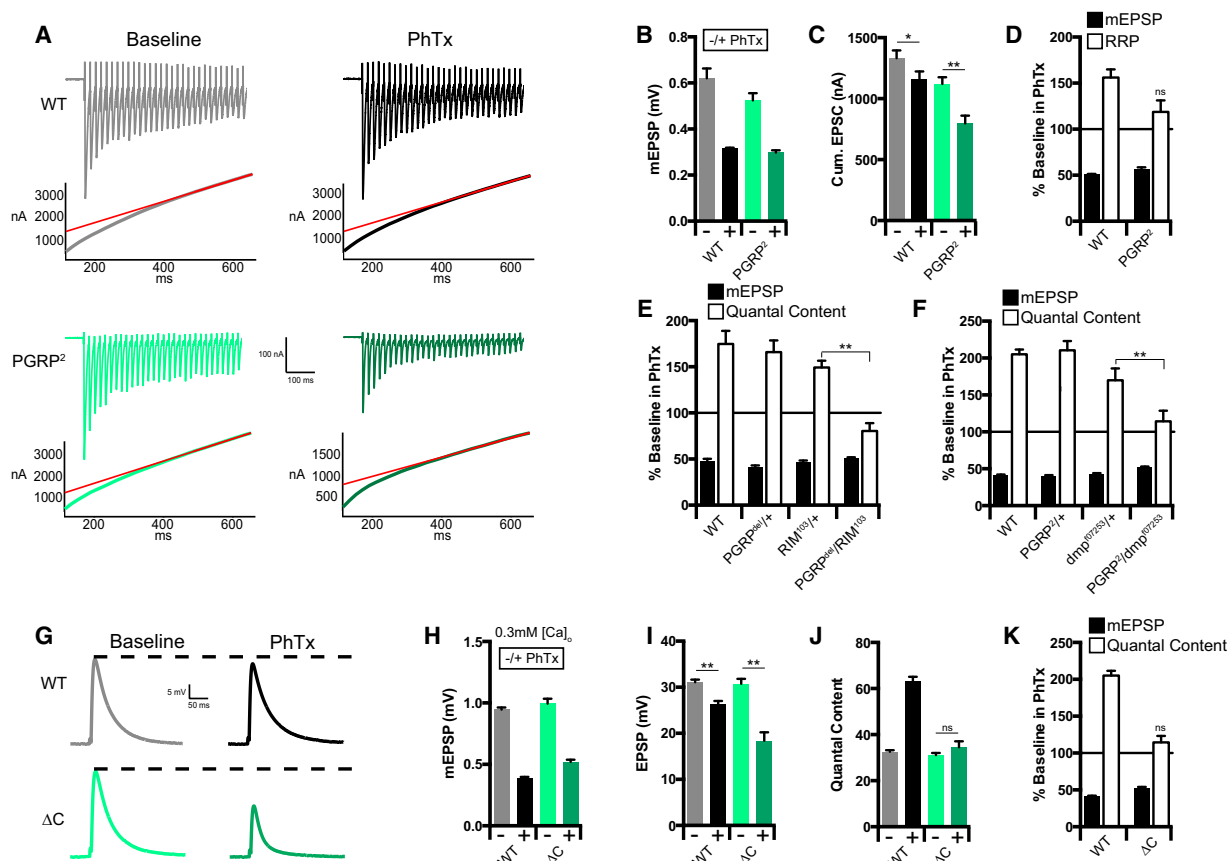
Figure 4. Loss of PGRP-LC Blocks the Homeostatic Expansion of the Readily Releasable Vesicle Pool

(A) Example EPSC traces (top) and cumulative EPSC amplitudes (bottom) minus and plus PhTx for WT and *PGRP^2*. Experiment used 60-Hz stimulation (30 stimuli) in 3 mM [Ca^{2+}]$_e$. The line fit to cumulative EPSC data that was back-extrapolated to time 0 is shown in red.

(B) Average data for mEPSP amplitudes for the indicated experiments. PhTx application reduces amplitudes in all genotypes ($p < 0.01$).

(C) Cumulative EPSC amplitudes as in (B) (*$p < 0.05$; **$p < 0.01$).

(D) Average percent change in mEPSP amplitude and RRP size in PhTx. There is not a significant PhTx-dependent increase in RRP in the *PGRP^2* mutant ($p > 0.05$). The percent change comparing WT and *PGRP^2* is significantly different ($p < 0.05$).

(E) Average percent change in mEPSP and quantal content caused by PhTx application to the indicated genotypes. *PGRP^{del}*/+ heterozygous mutants have normal presynaptic homeostasis compared to wild-type ($p > 0.2$). There is significant presynaptic homeostasis in *rim*/+ (± PhTx, $p < 0.01$) but a slight suppression of presynaptic homeostasis in *rim*/+ compared to wild-type ($p < 0.05$). The double heterozygous condition *PGRP^{del}*/+ ; *rim*/+ completely blocks homeostatic plasticity ($p > 0.5$).

(F) Average changes as in (E). Significant presynaptic homeostasis is observed in *PGRP^2*/+ and *dmp*/+ heterozygous animals ($p < 0.05$). No significant homeostasis is observed in the double heterozygous condition ($p > 0.05$).

(G) Example EPSP traces minus and plus PhTx for WT and ΔC rescue at 0.3mM [Ca^{2+}]$_e$. ΔC denotes expression of *UAS-PGRP* truncated transgene, see Supplemental Experimental Procedures.

(H) mEPSP amplitudes as in (B), all PhTx changes are significant ($p < 0.01$).

(I) Average data for EPSP amplitudes in the indicated experiments (**$p < 0.01$).

(J) Average data for quantal content in the indicated genotypes.

(K) Average changes as in (E). Student's t test.

consistent with quantification of baseline neurotransmission at a ten-fold lower [Ca^{2+}]$_e$ (Figures 1D–1F). Following application of PhTx, there is a statistically significant, homeostatic potentiation of the RRP in wild-type (Figure 4D; $p < 0.01$). However, there is no significant increase of the RRP in the *PGRP^2* mutant ($p > 0.1$). We conclude that PGRP-LC is necessary for the homeostatic expansion of the RRP. We also note that the failure to expand the RRP does not correlate with altered vesicle number or distribution (Figure 3).

We quantified two additional parameters based on delivery of stimulus trains to the NMJ (Figure 4A): (1) short-term synaptic depression during the stimulus train, and (2) an estimate of release probability at the onset of the stimulus train (P$_{train}$; estimated by dividing the first EPSC amplitude in the stimulus train by cumulative EPSC) (Thanawala and Regehr, 2013). Short-term depression was calculated as the percent change in EPSC amplitude comparing EPSC1 and EPSC4 of a stimulus train. There was no difference comparing wild-type in the

presence or absence of PhTx with $PGRP^2$ in the presence or absence of PhTx (p > 0.05; WT (−PhTx) = 59.7 ± 1.7%, WT(+PhTx) = 54.6 ± 1.5%, $PGRP^2$(−PhTx) = 57.8 ± 2.4%, $PGRP^2$ (+PhTx) = 55.0 ± 2.3%). There is a small, but significant, 2.7% increase in P_{train} comparing wild-type and $PGRP^2$ (p < 0.05). However, application of PhTx does not alter P_{train} in either genotype (wt = 0.25 ± 0.07; wt+PhTx = 0.26 ± 0.09; $PGRP^2$ = 0.27 ± 0.01; $PGRP^2$ + PhTx = 0.28 ± 0.01). These data argue that the primary consequence of the $PGRP^2$ mutation is not a change in presynaptic release probability, but rather the elimination of a homeostatic expansion of the RRP.

Finally, we sought additional genetic evidence that PGRP acts in concert with other established mechanisms that are responsible for the homeostatic potentiation of the RRP during presynaptic homeostasis. It was previously demonstrated that the homeostatic potentiation of the RRP requires the presence of the active-zone associated scaffold RIM (Rab3 Interacting Molecule) (Davis and Müller, 2015). Therefore, we tested whether $PGRP^{del}$ interacts, genetically, with a null mutation in rim. The heterozygous mutation in PGRP-LC ($PGRP^{del}$/+) shows robust homeostatic potentiation (Figure 4E). We then examined a heterozygous null mutation in rim (rim^{103}/+) and, again, document robust presynaptic homeostasis, although somewhat less pronounced compared to that observed in $PGRP^{del}$ alone (Figure 4E). But, when we examine the double heterozygous mutant combination we find a block of presynaptic homeostasis (p > 0.05; Figure 4E). This result underscores the likelihood PGRP is required for the RIM-dependent modulation of the RRP during presynaptic homeostasis. It is possible that RIM could be a target of signaling downstream of PGRP-LCx activation, but this remains speculative without additional information about the signaling system that acts downstream of PGRP-LC within the presynaptic terminal.

PGRP Interacts Genetically with Multiplexin/Endostatin

In innate immunity, PGRP-LCx recognizes a peptidoglycan sequence presented by invading bacteria. One interesting possibility is that PGRP-LCx might recognize molecular patterns caused by the cleavage of extracellular matrix proteins at the nerve terminal. Regulated proteolysis of synaptic proteins is becoming increasingly apparent as a mode of inter-cellular signaling (Peixoto et al., 2012). We recently demonstrated that Endostatin, a small peptide that is released following proteolytic cleavage of the Drosophila Collagen VIII homolog Multiplexin, is necessary for presynaptic homeostasis (Wang et al., 2014). Therefore, we tested whether PGRP interacts, genetically, with a strong loss of function mutation in multiplexin. The heterozygous mutation in PGRP-LC ($PGRP^2$/+) shows robust homeostatic potentiation, as does a heterozygous mutation in multiplexin (dmp^{f07253}/+) (Figure 4F). But, when we examine the double heterozygous mutant combination we find a complete block of presynaptic homeostasis (p > 0.05) (Figure 4F). These data are consistent with a model in which Endostatin could function as a ligand for PGRP, but additional work is necessary to prove that this interaction occurs biochemically.

If PGRP-LC functions as a receptor for intercellular signaling necessary for presynaptic homeostasis, then expression of PGRP-LC lacking the extracellular PGRP domain (termed $PGRP^{\Delta C}$) should fail to rescue presynaptic homeostasis in the $PGRP^{del}$ mutant background. We generated a Venus tagged PGRP-LC deletion transgene (UAS-$PGRP^{\Delta C}$-Venus), modeling it after a similarly truncated receptor that was previously used to study innate immunity (Choe et al., 2005). First, we document that neuronally expressed UAS-$PGRP^{\Delta C}$-Venus localizes to the presynaptic terminal (Figure S4). Then we show that UAS-$PGRP^{\Delta C}$-Venus fails to rescue presynaptic homeostatic plasticity (Figures 4G–4K). There is no effect of UAS-$PGRP^{\Delta C}$-Venus expression on baseline transmission and homeostatic plasticity remains blocked in the $PGRP^{del}$ mutant background. Importantly, the UAS-$PGRP^{\Delta C}$-Venus transgene is similar to the predicted protein truncation caused by the $PGRP^2$ mutation, which resides at the start of the PGRP domain. This result, therefore, further validates the functional blockade of presynaptic homeostasis observed in the $PGRP^2$ mutant.

DISCUSSION

Innate immune signaling has been found to participate in neural development and disease including a role for the C1q component of the complement cascade (Stevens et al., 2007), Toll-like receptor signaling (Okun et al., 2011), and tumor necrosis factor signaling (Stellwagen and Malenka, 2006). In these examples, however, the innate immune response is induced within microglia or astrocytes. Far less clear is the role of innate immune signaling within neurons, either centrally or peripherally, although there are clear examples of downstream signaling components such as Rel and NFκB having important functions during learning related neural plasticity (Meffert et al., 2003; Ahn et al., 2008). Here we place the innate immune receptor, PGRP-LC at the presynaptic terminal of Drosophila motoneurons and demonstrate that this receptor is essential for robust presynaptic homeostatic plasticity. We speculate, based on our data, that PGRP-LC could function as a receptor for the long-sought retrograde signal that mediates homeostatic signaling from muscle to nerve.

A Model for Innate Immune Signaling: PGRP-LC as a Feed-Forward Signaling Switch for Homeostatic Plasticity

A distinguishing feature of presynaptic homeostatic plasticity is that it can be both rapidly induced and sustained for prolonged periods (Davis and Müller, 2015). It is possible that innate immune signaling activity is adjusted to fit the requirements of presynaptic homeostasis in motoneurons. This would be consistent with the established diversity of innate immune signaling functions during development such as dorso-ventral patterning (Anderson et al., 1985). Alternatively, the induction of innate immune signaling might serve a unique function during presynaptic homeostasis. Many homeostatic signaling systems incorporate feed-forward signaling elements (Davis and Müller, 2015). By analogy, PGRP-LCx could function as a feed-forward signaling receptor that acts more like a "switch" to enable presynaptic homeostasis in the nerve terminal. The accuracy of the homeostatic response would be determined by other signaling elements. In favor of this idea, the induction of innate immune signaling is rapid, occurring in seconds to minutes (Stöven et al., 2000), and can be maintained for the duration of an inducing stimulus,

consistent with recent evidence that presynaptic homeostasis is rapidly and continually induced at synapses in the presence of a persistent postsynaptic perturbation (Younger et al., 2013). Finally, it remains formally possible that PGRP-dependent signaling is a permissive signal that allows expression presynaptic homeostasis (see supplemental discussion).

EXPERIMENTAL PROCEDURES

See the Supplemental Experimental Procedures for complete anatomical, electrophysiological, and genetic methods.

SUPPLEMENTAL INFORMATION

Supplemental information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.10.049.

AUTHOR CONTRIBUTIONS

N.H., D.J.B., and R.F. conducted experiments and analyses. N.H., D.J.B., and G.W.D. designed experiments and wrote the paper. D.K.D. conducted the genetic screen implicating PGRP-LC.

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