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PI3K Signaling and Stat92E Converge to Modulate Glial Responsiveness to Axonal Injury

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Abstract

Glial cells are extraordinarily sensitive to neuronal injury but mechanisms by which glia establish competence to respond to injury, continuously gauge neuronal health, and rapidly activate reactive responses remain poorly defined. Here, we show glial PI3K signaling in the uninjured brain regulates baseline levels of Draper, a receptor essential for Drosophila glia to sense and respond to axonal injury. After injury, Draper levels are up-regulated through a Stat92E-modulated, injury-responsive enhancer element within the draper gene. Surprisingly, canonical JAK/STAT signaling does not regulate draper expression. Rather, we find injury-induced draper activation is downstream of the Draper/Src42a/Shark/Rac1 engulfment signaling pathway. Thus, PI3K signaling and Stat92E are critical in vivo regulators of glial responsiveness to axonal injury. We provide evidence for a positive auto-regulatory mechanism whereby signaling through the injury-responsive Draper receptor leads to Stat92E-dependent, transcriptional activation of the draper gene. We propose that Drosophila glia use this auto-regulatory loop as a mechanism to adjust their reactive state following injury.

Introduction

Glial cells are extraordinarily sensitive to disruptions in central nervous system (CNS) homeostasis and exhibit an impressive ability to respond to a diversity of neural injuries including hypoxia, chemical insults, and mechanical injury (e.g., axotomy or traumatic brain injury) [1–3]. During injury responses, glia exhibit robust changes in gene expression, migrate or extend membranes to sites of trauma, and phagocytose degenerating neuronal debris. Glial reactive responses after injury can be beneficial and promote recovery. For example, glial clearance of degenerating neuronal debris is thought to suppress nervous system inflammation and facilitate remyelination [4–8]. However, glia can also exacerbate damage in the CNS by driving inflammation through the release of pro-inflammatory cytokines and actively destroying healthy cells [9–14]. Whether reactive gliosis is ultimately more beneficial or harmful to the nervous system remains an open question and is likely context dependent. However, mounting evidence points to reactive glial cells as an excellent target for therapeutically modifying CNS disorders [15].

Reactive glial cells have been studied in a variety of neurodegenerative diseases and injury models but surprisingly little is known about how glial sensitivity to neuronal health is initially established in the healthy brain, or how glial perception of neuron-derived “injury signals” is translated into dynamic reactive responses after trauma. A wealth of studies indicate that reactive gliosis is not an all-or-none response, but rather includes a wide range of graded responses that correlate with the severity of nervous system injury [15]. The ability of glia to “measure” the severity of trauma in the nervous system and respond accordingly implies a tight functional coupling between signaling pathways sensing neural injury and those executing glial reactive responses [16]. In some cases reactive glia can modulate the intensity of reactive gliosis in an autocrine way. For example, focal denervation leads to activation and release of endothelin-1 (ET-1) from astrocytes, which in turn can signal back to astrocytes (through ET-1 receptors) and further enhance astrocyte cellular hypertrophy, proliferation, and GFAP expression [17–22]. However, the initial injury signal which activates ET-1 expression remains to be identified. An intriguing possibility is that so-called “eat me” cues on degenerating neuronal debris directly activate receptors on glial cells, which in turn modulate initiation of reactive gliosis, but evidence for such molecular regulation is lacking.

We have previously shown that Draper, a glial-expressed immunoreceptor-like molecule, is a key surface receptor required for glial engulfment of degenerating axons after axotomy in the adult Drosophila brain [23–25]. Draper is the Drosophila ortholog of CED-1, an engulfment receptor that is essential for engulfment of cell corpses in Caenorhabditis elegans [26]. Within hours after axotomy of Drosophila antennal olfactory receptor neuron (ORN) axons, Draper protein, and mRNA levels are dramatically increased in glia surrounding degenerating axons. Glial mem-
branes are then recruited to severed axons, glia phagocytose axonal debris, and ultimately glia return to a resting state [24,27]. Loss of Draper function blocks all glial morphological and molecular responses to axonal injury and axonal debris lingers in the brain for the life of the animal. Similar phenotypes have been observed when the components of the Src-family kinase signaling cascade that acts downstream of Draper (i.e., Shark and Src42a) are eliminated specifically from glial cells [25]. These data argue that Draper acts very early in the activation of Draper ligands present on degenerating axonal debris, perhaps even in the recognition of axonal debris, and ultimately glia return to a resting state [24,27].

To further explore the cellular basis for this delayed axon clearance phenotype, we assayed glial expression of the engulfment receptor Draper. Surprisingly, glial knockdown of p38α, raptor, or pdk1—key components of the phosphoinositide 3-kinase (PI3K) signaling pathway—led to a decrease in glial clearance of axonal debris 5 days after axotomy (Figure 1A and 1B; Data S1). Similar results were found when we drove glial expression of a dominant-negative version of PI3K92e (UAS-p38α, raptor, or pdk1; Figure S1; Data S2). We note that while axon clearance was delayed in these backgrounds, ultimately all axonal debris was cleared from the brain 7–10 days after axotomy (unpublished data), indicating that glia exhibited a delay in clearance rather than a blockade.

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Results

Glia P3K Signaling Modulates Levels of the Engagement Receptor Draper in the Healthy Brain

In order to identify new glial engulfment genes we performed an in vivo RNAi screen using a previously established assay [24]. Briefly, ~300 candidate engulfment genes were knocked down specifically in glia by driving UAS-regulated RNAi constructs with the glial specific repo-Gal4 driver. To assay the ability of glia to clear degenerating axonal debris we labeled a subset of maxillary palp ORNs with green fluorescent protein (GFP), severed the axons by surgically removing the maxillary palps, and assayed for clearance of GFP-labeled ORN axonal debris at various time-points. Interestingly, we found glial-specific knockdown of p38α, raptor, or pdk1—key components of the phosphoinositide 3-kinase (PI3K) signaling pathway—led to a decrease in glial clearance of axonal debris 5 days after axotomy (Figure 1A and 1B; Data S1). Similar results were found when we drove glial expression of a dominant-negative version of PI3K92e (UAS-p38α, raptor, or pdk1; Figure S1; Data S2). We note that while axon clearance was delayed in these backgrounds, ultimately all axonal debris was cleared from the brain 7–10 days after axotomy (unpublished data), indicating that glia exhibited a delay in clearance rather than a blockade.

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basis of the consistency in results among the different components of the pathway we favor the notion that basal levels of Draper (i.e., those present in the healthy brain) are regulated by PI3K signaling and injury-induced up-regulation of Draper is regulated by alternate signaling pathways.

The Draper Locus Contains an Enhancer Element That Is Responsive to Axonal Injury through Stat92E

Our observations that basal and injury induced Draper expression were controlled by distinct molecular pathways prompted us to attempt to identify draper gene enhancer elements responsible for establishing basal levels of draper expression in adult brain glia, and/or increasing draper expression specifically after ORN axotomy. We focused our search on an ~40 kb region centered around the draper locus (Figure 2A). We cloned nine different potential draper enhancer elements (termed dee2-dee10) from 5', intronic, or 3' regions of the draper gene into the Gal4-based pBGW vector [30] and inserted these elements into identical genomic locations (Figure 2A). Each dee-Gal4 line was then used to drive two copies of UAS-mCD8::GFP in vivo and expression patterns were examined in the adult brain before and after injury. No expression in ensheathing or cortex glia was observed with any of the enhancer element lines in the healthy, uninjured brain (unpublished data). We therefore failed to identify any single enhancer element that was capable of driving glial expression of reporters in the adult brain in a pattern similar to endogenous Draper protein. This observation suggests that PI3K-dependent regulation of Draper levels might be governed by an enhancer element some distance from the draper gene, requires the convergent activity of multiple enhancers along the draper gene, or could be controlled through post-transcriptional mechanisms.
To determine whether any of these potential DEEs were responsive to axotomy, we ablated antennae or maxillary palps and assayed reporter activation in glia one day after injury. We did not observe glial expression after axonal injury with dee2-6- or dee8-10-Gal4 lines (unpublished data). However, one day after antennal ORN axotomy, we observed a striking increase in glial expression of mCD8::GFP in the dee7-Gal4 reporter background, and GFP levels were further increased four days after antennal ablation (Figure 2B). We note that in uninjured animals we observed low level expression of this element in astrocyte-like glia, which were randomly distributed in the neuropil (Figure 2B) but it did not drive GFP expression prior to axon injury in ensheathing or cortex glia, those adult brain glia which normally express Draper [23]. Interestingly, not only was injury-induced reporter expression strong in ensheathing glia surrounding the antennal lobe—those glia that normally engulf degenerating ORN axons [23]—but the reporter expression also increased robustly in cortex glia throughout the brain (Figure 2B and 2D). This widespread activation of the reporter supports the notion that glia, even at locations distant from the injury site, can respond molecularly to axonal damage.

Notably, we found the activation of the dee7-Gal4 element appeared to scale with the severity of the axonal injury: when we ablated one maxillary palp (~60 ORNs), two maxillary palps (~120 ORNs), one antenna (~600 ORNs), or both antennae (~1,200 ORNs), we observed a correlated increase in dee7-Gal4-driven mCD8::GFP (Figure 2D). We further note that ablation of maxillary palps, whose axons are found in the maxillary nerve and ventro-medial regions of the antennal lobe, resulted in a much reduced and more localized increase in dee7-Gal4-driven mCD8::GFP in cortex glia located in the ventral region of the antennal lobe (Figure 2D).

Sequence analysis of the 2619 bp dee7 element led to the discovery of three consensus Stat92E binding sites (TTC3n/4nGAA) [31], the sole member of the signal transducer and activator of transcription (STAT) family of molecules in Drosophila [31,32]. Of these three Stat92E sites, two were also present in the draper locus containing a Stat92E-regulated injury responsive element. (A) Schematic representation of the draper locus and regions used to generate DEEs 2–10. Blue box highlights dee7 region located in the first intron of the draper gene. Red lines indicate Stat92E binding sites in dee7. Asterisk indicates binding site in dee7mut containing two point mutations. (B) Single slice confocal images of antennal lobe regions; dee7-Gal4 or dee7mut-Gal4 driving two copies of UAS-mCD8::GFP (dee7>2XmCD8::GFP, dee7mut>2XmCD8::GFP). Uninjured, one day after antennal ablation and four days after antennal ablation are shown. Dashed circles outline antennal lobes, the site of injury. Asterisk indicates cortex glia responding to axotomy. (C) Quantification for (B), p-values were calculated using Student’s t test, n.s., not significant. Error bars represent SEM. (D) Single slice confocal images of antennal lobe regions stained with α-GFP and α-Repo; dee7-Gal4 was used to drive two copies of UAS-mCD8::GFP. Removal of one maxillary palp, two maxillary palps, one antenna, and two antennae are shown one day after injury.

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Glia1, which was not responsive to axonal injury (Figure 2A). We mutated the Stat92E binding site specific to the glia1-Gal4 element, integrated this glia1-Gal4 construct into the same genomic location used for the previously generated reporter lines, and examined its responsiveness to axonal injury. While baseline levels of mCD8::GFP expression were similar to glia1-Gal4, glia1-GAL4-exhibited an ~30%–40% decrease in the injury-induced expression of mCD8::GFP at both 1 and 4 days after axotomy (Figure 2B and 2C; Data S4). In contrast, simultaneous mutation of the two other Stat92E binding sites within the glia1 element had no effect on transcriptional activation of the glia1-Gal4 reporter (Figure S2A–S2C; Data S5). From these data we conclude that glia1 contains a glial transcriptional regulatory element that is responsive to axonal injury, and our data suggest that at least one Stat92E binding site is required for maximal activation of this element after axotomy.

Glia1 Loss of STAT92E Suppresses Clearance of Axonal Debris and Eliminates Expression of Draper

We next sought to determine whether Stat92E was required for modulating glial responses to axonal injury and clearance of degenerating axonal debris. Stat92E function was knocked down specifically in glia by expressing a UAS-stat92eRNAi construct with the pan-glial repo-Gal4 driver. In controls the majority of GFP+ maxillary palp ORN axon material was cleared from the brain 5 days after injury (Figure 3A–3C; Data S6). However, in stat92eRNAi animals, GFP+ axonal debris persisted 5 days after injury (Figure 3A–3C; Data S6). In contrast to depletion of the PI3K signaling cascade, glial stat92eRNAi suppressed clearance of antennal ORN axons even 15 days after injury (Figure 3A–3C; Data S6), indicating an essential requirement for STAT92E in glial clearance of axonal debris. We were able to confirm the UAS-Stat92eRNAi line efficiently targets stat92e as glial co-expression of a GFP-tagged stat92e molecule with the stat92eRNAi construct eliminated all Stat92e-GFP expression compared to controls (Figure S3B). These observations argue that Stat92E is an important regulator of glial engulfment activity in the adult brain.

On the basis of our identification of a Stat92E-dependent injury-responsive element in the draper gene, we predicted Stat92E would modulate glial phagocytic activity by regulating draper expression after axotomy. Draper is normally expressed in ensheathing and cortex glia throughout the uninjured brain and is up-regulated around the antennal lobe after antennal ORN axotomy (Figure 3D). Glial-specific knockdown of Stat92E led to nearly undetectable levels of Draper expression in the adult brain even prior to injury (Figure 3D and 3E; Data S7). We confirmed this widespread loss of Draper by performing Western blots on dissected adult brains from control, stat92eRNAi, and draperRNAi animals (Figures 3F, 3G, and S3; Data S8). In contrast to loss of PI3K signaling, knockdown of Stat92E in glia was also sufficient to potently suppress glial activation of draper after axotomy: stat92eRNAi animals exhibited no detectable increase in Draper levels after antennal ablation compared to controls. In addition, while Draper was localized specifically to severed ORN axons after maxillary palp ablation in controls, we found no detectable Draper localization to severed axons in stat92eRNAi animals (Figure 3D and 3E; Data S7). These data suggest that Stat92E is required to establish normal basal levels of Draper expression in glia, and dynamically regulates increased Draper levels as glia respond to axotomy.

Stat92E Transcriptional Activity Is Transiently Upregulated in Glia after Axonal Injury

To explore the dynamics of Stat92E signaling in adult brain glia we examined the expression patterns of transcriptional reporters for Stat92E activity [43]. These reporters have been previously shown to accurately reflect Stat92E transcriptional activity during development as well as in the adult [43–46]. We first used the 10xStat92eGFP, which harbors ten Stat92E binding sites driving expression of enhanced GFP. In co-stains with Dox-Draper and Dox-Repo (a glial nuclear marker) we observed quite specific glial expression of the Stat92E reporter in uninjured controls (Figure 4A). Moreover, after ablation of antennae we found strong GFP labeling of antennal lobe glia, and the GFP signal completely overlapped with Draper (Figure 4A). After ablation of maxillary
palps we found GFP signals co-localized with Draper in glomeruli housing severed axons (Figure 4A). These data argue that Stat92E is active at a transcriptional level in adult brain glia.

The GFP driven by the 10XStat92E-GFP reporter is quite stable and can perdure in cells for 20 hours after activation, which precludes our use of this construct to examine dynamic changes in Stat92E transcriptional activity. We therefore used a second reporter, 10XStat92E-dGFP, which drives the expression of a rapidly degraded, destabilized GFP (dGFP), thereby allowing for increased temporal resolution of Stat92E activity. Prior to injury, we were unable to detect any activation of this Stat92E transcriptional reporter in adult brains (Figure 4B). However, beginning ~16 hours after antennal ablation we detected 10XStat92E-dGFP expression in cells surrounding the antennal lobe (Figure 4B, arrows). GFP intensity peaked at ~24 hours after antennal ablation and disappeared by 48 hours after axotomy (Figure S6).

To confirm that activation of the 10XStat92E-dGFP reporter after axotomy is Stat92E-dependent and glial specific, we knocked down Stat92e specifically in glia, severed axons, and assayed 10XStat92E-dGFP activity. We found that glial-specific knock-down of Stat92E completely suppressed the axotomy-induced activation of the 10XStat92E-dGFP transcriptional reporter (Figure 4B). Consistent with our observations of widespread activation of the deet7-Gal4 driver in glia throughout the brain after injury, we also found that axonal injury led to broad...
activation of the 10XStat92E-dGFP reporter in glial cells (Figure 4C) and expression of these two reporters colocalize in glial cells after axotomy (Figure 4D), indicating they are active in the same cells. Together these data indicate that Stat92E can transiently increase the transcriptional activation of target genes in glial cells throughout the brain after axonal injury, with the strongest increases in target gene activation occurring adjacent to injury sites.

Stat92E-Dependent Activation of Draper Does Not Require Components of the Canonical JAK/STAT Signaling Pathway

STAT activity is generally regulated by the JAK signaling platform, and this pathway is conserved in all higher metazoans. The Drosophila JAK/STAT signaling pathway consists of a single JAK molecule, Hopscotch (hop) [47], and the cytokine like receptor Domeless (Dome) [48]. To determine if Stat92E-dependent activation of draper is mediated through canonical JAK/STAT signaling we drove RNAi constructs targeted against hop, and a dominant negative Domeless molecule, DomelessACVT [49], in glial cells, and assayed Draper expression and clearance of severed axons. Surprisingly, in each of these backgrounds we found Draper levels were similar to control animals and axons were efficiently cleared 5 days after injury (Figure S7A and S7B; Data S11). Reciprocally, we found a gain-of-function allele of hop, hopTUM, which has been shown in numerous assays to activate Stat92E transcriptional activity [43,50,51], failed to activate the 10XStat92E-dGFP reporter in adult brain glia (Figure S7C). Notably, expression of an activated version of Stat92E, Stat92EDNDC [52], led to strong activation of the 10XStat92E-dGFP reporter (Figure S7C). Stat92EDNDC has previously been shown to require phosphorylation at Y711 for activation [52]. We therefore expressed a version of Stat92EDNDC with a Y711F mutation in glia, and found it was insufficient for activation of the 10XStat92E-dGFP reporter (Figure S7C). Together these data argue that while phosphorylated Stat92E mediates activation of Stat92E reporters in glia, canonical JAK/STAT signaling is neither necessary nor sufficient to activate Stat92E-dependent glial responses to axon injury.
Signaling through the Draper Receptor Activates Stat92E-Dependent Transcriptional Up-regulation of draper

To date Domeless is the only receptor known to positively regulate Stat92E signaling under normal physiological conditions [48,53,54]. Considering we were unable to demonstrate a role for Domeless signaling in activation of Draper after injury, we sought to determine whether Draper itself might have a role in modulating glial gene expression. We assayed 10XStat92E-dGFP activation after axonal injury in draperD5 null mutants and, intriguingly, loss of Draper resulted in a complete lack of 10XStat92E-dGFP activation after axotomy (Figure 5A). Consistent with a direct requirement for Draper signaling in STAT92E-dependent activation of draper after axotomy, we also found a lack of activation of the dee7-Gal4 reporter after axonal injury in draperD5 animals (Figure 5B). These data demonstrate a direct role for the Draper receptor in modulating Stat92E-dependent changes in glial gene expression following local axonal injury.

We next explored whether other identified components of the Draper signaling pathway modulate Stat92E transcriptional activity after axon injury. Draper is thought to be phosphorylated by Src42a upon activation, initiating binding of the non-receptor tyrosine kinase Shark, which together, with Rac1 and dCed-6, promote engulfment [23,25]. Interestingly, we found that glial-specific knockdown of Shark, Src42a, or Rac1 blocked injury-induced activation of the 10XSTAT92E-dGFP transcriptional reporter (Figures 5A and S8A). This finding was confirmed using multiple RNAi lines and/or dominant negative alleles for each gene in the pathway (Figure S8A). However, while RNAi mediated knockdown of dCed-6 efficiently eliminated dCed-6 immunoreactivity (Figure S8B), axotomy-induced activation of 10XSTAT92E-dGFP was still detectable in dCed-6RNAi animals (Figure 5A). Thus Draper, Src42a, Shark, and Rac1, but not dCed-6, are essential for Stat92E-dependent activation of transcriptional targets in glia responding to axonal injury. These are the first data that demonstrate a functional divergence between dCed-6 and Draper/Src42a/Shark function during engulfment signaling.

Figure 5. Injury-induced activation of Stat92E is downstream of the Draper signaling cascade. (A–C) Single slice confocal images; (A) Glial-specific knockdown of components of the Draper pathway in a 10XStat92E-dGFP reporter background one day after antennal ORN axotomy; control (10XStat92E-dGFP/+; draperD5), sharkRNAi (sharkRNAi/10XStat92E-dGFP; repo-Gal4/+), rac1N17 (10XStat92E-dGFP/+; repo-Gal4/rac1N17), src42aRNAi (src42aRNAi/10XStat92E-dGFP; repo-Gal4/+), and dCed6RNAi (dCed6RNAi/10XStat92E-dGFP; repo-Gal4/+). (B) dee7-Gal4 activity in control (dee7-Gal4,UAS-mCD8::GFP/+) and draperD5 (dee7-Gal4,UAS-mCD8::GFP/+; draperD5) backgrounds. Uninjured, one day after antennal injury, and one day after maxillary palp injury are shown. Dotted circles indicate antennal lobes. (C) Glial-specific activation of components of the draper pathway in a 10XStat92E-dGFP reporter background; rac1V12 (10XStat92E-dGFP/+; repo-Gal4/rac1V12), and src42aRNAi (10XStat92E-dGFP/+; repo-Gal4/src42aRNAi).

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Since Src42a is known to signal downstream of Draper and has been shown to mediate Stat92E signaling in multiple contexts [25,35,56], we sought to determine whether Src42a activity was sufficient to activate Stat92E transcriptional reporters. Indeed, expression of a constitutively active Src42a molecule (Src42aCA) resulted in robust 10XStat92E-dGFP reporter activation throughout brain [Figure 5C, refer to Figure 4B for control]. Based on our previous genetic studies it appears that Src42a acts through modulating Rac1 activity. We therefore drove gial expression of a constitutively active Rac1 molecule (Rac1VT) and found this also robustly activated the 10XStat92E-dGFP reporter throughout the adult brain (Figure 5C, refer to Figure 4B for control). These data are consistent with the notion that Src42a acts in glia downstream of Draper to activate Stat92E signaling through Rac1 after axonal injury.

In summary, our data suggest that axonal injury leads to Stat92E-dependent transcriptional up-regulation of draper through a Draper/Src42a/Shark/Rac1-dependent signaling cascade. While activation of Stat92E, Src42A, or Rac1 led to robust activation of the 10XStat92E reporter, none were sufficient to increase basal Draper levels (unpublished data), suggesting additional transcriptional inputs are required for injury-induced transcriptional activation of draper.

### Draper Is a Primary Target for Stat92E during Glial Clearance of Degenerating Axons

Together our data argue that draper transcription is regulated by Stat92E after axotomy. Stat92E may regulate many genes after axotomy, or only a few critical targets essential for engulfment. To further explore the relationship between Stat92E and the draper gene, we over-expressed Draper in a stat92eRNAi background to determine whether resupplying Draper was sufficient to overcome the engulfment deficit observed in Stat92E knockdown animals. Remarkably, over-expression of Draper in stat92eRNAi animals led to a near complete rescue of the engulfment defect (Figure 6A and 6B; Data S12). As an alternate method to increase Draper levels we also drove gial expression of the activated PI3K molecule, PI3K92eCAAX, in the presence of stat92eRNAi or control dprerNAI animals. We found that activation of PI3K signaling was sufficient to increase Draper levels [Figures 6E, 6F, and S9; Data S13] and rescue engulfment defects in stat92eRNAi backgrounds [Figure 6C and 6D; Data S14].

Consistent with the ability of PI3K signaling to drive Draper expression independently of Stat92E, we also found that activated PI3K signaling was not sufficient to activate expression of the 10XStat92edGFP reporter [Figures 6E, 6F, and S9]. However, PI3K signaling appeared to sensitize brain glia to injury as gial PI3K92eCAAX enhanced 10XStat92E-dGFP activation after antennal ablation compared to controls (Figure S9), perhaps through driving increased Draper levels. From these data we conclude that draper is a critical target of Stat92E during gial responses to axonal injury. In addition, our data argue that activated PI3K signaling results in Stat92E-independent increases in Draper levels.

### Discussion

#### PI3K Signaling and Stat92E Converge to Regulate Draper Expression in the Adult Brain

Our work identifies two new signaling pathways important for regulating gial engulfment function in vivo. First, we show that the PI3K signaling pathway modulates Draper levels in the healthy, uninjured brain as reduced PI3K signaling leads to dramatically decreased gial Draper and constitutive activation of PI3K signaling leads to Draper up-regulation. However, depletion of PI3K signaling components delays but does not completely block the ability of glial cells to up-regulate Draper levels or clear axonal debris in response to axotomy, perhaps due to positive signaling through the small amount of Draper that remains under these conditions. Second, we identify Stat92E as a potent regulator of both basal and injury induced Draper levels in adult brain glia. Loss of Stat92E in mature glia results in significantly decreased draper transcript levels and a near complete loss of Draper protein in the uninjured brain.

What is the relationship between PI3K signaling and Stat92E in regulating basal levels of Draper in the healthy brain? On the basis of our analysis of draper mRNA levels we speculate that STAT92E regulates draper at least in part at the transcriptional level. It appears unlikely that Stat92E functions downstream of PI3K signaling since loss of Stat92E in a constitutively activated PI3K background did not suppress PI3K-dependent increases in Draper levels, and gain-of-function PI3K was sufficient to rescue reduced Draper levels and engulfment defects in stat92eRNAI animals. STAT molecules have been shown to be capable of acting as adaptor molecules for receptors that ultimately lead to activation of PI3K signaling [57–59], therefore Stat92E could function in adult brain glia upstream of PI3K signaling in a non-transcriptional manner to regulate basal levels of Draper. Finally, Stat92E might transcriptionally regulate key molecules required for activation or execution of PI3K signaling. In such a situation Stat92E and PI3K signaling could modulate Draper levels through parallel mechanisms, but both would be required for expression of appropriate levels of Draper in the adult brain.

A role for PI3K signaling in phagocytic function appears to be conserved from Drosophila to mammals. Activation of PI3K signaling occurs downstream of the Fcγ receptor [60–63]. This finding is intriguing in light of the fact that Draper appears to act as an ancient immunoreceptor, activating a Src family kinase signaling cascade through ITAM/ITIM-dependent mechanisms [25,27]. PI3K signaling is required for efficient formation of the phagocytic cup in macrophages [61,64,65], and loss of PI3K has been reported to lead to a delay in clearance of cell corpses and myelin [62,66,67]. This latter finding argues for conservation of a requirement for PI3K signaling even among glial cell types in Drosophila and mammals. In our study we also find a delay of axonal clearance when PI3K signaling is suppressed, but ultimately axons are cleared. We speculate this phenotype is a result of decreased expression of Draper since we can fully rescue loss of PI3K signaling phenotypes by resupplying Draper. This observation argues strongly that a key role for PI3K signaling in phagocytic function is the regulation of engulfment factors, and in particular Draper.

### Axonal Injury Causes Widespread Activation of Glial Cells throughout the Drosophila Brain

Ablation of Drosophila third antennal segments leads to axotomy of all antennal ORNs and Wallerian degeneration of ~1,200 ORN axons and their synapses within the antennal lobe of the brain. We previously observed a robust increase in Draper levels in glia surrounding the antennal lobe after antennal injury (but not elsewhere) and proposed that Draper increases were only local [23,24]. Using the dee7-Gal4 and 10XStat92E-dGFP reporters we have now shown that severe axonal injury (i.e., axotomy of nearly all ORNs) in the antennal lobe is sufficient to induce a transcriptional response in glial cells throughout the entire Drosophila brain (Figures 2B–2D, 4B–4D, 5A, and 5B). How can glial cells in distant parts of the brain receive signals that an injury has occurred? We can envision at
least two scenarios to explain this observation. First, severed axons could release signals that act at a long distance to activate glia. If so, the reception of this axon-derived signal by glia would be Draper-dependent, since we show that Draper signaling is required for activation of both the *dee7-Gal4* and *10XStat92E-dGFP* reporters in glia after injury. Alternatively, spreading of an injury signal throughout the brain could be accomplished by glial to glial signaling. Astrocytes are indeed heavily coupled in mammals [68] and the same could be true in *Drosophila* adult brain glia where signals could spread through gap junction-dependent mechanisms. In the future it will be very exciting to define the molecules that regulate the spreading of the injury signal to distant glial subtypes, and determine their functional role in brain recovery from trauma.

In mammals, it is widely accepted that reactive glial responses are graded according to the severity of the brain injury. Here we show that *Drosophila* glia also respond in a graded way to ORN injury: axotomy of a small number of ORN axons by maxillary palp ablation led to *dee7-Gal4* activity in a small subset of cells, while severing the majority of ORNs (85%) by antennal ablation led to a more dramatic increase in the activation of two separate reporters. We propose that relatively mild injuries promote signaling through the Draper pathway in a limited number of cells close to the site of injury while more severe injuries result in widespread activation of the Draper pathway in cells throughout the entire brain, even those distant from the injury site. Presumably up-regulation of engulfment factors enhances the ability of glia to clear neuronal debris (Figure 7). Such a scenario is likely to be implicated in the response of glial progenitors to axonal injury and neuronal death.

Figure 6. The Draper gene is a primary target of Stat92E after axonal injury. (A) Z-stack confocal images; OR85e* axons were labeled with GFP in control (OR85e-GFP/+;repo-gal4/+), stat92eRNAi (stat92eRNAi/OR85e-GFP;repo-Gal4/+), stat92eRNAi/UAS-drpr (stat92eRNAi/OR85e-GFP;repo-Gal4/UAS-drpr-I), and stat92eRNAi/UAS-mCherry (stat92eRNAi/OR85e-GFP;repo-Gal4/UAS-mCherry) backgrounds. Uninjured and 5 days after maxillary palp ablation are shown. (B) Quantification of GFP intensities in 85e* glomeruli from (A), *p*-values were calculated using one-way ANOVA followed by Tukey’s post hoc, n.s., not significant. Error bars represent SEM. (C) Z-stack confocal images of OR85e* axons labeled with GFP; controls (OR85e-GFP;repo-gal4/+), *stat92eRNAi* (UAS-stat92eCAAX/OR85e-GFP;repo-gal4/+), and *drprRNAi* (UAS-drpr/OR85e-GFP;repo-gal4/+;UAS-mCherry) backgrounds. Five days after maxillary palp ablation is shown. (D) Quantification of GFP intensities in 85e* glomeruli from (C), *p*-values were calculated using one-way ANOVA and Tukey’s post hoc analysis, n.s., not significant. Error bars represent SEM. (E) Western blot analysis of Draper protein levels in 3 adult central brain regions of controls (OR85e-GFP;Gal80DT/+;repo-gal4/+), and *UAS-pi3k92eCAAX* (OR85e-GFP;Gal80DT/+;UAS-Gal80DT;repo-gal4/+;UAS-pi3k92eCAAX), and *drprRNAi* (UAS-Gal80DT;UAS-drpr;repo-gal4/+;UAS-mCherry) backgrounds. α-Tubulin was used as a loading control. Full blot located in Figure S9. (F) Quantification of Draper protein levels shown on Western blot after normalization to α-Tubulin, *n* = 4, *p*-values were calculated using one-way ANOVA and Tukey’s post hoc analysis.

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mechanism whereby glial transcriptional responses are activated downstream of the very pathways that drive glial phagocytic activity would allow glia to directly modulate their engulfment capacity. Since it is likely that Draper ligands are present on engulfment targets, transcriptional activation of glial engulfment genes would ultimately be regulated by extracellular levels of "eat me" cues on degenerating axons.

Figure 7. Basal and injury-induced Draper expression are regulated through two distinct pathways. Basal levels of Draper expression are dependent upon the convergent activities of PI3K and Stat92E signaling. After injury Draper signals through the Src family kinase signaling cascade (Src and Shark), and Rac1, and transcriptionally activates its own expression in a Stat92E-dependent fashion. Increased levels of Draper (and perhaps other engulfment genes) likely enhance the ability of glia to engulf axonal debris.

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A Draper→Stat92E→draper Gene Auto-Regulatory Loop Increases Glial Envelopment Activity after Axotomy

We propose a novel injury-induced auto-regulatory loop whereby activation of Draper in glial cells responding to axonal injury leads to downstream signaling through Src42a/Shark/Rac1, and in turn Stat92E-dependent transcriptional activation of the draper locus. We provide multiple lines of evidence that activation of glial cells after local axotomy involves Stat92E-dependent transcriptional activation of draper: RNAi knockdown of Stat92E leads to a loss of injury-induced Draper expression, mutation of a single Stat92E binding site in the dee7 enhancer element leads to 40%–50% reduction in dee7-Gal4 activity upon injury, the 10XStat92E-dGFP transcriptional reporter is activated in glial cells upon injury, and activation of both 10XStat92E-dGFP and the dee7-Gal4 reporter are lost in draper mutant animals. We provide compelling evidence that the dee7 reporter activity resembles injury induced transcriptional activation of Draper: it expresses in the subtype of glia known to respond to injury and only after axotomy; it correlates with our data showing a larger increase in overall Draper up-regulation in the brain during the more severe antennal ablation; expression of the reporter is lost in drpr mutant animals; and its expression overlaps with the 10XStatDGFP reporter activity. As with all transcriptional reporters it remains to be determined whether it fully recapitulates endogenous draper regulation after injury, nevertheless, the dee7-Gal4 element will be an extremely useful tool with which to dissect pathways mediating axon injury-induced molecular changes in glial transcription. Finally, based on the presence of STAT92E binding sites in the draper locus, and the requirement of these for maximal activation of the injury-responsive dee7-Gal4 element, we suspect the draper gene may be a direct transcriptional target of STAT92E after axotomy.

Since mutation of the STAT92E binding sites in the dee7 element only led to a partial inhibition of dee7 activity after injury, it seems likely that Stat92E acts in cooperation with other factors. Evidence from mammals suggests that based on their weak transcriptional activation, STAT proteins often act in combination with other transcription factors for maximal target gene activation. Interestingly, we recently showed JNK signaling is required in glial cells for up-regulation of Draper after axotomy and that transcriptional reporters for JNK pathway signaling to dAP-1 increased in glia after axonal injury [69]. Mammalian STAT and AP-1 act together in reactive microglial cells to mediate the up-regulation of inflammatory proteins [70,71]. It therefore remains
an interesting possibility that combinations of Stat92E binding sites, AP-1 binding sites, and binding sites for other unidentified co-factors are involved in precisely modulating levels of Draper expression in the context of injury.

Our work has revealed a novel role for engulfment signaling pathways (i.e., Draper signaling) in the regulation of glial transcription after local axon injury, and revealed that Stat92E acts downstream. Despite the fact that Stat92E signaling is essential for a wide range of developmental processes in Drosophila, Draper is only the second receptor implicated in positively regulating the ability of Stat92E to activate transcriptional targets in vivo [48,53,54]. At the same time our work identifies the draper gene as a novel target for Stat92E—only three other genes, eve, crh, and dome, have been shown by in vivo analyses to be directly regulated by Stat92E [72–74]. Our work therefore enriches our understanding of the physiological roles for engulfment receptors and Stat92E signaling in the modulation of glial responsiveness to axonal injury.

We anticipate a similar role for mammalian engulfment receptors and STATs in reactive gliosis. Indeed, activation of STAT molecules in mammalian glial cells (determined primarily by using phospho-STAT-specific antibodies) has been widely reported in response to focal brain lesion [75,76], traumatic brain injury [77,78], and spinal cord injury (SCI) [79]. Stat3 is a critical modulator of reactive gliosis: loss of STAT3 from astrocytes in the context of SCI has been shown to lead to attenuated activation of GFAP expression, a lack of astrocyte hypertrophy, and reduced formation of the glial scar. This in turn results in increased demyelination, enhanced inflammation, and less efficient motor axon recovery [80,81]. IL-6 has been shown to act upstream of STAT3-dependent GFAP induction in Schwann cells during peripheral nerve regeneration, but both the source of IL-6 in vivo and how STATs are activated in glia after injury remains unclear [82]. Based on this study we speculate mammalian STATs may be activated in reactive glia by the Draper orthologs MEGF10 or Jedi.

Materials and Methods

Fly Strains and Molecular Biology

The following fly strains were used: (1) UAS-pdk1, VDRC 18736, (2) UAS-raptor^{RNAi}, VDRC 13112, (3) UAS-psk92^{RNAi}, VDRC 38986, (4) UAS-psk92^{RNAi}, Bloomington Stock 8295 (5) UAS-mCD8::GFP [83 (II)], (6) UAS-mCD8::GFP [83 (III)], (7) OR85e-mCD8::GFP [84], (8) UAS-stat92^{RNAi}, VDRC 43866, (9) UAS-drp9^{RNAi} [24] (10) repo-Gal4, (11) tubulin-Gal80^{u}, (12) repo-Gal4, UAS-mCD8::GFP, (13) 10XStat92E-GFP [43], (14) 10XStat92E-GFP [43], (15) UAS-rac^{112}, Bloomington Stock 6291, (16) UAS-Src42-G^{CA}, Bloomington Stock 6410, (17) UAS-src42^{RNAi} VDRC 26019 (18) UAS-src42^{RNAi} VDRC 100708, (19) UAS-rac^{RNAi} VDRC 49247, (20) UAS-rac^{RNAi}^{u}, Bloomington Stock 6292, (21) UAS-src42^{RNAi} [25] (22) UAS-src42^{RNAi}, Bloomington VDRC 105706 (23) UAS-Drpr-I [27], (24) drpr^{A1}, (25) UAS-mcherry, (26) and yw.

The drp9::GFP construct was made by PCR amplification of the 2.619 bp fragment from the Draper BACR17K18 clone using the following primers: forward 5’-caccagacctactcttagctctgatggagg-3’, reverse 5’-gggctggctttcagcatcctttg-3’. The PCR product was purified using a Qiagen Gel Purification kit, directionally cloned into the Invitrogen pENTR/D-TOPO vector and transformed into One Shot Competent Cells using the pENTR/D-TOPO Cloning Kit (Invitrogen catalog number K2400-20). Colonies were prepped using the Qiagen miniprep kit. The drp9 fragment was then shuttled into the pBGUw destination vector [15] using the Invitrogen Gateway LR Clonase Enzyme and transformed into heat shock competent DH5a cells. The construct was sequence verified and transgenic flies were generated by Best Gene Inc. using the PhiC31 targeted integration system.

To generate the deg^{MUT}-GAL4 construct, the deg7/TOPO construct was used as a template and PCR was carried out using Invitrogen Quick Change II Site-Directed Mutagenesis kit (catalog number 200523) with the following primers (mutation sites underlined); forward-5’ CTG TGC CGA ACA CGT TA ACAA TTG AAA AAC AAT CTC GC 3’, reverse-5’ GCG AGA TTT TTC AAT GGT TA ACG GTT CGG CCC ACG AG 3’. A DpnI digestion was performed and DNA was transformed into XL-1 Blue super competent cells and plated. Colonies were prepped using the Qiagen miniprep kit and the mutations were verified by sequencing. (Geneviz). The deg7^{MUT} enhancer fragment was then shuttled into the pBGUw vector using methods described above and transgenic flies were generated by Best Gene Inc. using PhiC3 targeted integration.

Olfactory Neuron Injury Protocol, Immunohistochemistry, and Confocal Microscopy

Maxillary palp and third antennal segment ablations, adult brain dissections, and antibody stainings were performed using previously described methods [24,85]. Samples were mounted in Vectashield (Vector Laboratories) anti-fade reagent and viewed on a Zeiss LSM5 Pascal confocal microscope. In all experiments, laser settings were kept identical for all brains imaged as part of that experiment. For Figure 2C, single slice confocal images of approximately the same depth in the brain were identified and total intensity of GFP in the brain was measured. The minimum threshold was set at 3 to eliminate most background and the maximum threshold was set at the maximum value of 255. Measures of GFP intensity within maxillary palp glomeruli were performed as previously described [24]. For Figure 3E, Draper expression after maxillary palp injury was measured from single confocal slices at the depth of the OR85e-innervated glomerulus. A circle was drawn around the area of the OR85e^{+} innervated glomerulus and total intensity of Draper was measured. Draper expression after antennal injury was measured from single confocal slices about half way through the antennal lobe. A fixed area rectangle at the edge of the antennal lobe was used to measure total intensity of Draper. All quantification of measurements was performed using Image J software. The following antisera were used: 1:200 mouse anti-GFP (Invitrogen), 1:500 rabbit anti-Draper [86], 1:500 rat anti-dCed6 [87], 1:200 FITC anti-mouse IgG, 1:200 Cy3 anti-rabbit IgG, 1:200 Cy3 anti-rat IgG (Jackson ImmunooResearch). Flies were raised and maintained at 25°C unless otherwise noted. In experiments utilizing Gal80^{u} flies were raised at 18°C and shifted to 30°C for at least 7 days prior to injury or dissection.

Western Blots

Drosophila brains of the indicated genotype were dissected in PBS and homogenized in SDS loading buffer (60 mM Tris [pH 6.8], 10% glycerol, 2% SDS, 1% mercaptoethanol, 0.01% bromophenol blue). For Western analysis, samples containing approximately three brains were loaded onto 10% SDS-PAGE gels (BioRad), transferred to nitrocellulose membranes (BioRad), and probed with rabbit z-Draper antibody [86] at 1:1,000 diluted in PBS/0.01% Tween-20/5% BSA. Blots were incubated overnight at 4°C, washed several times in PBS/0.01% Tween-20, and probed with the appropriate HRP conjugated secondary antibody for 2 hours at room temperature. Additional washes were performed and the blot was developed using chemiluminiscence (Amersham ECL Plus), and detected with a Fujifilm

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Drosophila central brain regions were dissected in Jan’s saline (1.8 mM Ca\(^{2+}\)) and immediately frozen on dry ice. Total RNA was extracted in Trizol and the aqueous phase was passed over an Omega Bio-Tek E.Z.N.A. MicroElute RNA Clean-Up Column with an on-column DNAse I treatment (OmegA). RNA concentration was determined on a Nanodrop 2000c spectrometer (Thermo Scientific). RNA was diluted to equal concentration and 125 ng of total RNA was reverse-transcribed with the SuperScript VILO cDNA synthesis Kit for 2 h at 42°C. Relative quantification of gene expression was carried out on an ABI 7000 Real-Time PCR machine. The following Taqman assays (Applied Biosystems) were used: (i) Ribosomal protein L32 (ABI pre-made assay Dm02135272_g1) (ii) Draper-1 custom assay, F-primer, TGGTATGCTGTTAGGGGAG; R-primer, CAGGCCGTTGGGCAA; probe, GCCTGCTGATATAA [27]. Assay efficiencies were experimentally determined (RpL32, 102%; Draper-I, 103%); using a 3-point dilution series of cDNA spanning a 20-fold range in concentration. The raw threshold cycle (Ct) of the normalization control (RpL32), did not vary by more than 0.5 cycles across all time points analyzed. Statistical analysis (ANOVA, with Dunnett’s Multiple Comparisons Test) was performed on 2\(^{\pm}\)SEM values. Draper Ct values were normalized to ribosomal protein L32 and results are presented as fold induction relative to uninjured levels.

Supporting Information

Figure S1 Glial specific expression of a dominant negative PI3K92E results in decreased Draper protein levels and delayed axon clearance 5 days after injury. (A) Single slice confocal images of adult brains stained with Draper antibody and Z-stack confocal images of OR85e\(^{+}\) axons labeled with GFP in control (OR85e-GFP, Gal80\(^{+/+}\); repo-gal4\(^{+/+}\)) and PI3K92E dominant negative backgrounds (OR85e-GFP, Gal80\(^{+/+}\); repo-gal4\(^{+/+}\); OR85e-GFP, UAS-ja392e\(^{12880}\)). Uninjured and 5 days after maxillary palp ablation are shown. (B) Quantification of GFP intensities in 85° glomeruli from (A), p-values were calculated using Student’s t test, Error bars represent SEM. (C) Image of full Western blot for bands shown in Figure S1. (TIF)

Figure S2 Simultaneous mutation of tandem Stat92E binding sites in the der7 enhancer element does not affect der7 activity after antennal ablation. (A) Schematic representation of the draper gene. Asterisks indicate location of the tandem Stat92E binding sites in the der7 enhancer region. Underlined letters indicate potential Stat92E binding motifs. Red letters show nucleotides mutated in the der7\(^{Mut2}\)-Gal4 element. (B) Single slice confocal images of antennal lobe regions; der7-Gal4 or der7\(^{Mut2}\)-Gal4 driving two copies of UAS-mCD8-GFP (der7\(^{>2}\)mCD8:GFP, der7\(^{Mut2}\)\>2\)mCD8:GFP). One day after antennal ablation and four days after antennal ablation are shown. (C) Quantification for (B), p-values were calculated using Student’s t test, n.s., not significant. Error bars represent SEM, n=5 for all. (TIF)

Figure S3 Glial specific knockdown of Stat92E inhibits axon clearance for at least 15 days after antennal ablation. (A) Z-stack confocal images of OR67b\(^{+}\) axons labeled with GFP in control (OR67b-GFP; repo-gal4\(^{+/+}\)) and stat92e\(^{RNAi}\) (OR67b-GFP/UAS-stat92e\(^{RNAi}\); repo-gal4\(^{+/+}\)) backgrounds. Uninjured and 15 days after antennal ablation are shown. (B) Single slice confocal images of adult brains stained with \\^{\pm}Draper and \^{\pm}GFP in control (UAS-stat92e-GFP/repo-Gal4\(^{+/+}\), and stat92e\(^{RNAi}\) (UAS-stat92e\(^{RNAi}\); repo-gal4\(^{+/+}\)) backgrounds. (C) Image of full Western blot for bands shown in Figure S3. (TIF)

Figure S4 Stat92E is required in the adult brain for draper expression and glial engulfment function. (A) Z-stack confocal images of OR85e-GFP axons, single slice confocal images of Draper antibody stain; UAS-stat92e\(^{RNAi}\) was driven in all glia using repo-Gal4 in a background containing the temperature sensitive Gal4 repressor, Gal80\(^{ct}\). Axons (OR85e-GFP, green) and Draper levels (red) are shown in control (OR85e-GFP, Gal80\(^{ct}\); repo-gal4\(^{+/+}\) or stat92e\(^{RNAi}\) knockdown (OR85e-GFP, Gal80\(^{ct}\); stat92e\(^{RNAi}\); repo-Gal4\(^{+/+}\)) animals. Temperature shifts were performed as follows: 18°C indicates that flies were raised and kept at 18°C throughout experiment. 18°C–30°C indicates that flies were raised at 18°C and then shifted to 30°C as adults for 7 days prior to and 5 days following maxillary palp ablation. 18°C–30°C–18°C indicates that flies were raised at 18°C, shifted to 30°C as adults for 7 days, and then shifted back to 18°C for 7 days prior to and 5 days following maxillary palp ablation. (B) Quantification of GFP intensities in 85° glomeruli from (A). Error bars represent SEM, n=10. (TIF)

Figure S5 Glial specific knockdown of STAT92E does not disrupt glial morphology or result in loss of dCed-6 protein levels. (A) Single slice confocal images of adult brains of indicated genotype, (UAS-stat92e\(^{RNAi}\); repo-Gal4, UAS-md8-GFP, Gal80\(^{ct}\)) one day after maxillary palp injury. Glial nuclei are labeled with anti-Repo (blue), glial membranes are labeled with GFP (green), and anti-Draper staining is shown (red). Flies were raised at 18°C and either kept at 18°C throughout the experiment or shifted to 29°C for 7 days prior to dissection. At the restrictive temperature of 18°C some repo-gal4 driven GFP is detectable but Draper staining looks grossly normal and glia are able to respond to injury one day after maxillary palp ablation. In flies shifted to the restrictive temperature, Draper staining is significantly reduced and no glial membranes are recruited to severed axons one day after maxillary palp injury. However, glial cell nuclei and membranes appear grossly normal in the stat92eRNAi flies. (B) Single slice confocal image of adult brain, high magnification view of cortex region of the brain. Asterisk indicates that while Draper is absent in the stat92e\(^{RNAi}\) animals, glial morphology is normal. (C) Adult brains one day after maxillary palp removal, stained with \\^{\pm}dCed6 or \\^{\pm}Draper in control (OR85e-GFP, Gal80ts/+;repo-Gal4\(^{+/+}\), and stat92e\(^{RNAi}\) (OR85e-GFP, Gal80ts/+;stat92e\(^{RNAi}\);repo-Gal4\(^{+/+}\)) backgrounds. (D) \\^{\pm}Draper Western blot performed in duplicate on ~3 adult central brain regions per lane in control (OR85e-GFP, Gal80ts/+;repo-Gal4\(^{+/+}\), and stat92e\(^{RNAi}\) (OR85e-GFP, Gal80ts/+;stat92e\(^{RNAi}\);repo-Gal4\(^{+/+}\)) backgrounds. \\^{\pm}Tubulin was used as a loading control. (TIF)

Figure S6 48 hour time course showing activation of the 10XStat92E-dGFP reporter following antennal ablation. (A) Single slice confocal images of adult brains: 10XStat92E-dGFP reporter activity in an uninjured brain and at various timepoints after injury, 12 hours, 16 hours, 24 hours, 36 hours, and 48 hours. (TIF)

Figure S7 Glial activation of Stat92E activity after axotomy is not mediated by canonical JAK/STAT signaling. (A) repo-gal4 was used to drive two independent UAS-hop\(^{RNAi}\) constructs and a UAS dominant negative domeless allele; single slice confocal images
of Draper antibody stain and Z-stack confocal images of OR85e-GFP axons in control (OR85e-GFP/hop<sup>RNAi</sup>;<repo-gal4/+), hop<sup>RNAi</sup> (OR85e-GFP+/repo-gal4/+; hop<sup>RNAi</sup> (OR85e-GFP/UAS-hop<sup>RNAi</sup>;<repo-gal4/+), domeless<sup>ANMC</sup> (OR85e-GFP/UAS-domeless<sup>ANMC</sup>; repo-gal4/+). (TIF)

Figure 8B RNAi mediated knockdown of rac1, shark, and src42a results in loss of Stat92E transcriptional reporter activity after injury. (A) Glial-specific knockdown of components of the draper pathway in a 10XStat92E-dGFP reporter background one day after antennal ORN axotomy; control (10XStat92E-dGFP/+; repo-gal4/+), hop<sup>TUM</sup> (hoptum/X);10XSTAT92E-dGFP/+; repo-Gal4/+; shark<sup>RNAi</sup>; repo-Gal4/+; draper<sup>RNAi</sup>; repo-Gal4/+; d-ced6<sup>RNAi</sup>; repo-Gal4/+; hop<sup>TUM</sup>;10XSTAT92E-dGFP/+; repo-Gal4/+). (B) Single slice confocal images of adult brains stained with z-dCed6 in control, (OR85e-GFP;Gal80ts/+;repo-gal4/+), and d-ced6<sup>RNAi</sup> (OR85e-GFP;Gal80ts/d-ced6<sup>RNAi</sup>;repo-gal4/+). backgrounds. Uninjured, one day after maxillary palp injury and one day after antennal injury are shown. (B) Image of full Western blot for bands shown in Figure 6. (TIF)

Data S1 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 1A and 1B. (XLSX)

Data S2 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure S1. (XLSX)

Data S3 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 1D and 1E. (XLSX)

Data S4 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 2B and 2C. (XLSX)

Data S5 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure S2B and S2C. (XLSX)

Data S6 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 3A–3C. (XLSX)

Data S7 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 3D and 3E. (XLSX)

Data S8 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 3F and 3G. (XLSX)

Data S9 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure S4. (XLSX)

Data S10 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 3H. (XLSX)

Data S11 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure S7. (XLSX)

Data S12 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 6A and 6B. (XLSX)

Data S13 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 6E and 6F. (XLSX)

Data S14 Excel spreadsheet containing the raw numerical data and statistical analyses for Figure 6C and 6D. (XLSX)

Methods S1 Supporting methods and references. (DOC)

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Author Contributions
The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: JD TSL AS NF RB MRF. Performed the experiments: JD TSL AS NF RB. Analyzed the data: JD TSL AS NF RB MRF. Contributed reagents/materials/analysis tools: JD TSL AS NF RB MRF. Wrote the paper: JD MRF.

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