Phospholipid homeostasis regulates dendrite morphogenesis in *Drosophila* sensory neurons

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Summary

Disruptions in lipid homeostasis have been observed in many neurodevelopmental disorders that are associated with dendrite morphogenesis defects. However, the molecular mechanisms of how lipid homeostasis affects dendrite morphogenesis are unclear. We find that *easily shocked* (*eas*), which encodes a kinase with a critical role in phospholipid phosphatidylethanolamine (PE) synthesis, and two other enzymes in this synthesis pathway are required cell-autonomously in sensory neurons for dendrite growth and stability. Further, we show that the level of Sterol Regulatory Element Binding Protein (SREBP) activity is important for dendrite development. SREBP activity increases in *eas* mutants, and decreasing the level of SREBP and its transcriptional targets in *eas* mutants largely suppresses the dendrite growth defects. Furthermore, reducing Ca^{2+} influx in neurons of *eas* mutants ameliorates the dendrite morphogenesis defects. Our study uncovers a role for EAS kinase and reveals the *in vivo* function of phospholipid homeostasis in dendrite morphogenesis.

eTOC Blurb

Meltzer et al. show that EAS, a conserved kinase in the phospholipid phosphatidylethanolamine synthesis pathway, regulates dendrite growth via SREBP signaling and Ca^{2+} influx. Their study reveals the role of phospholipid homeostasis in dendrite morphogenesis *in vivo*.
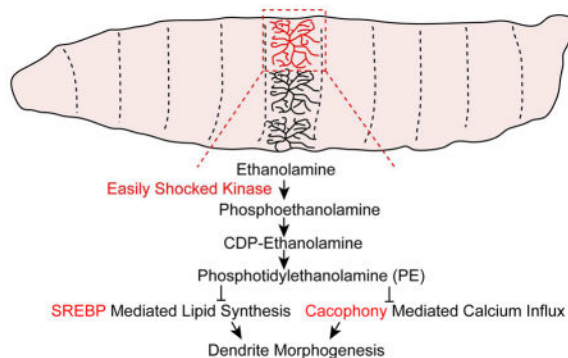
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Author Contributions

S.M., J.A.B., G.L.P., Y.G., L. D., and C.E.O., conducted experiments and analyses. S.M., L.Y.J. and Y.-N.J. designed experiments and wrote the paper. J.A.B. conducted the genetic screen.

Ethanolamine
Easily Shocked Kinase ↓
Phosphoethanolamine
CDP-Ethanolamine
Phosphotidylethanolamine (PE)
SREBP Mediated Lipid Synthesis   Cacophony Mediated Calcium Influx
Dendrite Morphogenesis

Introduction

How neurons achieve the proper wiring pattern during development is an important question, as the dendrite arborization pattern of each neuron is critical for the function of the nervous system (Jan and Jan, 2010; Lefebvre et al., 2015). Defects in dendrite morphogenesis are common anatomical features of many neurodevelopmental disorders (Kaufmann and Moser, 2000). Although abnormal lipid metabolism has been observed in mouse models as well as patients with neurodevelopmental disorders (Buchovecky et al., 2013; Tamiji and Crawford, 2010; Tint et al., 1994) and 60 percent of the human brain dry mass is lipids (Wong and Crawford, 2014), relatively little is known about the importance of lipid homeostasis for dendrite morphogenesis during neural development.

We have used the dendritic arborization (da) sensory neurons of the *Drosophila* larval peripheral nervous system (PNS) to identify the molecular mechanisms that regulate dendrite morphogenesis of these neurons, which fall into four distinct classes (I–IV) based on their dendrite morphologies and axon projections (Grueber et al., 2002; 2007). From a genetic screen for genes that affect dendritic growth in class IV da neurons, we identified *easily shocked* (*eas*) as an important gene that regulates dendrite morphogenesis in sensory neurons. The *eas* gene encodes a conserved ethanolamine kinase, the first enzyme in the cytidine 5′-diphosphate (CDP)-ethanolamine pathway for the synthesis of the membrane phospholipid phosphotidylethanolamine (PE) (Kennedy, 1957). PE is the predominant phospholipid in membranes in *Drosophila* (Jones et al., 1992), and the second most abundant phospholipid in mammals (Vance, 2014). In *Drosophila*, PE regulates the processing and activity of sterol regulatory element-binding protein (SREBP), a highly conserved basic helix-loop-helix leucine zipper transcription factor that is crucial for lipid homeostasis (Dobrosotskaya et al., 2002). Reduction of the PE level causes SREBP precursor proteins to be transported from the endoplasmic reticulum to the Golgi apparatus, where they are cleaved to release the transcriptionally active domain that translocates to the nucleus to activate the expression of lipogenic genes (Rawson, 2003). It was unknown how this phospholipid homeostasis process affects dendrite development.

Originally identified by Seymour Benzer, *eas* mutants respond to a mechanical jolt by exhibiting transient paralysis and then recover, whereas wild type flies are unaffected (Benzer, 1971). *eas* mutant flies were subsequently used as a *Drosophila* model of seizure;

their seizure-like phenotype could be suppressed by mutations that reduced the hyperexcitability of *eas* mutants (Parker et al., 2011; Pavlidis et al., 1994). It is unknown whether these mutations that reduce hyperexcitability also affect neural morphogenesis in *eas* mutants. Reducing the level of *cacophony* (*cac*), the *Drosophila* homolog of the Cav2 voltage-gated calcium channel genes and a major mediator of neuronal Ca^{2+} influx (Peng and Wu, 2007; Saras and Tanouye, 2016), suppresses the seizure phenotype present in *eas* mutants. We found that reducing *cac* gene activity ameliorated the dendrite morphogenesis defects of *eas* mutants.

Here, we show that the dendrite morphogenesis defects of *eas* mutants are attributable to increased lipogenesis and altered Ca^{2+} influx. Our results uncover a role for the conserved ethanolamine kinase and for SREBP signaling in dendrite morphogenesis and highlight an important role of phospholipid and lipid homeostasis during neuronal development.

Results

EAS Kinase Acts Cell-Autonomously to Regulate Dendrite Morphogenesis in da Neurons

From an RNA interference (RNAi) screen for regulators of dendrite development in *Drosophila*, we identified *eas*, which encodes ethanolamine kinase (Pavlidis et al., 1994), as a candidate. To corroborate the RNAi results, we first examined the existing *eas* allele, *eas^1*, which produces a truncated protein lacking kinase activity (Pavlidis et al., 1994). We further generated knockout mutants, *eas^{KO}* (Figures S1A and S1B), in which the entire coding region is removed via CRISPR/Cas9 (Port et al., 2014). Both alleles showed similar dendrite outgrowth defects at 120 hours (hrs) after egg laying (AEL), with dramatic decreases in the number of branches and the total dendrite length in class IV da neurons (Figures 1A–1C, 1E and 1F). Sholl analysis revealed that reductions in dendrite branching occurred uniformly throughout the dendritic arbor in the *eas^1* and *eas^{KO}* mutants (Figure 1G). In addition, we found decreases in the number of branches and the total dendrite length in class I and class III da neurons in the *eas^1* and *eas^{KO}* mutants (Figures S2A–S2H). We further examined the morphology of class IV da neurons at 48, 72, 96, and 120 hrs AEL in wildtype and *eas^{KO}* mutants. Dendrites of class IV da neurons normally establish their dendritic territories and completely tile the body wall by 48 hrs AEL, and then continue to grow throughout larval development (Parrish et al., 2007). Although the patterning of class IV da neurons in *eas^{KO}* mutants examined at 48 hrs AEL initially proceeded normally, dendrite growth defects became apparent at 72 hrs AEL (Figures 1E and 1F), suggesting that *eas* is required for dendrite outgrowth and/or stability after the initial dendrite territory is established. The overall pattern of axon projections in *eas^{KO}* animals appears to be similar to but much fainter than that of wildtype animals (Figures S2I and S2J), raising the possibility that axon morphogenesis may also be affected in *eas^{KO}* mutants.

We next asked whether *eas* was required cell-autonomously in class IV da neurons to regulate dendrite morphogenesis. We were able to rescue the *eas^{KO}* and *eas^1* dendrite morphogenesis defects by expressing *eas* in class IV da neurons (Figures 1H and 1I). Furthermore, knocking down *eas* only in da neurons led to a similar reduction in number of branches and total dendrite length (Figures 1D, 1H and 1I), suggesting that *eas* is required cell-autonomously in class IV da neurons for proper dendrite morphogenesis. Interestingly,

overexpressing *eas* in class IV da neurons did not strongly affect dendrite growth, suggesting that an increase in easily shocked kinase activity does not affect dendrite morphogenesis (Figures 1H and 1I).

Dendrites of class IV da neurons undergo complete pruning and regrowth during the pupal stage to reestablish dendrite territory (Kuo et al., 2005; Williams and Truman, 2005). We also found reductions in the number of dendrite branches and total dendritic length in 1 day old *eas*^KO^ adult flies (Figures S1C, S1D, S1F and S1G). Knocking down *eas* in the neurons also led to similar dendrite morphogenesis defects, suggesting that *eas* cell-autonomously regulates dendrite morphogenesis during the pupal stage (Figures S1E–S1G).

The *eas* gene encodes an ethanolamine kinase that catalyzes the phosphorylation of ethanolamine to phosphoethanolamine, which is modified by phosphoethanolamine cytidylytransferase (encoded by *pect*) to produce CDP–ethanolamine. CDP-ethanolamine donates phosphoethanolamine to diacylglycerol to generate PE, which is mediated by CDP-ethanolamine phosphotransferase (encoded by *bbc*) (Dobrosotskaya et al., 2002). In addition, the level of PE is reduced in *eas* whole flies and heads (Kliman et al., 2010; Nyako et al., 2001; Pavlidis et al., 1994). Therefore, we asked whether the dendrite morphogenesis defects were due to insufficient PE synthesis through the CDP–ethanolamine pathway. Compared with control animals, knocking down *pect* and *bbc* in neurons both led to *eas*^KO^-like decreases in the number of branches and dendrite length (Figures 1J–1N), suggesting that PE synthesis through the CDP–ethanolamine pathway is important for dendrite growth.

EAS Kinase Regulates Terminal Dendrite Dynamics

Dendrites in wildtype animals grow and branch extensively starting from 48 hrs AEL, while displaying dynamic growth and retraction of the terminal dendrites (Parrish et al., 2007). Since the dendrite defects of *eas*^KO^ mutant neurons became apparent during this period, we asked whether *eas* is required for normal terminal dendrite dynamics (Figures 2A–2F). By performing *in vivo* time-lapse analysis during larval development at 72 hrs and 76 hrs AEL, we detected terminal dendrite growths and retractions while these dendrites formed complete field coverage in wildtype animals (Figures 2A–2C). The fraction of retracting terminal dendrites in *eas*^KO^ mutant class IV da neurons was increased (Figure 2H), while the fraction of growing terminal dendrites was unaltered (Figure 2G). As a result, 77.5% of the terminal dendrites were dynamic in *eas*^KO^ mutants (n = 10 neurons), whereas 70.1% of the terminal dendrites were dynamic in wildtype animals (n = 10 neurons, p<0.05), suggesting that *eas* is required for regulating terminal dendrite stability. As a result, the growth/retraction ratio of terminal dendrites was decreased in class IV da neurons in *eas*^KO^ mutant (Figure 2I). Our findings suggest that *eas* is important for terminal dendrite stability and supporting dendrite growth after the initial dendrite patterning is established.

Level of SREBP Activity is Critical for Dendrite Morphogenesis in the Class IV da Neurons

Previous studies have established a link between low PE levels and increased SREBP activity (Dobrosotskaya et al., 2002; Lim et al., 2011), which is critical for turning on the transcription of downstream genes for lipogenesis (Rawson, 2003). However, it is unknown

whether SREBP, a highly conserved transcription factor that is essential for lipid homeostasis, regulates nervous system development.

To test whether the level of SREBP transcriptional activity is important for dendrite morphogenesis, we tested the consequence of increased SREBP activity in class IV da neurons by expressing constitutively active forms of SREBP. Indeed, expressing the N-terminal domain containing the first 452 amino acids of SREBP, which mimics the cleaved, active form of SREBP (SREBP.1-452), led to decreases in the number of branches and the total dendrite length of class IV da neurons (Figures 3A, 3B, 3D and 3E).

Conversely, knocking down *srebp* by expressing a previously-characterized SREBP RNAi in class IV da neurons (Figure 3C) (Song et al., 2014) also led to decreases in the number of branches (Figure 3E) and the total dendrite length (Figure 3D). Interestingly, both knocking down and expressing constitutively active SREBP in class IV da neurons led to many missing longitudinal and commissural projections (Figures S2K and S2L), suggesting that SREBP is also involved in regulating axon morphology. Taken together, our results demonstrate that SREBP signaling is critical for dendrite outgrowth during larval development.

Aberrant Lipid Homeostasis Mediated by SREBP Signaling Contributes to Dendrite Morphogenesis Deficits in *eas*^{KO} Mutants

Given that the level of SREBP activity is critical for dendrite growth, we tested the hypothesis that SREBP acts downstream of a deficiency in PE synthesis to alter dendrite morphogenesis in *eas*^{KO} mutants. First, we determined whether SREBP activity is altered in *eas*^{KO} mutants by examining the processing of endogenous SREBP proteins by Western blots using a previously characterized SREBP antibody (Lim et al., 2011). Most of the SREBP proteins were full-length, membrane-bound precursors (Fl-SREBP), and only a trace amount of the cleaved and mature form (m-SREBP) was detected in wildtype larval brains (Figure 3F). Compared to wild-type, activated SREBP protein level was increased by 3.6 ± 1.4 fold in *eas*^{KO} mutant brains (Figure 3G; n=3), suggesting that loss of *eas* results in greater activation of SREBP signaling.

To determine whether the increased transcriptional activity of SREBP contributes to dendrite morphogenesis defects in *eas*^{KO} mutants, we reduced the level of SREBP in *eas*^{KO} mutants to see whether the severity of dendrite growth defects would be ameliorated. Indeed, removing one copy of the SREBP gene in *eas*^{KO} mutants largely restored dendrite growth (Figures 3H and 3I) (n = 7). In *Drosophila*, activated SREBP protein turns on the expression of known lipogenic genes, including *acetyl-coA carboxylase* (*acc*), *fatty acid synthase* (*fas*), *acetyl CoA synthase* (*acs*) and *fatty acyl CoA synthetase* (*acsl*) (Rawson, 2003). Given that the SREBP processing is increased in *eas*^{KO} mutants, we reasoned that the dendrite morphogenesis defects could arise from increased expression of SREBP target genes. Indeed, expressing RNAi constructs targeting *acc* and *fas* in class IV da neurons of *eas*^{KO} mutants (Figures 3J and 3K) (n = 5 per genotype), and introduced one copy of the *acsl* or *acs* mutant alleles in *eas*^{KO} mutants (Figures 3L and 3M) (n = 6 per genotype) improved dendrite morphogenesis in *eas*^{KO} mutants (Figures 3O and 3P). While knocking down *fas* in class IV da neurons in *eas*^{KO} mutants also increased the number of branches, it did not

increase the dendrite length, possibly due to a low RNAi efficiency or the redundancy of the three *fas* genes in the *Drosophila* genome. Further, increasing SREBP levels in class IV da neurons of *eas*[KO] mutants does not enhance their dendrite growth defects (Figures 3N–3P) (n = 5). Similarly, increasing SREBP levels of class IV da neurons in wildtype animals did not lead to any defects in dendrite morphogenesis (Figures 3D and 3E) (n = 5), suggesting that the level of activated SREBP is not further increased by increasing the total amount of full-length SREBP in the neurons.

Together, these results show that hyperactivation of SREBP contributes to the dendrite growth defects in *eas*[KO] mutants.

Reducing the Level of *cacophony* Partially Suppresses Dendrite Morphogenesis Deficits in *eas*[KO] Mutants

Adult *eas* flies with characteristic seizure-like phenotypes have been used as a model for screening for mutations that could suppress neuronal hyperexcitability and hence bang sensitivity (Parker et al., 2011). To determine whether the seizure-like phenotype was associated with defects in dendrite development, we asked whether the altered lipid homeostasis mediated by SREBP also contributes to the seizure-like phenotype in *eas*[KO] mutants (Figure S3A). Interestingly, mutations in the SREBP and its transcriptional targets, *acs* and *acsl* did not suppress the bang-sensitive seizure-like behavior in *eas*[KO] mutants (Figure S3B), suggesting that dendrite morphogenesis defects mediated by the altered lipid homeostasis pathway likely do not contribute to the physiological defects that lead to seizure susceptibility in *eas*[KO] mutants.

We then asked whether genes that suppressed the seizure-like phenotype could ameliorate the dendrite morphogenesis defects in *eas*[KO] mutants. Mutations in *cacophony* (*cac*), a gene encoding the α1 subunit of the voltage-gated Ca^{2+} channel, potently suppress the seizure-like behavior in *eas* mutants (Saras and Tanouye, 2016). Interestingly, *cac* is also required to mediate dendrite Ca^{2+} influx and pruning of class IV da neurons during metamorphosis (Kanamori et al., 2013). We found that knocking down *cac* by expressing a previously characterized RNAi construct (Saras and Tanouye, 2016) in class IV da neurons ameliorated the dendrite outgrowth defects in *eas*[KO] mutants (Figures 4A, 4B, 4D and 4E). Meanwhile, knocking down *cac* in class IV da neurons in wildtype animals did not cause any significant dendrite growth defects, suggesting that low Ca^{2+} influx does not impair dendrite morphogenesis (Figure 4C). It thus appears that calcium signaling is abnormally enhanced in *eas*[KO] mutants. We then checked the localization of Cacophony by expressing a previously-characterized GFP-tagged Cacophony (Cac-GFP) in class IV da neurons (Kawasaki et al., 2004). We found that the distribution of Cac-GFP remains unchanged in wildtype and *eas*[KO] mutants (Figures S3G–S3L), suggesting that *eas* does not regulate the localization of Cacophony. Although overexpressing *escargot* (*esg*, a member of the snail family of transcription factors) (Hekmat-Scafe et al., 2005) or *kazachoc* (*kcc*, K^{+}-Cl^{−} cotransporter) (Hekmat-Scafe et al., 2010) was able to suppress seizure-like phenotype in *eas* mutants, their overexpression in class IV da neurons did not suppress the dendrite phenotype in *eas*[KO] mutants (Figures S3C–S3F). Therefore, the increased Ca^{2+} influx, rather than

hyperexcitability *per se*, likely contribute to dendrite morphogenesis defects in *eas*^{KO} mutants.

Discussion

A growing body of evidence suggests that lipid metabolism and homeostasis is important for nervous system development (Zhang and Liu, 2015). Here, we provide evidence that EAS kinase and SREBP signaling pathway are required for dendrite growth during development. Our results do not rule out the possibility that a reduction in PE levels could also independently contribute to the *eas* dendritic phenotype, due to a lack of PE supply for expanding the plasma membrane during dendrite growth. Interestingly, although both reduced and increased SREBP activity led to the same dendrite reduction phenotype, the underlying mechanism may be different. Reduced lipid production caused by low SREBP activity might impair the lipid supply for growing dendrites, while aberrantly increased lipid production by SREBP might lead to lipotoxicity (Liu et al., 2015). Since the homologues of both genes are highly conserved, it will be interesting to determine their roles in dendrite morphogenesis within the developing mammalian nervous system.

We observed that terminal dendrite dynamics, but not initial establishment of the dendritic territory, is perturbed in *eas* mutants. It is possible that terminal dendrite growth and stability rely more on PE, while the primary and secondary branches do not. It is also possible that although PE synthesis is impaired in *eas* mutants, neurons still have enough PE to support initial dendrite growth. As the animal grows, neurons cannot supply enough PE for the exuberant terminal dendrite growth. It would be of interest to identify how impairment in PE specifically affects terminal dendrite dynamics in future studies.

We find that reducing the level of *cacophony*, a subunit of the Cav2 voltage-gated Ca^{2+} channel, ameliorates the dendrite morphogenesis defects in *eas*^{KO} mutants. In contrast, reducing SREBP signaling largely rescues the dendrite phenotypes but does not suppress the adult seizure-like phenotype. It is conceivable that the part of the neural circuit that heavily relies on SREBP signaling for dendrite morphogenesis during development does not contribute to the seizure-like behavior. Consistent with this hypothesis, expressing *eas* acutely in neurons in adult *eas* flies suppresses the seizure-like phenotype (Kroll and Tanouye, 2013). It thus appears that alterations in neuronal Ca^{2+} influx contribute to not only seizure-like phenotypes but also dendrite morphogenesis defects during larval development.

In summary, our study uncovers an important role for phospholipid PE synthesis in neural development, and they further reveal a molecular pathway involving conserved molecules for phospholipid homeostasis as being important in the regulation of dendrite morphogenesis.

Experimental Procedures

Live Imaging

Animals were reared at 25°C in density-controlled v ials or at 29°C for *bbc* and *pect* RNAi experiments. Larvae at appropriate stages were mounted in glycerol and dendrites of da neurons were imaged using a Leica SP5 laser scanning confocal microscope as previously described (Meltzer et al., 2016). Using water as a mounting media, v'ada neuron of the 1-day old adult ventral abdomen was imaged with a Leica SP5 laser scanning confocal microscope. Dendritic length and branch number were calculated using the Simple Neurite Tracer plugin in ImageJ.

Statistical Analysis

Data were analyzed and plotted with Prism 6.0c software. Student's t test was to compare statistical significance of two independent groups. One-way ANOVA tests were used to compare statistical significance of more than two independent groups. The Tukey's multiple comparison test was used along with one-way ANOVA tests when multiple comparisons was required.

See Supplemental Experimental Procedures for details on fly stocks, Western blotting, generating *eas* knockout flies, immunohistochemistry, and bang-sensitive analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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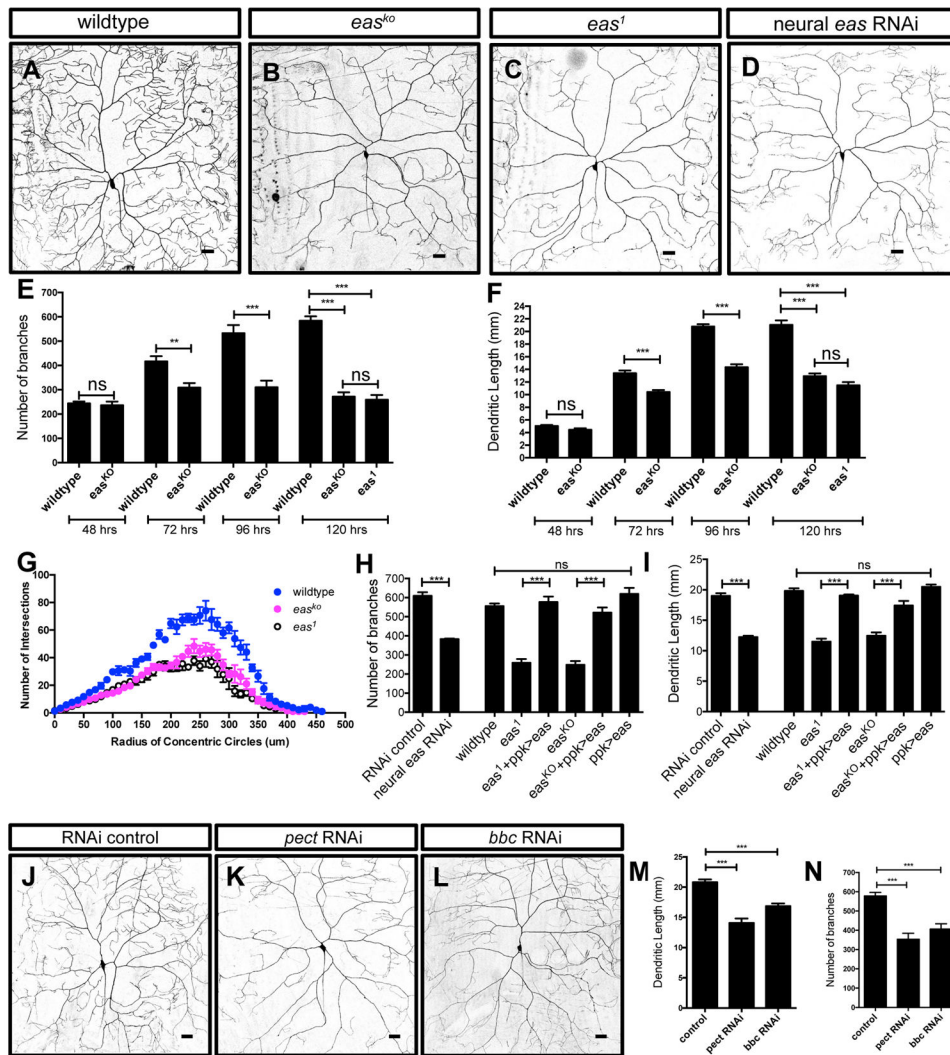
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Highlights

- A conserved ethanolamine kinase, EAS, regulates dendrite growth and stability

- Multiple enzymes involved in phospholipid PE synthesis affect dendrite growth

- EAS regulates dendrite morphogenesis via SREBP signaling

- Reduction of *cacophony* calcium channel partially rescues *eas* dendrite phenotype

Figure 1. EAS Is Required in Class IV da Neurons for Dendrite Morphogenesis

(A–D) Sample images showing dendrite morphology for the indicated genotypes.

(E and F) Quantification of number of branches (E) and total dendritic length (F) at 48 hrs, 72 hrs, 96 hrs, and 120 hrs AEL in wildtype (n = 6, 6, 6 and 10 respectively), eas^{KO} (n = 7, 6, 6 and 9 respectively) and eas^1 mutants (n = 6). Student's t tests were used to compare between wildtype and eas^{KO} mutants at 48 hrs, 72 hrs, and 96 hrs AEL. one-way ANOVA analysis was used for wildtype, eas^1, and eas^{KO} mutants at 120 hrs AEL.

(G) Sholl analysis showing decreased complexity of the dendritic arbor for the indicated genotypes. n = 5 per genotype.

(H and I) Quantification of number of branches (H) and total dendritic length (I) for the indicated genotypes. n = 7 and 5 for wildtype and $eas^{KO}+ppk>eas$, respectively. n = 6 for all of the other genotypes.

(J–L) Sample images showing dendrite morphology for the indicated genotypes.

(M and N) Quantification of total dendritic length (M) and number of branches (N) for neuroal *pect* and *bbc* RNAi, two other critical genes that act together with *eas* in the CDP–

ethanolamine pathway for PE synthesis. n= 6, 6, and 5 for control, *pect* RNAi, and *bbc* RNAi, respectively.

See also Figures S1 and S2. Scale bars represent 30 µm. ns, not significant; *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ for all figures.
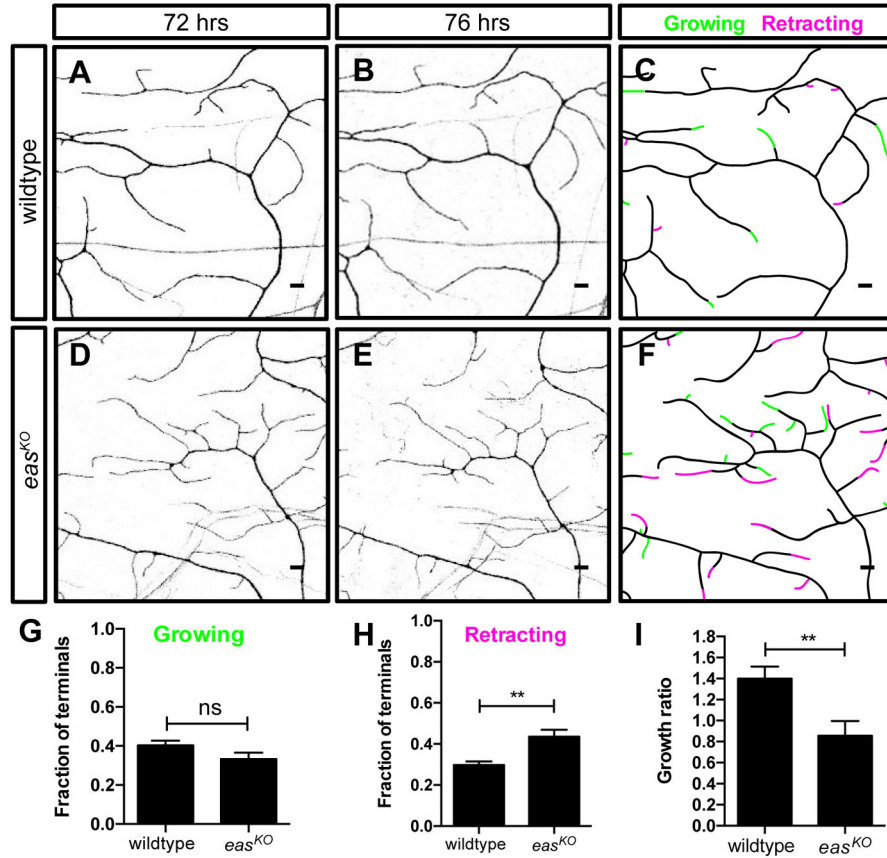
Figure 2. EAS Regulates Terminal Dendrite Growth Dynamics

(A and B) Sample images showing dendrite morphology for wildtype animals at 72 hrs (A) and 76 hrs (B) AEL.

(D and E) Sample images showing dendrite morphology for eas^{KO} mutants at 72 hrs (D) and 76 hrs (E) AEL.

(C and F) Branch dynamics are depicted in traces. Increased and decreased terminal branch region are marked in green and magenta, respectively.

(G–I) Quantification of fractions of growing (G) and retracting (I) terminal dendrites, as well as dendrite growth/retraction ratios for the indicated genotypes, showing decreases of dendrite stability and overall dendrite growth in eas^{KO} mutants.

Scale bars represent 5 μm. Student's t test was used. n = 10 neurons per genotype.
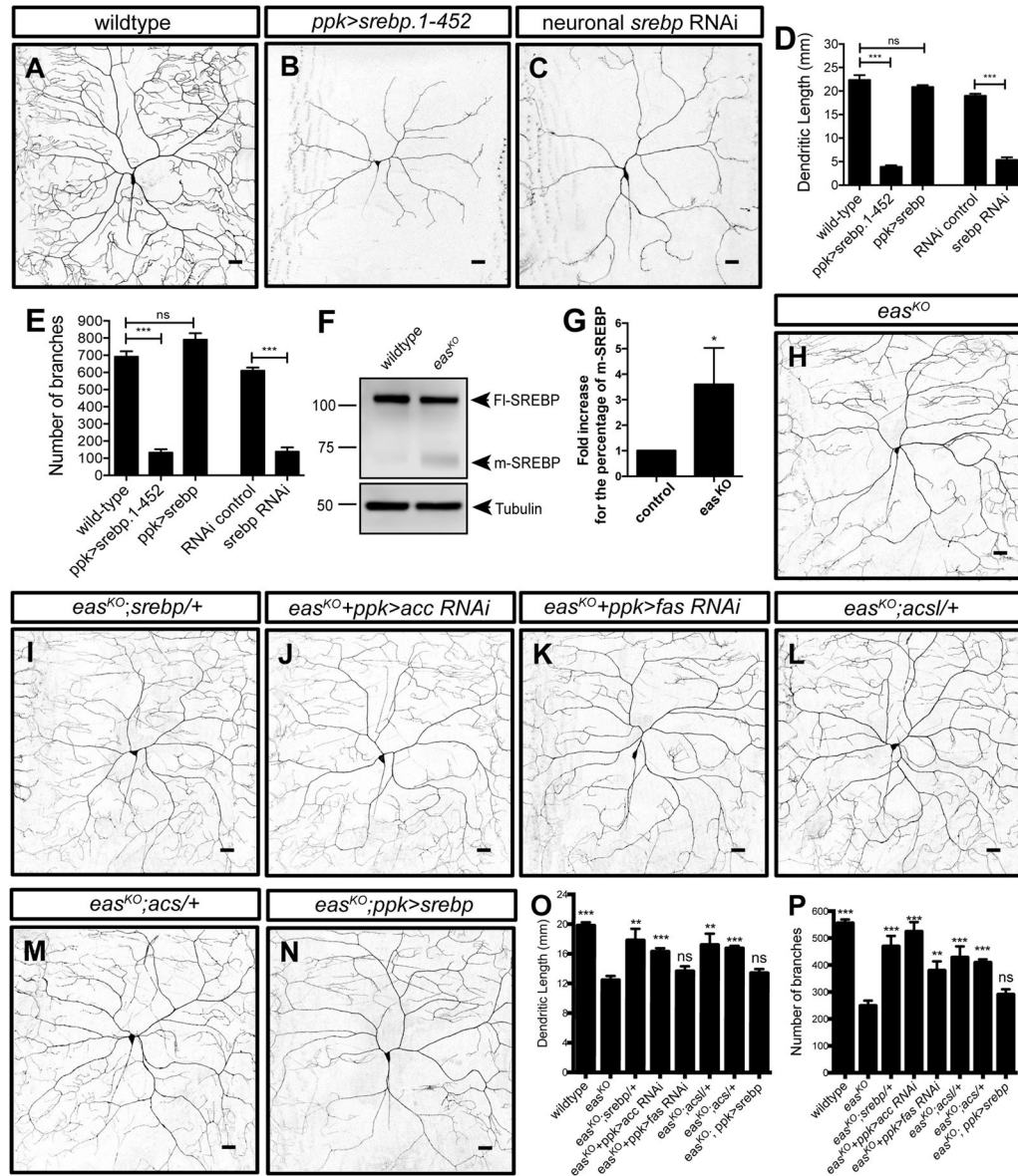
Figure 3. Aberrantly High Level of SREBP Transcriptional Activity Contributes to the Dendrite Morphogenesis Defects in *eas^KO* Mutants

(A–C) Sample images showing dendrite morphology for the indicated genotypes.

(D and E) Quantification of total dendritic length (D) and number of branches (E) for neurons expressing constitutively active forms of SREBP (*srebp.1-452*), full-length SREBP, and RNAi construct targeting SREBP. n = 5 for *ppk>srebp*. n = 6 for all of the other genotypes.

(F and G) Western blot of larval brains showing the full-length (Fl-SREBP) and constitutively active, mature (m-SREBP) forms of SREBP protein in wildtype and *eas^KO* mutants. Tubulin was used as a loading control.

(H–N) Sample images showing dendrite morphology for the indicated genotypes.

(O and P) Quantification of total dendritic length (O) and number of branches (P) for the indicated genotypes. One-way ANOVA tests were used. Results of comparison between

eas^{KO} mutants and each genotype are labeled on top of each column. n = 7 for wildtype and n = 6 for *eas^{KO}* mutants.
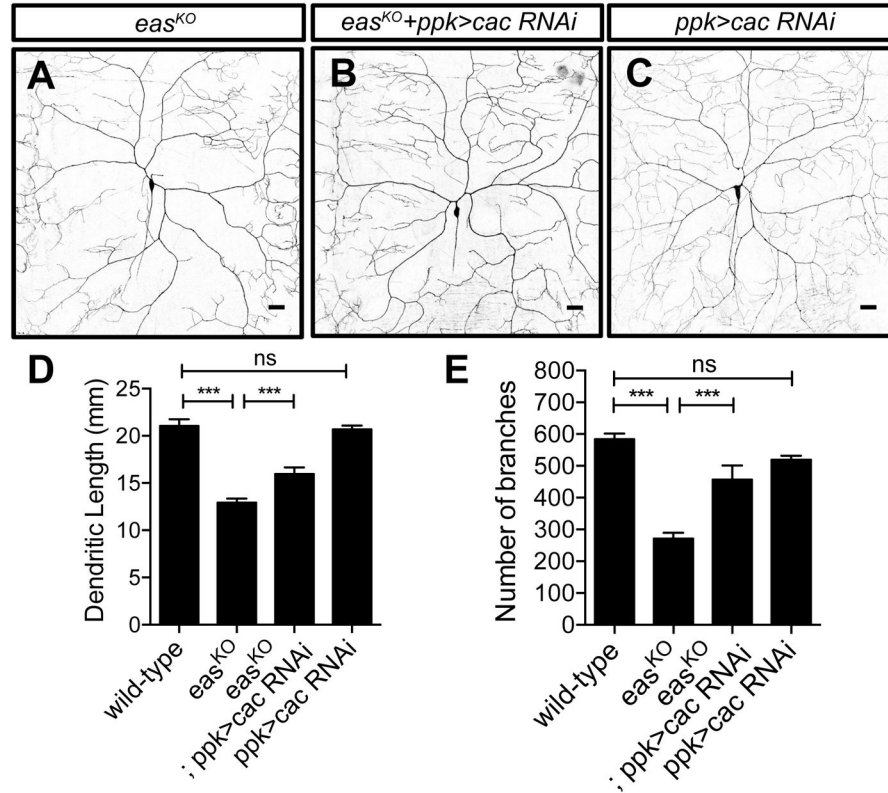
Scale bars represent 30 μm.

Figure 4. Reducing the level of *cacophony* partially suppresses dendrite morphogenesis defects in *eas^KO* mutants

(A and C) Sample images showing dendrite morphology for *eas^KO* mutants (A) (n = 9), *eas^KO* mutants with neuronal specific *cacophony* knocked down (B) (n = 6), and wildtype animals with neuronal specific *cacophony* knocked down (C) (n = 6). n = 10 for wildtype.

(D and E) Quantification of total dendritic length (D) and number of branches (E), showing that reducing the level of *cacophony* partially suppresses the dendrite growth defects in *eas^KO* mutants. One-way ANOVA tests were used.

See also Figure S3. Scale bars represent 30 μm.