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Dynein Is Required for Polarized Dendritic Transport and Uniform Microtubule Orientation in Axons

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Abstract

Axons and dendrites differ in both microtubule (MT) organization and in the organelles and proteins they contain. Here we show that the MT motor dynein plays a critical role in polarized transport and in controlling the orientation of axonal MTs in fly dendritic arborisation (da) neurons. Changes in organelle distribution within the dendritic arbors of dynein mutant neurons correlate with a proximal shift in dendritic branch position. Dynein is also necessary for the dendrite-specific localization of Golgi outposts and the ion channel Pickpocket. Axonal MTs are normally oriented uniformly plus end-distal, but without dynein axons contain both plus and minus end-distal MTs. These data suggest that dynein is required for the distinguishing properties of the axon and dendrites: without dynein, dendritic organelles and proteins enter the axon and the axonal MTs are no longer uniform in polarity.

The differential distribution of organelles and proteins to distinct compartments within cells is critical to their specialized functions. Proteins and organelles are transported to different subcellular compartments by the MT motors dynein and kinesin. The multi-subunit dynein complex travels towards MT minus ends whereas the majority of kinesins travel towards MT plus ends. Cargo localization depends on motor activity and MT organization¹. In neurons, the signal-sending axons contain MTs that are oriented uniformly plus end-distal, whereas the signal-receiving dendrites have MTs whose orientation is mixed². How might this difference in MT orientation be created? Dynein and kinesin not only move along MTs, but can also transport MTs³. Without the kinesin CHO1/MKLP the orientation of dendritic MTs are uniformly plus end-distal, rather than mixed, raising the possibility that MT motors may regulate MT polarity^{4, 5}. Whether dynein contributes to MT orientation in neurons remains an outstanding question.

Similar to most mammalian neurons, the fly dendritic arborisation (da) neurons have distinct axonal and dendritic compartments^{6–9}, and their MT organization resembles that in typical mammalian neurons^{6,7}. In a genetic screen we uncovered mutations in components of the dynein complex, *dynein light intermediate chain 2* (*dlic2*) and *dynein intermediate chain* (*dic*, also called *short wing*), that cause a proximal shift in both organelle distribution and branch position within mutant dendritic arbours. These dynein mutations cause dendritic cargo to be mislocalized to axons and result in mixed orientation of axonal MTs. Our results provide

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new insight into the function of dynein in neurons, including hitherto unrecognized roles in polarized dendritic targeting and in regulating MT polarity.

A forward genetic screen uncovered mutations in the dynein complex components *Dlic2* and *Dic* causing radical changes in da neuron dendritic arbour patterning (see Supplementary Information, Fig. S1). Mosaic analysis revealed that removing *Dlic2* specifically within da neurons resulted in a drastic reduction in dendrite arborisation with greatly reduced receptive field coverage (control: $16,903 \pm 3,292 \mu\text{m}$; *dlic2*¹¹⁵⁷: $3,671 \pm 681 \mu\text{m}$, $p < 0.001$; data represent mean $\pm$ standard deviation; $n=4$; Fig. 1a,b,f; Supplementary Information, Fig. S4, and data not shown). Not only was there a reduction in the total number of branch points (control: 499 ± 124 , $n=4$; *dlic2*¹¹⁵⁷: 140 ± 29 , $p < 0.01$; $n=4$; Fig. 1g), fewer branches were located distally (Fig. 1b') while the proximal dendrites branched profusely (Fig. 1b''), revealing a dramatic proximal shift in branch distribution (Fig. 1e). Also, most *dlic2*¹¹⁵⁷ axons are abnormally thick and many appear to have split, or branched, into multiple neurites a short distance from the soma (Fig. 1h; Supplementary Information, Fig. S2). The multiple neurites of the mutant da neurons bundle with other axons in the nerve, although only one neurite fully extends into the ventral nerve cord (VNC) (Supplementary Information, Fig. S2). These dendritic and axon phenotypes are rescued by neuronal expression of *UAS-dlic2-eGFP* (Fig. 1d). Likewise, a *dic*⁺ transgene rescues the lethality and phenotypes of *dic*¹²²⁹ animals (Supplementary Information, Fig. S3). In addition, *dynein heavy chain* (*dhc*) clones exhibited a similar, although less severe, phenotype (possibly due to differential perdurance of *Dhc* versus *Dlic2*; Supplementary Information, Fig. S3), and loss of *Lis1*, which interacts with dynein, phenocopies *dlic2*¹¹⁵⁷ (Fig. 1c, and Supplementary Information, Fig. S3). We therefore conclude that these phenotypes result from a loss of dynein function.

Dynein mediates the subcellular localization of Golgi¹⁰. In mammalian and fly neurons, Golgi structures in the form of "outposts" localize to dendrites and influence branching^{8, 11}. Indeed, distal *dlic2*¹¹⁵⁷ dendritic arbours displayed a nearly four-fold decrease in the number and size of Golgi outposts, which were marked by Mannosidase II-eGFP (ManII-eGFP) (number per 100 μm : control: 7.92 ± 3.11 , $n=6$; *dlic2*¹¹⁵⁷: 2.00 ± 2.00 , $p < 0.025$, $n=3$; total size per 100 μm : control: $1.12 \pm 0.36 \mu\text{m}^2$, $n=6$; *dlic2*¹¹⁵⁷: $0.25 \pm 0.25 \mu\text{m}^2$, $n=3$, $p < 0.01$; Fig. 2g,h,k,l). In contrast, there was a significant increase in the number and size of Golgi outposts in the proximal *dlic2*¹¹⁵⁷ dendritic arbours (number within 30 μm from soma: control: 10.00 ± 3.39 , $n=5$; *dlic2*¹¹⁵⁷: 15.45 ± 4.18 , $n=11$, $p < 0.025$; total size within 30 μm from soma: control: $3.31 \pm 1.08 \mu\text{m}^2$, $n=5$; *dlic2*¹¹⁵⁷: $6.00 \pm 1.75 \mu\text{m}^2$, $n=11$, $p < 0.01$; Fig. 2a,b,e,f,k,l). This change in Golgi outpost distribution (reduced distally, increased proximally) thus parallels the change in branch distribution.

In contrast to the scarcity of Golgi outposts in wild type axons⁸ (Fig. 2a,c,k), there was a striking increase in Golgi outpost number in *dlic2*¹¹⁵⁷ axons (number per 100 μm : control: 2.33 ± 2.71 , $n=6$; *dlic2*¹¹⁵⁷: 34.49 ± 14.55 , $n=5$, $p < 0.001$; Fig. 2a–d,k). Golgi outpost distribution was similarly altered in the axons of *dic*¹²²⁹/*dic*^{ts} larvae (control: 1.43 ± 1.69 per 100 μm , $n=19$; *dic*¹²²⁹/*dic*^{ts}: 15.36 ± 7.29 , $n=11$, $p < 0.001$), which survive to 3rd instar and whose da neurons exhibit similar, though milder, dendrite and axon defects (Supplementary Information, Fig. S3). Live imaging of ManII-eGFP in *dic*¹²²⁹/*dic*^{ts} neurons further revealed Golgi outposts moving from the soma into the axon and travelling anterogradely and retrogradely within the axon (Fig. 2i,j, Supplementary Information, Movies 3 and 4 and Supplementary Table 1). In contrast, in control axons, the Golgi outposts that were occasionally present were stationary, and we did not observe any Golgi outposts moving from the soma into the axon (Fig. 2i). These results suggest dynein actively prevents Golgi outposts from entering axons in wild type neurons.

Dynein also transports endosomes¹², which regulate dendritic membrane supply^{13, 14}. Indeed, disrupting endosomal function alters dendrite morphogenesis, including branch formation¹⁵. Normally, the recycling and early endosomal marker Rab4-RFP and the late endosome and lysosome marker Spinster (Spin)-RFP localize to both axons and dendrites (Fig. 3a,a',c,c'). In *dic¹²²⁹/dic^{ts}* and *dlic2¹¹⁵⁷* neurons, Rab4-RFP and Spin-RFP are virtually absent from dendrites (Fig. 3b,b',d,d' and Supplementary Information, Fig. S5) but are still present in axons, suggesting that dynein is required for the dendritic localization of endosomes.

We hypothesized that as Dlic2 decreases over time in *dlic2¹¹⁵⁷* clones, Golgi outposts and endosomes would fail to travel distally, and new branches would only be added proximally. Indeed, time lapse analysis revealed proximal branch dynamics increased in *dlic2¹¹⁵⁷* dendritic arbours and the change in branch dynamics correlated with Golgi outpost position (Supplementary Information, Fig S6), suggesting that machinery (including Golgi outposts) required for branch growth and dynamics accumulate proximally in dynein mutant neurons.

Next we examined the localization of Pickpocket (Ppk), which belongs to a family of conserved degenerin/epithelial sodium channels that likely function as sensory channels¹⁶. Whereas Ppk is normally detectable at low levels in dendrites but not in axons¹⁷ (Fig. 3e,e'), in *dlic2¹¹⁵⁷* clones Ppk was present in both dendrites and axons (Fig. 3f,f').

Besides transporting organelles and proteins, dynein also transports MTs within axons, leading us to investigate if axonal MT organization is impacted by the loss of dynein function. First we used the MT minus end marker Nod-βgal, a chimera comprised of the Nod motor domain fused to the Kinesin1 (Kin1) coiled-coiled domain and β-galactosidase¹⁸. Although full length Nod preferentially binds MT plus ends¹⁹, the Nod-βgal chimera localizes to MT minus ends in multiple cell types and is commonly used as a MT minus end marker¹⁸ (Supplementary Information, Fig. S7). In fly neurons, including da neurons, Nod-βgal localizes specifically to dendrites^{6–9, 18, 20, 21} (Fig. 4a,a'). In *dlic2¹¹⁵⁷* clones and in *dic¹²²⁹/dic^{ts}* neurons Nod-βgal was still present in dendrites but frequently localized to axons as well (36% of *dlic2¹¹⁵⁷* ddaC axons (n=11) and 30% of *dic¹²²⁹/dic^{ts}* ddaC axons (n=15) showed strong Nod-βgal signal; Fig. 4b,b',c,c'). The amount of Nod-βgal in the mutant axons was variable and did not correlate with the formation of ectopic neurites or axonal width. These results are consistent with the finding that Nod-βgal localizes to the axons of fly photoreceptor neurons expressing a dominant-negative form of *Glued*²², which is a component of the dynactin complex that is necessary for dynein function²³. Over-expressing another dynactin complex member, *dynamitin* (*dmn*), also interferes with dynein function²³. ddaC neurons over-expressing *dmn* exhibited dendritic and axonal morphology defects similar to *dlic2¹¹⁵⁷* and *dic¹²²⁹/dic^{ts}*, and Nod-βgal was ectopically localized to axons (50% of *ppk-Gal4 UAS-dmn* axons had strong Nod-βgal signal, n=30; Fig. 4d,d').

Next we employed the axon specific marker Kin-βgal^{18, 24}, which is comprised of the Kin1 motor and coiled-coiled domains together with β-galactosidase, and found it is localized exclusively to axons in all three different dynein loss-of-function scenarios as in control neurons (Fig. 4e–h). Additionally, pre-synaptic components such as Cystein string protein (Csp), Bruchpilot and Syntaxin localized normally in *dic¹²²⁹/dic^{ts}* neurons (data not shown), although there are axonal “cargo jams” indicative of defects in axonal transport (Supplementary Fig. S7).

The ectopic axonal localization of Nod-βgal in dynein loss-of-function neurons suggests a change in MT polarity. To further analyze MT orientation we utilized EB1-GFP, which binds growing MT plus ends and takes on a comet-like appearance as the MT grows. In fly PNS axons EB1-GFP always moves away from the soma (anterograde)⁷, indicating the axonal MTs are orientated with their plus ends-distal, as in mammalian neurons². Similar to control neurons

(Fig. 5a,b,e; Table 1 and Supplementary Information, Movie 1), in *dic¹²²⁹/dic^{ts}* axons EB1-GFP always moved anterogradely during 2nd instar (Table 1); however, during 3rd instar, a third of the EB1-GFP comets travelled retrogradely (Fig. 5c–e; Table 1 and Supplementary Information, Movie 2). Similar abnormal EB1-GFP movements occurred in *dlic2¹¹⁵⁷* clones (Supplementary Information, Movie 5) and in neurons over-expressing *dmn* (40% retrograde, 60% anterograde, n=177; Supplementary Information, Movie 6). The loss of dynein function also affects EB1-GFP movement in the axons of class I da neurons (control: 100% anterograde, n=96; *dic¹²²⁹/dic^{ts}* 19% retrograde, 81% anterograde, n=80; Fig. 5e), even though *dic¹²²⁹/dic^{ts}* class I axons are not noticeably enlarged, nor do they have ectopic branches. Whereas the loss of dynein function perturbed the orientation of axonal MTs, the orientation of dendritic MTs appeared normal. The direction and rate of EB1-GFP movement in dendrites was comparable between control (rate: 6.49 ± 2.16 $\mu\text{m}/\text{min}$; 96% retrograde, 4% anterograde; n=75) and *dic¹²²⁹/dic^{ts}* (rate: 6.37 ± 1.95 $\mu\text{m}/\text{min}$; 98% retrograde, 2% anterograde; n=64). Thus, the loss of dynein activity disrupts the uniform orientation of MTs in axons without causing a detectable effect on the orientation of dendritic MTs.

Proper cellular morphology and function depends on the polarized localization of organelles and proteins to specific subcellular compartments. In this study, we show that dynein plays a crucial role in dendrite arbour patterning and in organizing distinct functional compartments (the axon and dendrites) of a neuron. The position of branches within a dendritic arbour has a key role in determining the inputs a neuron receives from pre-synaptic axons or, in the case of sensory neurons, the local environment. We show here that dynein is necessary for proper positioning of dendritic branches relative to the soma. As a motor, dynein likely influences branch formation by mediating the distribution of cargos that affect branch growth and dynamics. Notwithstanding an overall decrease in dendrite extension and branching in dynein mutants, time-lapse analysis of a few dendrites revealed that they extend normally but have fewer and less stable terminal branches, suggesting that decreased terminal branching is not simply caused by a decrease in dendrite growth. One likely explanation is that “branching machinery” (including Golgi outposts, endosomes and potentially other proteins and/or organelles) that are normally transported distally for dendrite extension and maintenance become trapped in the proximal arbour in the dynein mutant neurons, resulting in decreased distal branching and the formation of ectopic branches close to the cell body.

Without dynein, Golgi outposts and Ppk are present ectopically in axons, revealing a previously unappreciated role for dynein in mediating the dendrite-specific localization of organelles and proteins. One possible explanation for axonal mislocalization is that Golgi outposts and Ppk interact with a MT plus end-directed motor (e.g., kinesin) that transports them into axons in the absence of dynein. Dynein might normally transports such cargo directly to dendrites; alternatively, it is also possible that cargo first enters axons but that dynein counter-acts kinesin and carries this cargo out of axons. Since we never observed Golgi outposts moving from the soma into the axon in wild type neurons, our data favour the former possibility. In contrast to the mislocalization of dendritic protein and organelles, Kin- β gal and proteins destined for the axonal terminal retain their polarized distribution, perhaps to be expected given that kinesin mediates the majority of anterograde axonal transport¹.

In mammalian and fly neurons, axonal MTs are arrayed plus end-distal whereas dendritic MT orientation is mixed^{2, 7}. A long-standing question concerns the mechanism(s) that establish and maintain different MT orientations in axons and dendrites. Loss of dynein function causes the axonal localization of Nod- β gal and retrograde movement of EB1-GFP, indicating that minus end-distal MTs are present in these mutant axons. How might dynein regulate the orientation of axonal MTs? The sliding filament model of axonal MT transport proposes that a subset of dynein in the axon is stationary (via an interaction with stable MTs and/or actin) and that dynein's motor domain interacts with short MT polymers, propelling plus end-distal

MTs down the axon as the motor moves to the MT minus end³. Our in vivo data support the idea that in addition to transporting MTs, dynein functions as a “gatekeeper” to move minus end-distal MTs towards the soma, excluding them from the axon. Neurons lacking functional dynein would still transport MTs, likely via kinesin³, but now minus end-distal MTs would infiltrate the axon. Proximal axons likely have unique properties¹, providing a possible explanation for how minus end distal MTs would be excluded from axons but not dendrites.

Recent studies indicate that the trans Golgi network (TGN), which comprises part of the Golgi outposts^{25, 26}, can also function as a MT organizing center (MTOC)^{27, 28} and influence MT organization. Although it is conceivable that Golgi outposts mislocalized to dynein mutant axons could alter MT polarity, expressing *lava lamp*^{dominant-negative}, which prevents Golgi from associating with dynein without affecting dynein function²⁹, causes Golgi outposts to mislocalize to axons without altering MT orientation (Ye et al., 2007 and unpublished observations). Moreover, the change in axonal MT orientation is not likely to be simply a consequence of altered axon morphology because the loss of dynein function also alters the MT orientation of class I neuron axons, which appear relatively normal. With our current level of understanding, the model in which dynein acts as a “gatekeeper” is most consistent with our results and the findings of others.

METHODS

Mutagenesis and Mapping

EMS mutagenesis was performed according to standard protocols. Briefly, male flies were fed 20 mM EMS to induce mutations on an isogenic *FRT 19A* chromosome. We screened the live embryonic and larval progeny of approximately 1,900 lethal lines and recovered 112 mutants that affect dendrite and/or axon morphogenesis. Mutations were mapped using X chromosome duplications and deficiencies from Bloomington, followed by complementation tests with known mutants (see Supplemental Information for additional details).

Fly Stocks

*dlic2*¹¹⁵⁷ and *dic*¹²²⁹ were generated by EMS mutagenesis as described above. Both mutations cause lethality during the 2nd larval instar. The following lines were generously shared: *FRT G13 lis1*^{G10.14} and *FRT 2A dhc64C*⁴⁻¹⁹ (L. Luo), *UAS-EB1-GFP* (T. Uemura), *UAS-dmn* (R. Warrior), *dic+* transgene (T. Hays), and *UAS-Spin-RFP* (S. Sweeney). *UAS-Rab4-RFP*, *dic*^{ts} and *lis1*⁴⁻¹⁹ are from Bloomington. To generate *UAS-dlic2-eGFP* flies, *dlic2* cDNA from the *Drosophila Genomics Resource Center* was cloned into a modified *pUAST* vector so that eGFP was fused in frame to the Dlic2 C-terminus; transgenic flies were generated according to standard protocols. *UAS-dmn* was over-expressed in class IV neurons using *ppk-Gal4* and *4-77-Gal4*.

Clonal Analysis

Clonal analysis (MARCM) was performed as previously described^{8, 30}. Briefly, embryos were collected on grape plates for 2 hr, allowed to develop for 2 hr at 25°C and heat shocked for 45 min twice at 38°C with a 30 min rest in between. Larvae with da neuron clones were selected and examined by either live imaging or immunohistochemistry. For da neuron clone analysis *yw hs-flp tub-Gal80 FRT 19A; 109(2)80-Gal4 UAS-mCD8-GFP* flies were mated with: (1) *yw FRT 19A* (control), (2) *yw dlic2*¹¹⁵⁷ *FRT 19A* and (3) *yw dlic2*¹¹⁵⁷ *FRT 19A; UAS-dlic2-eGFP* (*dlic2+* rescue). The location of *dic* between the centromere and *FRT 19A* has prevented us from generating loss-of-function clones, so we focused on the phenotypes displayed in *dlic2*¹¹⁵⁷ clones. *lis1* clones: *yw hs-flp elav-Gal4 UAS-mCD8-GFP; FRT G13 lis1*^{G10.14}/*FRT G13 hs-flp tub-Gal80*. *dhc64C* clones: *yw hs-flp elav-Gal4 UAS-mCD8-GFP; FRT 2A dhc64C*⁴⁻¹⁹/*FRT 2A tub-Gal80*. For Golgi outpost analysis *yw hs-flp tub-Gal80 FRT 19A; 109*

(2) *80-Gal4 UAS-ManII-eGFP* flies were mated with: (1) *yw FRT 19A; UAS-mCD8-dsRed* (control) and (2) *yw dlic2¹¹⁵⁷ FRT 19A; UAS-mCD8-dsRed*. The number and size of Golgi outposts in fixed (Fig. 3a–h,k,l) and live samples (Fig. 3i,j) were quantified using ImageJ. We sampled two domains of the wild type and mutant dendritic arbours: a proximal domain, which included all dendrites within a 30 μ m radius from the soma, and a distal domain, which encompassed an 100 μ m segment of the distal part of major dendrites that extended towards the dorsal midline.

Live Imaging and Analysis of EB1-GFP

2nd or 3rd instar larvae of the following genotypes were imaged: (1) *4-77-Gal4 UAS-mRFP; UAS-EB1-GFP* (control), (2) *dic¹²²⁹/dic^{ts}; 4-77-Gal4 UAS-mRFP; UAS-EB1-GFP*, (3) *2-21-Gal4/UAS-EB1-GFP* (control) and (4) *dic¹²²⁹/dic^{ts}; 2-21-Gal4/UAS-EB1-GFP*. Larvae were washed briefly in 1X PBS before mounting in halocarbon oil for live imaging of ddaC (class IV) and ddaE (class I) neurons. Larvae were imaged on average 5–15 min but no longer than 25 min on a Zeiss LSM 510 confocal microscope. Movies of EB1-GFP comets were made by imaging axons or dendrites every second for 1.5 to 6.5 min. For each genotype multiple larvae were imaged. EB1-GFP direction and rate were calculated using ImageJ (NIH), which was also used to generate the kymographs.

Immunohistochemistry and Dendrite Analysis

3rd instar larvae were fixed according to standard protocols³⁰. The following antibodies were used: rabbit anti- β gal, 1/5000 (Cappel); rat anti-mCD8, 1/100 (Invitrogen); rabbit anti-GFP, 1/3000; rabbit, anti-Ppk 1/800 (generously provided by W. Johnson); mouse 22C10, 1/100 (DSHB); Cy3-conjugated anti-HRP, 1/1000 (Jackson ImmunoResearch). In the Figures, we label the neurites that extend within the intersegmental nerve (ISN) as axons; however, as described in the text, these neurites contain proteins and organelles that are normally found specifically in dendrites. Dendrite analysis was performed using Neurolucida.

Statistical analysis

All statistical tests were performed with two-tailed Student t-test according to standard methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. *dlic2*¹¹⁵⁷ Functions Cell-Autonomously to Regulate Dendrite and Axon Development
Dendritic arbours are labelled by mCD8-GFP (anterior is to the left and dorsal is up). Scale bars: 30 μm (a–d) and 10 μm (a', a'', b', b'', h). Error bars represent Standard Deviation (S.D.) in this and all subsequent figures. (a–d) Clones of class IV ddaC neurons: (a) wt, (b) *dlic2*¹¹⁵⁷, (c) *lis1*^{G10.14} and (d) *dlic2*¹¹⁵⁷; *dlic2*-eGFP (*dlic2*⁺ rescue). The size and pattern of the *dlic2*¹¹⁵⁷ and *lis1*^{G10.14} arbours are grossly abnormal (b,c). The number of terminal branches in *dlic2*¹¹⁵⁷ is substantially reduced (compare a' to b') whereas the number of proximal branches is greatly increased (compare a'' to b''). Expressing *dlic2*-eGFP specifically in *dlic2*¹¹⁵⁷ clones fully rescues the dendrite and axon phenotypes (d).

(e) Sholl analysis of dendritic arbours of wt and *dlic2¹¹⁵⁷* ddaC clones. Concentric circles with 5 μm increments were drawn around the soma and the number of dendritic branches that intersected each circle was tallied. In control ddaC neurons the number of branches increases progressively from proximal to distal and the maximal number of dendrite branches are found between 225 and 250 μm from the cell body. The *dlic2¹¹⁵⁷* neurons exhibited a dramatic proximal shift in dendrite distribution such that the majority of dendrites are located within 100 μm of the soma. Blue diamonds: wt, red squares: *dlic2¹¹⁵⁷*.

(f) Dendrite length of wt ddaC ($16,902 \pm 3,292 \mu\text{m}$, $n=4$) and *dlic2¹¹⁵⁷* ddaC ($3,671 \pm 681 \mu\text{m}$, $n=4$, *** $p<0.001$, Student's unpaired t - test). The dendrite length of *dlic2¹¹⁵⁷* ddaC is greatly reduced.

(g) The number of dendritic branch points is decreased in *dlic2¹¹⁵⁷* ddaC (140 ± 29 , $n=4$, ** $p<0.01$, Student's unpaired t - test) compared to wt (499 ± 124 , $n=4$).

(h) Axons of individual wt (top) and *dlic2¹¹⁵⁷* (bottom) ddaC clones coursing through the intersegmental nerve (ISN). The wt axon is a single process whereas the *dlic2¹¹⁵⁷* axon has separated into multiple neurites, as revealed in cross sections of the ISN (rightmost panels) at the points indicated by the dashed line. Dotted lines in the rightmost panels delineate the boundary of the nerve. Green: mCD8; magenta: HRP, which labels all nerve processes.

Figure 2. Loss of Dynein Function Alters Golgi Outpost Distribution

Green: ManII-eGFP, magenta: mCD8. Arrowheads: axon, arrows: position of Golgi outposts. (a–h) Localization of Golgi outposts in wt and *dlic2*¹¹⁵⁷ ddaC clones. Boxes with dotted lines highlight axons (shown in c,d) and boxes with dashed lines highlight proximal dendrites (shown in e,f). Distal dendritic tips are shown (g,h). Scale bars: 30 μ m (a,b) and 15 μ m (c–h). (a–f) In (c) wt ddaC there are virtually no Golgi outposts in the axon (box with dotted lines in a). In contrast, many are present in (d) *dlic2*¹¹⁵⁷ axons (box with dotted lines in b). Golgi outposts are more numerous in the proximal dendritic arbour of *dlic2*¹¹⁵⁷ clones (compare e and f). The signal intensity is optimized for visualizing the smaller Golgi outposts in the proximal arbour; consequently, the outposts close to the soma are over-exposed (e,f).

(g,h) Along the distal dendrite there are fewer Golgi outposts in (h) *dlic2¹¹⁵⁷* compared to (g) wt.

(i,j) Frames from movies (Supplementary Information, Movies 3 and 4) following Golgi outposts in the soma and axon of (i) control and (j) *dic¹²²⁹/dic^{ts}* neurons. Time (in seconds) is as indicated. The single Golgi outpost (arrowhead) in the control axon does not move whereas in the *dic¹²²⁹/dic^{ts}* neuron there are many Golgi outposts in the axon and one Golgi outpost (arrow) moves from the soma into the axon. Scale bar: 12.5 μm .

(k) Number of Golgi outposts in proximal (control: 10.00 ± 3.39 , $n=5$; *dlic2¹¹⁵⁷*: 15.45 ± 4.18 , $n=11$, $*p<0.025$, Student's unpaired *t*- test) and distal (control: 7.92 ± 3.11 per 100 μm , $n=6$; *dlic2¹¹⁵⁷*: 2.00 ± 2.00 per 100 μm , $n=3$, $*p<0.025$, Student's unpaired *t*-test) dendritic arbours as well as axons (control: 2.33 ± 2.71 per 100 μm , $n=6$; *dlic2¹¹⁵⁷*: 34.49 ± 14.55 per 100 μm , $n=5$, $***p<0.001$, Student's unpaired *t*- test).

(l) Size of Golgi outposts in proximal (control: $3.31 \pm 1.08 \mu\text{m}^2$, $n=5$; *dlic2¹¹⁵⁷*: $6.00 \pm 1.75 \mu\text{m}^2$, $n=11$, $**p<0.01$, Student's unpaired *t*- test) and distal (control: $1.12 \pm 0.36 \mu\text{m}^2$, $n=6$; *dlic2¹¹⁵⁷*: $0.25 \pm 0.25 \mu\text{m}^2$, $n=3$; $**p<0.01$, Student's unpaired *t*- test) dendritic arbours as well as axons (control: 0.45 ± 0.51 , $n=6$; *dlic2¹¹⁵⁷*: 16.71 ± 6.09 , $n=5$, $***p<0.001$, Student's unpaired *t*- test).

Figure 3. Localization of Endosomes and the Ion Channel Ppk Depends on Dynein
Green: RFP (a–d) or Ppk (e,f), magenta: mCD8. *ppk-Gal4 UAS-mCD8-GFP* (a–f) was used to visualize dendrites and axons of *ddaC* neurons expressing the endosomal marker *UAS-Rab4-RFP* (a,b) or *UAS-Spin-RFP* (c,d). Arrows indicate dendrites, arrowheads point to axons. Scale bar: 30 μ m.
(a,b) Rab4-RFP is present in dendrites and axons in (a) wt; however, its dendritic localization is reduced in (b) *dic¹²²⁹/dic^{ts}*.
(c,d) Spinster-RFP localizes to dendrites and axons in (c) wt, but its dendritic distribution is reduced in (d) *dic¹²²⁹/dic^{ts}*.
(e,f) Ppk is found specifically in dendrites in (e) wt, but is present in both axons and dendrites of (f) *dlic2¹¹⁵⁷* *ddaC* clones.
(g) Table summarizing the axonal and dendritic localization of Golgi outposts, endosomal markers and Ppk in wt (dynein +) and dynein loss-of-function (dynein-) neurons.

Figure 4. Mislocalization of Nod-βgal, but not Kin-βgal, in Dynein Mutant Neurons

Green: βgal, magenta: mCD8. *UAS-nod-lacZ* (a–d) and *UAS-kin-lacZ* (e–h) driven by *ppk-Gal4*, which also drives *UAS-mCD8-GFP* expression. Open arrowhead: proximal axon, filled arrowhead: axon shaft; arrows: proximal dendrites. Scale bar: 30 μm.

(a–d) Localization of Nod-βgal in (a) wt, (b) *dic¹²²⁹/dic^{ts}*, (c) *dlic2¹¹⁵⁷* ddaC clone and (d) ddaC over-expressing *dmn* (*dmn* OE). The lower panels (a'–d') show the Nod-βgal channel at a slightly higher magnification. In wt axons Nod-βgal enters only the very proximal axon but is not present in the axon shaft. In *dic¹²²⁹/dic^{ts}*, *dlic2¹¹⁵⁷* and *dmn* OE neurons Nod-βgal extends into the axon shaft. The *dic¹²²⁹/dic^{ts}* axon shown in (b,b') is not unusually wide, yet it has strong Nod-βgal signal.

(e–h) Localization of Kin-βgal in (e) wt, (f) *dic¹²²⁹/dic^{ts}*, (g) *dlic2¹¹⁵⁷* ddaC clone and (h) *dmn* OE. Inserts show Kin-βgal channel alone. Kin-βgal is normally localized specifically to axons and this distribution is not changed by a reduction in dynein activity.

Figure 5. Mixed Orientation of Axonal MTs in *dic¹²²⁹/dic¹²²⁹* Neurons Revealed by EB1-GFP Kymographs (a,c) and movie frames (b,d) showing the trajectory of EB1-GFP comets in the axons of *ddaC* neurons. *UAS-EB1-GFP* was expressed in class IV neurons by *4-77-Gal4* (a–e) and in class I neurons by *2-21-Gal4* (e). Time (in seconds) is as indicated. The bar to the right of the kymograph indicates the portion of the movie from which the frames were taken. The soma is to the left.

(a,c) Kymographs showing that EB1-GFP always moves away from the soma in (a) control axons, but moves both towards and away from the soma in the axons of (c) *dic¹²²⁹/dic¹²²⁹* neurons. A portion of the *dic¹²²⁹/dic¹²²⁹* movie was out of focus (109–225 sec) and this section of the kymograph was removed for clarity.

(b,d) Single frames from movies showing the movement of individual EB1-GFP comets. Arrowheads and diamonds indicate comets moving anterogradely (yellow) or retrogradely (magenta). Scale bar: 5 μ m. In (b) control axons all EB1-GFP comets move anterogradely whereas in (d) *dic¹²²⁹/dic¹²²⁹* axons EB1-GFP moves both anterogradely and retrogradely. EB1-GFP comets moved in both directions in *dic¹²²⁹/dic¹²²⁹* axons with or without ectopic neurites, and within individual neurites EB1-GFP comets moved retrogradely (42%) and anterogradely (58%).

(e) Bar graph illustrating the percentage of EB1-GFP comets that move anterogradely (yellow) or retrogradely (magenta) in the axons of class IV and class I control and *dic¹²²⁹/dic^{ts}* neurons in 3rd instar larvae.

Table 1
Direction and Rate of EB1-GFP Comets in the Axons of Control and *dic¹²²⁹/dic⁴⁵ ddaC* Neurons
EB1-GFP rates were calculated from movies of EB1-GFP comets in *ddaC* axons in live larvae. Control genotype: *477-Gal4; UAS-EB1-GFP*; *dic¹²²⁹/dic⁴⁵* genotype: *dic¹²²⁹/dic⁴⁵; 477-Gal4; UAS-EB1-GFP*. Larval stage is indicated at top of table; n: number of EB1-GFP comets; rate: mean $\pm$ SD; N.C.: not calculated.

	direction	2 nd instar		3 rd instar	
		n (%)	rate (μ m/min)	n (%)	rate (μ m/min)
control	anterograde	64 (100%)	6.68 $\pm$ 2.34	26 (100%)	5.93 $\pm$ 2.13
	retrograde	0	N.C.	0	N.C.
<i>dic¹²²⁹/dic⁴⁵</i>	anterograde	45 (100%)	5.15 $\pm$ 2.38	133 (68%)	8.03 $\pm$ 3.11
	retrograde	0	N.C.	64 (32%)	6.54 $\pm$ 2.42