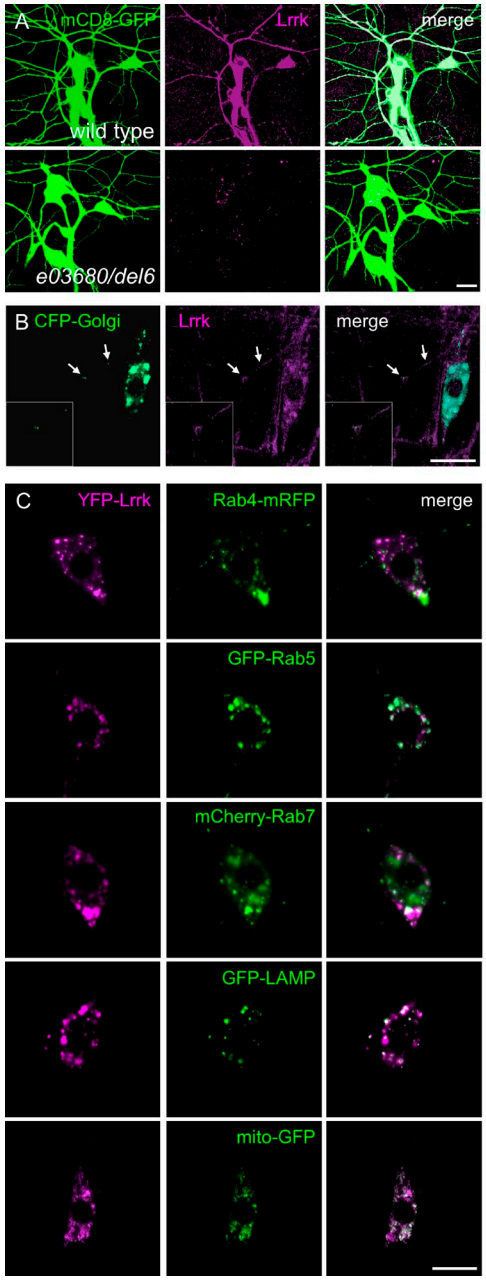
lin et al., http://www.jcb.org/cgi/content/full/jcb.201411033/DC1



D

Colocalization of YFP-Lrrk and organelle marker statistics

Organelle marker	mean ± SD (%)	n
ManII-GFP	62.80 ± 17.0	15
Rab4-mRFP	23.80 ± 8.6	10
GFP-Rab5	46.80 ± 9.8	10
mCherry-Rab7	39.13 ± 7.3	12
GFP-LAMP	42.10 ± 11.2	10
mito-GFP	44.80 ± 6.4	11

Figure S1. **Colocalization of Lrrk with organelle or vesicle markers in da neurons.** (A) Lrrk expression (magenta) in da neurons was detected by immunostaining in *109(2)80* control (wild type) and was absent in the *Lrrk* mutant *e03680/del6* (bottom). Expressed mCD8-GFP labels neuronal morphology (green). (B) Colocalization of Lrrk puncta with CFP-Golgi driven by *ppk-GAL4* was detected in cell bodies and dendrites (arrows) of da neurons by immunostaining with membrane permeation. (C) Colocalizations of YFP-Lrrk (magenta) with Rab4-mRFP, GFP-Rab5, mCherry-Rab7, GFP-LAMP, or mito-GFP (green) expressed by *ppk-GAL4* in class IV da neurons. Bars, 10 µm. (D) Table shows the percentage (±SD) of YFP-Lrrk colocalized with organelle markers and numbers of neurons scored. Colocalization % = colocalization area/YFP-Lrrk area × 100%.

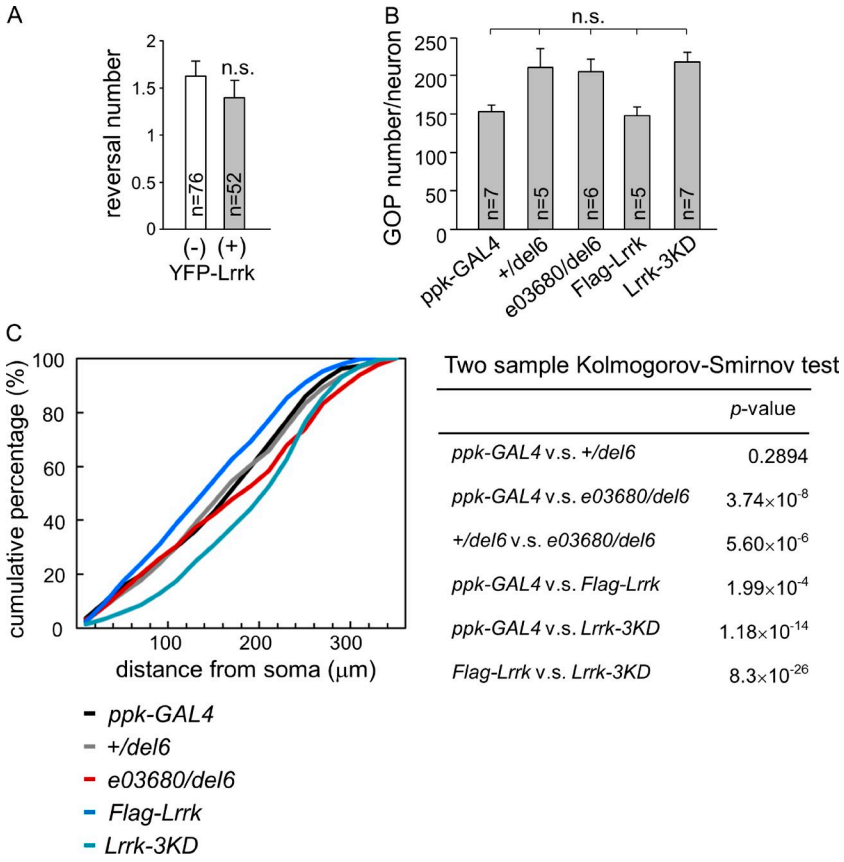


Figure S2. **Number and distribution of GOPs in da dendrites with different levels of Lrrk.** (A) Reversal of ManII-GFP puncta of with (shaded bar) or without (open bar) YFP-Lrrk colocalization in *ppk-GAL4* neurons was recorded when a punctum makes a change in direction between anterograde and retrograde. YFP-Lrrk(–) puncta make 1.6 ± 0.16, whereas YFP-Lrrk(+) puncta make 1.4 ± 0.18 reversals during the recording period of 300 s. No significant difference ($P > 0.05$) was detected in these two groups of ManII-GFP puncta by Student's *t* test. (B) Bar graph shows mean numbers of ManII-GFP puncta in dendrites of *ppk-GAL4* (152.9 ± 7.6), *+/del6* (209.6 ± 24.4), *e03680/del6* (205.2 ± 16.2), *Flag-Lrrk* (147.6 ± 11.3), and *Lrrk-3KD* overexpression (217.7 ± 11.1) by *ppk-GAL4*. No significant differences (n.s.; $P > 0.05$) compared with *ppk-GAL4* were detected using Student's *t* test. Error bars represent SEM. (C) Distributions of ManII-GFP puncta in dendrites of *ppk-GAL4*, *+/del6*, *e03680/del6*, *Flag-Lrrk*, and *Lrrk-3KD* overexpression by *ppk-GAL4* are shown as cumulative percentages (y axis) along the proximal-distal axis (x axis). Numbers of class IV da neurons are the same as in B. Statistical significances between two samples were assayed by two-sample Kolmogorov-Smirnov test and p-values are shown.

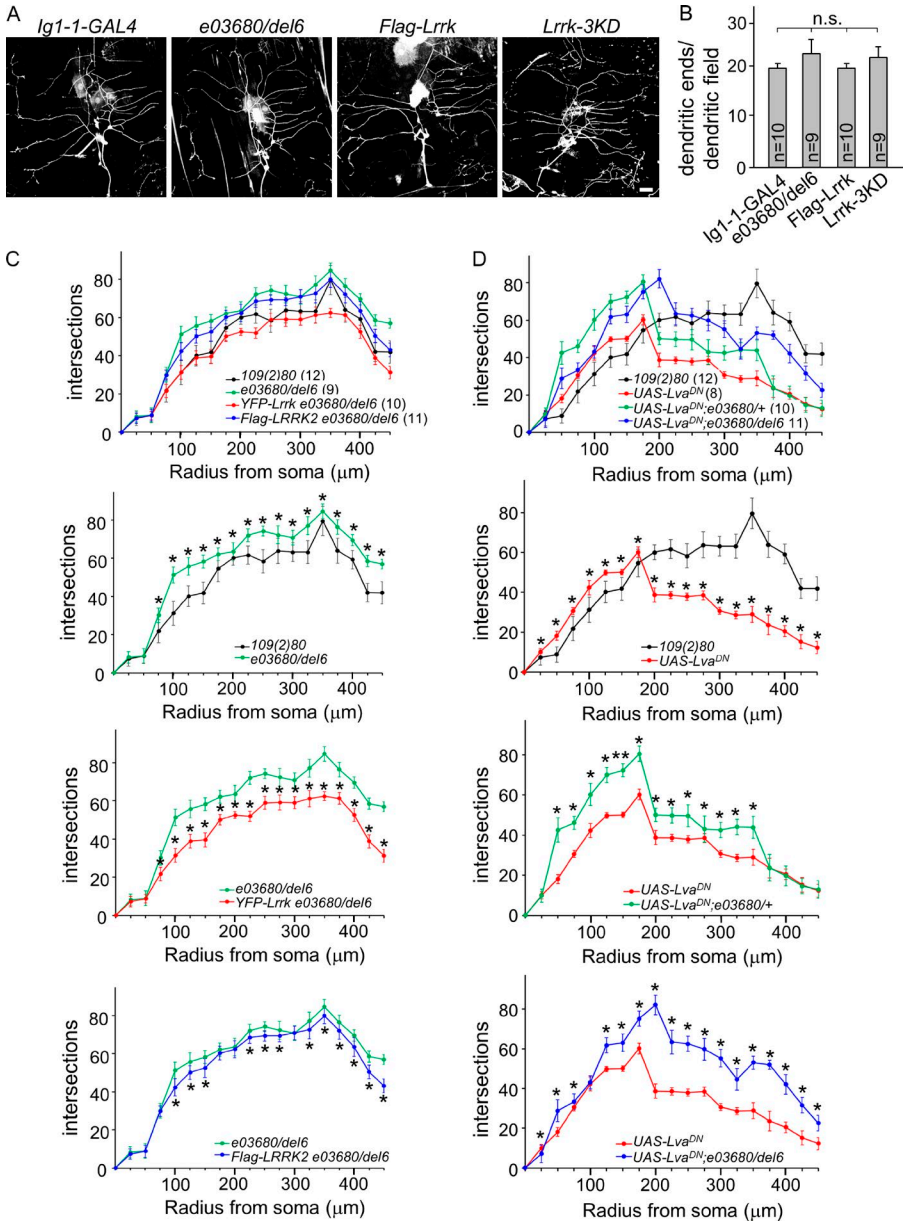
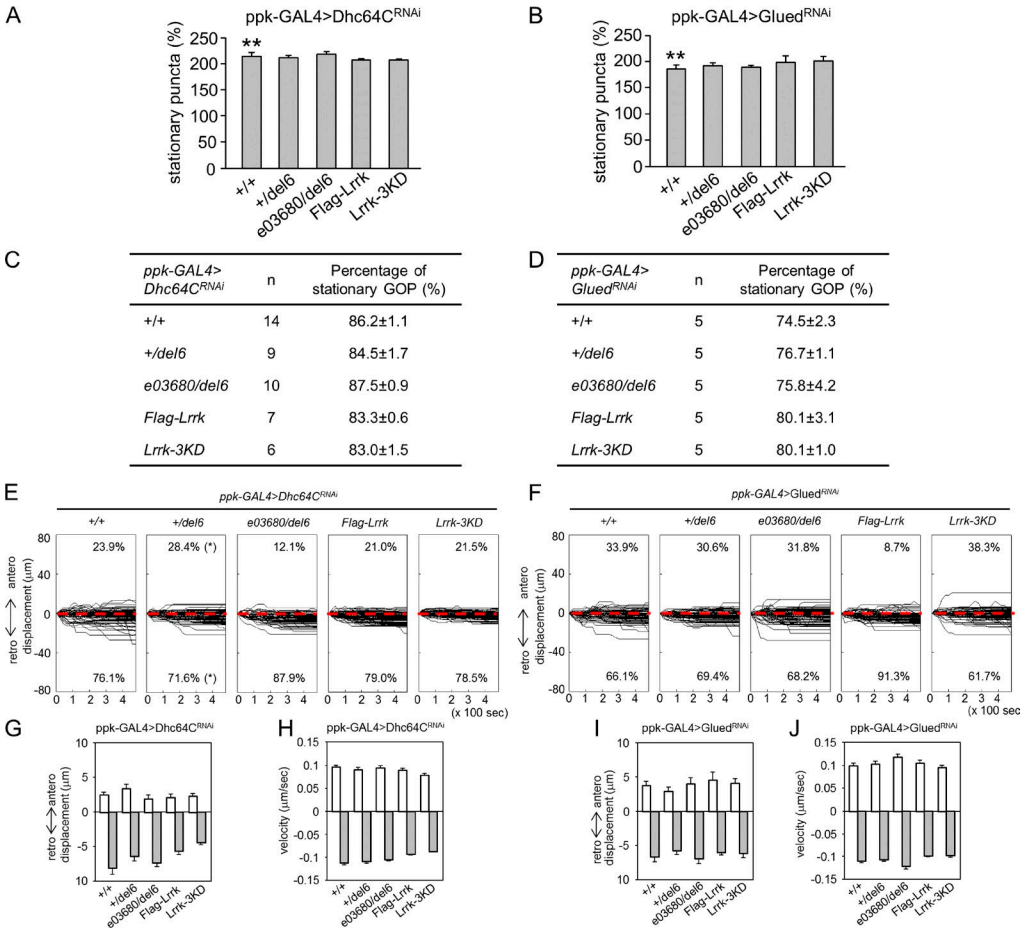


Figure S3. **Arborization of da dendrites regulated by Lrrk and Lva.** (A) Images for class I da dendrites with genotypes shown above the images. Dendrites in dorsal fields of A3–A6 segments were shown by mCD8-GFP driven by *lg1-1-GAL4*. Bar, 50 µm. (B) Bar graph shows numbers of dendritic ends per class I neuron of *lg1-1GAL4* (19.3 ± 3.2), *e03680/del6* (22.3 ± 5.3), *Flag-Lrrk* (19.4 ± 2.8), and *Lrrk-3KD* (21.5 ± 3.1). No significance (n.s.) was detected in comparison to *lg1-1-GAL4* by Student's *t* test. Error bars represent SEM. (C) Sholl analyses for dorsal dendritic fields show intersections (y axes) between dendritic processes and concentric rings of 25-µm intervals (x axes) with radial distances from da soma. The pair of genotypes for comparison by Student's *t* test are indicated: *, $P < 0.05$.



C

ppk-GAL4> Dhc64C^RNAi	n	Percentage of stationary GOP (%)
+/+	14	86.2±1.1
+/del6	9	84.5±1.7
e03680/del6	10	87.5±0.9
Flag-Lrrk	7	83.3±0.6
Lrrk-3KD	6	83.0±1.5

D

ppk-GAL4> Glued^RNAi	n	Percentage of stationary GOP (%)
+/+	5	74.5±2.3
+/del6	5	76.7±1.1
e03680/del6	5	75.8±4.2
Flag-Lrrk	5	80.1±3.1
Lrrk-3KD	5	80.1±1.0

K

ppk-GAL4> Dhc64C^RNAi	n	net displacement		velocity		Percentage	
		>0 (μm)	<0 (μm)	>0 (μm/sec)	<0 (μm/sec)	>0 (%)	<0 (%)
+/+	85	2.47±0.41	-8.12±0.88	0.10±0.003	-0.11±0.004	23.9±5.4	76.1±5.4
+/del6	69	3.43±0.58	-6.38±0.68	0.09±0.004	-0.11±0.004	28.4±3.4	71.6±3.4
e03680/del6	75	1.96±0.54	-7.31±0.59	0.10±0.004	-0.10±0.003	12.1±4.2†	87.9±4.2†
Flag-Lrrk	82	2.14±0.48	-5.64±0.50*	0.09±0.004	-0.09±0.002	21.0±2.1	79.0±2.1
Lrrk-3KD	81	2.33±0.37	-4.37±0.35**‡	0.08±0.003	-0.09±0.002	21.5±5.0	78.5±5.0

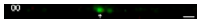
* compared to *Dhc64C^RNAi*; † compared to +/del6; ‡ compared to Lrrk

L

ppk-GAL4> Glued^RNAi	n	net displacement		velocity		Percentage	
		>0 (μm)	<0 (μm)	>0 (μm/sec)	<0 (μm/sec)	>0 (%)	<0 (%)
+/+	79	3.80±0.54	-6.57±0.70	0.10±0.005	-0.11±0.004	33.9±5.2	66.1±5.2
+/del6	72	2.94±0.55	-5.66±0.60	0.10±0.006	-0.11±0.004	30.6±4.1	69.4±4.1
e03680/del6	79	4.13±0.73	-6.83±0.74	0.12±0.007	-0.12±0.005	31.8±5.9	68.2±5.9
Flag-Lrrk	79	4.56±1.16	-5.94±0.41	0.11±0.006	-0.10±0.003	8.7±3.8*	91.3±3.8*
Lrrk-3KD	79	4.11±0.66	-6.03±0.70	0.10±0.005	-0.10±0.004	38.3±6.1‡	61.7±6.1‡

* compared to *Glued^RNAi*; † compared to +/del6; ‡ compared to Lrrk

Figure S4. **Disrupting the dynein–dynactin transport system stalls GOP movement in dendrites.** (A–D) Percentages of stationary GOPs in *ppk-GAL4*–driven *Dhc64C^RNAi* (A and C) or *Glued^RNAi* (B and D) in combination with genotypes of *+/+*, *+/del6*, *e03680/del6*, *Flag-Lrrk*, or *Lrrk-3KD* overexpression are shown as bar graphs (A and B) or tables (C and D). (A and B) Percentages of stationary GOPs in *Dhc64C^RNAi* or *Glued^RNAi* are significantly different from *ppk-GAL4* control (62.9 ± 2.8%; see Fig. 6 A; **, $P < 0.001$ in Student's *t* test), but are not significantly different ($P > 0.05$) to those in combination with *+/+*, *+/del6*, *e03680/del6*, *Flag-Lrrk*, or *Lrrk-3KD* overexpression. (E and F) Combined tracks of ManII-GFP puncta in the genotypes described above each panel, and graphs were done as in Fig. 2 D. Note that *Dhc64C^RNAi* shows increase in the percentage of retrograde movement (76.1 ± 5.4%) as compared with *ppk-GAL4* control (Fig. 6 B, 58.7 ± 7.7%). (G–J) Displacements (G and I) and velocities (H and J) in both anterograde (open bars) and retrograde (shaded bars) are shown as bar graphs. Error bars represent SEM. (K and L) The tables show displacements, velocities, and percentages of anterograde (>0) and retrograde (<0) displacements. Note that both *Dhc64C^RNAi* and *Glued^RNAi* show reduced anterograde and retrograde displacements and velocities to *ppk-GAL4* control (Fig. 6, C and D), consistent with dynein and dynactin being required in GOP transport in anterograde and retrograde directions. When combined with *e03680/del6* that shows enhanced anterograde movements, the GOP dynamics were suppressed by either of the RNAi transgenes (K and L), suggesting dependence on dynein and dynactin for GOP transport in the *Lrrk* mutant. Significant differences in pairwise comparisons are indicated in K and L.



Video 1. **GOP with colocalized YFP-Lrrk is stationary in dendrites.** Transgenes for expression of ManII-GFP (green) and YFP-Lrrk (red) were driven by *ppk-GAL4* in da neurons and imaged by laser-scanning confocal microscopy (LSM710; Carl Zeiss) in 20-s intervals during an 8-min period. One stationary GOP (arrow) labeled by both ManII-GFP and YFP-Lrrk stalls in the dendrite. Distal to the left and proximal to the right. Bar, 2 µm.

Video 2. **GOP free of YFP-Lrrk colocalization moves in anterograde direction.** Transgenes for expression of ManII-GFP (green) and YFP-Lrrk (red) were driven by *ppk-GAL4* in da neurons and imaged by laser-scanning confocal microscopy (LSM710; Carl Zeiss) in 20-s intervals during an 8-min period. One moving GOP (arrow) labeled only with ManII-GFP free of YFP-Lrrk moves bidirectionally and reaches a more distal position in the dendrite. Distal to the left and proximal to the right. Bar, 2 µm.

Video 3. **GOP free of YFP-Lrrk colocalization moves in retrograde direction.** Transgenes for expression of ManII-GFP (green) and YFP-Lrrk (red) were driven by *ppk-GAL4* in da neurons and imaged by laser-scanning confocal microscopy (LSM710; Carl Zeiss) in 20-s intervals during an 8-min period. One moving GOP (arrow) labeled only by ManII-GFP free of YFP-Lrrk moves bidirectionally and reaches a more proximal position in the dendrite. Distal to the left and proximal to the right. Bar, 2 µm.

Video 4. **GOP with YFP-Lrrk colocalization moves in anterograde direction.** Transgenes for expression of ManII-GFP (green) and YFP-Lrrk (red) were driven by *ppk-GAL4* in da neurons and imaged by laser-scanning confocal microscopy (LSM710; Carl Zeiss) in 20-s intervals during an 8-min period. One dynamic GOP (arrow) labeled by both ManII-GFP and YFP-Lrrk moves in the dendrite and reaches a more distal position. Distal to the left and proximal to the right. Bar, 2 µm.

Video 5. **GOP with YFP-Lrrk colocalization moves in retrograde direction.** Transgenes for expression of ManII-GFP (green) and YFP-Lrrk (red) were driven by *ppk-GAL4* in da neurons and imaged by laser-scanning confocal microscopy (LSM710; Carl Zeiss) in 20-s intervals during an 8-min period. One dynamic GOP (arrow) labeled by both ManII-GFP and YFP-Lrrk moves in the dendrite and reaches a more proximal position. Distal to the left and proximal to the right. Bar, 2 µm.