The Neuronal Kinesin UNC-104/KIF1A Is a Key Regulator of Synaptic Aging and Insulin Signaling-Regulated Memory

Highlights

- Aging neurons exhibit decreased activity of UNC-104/KIF1A, a neuronal kinesin
- UNC-104 plays a critical role in synaptic and behavioral decline in aged worms
- UNC-104 functions downstream of DAF-2 and is regulated by DAF-16

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In Brief

C. elegans exhibits an age-dependent decline of locomotion, learning, and memory. Li et al. demonstrate that decreased activity of UNC-104, a neuronal kinesin, plays a critical role in age-dependent degradation of neuronal functions. Overexpression of UNC-104 improves neural circuit functions in aged worms.
The Neuronal Kinesin UNC-104/KIF1A Is a Key Regulator of Synaptic Aging and Insulin Signaling-Regulated Memory

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http://dx.doi.org/10.1016/j.cub.2015.12.068

SUMMARY

Aging is the greatest risk factor for a number of neurodegenerative diseases, such as Alzheimer’s and Parkinson’s disease. Furthermore, normal aging is associated with a decline in sensory, motor, and cognitive functions. Emerging evidence suggests that synapse alterations, rather than neuronal cell death, are the causes of neuronal dysfunctions in normal aging and in early stages of neurodegenerative diseases. However, little is known about the mechanisms underlying age-related synaptic decline. Here, we uncover a surprising role of the anterograde molecular motor UNC-104/KIF1A as a key regulator of neural circuit deterioration in aging C. elegans. Through analyses of synapse protein localization, synaptic transmission, and animal behaviors, we find that reduced function of UNC-104 accelerates motor circuit dysfunction with age, whereas upregulation of UNC-104 significantly improves motor function at advanced ages and also mildly extends lifespan. In addition, UNC-104-overexpressing animals outperform wild-type controls in associative learning and memory tests. Further genetic analyses suggest that UNC-104 functions downstream of the DAF-2-signaling pathway and is regulated by the FOXO transcription factor DAF-16, which contributes to the effects of DAF-2 in neuronal aging. Together, our cellular, electrophysiological, and behavioral analyses highlight the importance of axonal transport in the maintenance of synaptic structural integrity and function during aging and raise the possibility of targeting kinesins to slow age-related neural circuit dysfunction.

INTRODUCTION

Both genes and environmental cues affect lifespan [1], and increasing evidence suggests that different tissues decline through distinct mechanisms [2]. Neurons are particularly vulnerable to the aging process, given their long lifespan, lack of cell renewal, and high degree of complexity [3].

Progressive neuronal dysfunction during normal aging is associated with a number of subtle changes at the functional and morphological levels, rather than the loss of neuronal cells [4]. For example, in aged mice and C. elegans, a subset of motor neurons exhibit abnormal sprouting of neurite branches and synapse deterioration, correlating with age-associated motility decline [5–8]. Moreover, changes in synapse density and morphology are closely associated with the degree of cognitive impairment in aged primates [9, 10], as well as cognitive decline in aged rats [11] and retinal defects in old mice [12]. At the cellular and molecular level, the decline of synaptic function during aging involves various aspects of synapse biology, including changes in synapse structure [8], changes of lipid and protein components of synaptic vesicles (SVs) [13, 14], and defects in synaptic release [15] and axonal transport [16, 17]. Whereas these findings emphasize the importance of dissecting cellular and molecular events in aging synapses that contribute to age-associated neural dysfunction, the multitude of age-dependent synapse changes poses difficulties for the development of therapeutic interventions. Therefore, it is critical to identify key components in the maintenance of neuronal function with age.

Furthermore, maintenance of neuronal activity is also critical for the maintenance of non-neuronal tissue functions and longevity. For instance, neuronal activation of the unfolded protein response (UPR) extends C. elegans lifespan [18] and improves metabolic homeostasis in mice [19]. In addition, neuronal function of the FOXO transcription factor DAF-16 contributes to lifespan regulation in C. elegans [20]. However, the underlying molecular mechanisms by which these lifespan- and homeostatic-regulatory pathways affect neuronal functions remain unclear.

To gain further understanding of the mechanisms that determine synaptic functions during aging, we performed candidate gene screens to identify regulators of synaptic function in motor neurons of aging C. elegans. We found that the anterograde kinesin motor UNC-104/KIF1A is a critical regulator of aging-associated neural circuit dysfunction. UNC-104 is required for the...
Figure 1. UNC-104 Modulates DA9 Synapse Distribution with Age

(A) C. elegans undergoes aging-associated motility decay. The solid black curve is drawn according to the y axis on the left, representing the percentage of animals that remain alive at a particular age (x axis), and the gray bars are drawn according to the y axis on the right, representing the body bends behavior of an animal at a certain age.
protective effects of insulin/IGF signaling (IIS) pathway downregulation in the maintenance of learning and memory as well as motor activity with age. These findings provide the first information about possible neuronal targets that mediate the protective effects of insulin signaling in neural circuit functions during aging and highlight the unexpected role of axonal transport as a potential target for pharmaceutical interventions in the maintenance of health span.

RESULTS

UNC-104 Modifies Age-Dependent Changes in SV Distribution

Motility decline is one of the most-prominent functional declines in aging animals. Consistent with previous reports [21–23], we found that the motor activity of aging worms gradually declines. For example, 18-day-old (day 18) animals had severe motility defects compared with young (day 1) adults, as indicated by reduced body bend movements in liquid medium (Figure 1A). Recent studies in aged mammals and worms have suggested that this age-associated motility decay results from synaptic alterations at neuromuscular junctions (NMJs) [8, 15], although the underlying molecular pathways are not well understood.

To explore the cellular and molecular basis of synaptic aging, we examined the synaptic morphology in the DA9 motor neurons, which form approximately 25 en passant presynaptic specializations within a discrete and stereotyped location along its axon (Figure 1B). Our previous studies showed that the DA9 synapses can be reliably labeled by GFP-tagged SV proteins such as RAB-3 [24] (Figures 1B and 1C). Aging wild-type animals display gradually reduced SV density in the DA9 presynaptic region and ectopic accumulation of synaptic vesicle proteins such as RAB-3 and SNB-1 in the dendritic and asynaptic axonal regions (Figures 1C, 1F, 1G, and S1A–S1C). These observations suggest that presynaptic integrity is compromised in the motor neurons of aging C. elegans.

We next performed a small-scale candidate screen to identify presynaptic-related mutants that show enhanced DA9 synaptic defects during aging. From this screen, we identified two molecules that affect SV transport: the anterograde molecular motor for SV transport UNC-104/KIF1A and its regulator, a small GTPase ARL-8 [25] (Figures 1B and 1C). Aging wild-type animals display gradually reduced SV density in the DA9 presynaptic region and ectopic accumulation of synaptic vesicle proteins such as RAB-3 and SNB-1 in the dendritic and asynaptic axonal regions (Figures 1C, 1F, 1G, and S1A–S1C). These observations suggest that presynaptic integrity is compromised in the motor neurons of aging C. elegans.

UNC-104 Mediates Age-Dependent Motor Function Decline by Regulating Presynaptic Transmission

We next asked whether the unc-104-dependent SV trafficking phenotype is relevant for age-related behavioral declines. As described previously [32], we found that worm thrashing activity declined in an age-dependent manner. Furthermore, mutants of UNC-104 (wy711 and e1265(+)) exacerbated this decline of motility with age (Figure 2A). By contrast, overexpression of UNC-104 in neurons either from a pan-neuronal promoter or from the endogenous UNC-104 promoter significantly rescued the thrashing defects of aged worms (Figures 2A and S3B).

*References*

1. Li et al., The Neuronal Kinesin UNC-104/KIF1A is a Key Regulator of Synaptic Aging and Insulin Signaling-Regulated Memory, Current Biology (2016), http://dx.doi.org/10.1016/j.cub.2015.12.068

**B** A schematic showing the morphology and synaptic pattern of the C. elegans DA9 neuron. A, anterior; D, dorsal.

**C–E** Confocal microscopy images of the GFP::RAB-3 distribution in DA9 neurons. The scale bar represents 20 μm.

**F** Quantification of the RAB-3 puncta density.

**G** Quantification of the population of the worms that develop ectopic accumulation of RAB-3 in the dendrite.

*p < 0.05; **p < 0.01; ***p < 0.001. One-way ANOVA, with post-test: Tukey’s multiple comparison in (F), chi-square test in (G). The error bars stand for SD. See also Figures S1 and S2.
motility decline, we expressed UNC-104 in different combinations of motor neurons that directly synapse onto muscles (Figure S3A). Expression of UNC-104 in all of the six classes of motor neuron had protective effects that were similar to pan-neuronal expression of UNC-104 (Figure 2B), whereas expressing UNC-104 in the cholinergic or GABAergic neurons alone had no beneficial effects (Figure S3B). Furthermore, we found that UNC-104-overexpressing animals showed not only improved swimming behaviors but also enhanced motility on solid plates (Figures 2C and 2D).

To test the specificity of the protective effects of UNC-104 with age, we asked whether mutations that cause hyperactive behaviors or affect other components of the SV transport pathways also improve motility at advanced ages. Unlike strains overexpressing UNC-104, hyperactive mutants such as diacylglycerol kinase theta (dgk-1(nu62)) and the G protein alpha subunit Go (goa-1(sa734)) showed exacerbated motility defects in aged worms (Figure S3C). In addition, reduced function of DHC-1 (dhc-1(or195ts)), a component of the cytoplasmic dynein complexes that antagonizes the anterograde trafficking of SVs mediated by UNC-104 [33], caused mild motility defects in young adults (day 1). However, this defect did not change with age, suggesting that reduced function of DHC-1 does not affect the age-associated motility decline (Figure S3D). Together, these results highlight a specific role of UNC-104 in motor neurons in the maintenance of presynaptic integrity and motor circuit function with age.

To directly test whether UNC-104 modulates synaptic transmission during aging, we recorded synaptic currents at the NMJs of various genotypes at different ages by patch-clamp. Our previous studies showed that, during aging, the worm motor circuit first developed a presynaptic defect in synaptic transmission in early–mid ages (days 7–12), as indicated by a decrease in the frequency of spontaneous post-synaptic currents (PSCs) [15]. This is followed by body-wall muscle deterioration in the mid–late ages (~day 12). Reduced function or upregulation of UNC-104 did not affect the frequency or the amplitude of spontaneous PSCs or the amplitude of evoked PSCs in day 1 adults, suggesting that synaptic transmission is not affected by these manipulations of UNC-104 dosage in young animals (Figures 3A–3D). At days 12 and 16, wy711 mutation further reduced the frequency of spontaneous PSCs and the amplitude of evoked responses, whereas UNC-104-overexpressing animals showed an increased frequency of spontaneous PSCs and increased amplitude of evoked responses compared to wild-type of the same ages (Figures 3A–3D). These effects of UNC-104 are likely to be caused by changes in presynaptic function, because the amplitude of spontaneous PSCs was not affected in unc-104 mutants at days 12 or 16 compared to wild-type (Figure 3D). Together with the motor neuron rescue data, these results support the notion that reduced presynaptic function in motor neurons is primarily responsible for the motor defects observed in aging worms. Furthermore, upregulation of UNC-104 is sufficient to improve the presynaptic structural integrity and function in aged animals.

**UNC-104 Activity Is Downregulated with Age**

To further understand the mechanisms by which UNC-104 alterations affect neuronal function, we next examined the steady-state level of UNC-104 mRNA in young (day 1) and aged (days 5 and 12) animals. UNC-104 mRNA at the whole-organism level remains similar between young and older worms (Figure 7A), suggesting that aging does not affect UNC-104 at the mRNA
DNC, consistent with previous reports [7]. In addition, we also examine the UNC-104 protein localization by analyzing the effects of age on a UNC-104::GFP reporter expressed specifically in the cholinergic DA9 neuron. Consistent with the results observed in the DNC, we found that presynaptic localization of UNC-104::GFP is decreased in day 12 animals compared to day 1 animals, whereas the control DsRed intensity is increased over time (Figure S3). These results suggest that aging specifically reduces the activity, but not the overall level, of UNC-104 mRNA or protein.

To further understand the effects of aging on UNC-104-mediated SV transport, we next examined the dynamics of SV movement in DA9 neuron in aged animals. Compared with day 1 adults, day 8 and day 12 wild-type adults displayed slower anterograde trafficking speed of synaptic vesicle markers (Figures S6A, S6D, and S6E). In UNC-104-overexpressing animals, this age-dependent decline is absent, suggesting that the dosage of UNC-104 in aging animals might be responsible for the reduction of transport speed. Consistent with previous findings [25], although unc-104(wy711) mutants do not have obvious SV distribution defects at day 1, the speed of anterograde trafficking is already severely reduced (Figures S6B, S6D, and S6E). Although the speed defect of unc-104(wy711) is not exacerbated by age, the total anterograde moving events are reduced by day 12, which may contribute to the exacerbation of synapse abnormalities in wy711 mutants with age (Figure S6F). These results further suggest that UNC-104 activity is gradually decreased over age, which leads to defective SV trafficking over time and eventually causes synaptic dysfunction.

**UNC-104 Regulates the Age-Related Decline of Learning and Memory**

To understand whether the effects of UNC-104 in neuronal aging extend to other neural circuits in addition to motor circuits, we next assessed learning and memory behaviors of unc-104(wy711) mutant and overexpression animals at different ages (Figure 5A). We previously found that, similar to Drosophila, mice, and other higher organisms [3], *C. elegans* associative learning and memory also decline with age; this happens at a much-faster rate than many other types of aging-associated neuronal dysfunctions [34, 35]. For example, unlike chemotaxis (Figures 5B and 5C, naive) and motility behaviors, which remain intact during the first 7 days of adulthood, long-term associative memory (16 hr) declines as early as day 4 in wild-type animals [34]. Short-term memory (2 hr) becomes defective with age as well [34]. Because short-term memory requires translation [36] (likely at the synapse) rather than CREB-dependent transcription as in long-term memory [34, 36], we explored the impact of UNC-104 dosage on short-term memory and its maintenance with age by examining day 1 and day 4 animals when short-term memory of wild-type animals have not severely deteriorated.

UNC-104 alterations did not affect the naive (untrained) chemotaxis behaviors of day 1 or day 4 adults (Figures 5B and 5C, naive), suggesting that the primary functions of sensory and interneurons involved in this behavior are not affected. Whereas reduced function of unc-104 did not affect learning or memory, we found that overexpression of UNC-104 significantly improved short-term memory, in both young and older animals. On day 1, overexpression of unc-104 caused a slight defect in learning (Figure 5B, 0 hr), which may be caused by excessive expression of unc-104. However, it significantly

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**Figure 3. UNC-104 Regulates Synaptic Transmission in Aged Worms**

(A) Representative traces of spontaneous PSCs recorded from the NMJs at the ventral nerve cord. Membrane voltage was clamped at ~60 mV during the recording.

(B) Quantification of the frequency of spontaneous PSCs.

(C) Quantification of the amplitude of evoked PSCs.

(D) Quantification of the amplitude of spontaneous PSCs.

*p < 0.05; **p < 0.01; one-way ANOVA, with post-test: Tukey’s multiple comparison. Total animals analyzed: day 1: 17 (wild-type), 17 (unc-104(wy711)), 16 (Prab-3::unc-104); day 12: 10 (wild-type), 12 (unc-104(wy711)), 12 (Prab-3::unc-104); day 16: 10 (wild-type), 10 (unc-104(wy711)), 10 (Prab-3::unc-104). The error bars stand for 95% CIs. See also Figure S4.
increased short-term memory; the animals exhibited no decline in chemotaxis by 2 hr post-training (Figure 5B, 2 hr). Overexpression of UNC-104 dramatically improved maintenance of short-term memory on day 4, showing no decline at either 1 or 2 hr post-training (Figure 5C). These results suggest that, similar to UNC-104’s role in maintenance of motor function, upregulation of UNC-104 alone is sufficient to improve the short-term memory of both young and aging animals.

UNC-104 Functions Downstream of DAF-2 in Regulation of Motility Decline, Longevity, and Short-Term Memory

We next investigated the interactions of UNC-104 with other aging-regulatory pathways. daf-2/insulin-IGF-1-like receptor mutants reduce insulin signaling, subsequently extending lifespan [37] and improving maintenance with age, including slowing age-associated motility decay [37–39]. Although unc-104 alterations did not affect the locomotory behaviors of daf-2 up to 22 days, changes in unc-104 dosage did modulate motor activities of daf-2 at later ages (days 26–34; Figure 6A): reduction of unc-104 reduced motility of aged daf-2 mutants, whereas overexpression of unc-104 in daf-2 mutants improved motility. Thus, the dosage effect of unc-104 is extended to old animals in the long-lived daf-2 mutants.

We also examined the effects of UNC-104 on longevity. In contrast to their dramatic effects on age-associated motility decline, unc-104 partial loss-of-function alleles (wy711 or e1265+/+) only had subtle effects on lifespan (Figure 6B; Table S1). By contrast, upregulation of unc-104 in the wild-type background caused a significant increase in lifespan, suggesting that maintenance of neuronal function during aging promotes longevity. The lifespan extension phenotype of UNC-104 upregulation is abolishe in daf-16 mutants (Figure 6B; Table S1) but is maintained in hsf-1 mutants (Figure 6C). In addition, reduced function of unc-104 did not affect the lifespan of daf-16 but strongly enhanced the short lifespan phenotype of hsf-1 mutant (Figures 6C and 6D; Table S1). Together, these results suggest that unc-104 functions in the same pathway as daf-16 in regulation of lifespan but independent of hsf-1. Moreover, in a daf-2 mutant that is already long lived, overexpression of unc-104 only had a subtle beneficial effect on the median lifespan (Figure 6D; Table S1).
Together, these results suggested that **unc-104** is an important neuronal target of the insulin signaling pathway in the regulation of aging-associated neuronal dysfunction and contributes to longevity as well. Furthermore, whereas lifespan and tissue functions are correlated, they can be distinct and separable phenotypes during the aging process.

We next examined the potential interactions between **UNC-104** and **DAF-2** in the regulation of learning and memory. **daf-2** mutants extend short-term memory 3-fold in day 1 animals [34] and slow age-related decline in short-term memory [35]. Interestingly, we found that **UNC-104** is required both for **daf-2** mutants’ improvement in learning and short-term memory on both day 1 and day 5 (Figures 6E and 6F). These results are consistent with the hypothesis that **UNC-104** functions downstream of **DAF-2** to generate beneficial effects on short-term memory in aged animals (Figure 6F, 1 hr). At the same time, we found that, unexpectedly, **unc-104** is also required for normal learning and memory behaviors of day 1 **daf-2** animals. It raised the possibility that reduced **UNC-104** motor activity causes detrimental effects on learning behavior. Remarkably, overexpression of **unc-104** extends short-term memory to more than 4 hr even in day 5 animals, suggesting that the decline of short-term memory of **daf-2** animals in young and older animals may be due to reduction of **UNC-104** function. Thus, **UNC-104** functions as a neural target that at least partially mediates the protective effects of **DAF-2** in the nervous system.

**UNC-104 mRNA and Protein Is Maintained in daf-2 Mutants with Age**

The above results, together with a recent study showing that **daf-2** ameliorates motor circuit function decline by affecting presynaptic transmission [15], raise the possibility that **unc-104** functions downstream of **DAF-2** in the regulation of presynaptic transmission during aging. Consistent with this hypothesis, we found that the steady-state level of **unc-104** mRNA is increased in **daf-2** mutants and to a much less degree in eat-2 mutants, which regulate longevity through calorie restriction (Figure 7A).

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**DISCUSSION**

**Aging Neurons Become More Susceptible to UNC-104 Dosage Alterations**

In this study, we discovered an unconventional role of the neuronal kinesin **UNC-104/KIF1A** as a key regulator of aging-associated neuronal circuit decline in *C. elegans*. The **UNC-104/KIF1A** family...
of kinesins specifically transports both SV precursors and active zone proteins, the essential components for the presynaptic nervous system function. Consistent with the latter possibility, we found that the level of UNC-104mCherry is specifically decreased in the axon-rich DNC in aged animals, but not VNC. These results suggest that the aging process is likely to affect the motor activity of UNC-104 and prevent its localization in the axons, thereby resulting in higher vulnerability of aged neural circuits. Consistent with this hypothesis, kymograph analyses of SV trafficking of aged animals revealed that the speed of anterograde trafficking, the percentage of anterograde events, and the total number of anterograde movements are decreased during aging, further suggesting a decrease of the motor activity. In addition, a recent study found that the synaptosome component phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2) is substantially reduced in hippocampal synaptic membranes in old mice, contributing to the reduced cognition [14]. Interestingly, the binding between SV PI(4,5)P2 to the PH domain of UNC-104 is required to activate the motor and initiate the vesicle transport [47]. Thus, the UNC-104 protein activity may also be decreased in aged animals because of altered lipid metabolism on synaptic vesicle cargoes.

**UNC-104 Slows Down the Age-Dependent Deterioration of Neural Circuit Function and Is Required for the Protective Effects of DAF-2**

Remarkably, increasing the dosage of unc-104 can significantly slow aging-associated synapse dysfunction, as well as motility decline and the loss of short-term memory. These results suggest that UNC-104 is one of the critical rate-limiting factors for motor activity.

**Figure 6. unc-104 Is Regulated by daf-16 and Modulates the Aging-Associated Motility Decline of daf-2, Longevity, and Short-Term Memory**

(A) Alterations of unc-104 do not affect the body-bend behaviors of daf-2 in early-mid ages but regulate the further motility decline in mid-late stages. Total animals analyzed were day 1: 6 (daf-2), 7 (daf-2; unc-104), 8 (daf-2; Ex[Prab-3::unc-104]); day 22: 7 (daf-2), 11 (daf-2; unc-104), 7 (daf-2; Ex[Prab-3::unc-104]); day 26: 18 (daf-2), 18 (daf-2; unc-104), 16 (daf-2; Ex[Prab-3::unc-104]); day 30: 21 (daf-2), 15 (daf-2; unc-104), 19 (daf-2; Ex[Prab-3::unc-104]); day 34: 20 (daf-2), 20 (daf-2; unc-104), 11 (daf-2; Ex[Prab-3::unc-104]); *p < 0.05; **p < 0.01; ***p < 0.001. One-way ANOVA. The error bars stand for 95% CIs.

(B–D) Lifespan analyses.

(B) Upregulation of unc-104 in the nervous system causes medium extension of the lifespan of wild-type worms, which is abolished in daf-16 mutants. Total animals analyzed were wild-type: 187, unc-104(wy711): 153, Prab-3::unc-104: 93, daf-16(mu86): 254, daf-16;unc-104: 200, and daf-16;Prab-3::unc-104: 109.

(C) Alterations of unc-104 still modify the shortened lifespan phenotypes of hsf-1. Total animals analyzed were hsf-1(y441): 244, Prab-3::unc-104: 194, and hsf-1;Prab-3::unc-104: 182.

(D) Alterations of unc-104 have subtle effects on the long lifespan phenotypes of daf-2 mutants. Total animals analyzed were daf-2(1370): 113, daf-2;unc-104: 176, and Prab-3::unc-104: 123. Log rank analysis.

(E and F) UNC-104 is required for the effects of DAF-2 in the maintenance of short-term memory in both day 1 (E) and day 5 (F) animals. Upregulation of UNC-104 improves the short-term memory of daf-2 animals at d5 (F). Total replicates were day 1: daf-2(1370): 8, daf-2;unc-104: 8, and daf-2; Prab-3::unc-104: 8 and day 5: daf-2(1370): 12, daf-2;unc-104: 12, and daf-2; Prab-3::unc-104: 12. *p < 0.05; **p < 0.01; ***p < 0.001. One-way ANOVA.

See also Table S1.
synaptic aging. It is possible that upregulation of UNC-104 preserves synapse function by promoting the transport of SV precursors and active zone proteins during aging. Interestingly, we also found that UNC-104-overexpressing animals behave similarly to *daf-2* mutants in the maintenance of short-term memory and motility and that UNC-104 is required for the protective effects of DAF-2 in these behavioral tests. In addition, aged *daf-2* mutants have higher levels of unc-104 mRNA and UNC-104 protein in motor neuron axons than the wild-type animals, suggesting that UNC-104 protein is maintained at both the mRNA and protein levels in aged *daf-2* mutants. These results raise the possibility that UNC-104 functions downstream of the DAF-2-signaling pathway in mediating the beneficial effects in aging neurons, although other cellular factors are likely to contribute *daf-2* effects as well, because unc-104 manipulation did not show an effect until 26 days in *daf-2* mutants.

Consistent with this hypothesis, UNC-104 mRNA is upregulated in *daf-2* mutants, which is partially suppressed by loss of function of the FOXO transcription factor *daf-16* in day 1 animals and completely abolished by a *daf-16* mutation in day 5 and day 12 animals. These results suggest that unc-104 is specifically regulated by DAF-16 at the transcriptional level during aging. Sequence analyses of the unc-104 promoter region identified two potential DAF-16-binding sites: TGTTTAC [48] and GTAAATA [49], at positions of −1,420 b.p. and −3,835 b.p., respectively, raising the possibility that UNC-104 might be a direct target of DAF-16. However, genome-wide chromatin profiling (modENCODE database) [50] did not report DAF-16 binding directly to these regions. Thus, whether UNC-104 is directly regulated by DAF-16 needs to be studied further.

In addition to transcriptional regulation, more UNC-104 protein accumulates in distal axons in aging *daf-2* mutants, suggesting that DAF-2-dependent mechanisms might be also required to stimulate the activity of UNC-104 in aging animals. Surprisingly, upregulation of unc-104 in the neurons alone can increase lifespan, suggesting that maintenance of neuronal functions significantly promotes longevity. Together, these findings identify a novel regulator in aging-associated motor circuit dysfunction and highlight the importance of axonal transport in age-related behavioral changes, as well as longevity.

**EXPERIMENTAL PROCEDURES**

Worms were raised on NGM plates at 20°C, using OP50 E.coli as a food source. N2 Bristol was used as the wild-type reference strain. The mutant strains CB1265 *unc-104*(e1265), CB1370 *daf-2*(e1370), syd-2(mu37), unc-10(e102), nab-1(ok943), unc-57(ok310), unc-11(e47), dsh-1(sy1445), lin-44(e1792), *daf-16*(mu86), *hsf-1* (sy441), *skn-1* (sy76), *let-363* (n754), *daf-2* (n754), *daf-16* (n754), *hep-1* (sy421), and *daf-2* (e1370) were obtained through the Caenorhabditis Genetics Center. The *wy711* mutant was described as previously [25].

For aging experiments, worms were transferred every 2 or 3 days to fresh NGM plates to separate the adults from the larvae. Transgenic constructs were cloned into the pSM vector, a derivative of pPD49.26 (A.Fire) with extra cloning sites. The transgenic strains were generated using standard techniques. Images of fluorescently tagged fusion proteins were captured in live *C. elegans* using a plan-Apochromat 40x 1.3 objective on a Zeiss LSM710 confocal microscope, using identical image and laser settings for each genotype. The electrophysiology recordings were performed as previously described [16].

See also Figure S7.
The details of the above and other experiments are included in the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.12.068.

AUTHOR CONTRIBUTIONS


ACKNOWLEDGMENTS

We thank the international Caenorhabditis Genetics Center for strains. We also thank C. Gao for technical assistance. This work is supported by the Howard Hughes Medical Institute and the NIH (K5) and by the American Federation for Aging Research (L.-B.L.). R.N.A. is supported by a National Research Service Award, and C.T.M. is the Director of the Glenn Center for Aging Research (L.-B.L.). R.N.A. is supported by a National Research Service Award, and C.T.M. is the Director of the Glenn Center for Aging Research at Princeton.

Received: July 30, 2015
Revised: November 23, 2015
Accepted: December 23, 2015
Published: February 11, 2016

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Neuron 40, 749–762.
Supplemental Information

The Neuronal Kinesin UNC-104/KIF1A
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Supplemental Figures and Legends

Figure S1, related to Figure 1 RAB-3 and SNB-1 are co-localized in aged animals, and is affected by unc-104 heterozygous mutants and mutations of UNC-104 regulator arl-8. (A-C) In DA9 neurons of day 18 animals, the SV-associated protein RAB-3 (A, red) and the SV transmembrane protein SNB-1 (B, green) are co-localized in both the presynaptic region and asynaptic region (arrows). (D-J) Confocal microscopy of distribution of the GFP::RAB-3 puncta in wildtype (D, J), unc-104(e1265/+)(E, H), and arl-8(wy271) (F, I). Middle row: day 1; Bottom row: day 12. (J) Quantification of the percentage of worms that have RAB-3 mislocalization in either the dendritic or ventral axonal regions. Total animals analyzed: day 1: wildtype:15, unc-104(e1265/+):19, arl-8(wy271):17; day 12: wildtype:24, unc-104(e1265/+):18, arl-8(wy271):22. There is a significant increase in the percentage of worms that have RAB-3 mislocalization in unc-104(e1265/) and arl-8(wy271) backgrounds during aging.
Figure S2, related to Figure 1 UNC-104 rescues aging-associated synapse loss in DB presynaptic regions cell autonomously. (A–C) Confocal images of SNB-1 puncta in the DB presynaptic region with age. upper row, wildtype; middle row, unc-104(wy711); bottom row, expression of UNC-104 specifically in DB neurons (Punc-129::unc-104) can rescue the loss of SV density over age. (D) Quantification of the SNB-1 synapse density with age. Total animals analyzed: day 1: 16(wildtype), 11(unc-104(wy711)), 13(Punc-129::unc-104); day 12: 14(wildtype), 17(unc-104(wy711)), 15(Punc-129::unc-104); day 18: 11(wildtype), 15(unc-104(wy711)), 18(Punc-129::unc-104). *, P<0.05; **, P<0.01. One-way ANOVA.
Figure S3, related to Figure 2 UNC-104 functions in motor neurons to maintain motor function during aging, and has distinctive effects from the hyperactive and SV transport mutants. (A) A schematic of the C. elegans motor circuit. DB, VB, DA, VA: cholinergic neurons; DD, VD: GABAergic neurons. (B) Expressing UNC-104 from a pan-neuronal promoter (Prab-3), its endogenous promoter (Punc-104), or motor neurons alone (Punc-17 and Punc-47), had similar effects in the improvement of body bend movements. In contrast, expressing UNC-104 in cholinergic neurons (Punc-17, DB, VB, DA, VA), or GABAergic neurons (Punc-47, DD, VD), or DB, DD, VD neurons (Punc-129 and Punc-47), did not affect the motility defects in day 18 animals. Total animals analyzed: wildtype:25, Prab-3::unc-104 line #1:16, Prab-3::unc-104 line #2:16, Punc-104::unc-104 line #1:15, Punc-104::unc-104 line #2:14, Punc-17::unc-104, Punc-47::unc-104:11, Punc-17::unc-104:17, Punc-47::unc-104:18, Punc-129::unc-104, Punc-47::unc-104:13. (C) The hyperactive mutants dgk-1 and goa-1 show increased body bend activities in bouts of 5-10 seconds and have slightly increased body bend activities over the period of 30s. Nevertheless, these mutants have more severe motility decline with age. Total animals analyzed: day 1: wildtype:15, dgk-1(nu62):7, goa-1(sa734):9; day 12: wildtype:25, dgk-1(nu62):16, goa-1(sa734):8. (D) Reduced function of DHC-1, a cytoplasmic dynein complex component, causes slight motility defects at day 1, but does not affect the movements in day 18 animals compared to the wildtype. Total animals analyzed: dhc-1(or195ts): day 1:6, day 18:6. *, P<0.05; ***, P<0.001. n.s., not significant. One-way ANNOVA. The error bar stands for 95% confidence intervals (CI).
Figure S4, related to Figure 3. Representative traces of evoked PSCs of wildtype, *unc-104*(wy711), *Prab-3::unc-104* over time.
Figure S5, related to Figure 4. The steady state level of UNC-104 protein decreases in DA9 synaptic region with age. (A-D) Confocal images of expression of UNC-104::GFP in DA9 neuron, which are labeled with DsRED (Pitr-1::unc-104::gfp, Pitr-1::dsRed). (E) Quantification of UNC-104::GFP and DsRED fluorescence in day 1 and day 12 animals. The steady state level of UNC-104 is specifically decreased in the synaptic region (marked by a bracket) in day 12 animals. Total animals analyzed: day 1:9, day 12:10. **: P<0.01. ***: P<0.001. Unpaired Student’s t test.
Figure S6, related to Figure 4 Kymograph analyses of synaptic vesicle trafficking in young and aged worms. (A-C) Representative kymograph of wildtype (A), unc-104(wy711) (B), and Prab-3::unc-104 (C). (D-F) Quantification of the speed of anterograde trafficking (D), the percentage of anterograde events (E), and the number of anterograde movements (F) in young and aged animals. Total animals analyzed: day 1: wildtype:13, unc-104(wy711):21, Prab-3::unc-104:12; day 8: wildtype:24; unc-104(wy711):22; Prab-3::unc-104:16; day 12: wildtype:17, unc-104(wy711):9, Prab-3::unc-104:11. *: P<0.05. **: P< 0.01. ***: P<0.001. n.s.: not significant. One-way ANNOVA.
Figure S7, related to Figure 7 DA9 synapses are preserved in aged daf-2 mutants. (A-D) Confocal images of DA9 RAB-3 puncta of wildtype (A) and young (B) and aged (C, D) daf-2 mutants. (E-F) Quantification of RAB-3 puncta in the synaptic region (E) and mislocalization (F) in daf-2 animals. Student’s t test. ***: P<0.001. Total daf-2 animals analyzed: day 1:8, day 18:11, day 30:12.
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Table S1, related to Figure 6 lifespan analyses
Supplemental Experimental Procedures

Culture and strains
Worms were raised on NGM plates at 20°C, using OP50 E.coli as a food source. N2 Bristol was used as the wildtype reference strain. The mutant strains CB1265 unc-104(e1265), CB1370 daf-2(e1370), syd-2(mu37), unc-10(e102), nab-1(ok943), unc-57(ok310),unc-11(e47),dsh-1(ok1445),lin-44(n1792), daf-16(mu86), hsf-1(sy441), skn-1(ze67)/nT1[unc-(n754)let-1](IV;V), daf-2(e1370), were obtained through the Caenorhabditis Genetics Center. The wy711 mutant was described as previously[S1].

For aging experiments, worms were transferred every 2–3 days to fresh NGM plates to separate the adults from the larvae. Transgenic constructs were cloned into the pSM vector, a derivative of pPD49.26 (A.Fire) with extra cloning sites. The transgenic strains were generated using standard techniques.

Transgenic Lines and Molecular Cloning
wyEx3296 (Pitr-1::snb-1::yfp, Pitr-1:: cfp::rab-3): The plasmid was injected at 1 ng µL⁻¹ with 40ng Podr-1::rfp into N2 worms.

wyEx5554 (Punc-129::snb-1::yfp, Punc-129:: cfp::rab-3): The Pitr-1 promoter sequences in the plasmids Pitr-1::snb-1::yfp and Pitr-1:: cfp::rab-3 was replaced by the Punc-129 promoter between the restriction enzyme sites SphI and Ascl. The plasmid was injected at 1.0 ng µL⁻¹ with 40ng Podr-1::rfp into N2 worms.

wyEx6415 (Prab-3::unc-104::mcherry): The plasmid was injected at 20 ng µL⁻¹ with 40ng Podr-1::gfp into N2 worms.

wyEx6187 (Punc-104::unc-104::mcherry): The Prab-3 promoter in Prab-3::mcherry::unc-104 was replaced by the Punc-104 promoter (4.5 kb upstream of the unc-104 transcript) between the SpI and NheI sites. The plasmid was injected at 20 ng µL⁻¹ with 40ng Podr-1::gfp into N2 worms.

wyEx6164 (Punc-17::unc-104::mcherry): The Prab-3 promoter in Prab-3::mcherry::unc-104 was replaced by the Punc-17 promoter between the SpI and NheI sites. The plasmid was injected at 20 ng µL⁻¹ with 40ng Podr-1::gfp into N2 worms.

wyEx6461 (Punc-104::myrGFP, Punc-104::unc-104::mcherry) The Prab-3 promoter in Prab-3::mcherry::unc-104, and the Punc-17 promoter in Punc-17::myrGFP was replaced by the Punc-104 promoter (4.5 kb upstream of the unc-104 transcript) between the SpI and NheI sites. The plasmid was injected at 12 and 16 ng µL⁻¹ into N2 worms.

Fluorescence Confocal Imaging and Quantification
Images of fluorescently tagged fusion proteins were captured in live C. elegans using a plan-Apochromat 40X 1.3 objective on a Zeiss LSM710 confocal microscope, using identical image and laser setting for each genotype. The distribution of GFP::RAB-3 puncta along the DA9 dorsal nerve cords were extracted from the confocal images with the “straighten to line” program in ImagJ64. The puncta number was calculated using the “analyze particles” function, with the threshold of 1 pixels.

Body bend assays
Individual worms were placed in a droplet (10 µl) of M9 buffer and allowed to recover for 20-30s. The number of body bends was calculated as the average of body bend movements counted
in two continuous 30s intervals. A body bend was defined as a change in the direction of the
segment from the head to the midbody of an animal.

**Learning and memory assays**
The learning and memory behaviors were performed as previously described[S2].

**Real-time RT-PCR**
Total RNA was isolated from 50-100 adult worms by Trizol Reagent (Ambion #15596). cDNA was
synthesized in a 20µl reaction volume from 1-2 µg of total RNA using the SuperScript III first-
strand synthesis Super Mix (Invitrogen #11752). 1 µl of 1:20 dilution of cDNA was used as the
template in a 10µl reaction volume from the SsoFast EvaGreen Supermix with Low ROX (Bio-Rad,
#172-5211). The real-time PCR primers were designed using the probe-design software form Roche.
Real-time PCR was performed in duplicates using the Bio-Rad CFX96 Real-Time System. Data
were analyzed using the standard curve method. The entire experiments were repeated 6-10 times on
independent RNA preparations.

**Supplemental References**
capture and dissociation of presynaptic proteins controls the spatial distribution of
(2010). Insulin signaling and dietary restriction differentially influence the decline