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Nmnat exerts neuroprotective effects in dendrites and axons

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ABSTRACT

Dendrites can be maintained for extended periods of time after they initially establish coverage of their receptive field. The long-term maintenance of dendrites underlies synaptic connectivity, but how neurons establish and then maintain their dendritic arborization patterns throughout development is not well understood. Here, we show that the NAD synthase Nicotinamide mononucleotide adenylyltransferase (Nmnat) is cell-autonomously required for maintaining type-specific dendritic coverage of *Drosophila* dendritic arborization (da) sensory neurons. In *nmnat* heterozygous mutants, dendritic arborization patterns of class IV da neurons are properly established before increased retraction and decreased growth of terminal branches lead to progressive defects in dendritic coverage during later stages of development. Although sensory axons are largely intact in *nmnat* heterozygotes, complete loss of *nmnat* function causes severe axonal degeneration, demonstrating differential requirements for *nmnat* dosage in the maintenance of dendritic arborization patterns and axonal integrity. Overexpression of Nmnat suppresses dendrite maintenance defects associated with loss of the tumor suppressor kinase Warts (Wts), providing evidence that Nmnat, in addition to its neuroprotective role in axons, can function as a protective factor against progressive dendritic loss. Moreover, motor neurons deficient for *nmnat* show progressive defects in both dendrites and axons. Our studies reveal an essential role for endogenous Nmnat function in the maintenance of both axonal and dendritic integrity and present evidence of a broad neuroprotective role for Nmnat in the central nervous system.

Introduction

Dendrite arborization patterns have a direct influence on the types and numbers of synaptic or sensory input a neuron receives, and consequently, the way in which a neuron integrates and processes these impinging signals (Hausser et al., 2000; Magee, 2000). Dendritic abnormalities are the most consistent pathological correlate of cognitive decline in the aging human cortex as the progressive loss of dendritic branches is thought to underlie altered patterns of cortical activity in Alzheimer's disease (AD) (Coleman and Flood, 1987; Scheibel et al., 1975). Likewise, progressive defects in dendritic arborization have been associated with a number of neurodevelopmental disorders, including Down syndrome (DS) (Kaufmann and Moser, 2000). Although dendrite arborization patterns are often established early in development and are largely maintained throughout the lifetime of a neuron (Lin and Koleske, 2010), the specific mechanisms that are required for the maintenance of dendritic arbors are not well understood.

Drosophila da neurons are multidendritic (md) neurons that have emerged as powerful model neurons for studying establishment and maintenance of type-specific dendritic coverage (Parrish et al., 2007b). Each of the four classes of da neurons (I–IV) has a class-specific dendritic morphology and covers a stereotyped region of the body wall (Grueber et al., 2002). Dendritic arbors of class IV da neurons establish complete and non-redundant coverage of the body wall by the end of the first larval instar and maintain this coverage for the remainder of larval development (Emoto et al., 2006). Recent findings that Polycomb group (PcG) genes and the nuclear Dbf2-related (NDR) family kinase Wts are required for proper maintenance of class IV dendrites provide evidence that intrinsic mechanisms are important for maintaining dendritic coverage of receptive fields throughout development (Emoto et al., 2006; Parrish et al., 2007a).

Nmnat catalyzes a key step of NAD synthesis and is an essential component of the Wallerian degeneration slow (Wld^S) chimeric protein, a neuroprotective factor that delays axonal degeneration (Conforti et al., 2000; Mack et al., 2001). In mouse superior cervical ganglia (SCG) explants, specific knockdown of Nmnat2, one of three mammalian Nmnat isoforms, is sufficient to induce Wallerian-like degeneration, supporting a role for endogenous Nmnat2 in the maintenance of neuronal integrity (Gilley and Coleman, 2010). Studies from *Drosophila* suggest that Nmnat plays an evolutionarily conserved role in neuronal maintenance as overexpression of

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Drosophila Nmnat is sufficient to delay both injury-induced axonal degeneration (MacDonald et al., 2006) and spinocerebellar ataxia 1 (SCA1)-induced neurodegeneration (Zhai et al., 2008). Furthermore, *Drosophila* Nmnat can function endogenously as a maintenance factor to protect against activity-induced neurodegeneration (Zhai et al., 2006). Despite its well-documented role in protecting against axonal degeneration, whether Nmnat functions in a similar capacity to maintain dendritic integrity remains largely unknown. In this study, we show that Nmnat acts cell-autonomously to maintain dendritic arborization patterns of both da sensory neurons and motor neurons and that Nmnat can serve a neuroprotective role in dendrites as shown by the ability of Nmnat overexpression to suppress dendrite maintenance defects associated with loss of *wts*. We further show that loss of *nmnat* causes axonal degeneration and that axons and dendrites of class IV neurons show disparate phenotypes in response to reduction of *nmnat* copy number. Collectively, these studies support an evolutionarily conserved function for Nmnat in axon maintenance and reveal a novel role for Nmnat as a neuroprotective factor against progressive dendritic loss.

Results

nmnat mutants display progressive defects in terminal dendritic branching

We examined dendrites of class IV neurons in mutants homozygous for the loss-of-function $nmnat^{\Delta 4792}$ allele using the class IV-specific *pickpocket (ppk)-EGFP* reporter (Grueber et al., 2003). At 24–28 h after egg laying (AEL), dendrites of the dorsal class IV neuron (ddaC) in homozygous $nmnat^{\Delta 4792}$ mutants did not show any patterning defects and were largely indistinguishable from those of wild-type controls (data not shown). Because $nmnat^{\Delta 4792}$ mutants do not survive beyond this stage, we next turned to heterozygous mutants to further examine the role of *nmnat* in dendrite development. Although heterozygosity for $nmnat^{\Delta 4792}$ had no effect on dendritic outgrowth or branching of ddaC at 48 h AEL, we observed a significant reduction in the total number of terminal dendritic branches and total dendrite length at 120 h AEL (Fig. 1A–D). To gain a better understanding of the basis for defects in dendritic coverage in *nmnat* heterozygotes, we conducted time-lapse imaging of ddaC dendrites at 48 h and 72 h AEL (Fig. 1E–J). At 48 h AEL, ddaC neurons in wild-type and $nmnat^{\Delta 4792}$ heterozygotes were indistinguishable (Fig. 1E,H). At 72 h AEL, $nmnat^{\Delta 4792}$ heterozygotes tended to have fewer terminal dendrites (Fig. 1K), although the average number of terminal dendritic branches was not significantly different from wild-type controls until after 72 h AEL. Nevertheless, time-lapse analysis between 48 h and 72 h AEL revealed significantly fewer branch extension and more terminal branch retraction events in $nmnat^{\Delta 4792}$ heterozygotes compared to wild-type controls (Fig. 1L,M). Therefore, it seems likely that the progressive dendrite defects observed in *nmnat* heterozygotes are a consequence of both increased retraction and reduced growth of terminal dendritic branches.

We next examined axons of class IV neurons in $nmnat^{\Delta 4792}$ heterozygotes and found that they were largely intact at 120 h AEL (Fig. 2A,B,E). Axons entered the CNS appropriately and their projection patterns in the ventral nerve cord (VNC) were morphologically indistinguishable from wild-type controls (Fig. 2A,B). Although it is possible that *nmnat* is important for maintenance of axon terminal branching, the lack of an axon degeneration phenotype in $nmnat^{\Delta 4792}$ heterozygotes suggests that a single copy of *nmnat* is sufficient to maintain the axonal integrity of class IV neurons.

nmnat functions cell-autonomously to maintain dendritic coverage

To determine whether the dendrite maintenance defects reflect a cell-autonomous function for Nmnat in da neurons, we used MARCM (mosaic analysis with a repressible cell marker) (Lee and Luo, 1999) to generate mCD8::GFP-labeled single-cell homozygous $nmnat^{\Delta 4792}$ sensory neuron clones. Whereas wild-type ddaC clones elaborated highly branched dendrites (Fig. 3A), $nmnat^{\Delta 4792}$ ddaC clones showed a significant reduction in the total number of terminal dendritic branches, effectively reducing dendritic field coverage (Fig. 3B,F). Unlike ddaC neurons mutant for some PcG genes (Parrish et al., 2007a), there was no evidence of severed or degenerated dendrites in $nmnat^{\Delta 4792}$ clones as dendritic trunks and branches were largely intact. Postmitotic expression of wild-type Nmnat was sufficient to rescue dendritic phenotypes observed in $nmnat^{\Delta 4792}$ ddaC clones (Fig. 3C,F), further demonstrating a cell-autonomous function for Nmnat in the proper maintenance of class IV dendrites. Previous studies have shown that an enzymatically inactive form of Nmnat can rescue degeneration phenotypes induced by loss of *nmnat* or overexpression of ataxin-1 (Zhai et al., 2006, 2008). We similarly found that overexpression of an enzymatically inactive Nmnat (Nmnat-WR) (Zhai et al., 2006) could rescue terminal branching defects associated with loss of *nmnat* (Fig. 3F), suggesting that the NAD synthesis activity of Nmnat is dispensable for its function in dendrite maintenance in these neurons. Dendritic phenotypes were not limited to class IV neurons as we found that class III neurons, which normally elaborate actin-rich protrusions along their dendrites, were largely devoid of terminal branches in $nmnat^{\Delta 4792}$ clones (Supplementary Fig. 1). On the other hand, we found that class I neurons, which normally elaborate few terminal branches that cover a small receptive field, exhibited a minor, but statistically insignificant decrease in the total number of dendritic branches in $nmnat^{\Delta 4792}$ clones (Supplementary Fig. 2). Together with our time-lapse observations, these data suggest that one manner by which *nmnat* maintains dendritic coverage is by preventing the loss of terminal dendritic branches.

Although class IV neurons did not exhibit an axon degeneration phenotype in $nmnat^{\Delta 4792}$ heterozygotes (Fig. 2A,B), we found that 96% of homozygous $nmnat^{\Delta 4792}$ sensory neuron clones showed extensive fragmentation of axons and a near complete loss of axon terminals in the VNC (Fig. 2C,D,F). Loss of *nmnat* affected the axons of all classes of da neurons demonstrating its function in maintaining axonal integrity is not restricted to distinct neuronal subtypes. Collectively, these data suggest that neurons with highly branched dendritic arbors (e.g. class IV neurons) have different dosage requirements for *nmnat* in the maintenance of dendritic coverage and axonal integrity.

Overexpression of Nmnat can suppress dendrite maintenance defects in *wts* mutants

We investigated the possibility that Nmnat functions as a neuroprotective factor against dendritic loss by examining the effects of Nmnat overexpression in wts^{latsX1} mutants, which show a progressive loss of dendritic branches in class IV neurons without

Fig. 1. *nmnat* mutants display defects in the maintenance of terminal dendritic branches. (A,B) Dendrites of class IV ddaC neuron in a late third instar larva (120 h AEL) for wild type (A) and $nmnat^{\Delta 4792}$ heterozygote (*nmnat*/+) (B). (C,D) Quantification of total number of terminal dendritic branches (C) and total dendrite length (D) per 9.6×10^4 μm^2 (mean ± SD) in ddaC neurons for wild type and $nmnat^{\Delta 4792}$ heterozygotes at 48 h AEL (wild type, $n=8$; *nmnat*/+, $n=13$) and 120 h AEL (wild type, $n=9$; *nmnat*/+, $n=10$). Double asterisk denotes $p<0.001$ relative to wild-type controls (Student's *t*-test). (E,F) Time-lapse imaging of a representative wild-type ddaC neuron at 48 h AEL (E) and at 72 h AEL (F). (G) Tracing of a wild-type ddaC neuron indicates new branch growth (green) over the 24 h interval. (H,I) Time-lapse imaging of a representative ddaC neuron in a $nmnat^{\Delta 4792}$ heterozygote at 48 h AEL (H) and at 72 h AEL (I). (J) Tracing of a ddaC neuron in a $nmnat^{\Delta 4792}$ heterozygote indicates instances of dendrite retraction (red) and less new branch growth (green) over the 24 h interval compared to wild-type (G). Arrows indicate cell body. Anterior is left and dorsal is up. Scale bar, 50 μm. (K–M) Quantification of total number of terminal dendritic branches (mean ± SD) (K), fraction of extending terminal branches (L), and fraction of retracting terminal branches (M) per 3×10^4 μm^2 in ddaC neurons for wild-type ($n=7$) and $nmnat^{\Delta 4792}$ heterozygotes ($n=6$) at the 72 h time point (asterisk, $p<0.01$; double asterisk, $p<0.001$).