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Dendrite architecture determines mitochondrial distribution patterns *in vivo*

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SUMMARY

Mitochondria are critical for neuronal function and must be reliably distributed through complex neuronal architectures. By quantifying *in vivo* mitochondrial transport and localization patterns in the dendrites of *Drosophila* visual system neurons, we show that mitochondria make up a dynamic system at steady-state, with significant transport of individual mitochondria within a stable global pattern. Mitochondrial motility patterns are unaffected by visual input, suggesting that neuronal activity does not directly regulate mitochondrial localization *in vivo*. Instead, we present a mathematical model in which four simple scaling rules enable the robust self-organization of the mitochondrial population. Experimental measurements of dendrite morphology validate key model predictions: to maintain equitable distribution of mitochondria across asymmetrically branched subtrees, dendritic branch points obey a parent-daughter power law that preserves cross-sectional area, and thicker trunks support proportionally bushier subtrees. Altogether, we propose that “housekeeping” requirements, including the need to maintain steady-state mitochondrial distributions, impose constraints on neuronal architecture.

1 INTRODUCTION

2 Neurons are energetically demanding cells with complex morphologies (Cajal,
3 1888; Attwell and Laughlin, 2001; Harris et al., 2012). Mitochondria produce most of the
4 ATP in neurons (Hall et al., 2012) and must be distributed throughout all neuronal
5 compartments to meet energetic demands. Mitochondria are also highly dynamic: they
6 grow and degrade, divide and fuse, and move through the cell (Morris and Hollenbeck,
7 1995; Amiri and Hollenbeck, 2008; Plucinska et al., 2012; Cagalinec et al., 2013; Ashrafi
8 et al., 2014; Misgeld and Schwarz, 2017; Lewis et al., 2018). Thus, to maintain stable
9 mitochondrial distribution patterns over time, neurons must coordinate mitochondrial
10 dynamics over large, elaborately branched axonal and dendritic arbors.

11 Mitochondrial motility, in particular, has been extensively studied in neurons
12 (Morris and Hollenbeck, 1995; Pilling et al., 2006; Wang et al., 2011; Plucinska et al.,
13 2012; Lewis et al., 2016; Vagnoni and Bullock, 2018; Mandal and Drerup, 2019). For an
14 individual mitochondrion, the mechanics governing motility are relatively clear
15 (Barnhart, 2016). In brief, the motor proteins kinesin and dynein transport mitochondria
16 along microtubules (Pilling et al., 2006). Adaptor proteins link mitochondria to motor
17 proteins, and anchoring proteins oppose mitochondrial movement (Kang et al., 2008;
18 Pathak et al., 2010; Schwarz, 2013). Whether a particular mitochondrion moves, and in
19 which direction, depends on the number and orientation of microtubule tracks, as well
20 as the relative amount of force generated by populations of motor proteins versus
21 anchoring interactions (Barnhart, 2016). It is unclear, however, how neurons regulate
22 these molecular-scale interactions across space and time in order to maintain large-scale
23 mitochondrial distribution patterns.

24 In principle, neurons could regulate mitochondrial localization patterns by
25 controlling the relative flux of mitochondria into subcellular compartments. In this
26 scenario, mitochondria would be enriched in regions where more microtubule tracks
27 allow for higher rates of mitochondrial delivery. EM studies have shown that

1 microtubule numbers are proportional to the thickness of neuronal processes in various
2 cell types (Kubota et al., 2011; Katrukha et al., 2021), so the shape of a neuron is likely to
3 play a role in mitochondrial transport patterns. The relationship between transport rates
4 and morphological scaling across branch points in tree-like structures — i.e. the relative
5 thickness of parent and daughter branches, as well as the relative sizes of sister subtrees
6 that sprout from each branch point — has been studied in various contexts, from
7 botanical trees (Leonardo et al., 1970; Eloy, 2011; Lehnebach et al., 2018) to the vascular
8 system (Murray, 1926). In neurons, theoretical work has defined various scaling rules
9 that optimize wiring economy (Cherniak et al., 1999; Wen and Chklovskii, 2008; Cuntz
10 et al., 2010), electrical signaling (Rall, 1959; van Ooyen et al., 2002; Chklovskii and
11 Stepanyants, 2003; Wen and Chklovskii, 2008), and, to a lesser extent, transport
12 (Hillman, 1979; Liao et al., 2021). Proposed rules include Da Vinci’s Rule (Leonardo et
13 al., 1970; Hillman, 1979), Rall’s Law (Rall, 1959), and Murray’s Law (Murray, 1926;
14 Cherniak et al., 1999; Chklovskii and Stepanyants, 2003), which conserve, narrow, or
15 expand the total cross-sectional area of the branches at each junction, respectively.
16 However, there is no comprehensive theory or experimental evidence that defines the
17 relationship between neuronal architecture, mitochondrial transport, and large-scale
18 mitochondrial distribution patterns.

19 To interrogate the relationship between neuronal architecture and dynamic
20 mitochondrial localization patterns *in vivo*, we used neurons in the *Drosophila* visual
21 system called horizontal system (HS) neurons as a model system. There are three HS
22 neurons per optic lobe of the fly (six in total), all of which are functionally equivalent
23 (Schnell et al., 2010). HS neurons have highly branched dendritic arbors, which detect
24 global optic flow patterns by integrating local motion input signals (Barnhart et al.,
25 2018). ADD summary of RF and wiring constraints (Cuntz 2010). ADD a schematic
26 showing HS neurons in a fly brain and an MCFO image to Figure 1.

27

1 To determine how dendritic branching patterns contribute to steady-state
2 mitochondrial localization patterns in HS cells, we combined experimental
3 measurements of *in vivo* mitochondrial transport, mitochondrial localization patterns,
4 and dendrite architecture with mathematical modeling. We found that although
5 mitochondria in HS dendrites are highly motile, large-scale mitochondrial distribution
6 patterns are conserved across HS cells: mitochondria are consistently enriched in distal
7 dendrites, relative to primary dendrites, and equitably distributed across asymmetric
8 sister subtrees. Our experimental measurements are consistent with a mathematical
9 model in which simple dendritic scaling rules enable robust self-organization of steady-
10 state mitochondrial localization patterns. Consistent with previously published work
11 (Faits et al., 2016; Smit-Rigter et al., 2016; Silva et al., 2021), we also found that
12 physiological neuronal activity does not affect mitochondrial motility. Altogether, this
13 work demonstrates that in HS neurons, dendritic architecture, not neuronal activity,
14 determines mitochondrial localization patterns *in vivo*.

15

16 **RESULTS**

17 **Mitochondrial localization patterns in HS neurons**

18 To measure mitochondrial distribution patterns in HS neurons, we took
19 advantage of publicly available serial section transmission electron microscopy (ssTEM)
20 images of an entire fly brain (“Female Adult Fly Brain,” or FAFB (Zheng et al., 2018)).
21 We used existing HS skeletons, traced throughout the three-dimensional FAFB image
22 volume (Michael Reiser, unpublished data), to identify mitochondria within HS
23 neurons (Figure 1A-B, S1A-B). Then, we measured mitochondrial morphology as a
24 function of subcellular compartment by manually reconstructing whole mitochondria
25 within small portions of the axon or dendrite (Figure S1A-B). We found that the median
26 volume of an individual mitochondrion was $\sim 0.5 \mu\text{m}^3$ in both HS dendrites and axons
27 (Figure S1C). We also found that dendrites, but not axons, often contained large,

1 branched mitochondria that spanned multiple dendritic branches (Figure S1C),
2 consistent with previously published measurements of mitochondrial morphology in
3 vertebrate neurons (Popov et al., 2005; Turner et al., 2022).

4 Next, rather than measuring the size of individual mitochondria within small
5 portions of the cell, we sought to quantify mitochondrial distribution patterns across the
6 entire cell. Manual segmentation of mitochondria in three dimensions, however, is
7 prohibitively labor- and time-intensive. To rapidly measure mitochondrial densities (i.e.
8 the fraction of the neuron occupied by mitochondria) for whole cells, we resampled
9 each HS skeleton such that skeleton nodes were placed at regular 5 μm intervals along
10 the skeleton. Then we extracted two-dimensional image slices centered around each
11 node and manually reconstructed the HS neuronal segment and all mitochondria
12 within it in each image (Figure 1A). We calculated total mitochondrial densities for each
13 HS neuron by dividing the total mitochondrial area by the total neuronal area and
14 found that, on average, the total mitochondrial density in HS neurons is ~20% (Figure
15 S1D; mitochondrial density = 0.19 +/- 0.04 STE). Although we measured significant
16 variation in the total mitochondrial density across the six HS neurons in the FAFB
17 dataset (Figure S1D), we found that mitochondrial localization patterns were conserved
18 across all six cells (Figure 1C,E and S1E-F).

19 First, we measured mitochondrial densities as a function of subcellular
20 compartment and found consistently higher densities in dendrites than in axons (Figure
21 S1E). Next, in dendrites but not axons, mitochondrial densities increased with distance
22 from the soma, such that mitochondrial densities were approximately four times higher
23 in the distal-most dendrites compared to the primary dendrite (Figure 1C, S1F-G).
24 Finally, we measured mitochondrial densities across dendritic subtrees. At each branch
25 point within a dendritic arbor, a parent branch splits into two daughter branches, and
26 the entire arbor can be decomposed into successive pairs of sister subtrees (Figure 1D).
27 Although in some cases the dendritic arbor splits in a symmetric fashion, we found that

1 sister subtrees are often asymmetric (Figure 1E). Despite this asymmetry in sister
2 subtree size, we found that mitochondria are equitably distributed across branch points
3 such that sister subtrees have equivalent mitochondrial densities (Figure 1D-E).
4 Altogether, these results show that mitochondria in HS dendrites follow a specific
5 distribution pattern, with equitable distribution across sister subtrees and
6 mitochondrial enrichment in distal dendrites.

7

8 ***In vivo* mitochondrial motility in HS dendrites**

9 Mitochondria in neurons are motile, both in cell culture (Morris and Hollenbeck,
10 1995; Overly et al., 1996; Wang and Schwarz, 2009) and *in vivo* (Plucinska et al., 2012;
11 Lewis et al., 2016; Mandal and Drerup, 2019; Silva et al., 2021). We therefore
12 hypothesized that the specific mitochondrial localization pattern we measured in HS
13 dendrites reflects a dynamic steady-state in which individual mitochondria are
14 continually reorganizing within a stable, global pattern. To test this hypothesis, we used
15 the GAL4/UAS binary system to drive expression of GFP-tagged mitochondria
16 (mitoGFP) and a cytosolic volume marker (tdTomato) in HS neurons (Brand and
17 Perrimon, 1993). Then, we used *in vivo* confocal microscopy to image HS dendrites in
18 living, head-fixed *Drosophila* (Figure 2A). Mitochondria are densely packed within HS
19 dendrites, so we bleached stationary mitochondria in a small portion of the dendrite to
20 resolve motile mitochondria moving through the bleached region. We measured
21 mitochondrial motility in primary and distal dendritic branches (Figure 2B-D). In the
22 primary dendrite, mitochondria moved in both anterograde (into the arbor) and
23 retrograde (out of the arbor) directions (Figure 2D). Motile mitochondria were highly
24 persistent, with less than 20% of mitochondria arresting or pausing as they moved
25 through the primary dendrite (Figure S2A). Individual mitochondria exhibited a broad
26 range of speeds and lengths, but there were no significant differences between
27 mitochondria moving in the anterograde versus retrograde directions in the primary

1 dendrite (Figure 2E-F). In the distal dendrites, mitochondria moving in the anterograde
2 direction were significantly slower than in the primary dendrite (Figure 2E). Linear flux
3 rates were also significantly lower in the distal dendrites (Figure S2B). Finally, the
4 average flux of anterograde mitochondria moving through the primary dendrite into
5 the arbor was equivalent to the average flux of retrograde mitochondria moving back
6 out of the arbor (Figure 2G). This balanced anterograde and retrograde transport
7 through the primary dendrite suggests that the total amount of mitochondria in the HS
8 arbor remains constant over time.

9 Notably, our measurements of mitochondrial density and motility allow us to
10 estimate the fraction of dendritic mitochondrial volume that is motile versus stationary
11 at any given point in time. In the primary dendrite, approximately two mitochondria
12 pass a cross-section per minute, in each direction. Based on our measurements of
13 mitochondrial length, we estimate that the volume of each motile mitochondrion is ~ 0.5
14 μm^3 (see Methods), consistent with the median mitochondrial volume in our EM
15 reconstructions (Figure S1C). Thus, $\sim 1 \mu\text{m}^3$ of mitochondrial mass exchanges through
16 the primary dendrite every minute. Based on this rate of mitochondrial volume
17 exchange ($J \sim 1 \mu\text{m}^3/\text{min}$), the typical speed of motile mitochondria ($v \sim 0.4 \mu\text{m}/\text{s}$), the
18 mitochondrial volume density ($c \sim 10\%$), and the cross-sectional area ($A_d \sim 30 \mu\text{m}^2$) in the
19 primary dendrite, we estimate that the fraction of motile mitochondria mass in this
20 proximal region is $f_m = 2J/(v c A_d) \sim 3\%$. Mitochondrial density is higher in the distal
21 dendrites ($c \sim 30\%$), and we therefore estimate an even lower motile fraction for the
22 distal portions of the dendritic arbor ($f_m \sim 1\%$). Thus, the majority of the mitochondrial
23 population is stationary at any given moment. However, we also estimate that the total
24 volume of mitochondria in the dendritic arbor exchanges through the primary dendrite
25 many times over the lifetime of the neuron. Based on the mitochondrial density in the
26 whole dendrite ($c \sim 20\%$), the total volume of the dendrite ($V_d \sim 2000 \mu\text{m}^3$ (Cuntz et al.,
27 2013) and the rate of mitochondrial volume exchange ($J \sim 1 \mu\text{m}^3/\text{min}$), we estimate the

1 fraction of mitochondrial volume exchanged per unit time in the dendrite to be: $J_{\text{norm}} =$
2 $J/(c V_d) \sim 15\% \text{ hr}^{-1}$. At this rate, the entire mitochondrial volume in an HS arbor
3 reorganizes in less than ten hours, or more than one hundred times over the course of a
4 fly's lifetime. Altogether, these results are consistent with the idea that the mitochondria
5 in HS dendrites make up a dynamic system at steady-state.

6 7 **Visual stimulus-evoked calcium signals do not affect mitochondrial motility in HS** 8 **dendrites**

9 How do HS dendrites maintain steady-state mitochondrial distribution patterns,
10 given rapid reorganization of mitochondrial mass? HS neurons selectively respond to
11 specific patterns of global optic flow (Schnell et al., 2010; Fujiwara et al., 2017; Barnhart
12 et al., 2018), which drive large calcium increases in the distal dendrites of HS neurons
13 (Barnhart et al., 2018). Calcium arrests mitochondrial motility in cultured neurons
14 (MacAskill et al., 2009; Wang and Schwarz, 2009), so we reasoned that if stimulus-
15 evoked calcium signals are stronger in the distal dendrites than in the primary dendrite,
16 then calcium-dependent arrest of mitochondrial transport could increase the density of
17 mitochondria in the distal dendrites, relative to the primary dendrite. To test this idea,
18 we projected the preferred visual stimulus for HS neurons — a global motion stimulus
19 moving from front to back across one eye — on a screen positioned in front of the fly
20 while simultaneously imaging a genetically-encoded calcium reporter (RGECO1a) in
21 HS dendrites (Figure 3A, S3A). Consistent with our previously published work, front-
22 to-back global motion stimuli drove robust calcium responses in HS distal dendrites
23 (Figure S3A-C). In contrast, we measured significantly smaller calcium responses to the
24 same stimuli in primary dendrites (Figure S3A-C). Thus, stimulus-evoked calcium
25 signals correlate with mitochondrial density, with larger calcium response amplitudes
26 and mitochondrial densities in the distal dendrites compared to the primary dendrites.

1 However, we also found that visual stimulus-evoked calcium signals are not
2 sufficient to arrest mitochondrial motility in HS distal dendrites (Figure 3B-D). When
3 we simultaneously imaged a green calcium reporter (GCaMP6f) and mitochondrially-
4 targeted DsRed (mitoDsRed) while presenting global motion stimuli to the fly, we again
5 measured large, stimulus-evoked calcium signals in the distal dendrites (Figure 3B). We
6 observed mitochondria moving through distal dendritic branches, regardless of
7 whether the stimulus was on or off (Figure 3B). The stimulus did not affect
8 mitochondrial speeds (Figure 3C), nor did we measure a difference in mitochondrial
9 transport rates when the stimulus was on versus off (Figure 3D). Thus, physiologically-
10 relevant calcium signals do not directly affect mitochondrial motility in HS dendrites *in*
11 *vivo*.

12

13 **Simple scaling rules recapitulate experimental measurements of mitochondrial** 14 **localization patterns**

15 A mean field model of mitochondrial distributions in branched dendrites

16 We next hypothesized that dendritic architecture, rather than neuronal activity,
17 determines steady-state mitochondrial localization patterns in HS cells. To test this idea,
18 we developed a quantitative model of mitochondrial transport and localization patterns
19 in HS dendrites. In developing this model, we assumed that mitochondrial distributions
20 are governed by the local relationship between mitochondrial transport rates and
21 dendrite thickness as well as the morphology of the dendritic arbor. Specifically, we
22 defined four simple scaling relationships governing mitochondrial localization patterns
23 (Figure 4A):

24 1) *Scaling of mitochondrial transport with dendrite radius.* Our *in vivo* measurements
25 of mitochondrial transport show that mitochondria are significantly more likely to
26 arrest motility in thin distal dendrites than in the thick primary dendrite (Figure S2A).
27 Based on this, we assume that the rate of mitochondrial arrest, k_s , scales with dendrite

1 radius according to $k_s \sim 1/r^\beta$. This relationship incorporates a width-dependence for the
2 population-averaged speed of mitochondrial transport in different dendritic branches.

3 2) *Splitting of mitochondria at branch points.* Motor proteins transport mitochondria
4 along microtubules in neurons (Pilling et al., 2006), and microtubule numbers scale with
5 branch thickness in other neuronal cell types (Kubota et al., 2011; Katrukha et al., 2021).
6 We therefore assume that the probability of a mitochondrion moving into a given
7 daughter branch is proportional to the cross-sectional area of the branch, according to
8 $p_1/p_2 = r_1^2/r_2^2$, where p_1 and p_2 are the probabilities that a mitochondrion moves from the
9 parent into daughter 1 or 2, and r_1 and r_2 are the radii of the two daughters.

10 3) *Power law scaling of parent and daughter branch widths.* Consistent with several
11 previous studies (Hillman, 1979; Cherniak et al., 1999; van Ooyen et al., 2002;
12 Chklovskii and Stepanyants, 2003), we assume that parent and daughter radii scale
13 according to the power law $r_0^\alpha = r_1^\alpha + r_2^\alpha$, where r_0 is the radius of the parent branch and
14 r_1 and r_2 are the radii of the daughter branches. The exponent α determines the extent to
15 which the total dendritic cross-sectional area increases or decreases in the daughter
16 branches, relative to the parent: area is conserved for $\alpha = 2$, decreases for $\alpha < 2$, and
17 increases for $\alpha > 2$. Optimal values for α have been derived based on theoretical
18 arguments for preservation of graded electrical signals across dendritic branch points (α
19 $= 3/2$, often called “Rall’s Law” after the neuroscientist Wilfrid Rall (Rall, 1959)), action
20 potential propagation in axons ($\alpha = 3$, often called “Murray’s Law” and first derived for
21 the vasculature system) (Murray, 1926; Cherniak et al., 1999; Chklovskii and
22 Stepanyants, 2003; Mandal and Drerup, 2019), or efficient intracellular transport ($\alpha = 2$,
23 often called “Da Vinci scaling” after Da Vinci’s rule for trees) (Leonardo et al., 1970;
24 Hillman, 1979).

25 4) *Scaling of sister subtree size with trunk thickness.* Finally, we assume that larger
26 subtrees are supported by proportionally thicker trunks, with trunk radii scaling with
27 either total subtree length, depth, or some other measure of subtree size. This scaling,

1 along with proportional mitochondrial transport at branch points, should ensure that
2 larger subtrees receive a proportionally larger supply of mitochondria.

3 To determine how these scaling rules contribute to steady-state mitochondrial
4 localization patterns, we developed a mathematical model of mitochondrial transport in
5 dendritic trees. In this model, each dendrite is a binary tree, with each junction node
6 connecting a parent edge to two daughter edges (Figure 4B). We assume that each edge
7 is a cylinder with fixed radius r_i along its entire length l_i . In our model, the connectivity
8 and length of each edge in a tree are known attributes, either extracted from skeletons
9 of real HS neurons (Figure 4B) or from synthetic trees with varying branching patterns
10 (Figure S4A). Radii of each branch are set according to various rules for parent-
11 daughter and sister subtree scaling (Rules 3 and 4, Figure 4A).

12 Within this dendritic structure, we assume that discrete mitochondrial units
13 undergo active transport, which includes processive motion in the anterograde and
14 retrograde directions, as well as arrest and restarting (Figure 4B). The ratio of arrest and
15 restarting rates (k_s/k_w) determines the local fraction of stationary mitochondria in each
16 dendritic branch. We assume the restarting rate is constant throughout the arbor, but
17 allow the arrest rate to vary in dendrites of different widths (Rule 1, Figure 4A). Finally,
18 we assume that the anterograde mitochondrial linear flux entering a junction node is
19 split in proportion to the cross-sectional area of the daughter branches, so that wider
20 daughter branches receive proportionally more mitochondria (Rule 2, Figure 4A),

21 We implemented several versions of this model, using different forms of parent-
22 daughter, sister subtree, and transport scaling rules, through analytical solutions of
23 mean-field equations for mitochondrial density (Figures 4, 7, S4, and S7) and stochastic
24 simulations (Figure S5). For all model versions, we calculated mitochondrial densities as
25 a function of distance from the soma and across sister subtrees to determine which
26 scaling rules recapitulate our experimental measurements (i.e. distal enrichment and
27 equitable distribution of mitochondria across sister subtrees).

1

2 Model results with uniform transport parameters

3 In the first, simplest version of our model, we assumed that mitochondrial arrest
4 rates and transport speeds are independent of dendrite thickness ($\beta = 0$ in Rule 1), such
5 that the fraction of stationary versus motile mitochondria is constant throughout the
6 arbor, and that the linear flux of mitochondria splits in proportion to daughter branch
7 thickness at each branch point (Rule 2). We also assumed that parent and daughter radii
8 follow power law scaling (Rule 3), either Rall's law ($\alpha = 3/2$), Da Vinci's rule ($\alpha = 2$), or
9 Murray's law ($\alpha = 3$). When $\alpha = 2$, the total cross-sectional area is conserved at each
10 junction, giving rise to constant mitochondrial volume densities throughout the entire
11 arbor, regardless of the tree topology and the relative thickness of sister radii (as set by
12 various forms of Rule 4). Thus, Da Vinci parent-daughter scaling results in equitable
13 distribution of mitochondria across sister subtrees, but not distal enrichment in this
14 minimal version of our model (Figure 4C,E, S4B,D).

15 In contrast, when the narrowing of daughter branches is governed by Rall's Law
16 ($\alpha = 3/2$), the reduction in total cross-sectional area at each branch point results in distal
17 enrichment of mitochondria (Figure 4E, S4D). When the narrowing of daughter
18 branches is governed by Murray's Law ($\alpha = 3$), the expansion in total cross-sectional
19 area at each branch point results in distal dilution of mitochondria (Figure 4E, S4D). In
20 addition, when $\alpha = 3/2$ or 3 (or any value other than 2), the relative volume density of
21 mitochondria in sister subtrees depends on the relative thicknesses of sister trunks at
22 each branch point (as set by various forms of Rule 4), and different forms of sister
23 subtree scaling all result in significant mitochondrial density asymmetries unless
24 dendritic branching patterns are perfectly symmetric (Figure 4C, S4B). In particular,
25 subtrees with more branch points tend to accumulate higher or lower mitochondrial
26 densities due to the reduction or expansion of dendritic cross-sectional area below each
27 branch points when $\alpha = 3/2$ or 3, respectively. Moreover, we show that for an arbor

1 obeying Rall's Law, it is impossible to establish equitable mitochondrial distributions
2 between asymmetric sister subtrees with any single function that sets sister subtree
3 trunk thicknesses based on subtree morphology (Figure S4F and Supplemental
4 Methods). Altogether, when mitochondrial transport parameters are spatially uniform,
5 our model recapitulates either equitable distribution of mitochondrial across sister
6 subtrees (for Da Vinci-scaled dendrites) or distal enrichment of mitochondria (for Rall-
7 scaled dendrites), but not both.

8

9 Model results with non-uniform transport parameters

10 Our experimental measurements indicate that in thin distal dendrites, average
11 mitochondrial speeds are lower and arrest rates are higher than in the thick primary
12 dendrite (Figure 2E and S2A), and we reasoned that scaling of mitochondrial transport
13 with dendrite radius could result in increased mitochondrial densities in thin distal
14 dendrites. We therefore updated our initial model such that mitochondrial transport
15 rates scale with dendrite radii. Specifically, we assumed that mitochondrial arrest rates
16 are inversely proportional to dendrite cross-sectional area, $k_s \sim 1/r^2$ ($\beta = 2$ in Rule 1). For
17 simplicity, we assumed that mitochondrial motility initiation rates and speeds are still
18 spatially uniform; different assumptions about how mitochondrial transport changes
19 with branch thickness (e.g. scaling of mitochondrial speed with dendrite radius) give
20 the same results when the fraction of motile mitochondria is very small (see
21 Supplemental Methods), as we observed experimentally.

22 In dendrites that follow Da Vinci scaling of parent and daughter branch widths
23 ($\alpha = 2$ in Rule 3), this model predicts that motile mitochondria densities will be constant
24 throughout the dendrite, while stationary mitochondrial densities will be higher in
25 distal dendrites (Figure 4F, S4E). If we assume that daughter branch radii are split
26 equally ($r_1 = r_2$) at each junction, then asymmetric topologies of sister subtrees lead to

1 asymmetry in the mitochondrial distribution, with more-branched subtrees acquiring a
2 higher mitochondrial density (Figure 4E, S4D).

3 Next, we investigated whether an alternate relationship between the trunk
4 widths of sister subtrees could restore equitable distribution of mitochondria across
5 subtree pairs. According to our analytical calculations for Da Vinci trees with $k_s \sim 1/r^2$
6 (see Supplemental Methods), the ratio of stationary mitochondrial densities in a pair of
7 sister subtrees is given by $c^{(s)1}/c^{(s)2} = (L_1/V_1)/(L_2/V_2)$, where $c^{(s)}$ is the density of stationary
8 mitochondria and L and V are subtree length and volume. Equitable distribution of
9 mitochondria therefore depends on a specific morphological relationship between sister
10 subtrees: the total subtree volume must be proportional to its length, such that $L_1/V_1 =$
11 L_2/V_2 .

12 For an arbor that follows the Da Vinci scaling law, the preservation of cross-
13 sectional area across branch points means that the total volume of a tree can be
14 expressed as $V = r_0^2 D$, where r_0 is the radius of the trunk of the tree and D describes the
15 effective depth of the tree. For a tree where all terminal dendritic branch tips are the
16 same distance d from the trunk, the effective tree depth is $D = d$. For trees with distal
17 tips at varying distances from the trunk, we compute the effective depth D recursively
18 from the branch lengths in each subtree (see Supplemental Methods). Notably, the
19 dependence of subtree volume on the trunk radius implies that for subtrees with
20 different depths, it is not possible to split sister subtree trunks in a way that would
21 allow their cross-sectional area to be proportional to volume. We thus do not consider r^2
22 $\sim V$ splitting behaviors in our calculations.

23 In a tree with Da Vinci parent-daughter scaling, all subtrees will exhibit a volume
24 proportional to their total branch length if and only if the sister subtree trunk areas scale
25 according to the following relation: $r_1^2/r_2^2 = (L_1/D_1)/(L_2/D_2)$. The ratio of total length over
26 depth (L/D) can be thought of as the “bushiness” of a tree. In trees with no branch

1 points, $L = D$ and thus $L/D = 1$. In trees with many branch points (i.e. bushier trees), $L \gg$
2 D and $L/D \gg 1$.

3 In dendrites that follow Da Vinci parent-daughter scaling and sister subtree
4 scaling with $r^2 \sim L/D$, mitochondria are equitably distributed across subtrees (Figure 4D,
5 S4C). Trees that follow different sister subtree scaling rules (e.g. with radii splitting
6 proportional to subtree length) do not exhibit such equitable distributions across
7 subtrees (Figure 4D, S4C). Regardless of the choice of parent-daughter or sister subtree
8 scaling rule, increased mitochondrial arrest rates in thinner distal branches leads to
9 distal mitochondrial enrichment (Figure 4F). We note that the predicted distal
10 enrichment of mitochondria in these simple models is unrealistically high. This effect
11 can be mitigated by assuming a smaller value for β , such that mitochondrial arrest rates
12 increase less as dendrite radii decrease, or setting a lower limit on dendrite radii (Liao et
13 al., 2021), as discussed below.

14 Our analytical mean-field calculations are supplemented by stochastic
15 simulations of point-like mitochondria moving through small synthetic dendritic arbors
16 (Supplemental Figure S5). These simulations show average densities similar to those
17 predicted by the mathematical model, although higher asymmetries are found between
18 sister subtrees due to stochastic variation in the simulations. Both simulations and
19 mean-field calculations demonstrate that this model — with dendritic trees obeying Da
20 Vinci parent-daughter scaling, inverse scaling of mitochondrial arrest and dendrite
21 thickness, and sister subtree trunk areas proportional to tree bushiness — successfully
22 recapitulates the key features of experimentally observed mitochondrial distributions:
23 equitable densities between sister subtrees and increased density in distal branches.

24

25 **Mitochondria split according to dendrite thickness at asymmetric branch points**

26 Our model rests on the assumption that mitochondria traversing branch points
27 in the anterograde direction split according to the cross-sectional areas of the daughter

1 branches. To test this assumption, we used our *in vivo* imaging setup to measure
2 mitochondrial transport across primary branch points in HS dendrites (Figure 5A). We
3 found, first, that most mitochondria moving through the branch in the anterograde
4 direction moved persistently from the parent into one of the two daughter branches
5 without reversing direction or arresting motility (Table S1). In the retrograde direction,
6 the vast majority of mitochondria moved from a daughter branch into the parent
7 branch, with infrequent reversals or arrests and almost no mitochondria moving from
8 one daughter to the other (Table S1). At each branch point, the population of
9 mitochondria moving in the anterograde direction splits, with some mitochondria
10 moving from the parent branch into one daughter branch and the rest into the other
11 (Figure 5A). By comparing mitochondrial linear flux rates to daughter branch cross-
12 sectional areas (Figure 5B), we found that asymmetric linear flux rates (where more
13 mitochondria are transported into one daughter than the other) correlate with
14 asymmetric daughter branch cross-sectional areas (where one daughter is thicker than
15 the other). Thus, proportionally more mitochondria are transported into thicker
16 daughter branches, consistent with our assumption that mitochondria split at branch
17 points according to daughter branch cross-sectional area.

18 In addition, we found that mitochondrial flux rates (the linear flux rate divided
19 by branch cross-sectional area) are conserved across branch points, such that flux in the
20 parent branch is roughly equivalent to flux in the daughter branches (Figure 5C,
21 average flux = 0.07 ± 0.01 (STE) mitochondria/min/ μm^2 in both parent and daughter
22 branches). We measured similar mitochondrial flux rates in primary (0.08 ± 0.01
23 mitochondria/min/ μm^2) and distal (0.06 ± 0.01 mitochondria/min/ μm^2) dendrites as
24 well (Figure S2D). Conservation of mitochondrial flux rates throughout the arbor is
25 consistent with spatially constant motile mitochondrial volume densities which,
26 according to our model, occurs in dendrites that follow Da Vinci parent-daughter
27 scaling.

1

2 **HS dendrites follow simple dendritic scaling rules**

3 According to our model, HS dendrites maintain realistic mitochondrial
4 distributions if parent and daughter radii scale according to Da Vinci's rule ($\alpha = 2$), and
5 sister subtrees scale with trunk thickness proportional to subtree bushiness ($r^2 \sim L/D$). To
6 determine whether HS dendrites obey these morphological scaling rules, we measured
7 HS dendritic architecture. We used stochastic multicolor FlpOut (MCFO) labeling (Nern
8 et al., 2015) to label individual HS dendrites, which we then imaged by confocal
9 microscopy (Figure 6A-B). Next, we segmented and skeletonized each dendrite before
10 measuring parent and daughter branch radii and the length, volume, and bushiness of
11 the subtrees sprouting from each branch point (Figure 6B). We found, first, that HS
12 dendrites are asymmetrically branched, with significant asymmetry in daughter branch
13 thickness and subtree length, volume, and bushiness (Figure 6C). Second, we fit our
14 measurements of parent and daughter radii to the power law $r_0^\alpha = r_1^\alpha + r_2^\alpha$ for a range of
15 values for the exponent α , and we found HS dendrites are approximately Da Vinci-
16 scaled, with $\alpha = 2.2$ giving the best fit (Figure 6D, $R^2 = 0.87$, 95% bootstrap confidence
17 interval = 1.98-2.44).

18 Next, our model predicts that Da Vinci-scaled dendrites can only achieve
19 equitable distribution of mitochondria if sister subtrees have volumes proportional to
20 their length. To test this prediction, we compared the asymmetry in subtree lengths ($(L_1 -$
21 $L_2)/(L_1 + L_2)$) with the asymmetry in volumes ($(V_1 - V_2)/(V_1 + V_2)$) for sister subtree pairs
22 emerging from the same branch point. We found that length asymmetry is equal to
23 volume asymmetry (Figure 6E, $R^2 = 0.94$), indicating that longer sister subtrees have
24 proportionally larger volumes, as predicted. Thus, HS dendrites obey two separate
25 morphological rules: power law scaling of parent and daughter branches with $\alpha \sim 2$,
26 and sister subtree splitting with volume proportional to length ($L_1/V_1 = L_2/V_2$). According
27 to our model, for dendrites that follow these two rules, daughter branch cross-sectional

1 areas must be proportional to subtree bushiness: $r_1^2/r_2^2 = (L_1/D_1)/(L_2/D_2)$. To test this
2 prediction, we compared asymmetry in branch cross-sectional area $(r_1^2-r_2^2)/(r_1^2+r_2^2)$ to
3 asymmetry in subtree bushiness $((L_1/D_1)-(L_2/D_2))/((L_1/D_1)+(L_2/D_2))$. We found that trunk
4 cross-sectional area and bushiness asymmetry are correlated (Figure 6F, $R^2=0.70$), and
5 that cross-sectional area asymmetry was only weakly correlated with subtree length or
6 depth asymmetry (Figure S6A-B). Finally, we measured similar correlations for
7 synthetic tree structures with an imposed error in radii measurements (Supplemental
8 Figure S6).

9 Altogether, these experimental results support our model and strongly suggest
10 that dendrite morphology plays a key role in determining steady-state mitochondrial
11 localization patterns in neurons *in vivo*.

12

13 **Robust self-organization of equitably distributed mitochondria for a range of** 14 **transport parameters**

15 In our model, we assume that mitochondrial arrest rates are inversely
16 proportional to the cross-sectional area of each dendritic branch ($k_s \sim 1/r^\beta$, where $\beta = 2$),
17 resulting in increased mitochondrial densities in thinner distal dendrites. However,
18 distal enrichment was orders of magnitude higher in our model (~300 fold enrichment)
19 compared to in our experimental measurements (~4 fold enrichment). Smaller values of
20 β should result in more reasonable distal enrichment, so we estimated β from *in vivo*
21 images of mitochondria moving through dendritic branches with a range of radii (see
22 Methods). Our measurements show that mitochondrial arrest is indeed more frequent
23 in thinner branches, and that the rate of arrest scales with dendrite radius according to
24 $k_s \sim 1/r^{1.3}$ ($\beta = 1.3$, $R^2 = 0.54$, 95% confidence interval = 0.93-1.67) (Figure 7A). In our
25 model, values for α and β in the range of our experimental measurements result in both
26 realistic distal enrichment (~10 fold enrichment) and equitable mitochondrial densities
27 in dendrites that obey $r^2 \sim L/D$ sister subtree scaling (Figure 7B-C). Incorporating a

1 minimum radius (r_m) such that parent and daughter dendrites scale according to $r_0^2+r_m^2$
2 = $r_1^2+r_2^2$ (as recently described for sensory neurons in *Drosophila* larva) (Liao et al., 2021)
3 reduces distal enrichment and introduces small asymmetries in mitochondrial densities
4 across sister subtrees (Figure S7A-B). Interestingly, in Da Vinci-scaled dendrites ($\alpha = 2$)
5 without a minimum radius, equitable distribution is highly robust to changes in β
6 (Figure 7C), indicating that changes in transport parameters have a dramatic effect on
7 distal enrichment without affecting equitable distribution. In contrast, Rall's ($\alpha = 3/2$)
8 and Murray's ($\alpha = 3$) laws failed to yield equitable mitochondrial distributions (Figure
9 7C), and different sister subtree scaling rules (e.g. trunks splitting according the subtree
10 length, rather than bushiness) yielded equitable distributions in Da Vinci-scaled
11 dendrites only when $\beta = 0$ (Figure S7D,F). Thus, equitable mitochondrial distributions
12 are robust to changes in mitochondrial transport parameters only in dendrites that
13 follow specific morphological scaling rules.

14

15 DISCUSSION

16 Neuronal function is inextricably linked to neuronal form: dendrite size limits
17 the formation of synaptic connections to a particular receptive field (Peichl and Wassle,
18 1983); branching patterns affect dendritic integration of input signals (van Elburg and
19 van Ooyen, 2010; Lefebvre et al., 2015); and axon thickness regulates action potential
20 propagation (Waxman and Bennett, 1972; Seidl, 2014). Our work suggests that HS
21 neurons in the *Drosophila* visual system also maintain a dendritic architecture that
22 allows reliable distribution of mitochondria through asymmetrically branched arbors.
23 We present a model in which four simple scaling rules determine steady-state
24 mitochondrial distribution patterns (Figure 4). The first two rules — scaling of
25 mitochondrial transport with dendrite radius and proportional splitting of
26 mitochondria at branch points — relate local mitochondrial motility rates to dendritic
27 branch radii. The third and fourth rules — power law scaling of parent and daughter

1 radii and scaling of trunk thickness with sister subtree size — are morphological rules
2 that determine the architecture of the dendrite. There are many possible forms of these
3 dendritic scaling rules, but only a subset of the rules we examined — Da Vinci scaling
4 of parent-daughter radii at branch points and sister subtree scaling with trunk thickness
5 proportional to subtree bushiness — predict realistic mitochondrial localization patterns
6 in our model. Our experimental measurements demonstrate that HS dendrites do in
7 fact obey these morphological scaling rules (Figure 6). Thus, our work suggests that
8 intracellular transport, and the need to distribute mitochondria throughout elaborately
9 branched dendritic arbors, acts as an important constraint on dendrite morphology.

10

11 We have shown that mitochondria are equitably distributed across sister
12 subtrees and enriched in the distal dendrites in HS cells (Figure 1). Distribution of
13 mitochondria throughout the cell is critical for neuronal stability (Griparic et al., 2004;
14 Verhoeven et al., 2006; Baloh et al., 2007; Nunnari and Suomalainen, 2012) but the
15 relationship between specific mitochondrial localization patterns (e.g. distal
16 enrichment) and neuronal function is unclear. One possibility is that mitochondrial
17 densities simply reflect local energetic demands, with mitochondrial enrichment in
18 subcellular regions that require relatively higher levels of ATP production. The reversal
19 of ion fluxes near synapses is thought to account for a large fraction of the neuron's
20 energy budget (Attwell and Laughlin, 2001; Harris et al., 2012), and mitochondrial
21 densities weakly correlate with synaptic densities in the dendrites of mouse cortical
22 pyramidal neurons (Turner et al., 2022). Mitochondria also buffer calcium (Werth and
23 Thayer, 1994), and variations in mitochondrial densities may contribute to
24 compartment-dependent differences in calcium buffering capacities, which have
25 recently been shown to contribute to place field formation in awake and behaving mice
26 (O'Hare et al., 2022).

27

1 In addition to supporting dendritic function, enrichment of mitochondria in
2 specific neuronal compartments may play a role in supporting mitochondrial function
3 (Chang et al., 2006; Thomas et al., 2019; Turner et al., 2022). In active mitochondria,
4 damaging reactive oxygen species are a by-product of the electron transport chain
5 (Miwa and Brand, 2003). Mitochondria are thought to compensate for ROS-induced
6 damage in two ways: by degrading and replacing damaged proteins (Wang et al., 2012),
7 and by homogenizing the mitochondrial population — diluting damaged proteins and
8 sharing freshly synthesized proteins — via mitochondrial fusion and fission (Willems et
9 al., 2015). The majority of mitochondrial proteins are thought to be synthesized and
10 transported into mitochondria in the cell body which are then trafficked out into the
11 axons and dendrites (Misgeld and Schwarz, 2017). Theoretical work suggests that
12 fusion with stationary mitochondria depletes freshly-synthesized mitochondrial
13 proteins from motile mitochondria as they move in the anterograde direction (Agrawal
14 and Koslover, 2021). A graded distribution of stationary mitochondria, with higher
15 densities in distal dendrites, may allow neurons to ensure adequate delivery of fresh
16 mitochondrial proteins to distal axons and dendrites while also maximizing
17 complementation across mitochondria in distal compartments. Moreover, mitochondria
18 in HS distal dendrites are large, often spanning multiple dendritic branch points (Figure
19 S1). If young, healthy mitochondria fuse with stationary mitochondria upon arrival in
20 the distal dendrites, passive transport within these elongated mitochondria would
21 ensure uniform local distributions of freshly-synthesized mitochondrial proteins. Future
22 versions of our model will include mitochondrial fusion and fission rates, as well as
23 mitochondrial motility.

24

25 In our model, equitable distribution of mitochondria across sister subtrees is
26 robust to variation in mitochondrial transport parameters in Da Vinci-scaled dendrites
27 (Figures 7C). Distal enrichment, on the other hand, depends on inverse scaling of

1 motility arrest with dendrite thickness (Figure 7B). The mechanism underlying inverse
2 scaling of motility arrest and branch thickness remains undetermined. In principle,
3 narrowing of dendrite branches, on its own, could be sufficient to increase motility
4 arrest. In cylindrical dendrites, the surface area-to-volume ratio (A_s/V) increases as
5 radius decreases, with $A_s/V \sim 1/r$. Microtubule densities are conserved throughout
6 dendritic arbors (Hillman, 1979; Kubota et al., 2011; Katrukha et al., 2021) and the
7 amount of microtubule-based transport should scale with dendrite volume. In contrast,
8 some mechanical interactions that oppose mitochondrial transport should scale with
9 surface area. Non-specific viscous friction between motile mitochondria and the cell
10 membrane could oppose motility in thin neuronal processes (Narayanareddy et al.,
11 2014). Actin localizes to the cell membrane in neurons (Leiss et al., 2009), and actin-
12 based anchoring opposes microtubule-based transport in several contexts (Kapitein et
13 al., 2013; Lu et al., 2020), including myosin V-dependent opposition to mitochondrial
14 movement in cultured neurons (Pathak et al., 2010). Biochemical signals generated at
15 the cell membrane could also contribute to inverse scaling of motility arrest and
16 dendrite radius. For example, high glucose levels trigger mitochondrial arrest in
17 cultured neurons via post-translational modification of the Milton adaptor protein
18 (Pekkurnaz et al., 2014), and quantitative modeling indicates glucose-mediated motility
19 should be sufficient to affect mitochondrial localization patterns (Agrawal and
20 Koslover, 2021). Neurons take up glucose via transporters in the cell membrane
21 (Ferreira et al., 2011; Lundgaard et al., 2015; Ashrafi et al., 2017) and, assuming a
22 constant areal density of these transporters, glucose concentrations in the cytosol will
23 increase as the surface area-to-volume ratio increases, thereby promoting increased
24 mitochondrial arrest in thin distal dendrites. Altogether, the relative weight of
25 mechanical and biochemical signals generated at the cell membrane versus in the
26 cytosol should increase as neuronal processes narrow, and surface area-to-volume
27 ratios may play a general role in regulating intracellular transport in neurons.

1 Finally, our results suggest that neuronal signal processing and housekeeping
2 requirements may act as competing constraints on neuronal architecture. Specifically,
3 HS dendrites are approximately Da Vinci-scaled ($\alpha \sim 2$) which, according to our model,
4 allows for equitable distribution of mitochondria across sister subtrees for a broad
5 range of transport parameters. However, according to cable theory, parent-daughter
6 scaling according to Rall's law ($\alpha = 3/2$) is optimal for dendritic function, as it allows for
7 efficient propagation of electrical signals across branch points in passive dendrites (Rall,
8 1959). Interestingly, unlike many neurons in the *Drosophila* visual system, HS neurons
9 are not purely graded (Schnell et al., 2010), and active dendritic conductances may
10 allow Da Vinci-scaled dendrites to efficiently integrate dendritic inputs while also
11 maintaining steady-state mitochondrial localization patterns. Parent-daughter scaling
12 may be more likely to be constrained by Rall's law in neurons with purely passive
13 dendrites. Strikingly, though, our model strongly suggests that Rall's law is
14 incompatible with equitable distribution of mitochondria in asymmetrically branched
15 dendrites; for equitable mitochondrial distribution, Rall-scaled dendrites must also be
16 symmetrically branched. Altogether, our work suggests that neuronal morphologies are
17 constrained not only by functional requirements and wiring economy, but also by the
18 need to efficiently distribute subcellular constituent elements like mitochondria
19 throughout the cell.

20

21 **METHODS**

22

23 *Drosophila* strains and husbandry

24 The *Drosophila* stocks used in this study are listed in the Key Resources table. All flies
25 were reared at 25°C on standard cornmeal-agar food in 12 h light: dark cycle. Crosses
26 were flipped into fresh vials every 3 days and progeny were imaged 4-7 days after
27 eclosion.

1

2 *In vivo* imaging

3 Female flies were cold anesthetized and positioned in a key-hole cut in a thin metal
4 shim, with the back of the head exposed above the shim and the eyes below the shim.
5 The fly was secured in place with fast-curing glue (Bondic) and the brain was exposed
6 using fine forceps to dissect a hole in the cuticle and remove overlying fat and trachea.
7 The brain was perfused with a sugar saline solution (103 mM NaCl, 3 mM KCl, 5 mM
8 TES, 1 mM NaH₂PO₄, 26 mM NaHCO₂, 4 mM MgCl₂, 1.5 mM CaCl₂, 10 mM trehalose,
9 10 mM glucose, and 7 mM sucrose). Neurons were imaged using an integrated confocal
10 and two-photon microscope (Leica SP8 CSU MP Dual) and a 25x 1.0 NA water
11 immersion objective (Leica). For confocal imaging of motile mitochondria, stationary
12 GFP-tagged mitochondria in the field of view were photobleached prior to time lapse
13 imaging, allowing for resolution of individual motile mitochondria as they moved into
14 the field of view. Confocal z-stacks (voxel size = 108.54 nm x 108.54 nm x 22 microns)
15 were collected every 4.4 seconds for 10-20 minutes after photobleaching. For two-
16 photon imaging of visual stimulus-evoked calcium signals, a fixed 1045nm femtosecond
17 laser beam (Spectra-Physics Insight X3 DUAL) was used to excite RGECO1a, and 256 x
18 256 images (pixel size = 139.09nm x 139.09nm) were collected at a frame rate of 10 Hz.

19

20 Visual stimulus presentation

21 Visual stimuli were generated using PsychoPy (Python) and presented on a white
22 screen (Da-Lite Dual-Vision vinyl, AV Outlet) using a digital light projector (DLP
23 LightCrafter, Texas Instruments). The stimulus screen spanned ~60° of the fly's visual
24 field horizontally and ~60° vertically, and the stimulus was updated at 60 Hz. To avoid
25 detection of light from the stimulus by the microscope, the stimulus was filtered using a
26 472/30 nm bandpass filter (Semrock). Voltage signals from the imaging software were
27 relayed to PsychoPy via a LabJack device, in order to synchronize the stimulus and the

1 imaging frames. The visual stimuli were full contrast square wave gratings ($\lambda = 30^\circ$) that
2 filled the entire stimulus screen. When the stimulus was on, the gratings moved in the
3 preferred direction for HS neurons (front-to-back across one eye) at $30^\circ/s$; when the
4 stimulus was off, the gratings remained stationary.

5

6 MultiColor FlpOut Labeling

7 HS Gal4 driver lines were crossed with MCFO virgins. Offspring were collected 1-2
8 days after eclosion, heat shocked at 38°C for 25 min, and dissected three days later. Fly
9 brains were dissected in cold PBS solution and fixed in 4% formaldehyde for 25 min at
10 room temperature. Brains were subsequently rinsed with PBST (PBS with 0.5% Triton)
11 and blocked in PBST-NGS (PBST with 5% normal goat serum) at room temperature for
12 1.5 hr. Brains were incubated for two nights in primary antibodies diluted in PBST-
13 NGS, then incubated for two nights in secondary antibodies in PBST-NGS, and finally
14 incubated overnight in tertiary antibodies in PBST-NGS. Prior to each antibody
15 incubation, brains were washed 3 times for 10 min each in PBST. All antibody
16 incubations were performed at 4°C . Brains were mounted on their ventral end in
17 VectaShield media (Vector Laboratories) and imaged using confocal microscopy. All
18 antibodies are listed in the Key Resources Table.

19

20 Quantification of mitochondrial morphologies and localization patterns

21 Mitochondrial morphologies and distribution patterns in HS neurons were measured
22 using the publicly available fully aligned fly brain (FAFB) dataset, serial section
23 transmission electron microscopy (ssTEM) images of the entire brain of an adult female
24 fly (Zheng et al., 2018). Previously traced HS skeletons (Michael Reiser, unpublished
25 data), available on the CATMAID (Collaborative Annotation Toolkit for Massive
26 Amounts of Image Data) server (Saalfeld et al., 2009), were used to identify HS neurons
27 within the larger FAFB image volume. To measure the size of individual mitochondria,

1 small image volumes centered around HS dendritic segments were cropped out of the
2 FAFB dataset, and mitochondria within HS dendrites were manually segmented in
3 three dimensions using the TrakEM Fiji plug-in. To measure mitochondrial localization
4 patterns throughout HS neurons, all HS skeletons were resampled using a python-
5 CATMAID interface library, pymaid, such that the graph distance between skeleton
6 nodes was 5 microns. All branch points and end points were preserved during
7 resampling. Two dimensional image slices centered around each node in the resampled
8 skeleton were then cropped out of the FAFB dataset, and HS neurons and the
9 mitochondria within them were manually segmented in each image using TrakEM.
10 Mitochondrial density (total mitochondrial area/total neurite area) was measured as a
11 function of neuronal compartment (axons versus dendrites), distance from the soma,
12 and across sister subtree pairs.

13

14 Quantification of mitochondrial motility patterns

15 Mitochondrial speeds, flux rates, arrest rates, and lengths were measured from max
16 projections of confocal z-stacks of mitoGFP and cytosolic tdTomato expressed in HS
17 neurons. Max projections were aligned using the Turboreg Fiji plugin. Individual motile
18 mitochondria were hand-tracked using the Tracking Fiji plugin, and mitochondrial
19 speeds were measured from mitochondrial tracks using custom-written Python code.
20 Linear mitochondrial flux rates were measured by counting the number of motile
21 mitochondria that moved through a particular cross-section of a dendritic branch in
22 either the anterograde or retrograde direction per unit time. Mitochondrial arrest rates
23 were calculated per dendritic branch, as the fraction of mitochondria that either stopped
24 or paused motility within each branch. Lengths of motile mitochondria were measured
25 using Celltool (Pincus and Therriott, 2007), and the average volume of motile
26 mitochondria was estimated as $V = l \pi r^2$, where l is ~ 2 microns, measured from our *in*

1 *in vivo* images, and r is assumed to be ~ 0.3 microns. Dendrite diameters were measured
2 based on the cytosolic tdTomato signal, using the line scan tool in Fiji.

3

4 Quantification of dendritic branching parameters

5 Dendritic arbors for individual HS neurons were segmented from MCFO images using
6 ilastik (Berg et al., 2019), publicly available interactive machine learning software, and
7 custom-written Python code. Unique pixel classifiers were trained in ilastik for each
8 MCFO z-stack, and binary masks were generated from the resulting probability maps in
9 Python by using thresholding and connected component analysis. Binary masks were
10 then manually cleaned up in Fiji and skeletonized using the Skeletonize (2D/3D) Fiji
11 built in plugin.

12 Skeleton data was translated into a set of nodes (including junction nodes, parent node,
13 and distal tips) with three-dimensional coordinates, and curved edge paths connecting
14 the nodes. Once the initial network structure was extracted, manual clean-up was
15 carried out with a custom Matlab GUI, involving the removal of short spurious
16 branches ('shrubs') from the network. A combination of percentile and asymmetry
17 cutoffs were used to quantitatively remove shrubs that would not contribute to the total
18 length in subsequent analysis. A degree of manual editing was performed for each cell,
19 such that any branch without a discernible thickness was removed from the network
20 object. The widths of network branches were also calculated with the aid of the Matlab
21 GUI. The interface allows the user to add and adjust width measurements across a
22 given edge. For longer edges, one measurement point close to the branching point and
23 the other closer to the end of the edge are chosen. Total subtree length, volume, depth,
24 and bushiness following each branch was calculated using Matlab written code.
25 Volume was measured using the diameter and length of each edge bounded between
26 two nodes. Bushiness is defined as the total subtree length (L) over the subtree depth
27 (D), where D is the path length from the base of the subtree to each distal tip, weighted

1 by the length of each subtree. Lastly, asymmetry for each parameter was measured
2 using our asymmetry metric, $(ST1-ST2)^2/(ST1+ST2)^2$, where ST1 and ST2 are the
3 indicated measurements (radius, length, volume, or bushiness) for sister subtree 1 and
4 2, respectively.

5

6 Synthetic tree construction

7 Synthetic binary trees were constructed in Python 3.7.6 using the NetworkX library
8 (Aric A. Hagberg, 2008). First, the skeleton of a binary tree was created, starting with a
9 single junction consisting of a parent branch and two daughter branches of unit length.
10 Moving downstream along the tree, each daughter branch either terminated as a distal
11 tip (with probability $\frac{1}{3}$), increased in length by an additional unit (probability $\frac{1}{3}$), or
12 branched into two more daughter branches (probability $\frac{1}{3}$). This process was repeated
13 up to a preset maximum path distance (40 unit branch lengths) from the arbor parent
14 node to the distal tips. The resulting random-topology binary tree structures were used
15 as the ensemble of synthetic arbors in Supplemental Figures S5 and S6.

16

17 Computing imposed radii

18 Skeletons with well-defined branch lengths and connectivity were obtained either from
19 MCFO images of *Drosophila* HS neurons, from published data (Cuntz et al., 2013), from
20 HS skeletons traced through a ssTEM dataset (Zheng et al., 2018) (Michael Reiser lab
21 unpublished data or from synthetically constructed trees.

22 For each skeleton, the widths of the branches (r_i) were calculated, starting with $r_0=1$ at
23 the parent trunk (in dimensionless units). At each junction node, the daughter branch
24 radii were defined by a combination of scaling rule (1) for parent and daughter radii: r_1^α
25 $+ r_2^\alpha = r_0^\alpha$ and rule (4) for sister subtree radii $r_1^2/ r_2^2 = \mu_{12}$. Da Vinci arbors have $\alpha = 2$,
26 Rall's Law arbors have $\alpha = 3/2$, and Murray's Law arbors have $\alpha = 3$.

27

1 The daughter branch splitting rules included equal splitting ($\mu_{12} = 1$), splitting in
2 proportion to total subtree length ($\mu_{12} = \sum_{i \in ST1} l_i / \sum_{i \in ST2} l_i$), and splitting in proportion to
3 subtree bushiness (total branch length over depth), defined in Supplemental Methods 1.

4
5 To impose radii with a minimum radius (Liao et al., 2021) on an MCFO HS skeleton
6 (Figure S7), we assumed the primary dendrite radius = 3 μm and the minimum radius
7 $R_m = 0.2 \mu\text{m}$ (Liao et al., 2021). At each branching point, the parent-daughter radii
8 relationship was modified to $r_0^2 + r_m^2 = r_1^2 + r_2^2$ to incorporate the effect of this
9 minimum radius. A 'minimum area' is imposed to take into account the finite area
10 occupied by a microtubule, so the distal dendrites have a greater predicted diameter in
11 this case.

12
13 To estimate the effect of measurement error in branch width (Supplemental Figure S6),
14 we added a Gaussian noise term with standard deviation equal to 30% of the computed
15 radius to all branch widths r_i .

16

17 Mean-field model for mitochondrial distribution

18 Our minimal mean-field model allows the calculation of steady-state mitochondrial
19 densities along each branch of a dendritic arbor with prescribed topology, branch lengths,
20 and branch radii. We assume punctate mitochondria are produced at the soma (parent
21 node of the arbor), and undergo processive bidirectional motion with pause free velocity
22 v_i (where i is the branch index), pausing rate k_i^s , and constant restarting rate k_w . The linear
23 densities of anterograde, retrograde, and stationary mitochondria in each branch are
24 given by ρ_i^+ , ρ_i^- , ρ_i^s , respectively. At steady-state, these densities obey the transport
25 equations:

$$\begin{aligned}\frac{d\rho_i^\pm}{dt} &= \mp v_i \frac{d\rho_i^\pm}{dx} - k_i^s \rho_i^\pm + \frac{k_w}{2} \rho_i^s = 0 \\ \frac{d\rho_i^s}{dt} &= k_i^s (\rho_i^+ + \rho_i^-) - k_w \rho_i^s = 0\end{aligned}$$

1
2 The steady-state solutions have densities that are constant along each individual
3 branch, with the relationship between different branch densities determined by
4 boundary conditions at the junctions. Distal tips are treated as reflecting boundaries
5 (yielding $\rho_i^+ = \rho_i^-$). At the branch junctions, the boundary conditions are set by the
6 conservation of incoming and outgoing mitochondrial flux, together with the
7 assumption that anterograde mitochondria split in proportion to the cross sectional area
8 of the daughter branches:

$$v_i \rho_i^\pm = v_j \rho_j^\pm + v_k \rho_k^\pm, \quad \frac{v_j \rho_j^+}{v_k \rho_k^+} = \frac{r_j^2}{r_k^2}$$

9
10 where i is the parent branch, and j, k the two daughter branches at the junction. Finally,
11 we set the boundary condition at the soma by fixing the motile mitochondria linear
12 density in the parent trunk to a constant, ρ_0 .

13
14 The steady-state linear densities of mitochondria in each branch are then found by
15 solving this set of linear equations. The density of stationary mitochondria is given by:

$$\rho_i^s = \frac{k_i^s}{k_w} (\rho_i^w)$$

16
17 where $\rho_i^w = \rho_i^+ + \rho_i^-$ is the motile mitochondrial density.

18
19 Volume densities and equitability metric

20 Volume density on each branch is computed as $c_i = \rho_i/r_i^2$. The average volume density in
21 a subtree is given by:

$$\langle c \rangle_{ST} = \frac{\sum_{i \in ST} \rho_i \ell_i}{\sum_{i \in ST} r_i^2 \ell_i}$$

1

2 Where the summation is carried out over all branches i of the subtree, with

3 corresponding length ℓ_i and radius r_i .

4

5 We define a single metric ζ for the equitability of mitochondrial distribution throughout

6 the entire tree. Specifically, this metric gives the root-mean-squared asymmetry of

7 mitochondrial densities in sister subtrees, averaged over all junctions in the arbor. We

8 focus on the regime where most mitochondria are in the stationary state, and hence

9 compute the asymmetry metric based specifically on distributions of stationary

10 mitochondria:

$$\zeta = \sqrt{\frac{1}{N_b} \sum_b \left(\frac{\langle c^s \rangle_{b,1} - \langle c^s \rangle_{b,2}}{\langle c^s \rangle_{b,1} + \langle c^s \rangle_{b,2}} \right)^2}.$$

11

12

13 Here, the index b enumerates the junctions, N_b is the total number of junctions in the

14 tree, and subscripts 1 and 2 refer to the two daughter subtrees emanating from a

15 junction.

16

17 Agent-based simulations of mitochondrial transport

18 Stochastic simulations of mitochondrial transport in a network were carried out using

19 custom code written in Fortran 90. An initial tree structure is initialized to contain

20 information on the junction connectivity, as well as lengths and radii of individual

21 branches. The tree skeletons were generated using the NetworkX library as described in

22 (Synthetic tree construction). A typical example is presented in Supplemental Figure

23 S5B. The widths of these synthetic trees were calculated by imposing either Da-Vinci

1 scaling law with $r^2 \sim L/D$, or Rall's law with $L \sim V$ scaling applied. For demonstrating
2 the simulated results showing the difference in equitability of mitochondrial
3 distribution in trees following Da-Vinci vs Rall's scaling, $N = 10$ synthetic trees were
4 used. The edge lengths for the synthetic tree were fixed to $l_0 = 10 \mu m$, consistent with
5 average edge length measurements observed in HS dendrites.

6 While these synthetic trees were shorter in extent compared to real HS neurons due to
7 computational limitations (going a maximum of $D/l_0 = 4$ levels down), the synthetic
8 trees incorporated a range of heterogeneity in branching structure.

9

10 The tree is then populated by a fixed number ($N = 1000$) of punctate of mitochondria,
11 distributed uniformly throughout. The position of each mitochondrion is tracked in
12 terms of the branch on which it is located and its position along the branch. Each
13 mitochondrion is associated with a transport state (anterograde, retrograde, or
14 stationary).

15

16 On every timestep, an anterograde mitochondrion steps distance $v\Delta t$ downstream
17 along the branch, and a retrograde mitochondrion steps distance $v\Delta t$ upstream. The
18 velocity of an individual mitochondrion was assumed to be $v = 0.4 \mu m/s$. One time
19 step corresponds to $\Delta t = 2.5 s$, so that the stepping distance $v\Delta t = 1 \mu m$. A motile
20 mitochondrion switches to a stationary state with probability P_{stop} , whereas a stationary
21 mitochondrion becomes motile with probability P_{start} , according to the corresponding
22 rates:

23

$$P_{stop} = 1 - e^{-k_i^s \Delta t}$$
$$P_{start} = 1 - e^{-k_w \Delta t}$$

24
25

1 When becoming motile, the mitochondrion is equally likely to enter the anterograde or
2 retrograde state. The rate of switching from motile to stationary state was fixed
3 according to measurements of mitochondrial motility in HS dendrites. From the
4 measurements of mitochondrial densities and exchange rate, we obtain a $\sim 3\%$ motile
5 fraction in the primary dendrite. Plugging in to the expression for motile fraction
6 $f_{motile} = 1 + k_s/k_w$, we obtain the dimensionless ratio $k_s/k_w = 35$ for the primary
7 dendrite. The rate k_s for a primary dendrite was set equal to $0.15/s$ and the rate k_w was
8 set equal to $2 \times 10^{-3}/s$, so that on an average a mitochondrion had a probability $P_{stop} =$
9 0.31 of stopping in a primary dendrite at each timestep. The stopping to walking rate
10 was tuned as a function of branch radii according to the rule $k_s \sim 1/r^2$.

11
12 At each junction, an anterograde mitochondrion chooses which daughter branch to
13 enter with probability proportional to the cross-sectional area of the branch: $p_1/p_2 =$
14 r_1^2/r_2^2 . When an anterograde mitochondrion reaches the distal tip, it reverses and
15 becomes retrograde. When a retrograde mitochondrion reaches the soma, it becomes
16 anterograde again. The simulations were run for $N_{steps} = 10^8$ to ensure convergence in
17 observed mitochondrial distributions, as monitored by the edge density autocorrelation
18 function over time.

19 20 Estimating the power law rule for dependence of motility arrest on dendritic branch 21 thickness

22 Figure 7A shows the dependence of the pausing rate k_s on dendritic diameter, from
23 experimental mitochondrial motility data. Each field in the motility data gave the
24 number of transport events observed for a specific dendritic branch in the entire
25 imaging duration, classified as (i) anterograde-persistent (ii) anterograde-stop (iii)
26 anterograde-pause (iv) anterograde-reverse (v) retrograde-persistent (vi) retrograde-

1 stop (vii) retrograde-pause and (viii) retrograde-reverse. The probability of stopping
2 ($P_{stop,i}$) for the branch i was calculated as the fraction of total stopping events (antero-
3 stop + retro-stop + antero-pause + retro-pause) and total transport events recorded.
4 From the probability of stopping, the effective stopping rate $k_{s,i}$ for each branch i was
5 calculated from the formula $P_{stop,i} = 1 - e^{-k_{s,i}\Delta t}$, where Δt is the time spent by
6 mitochondria in that branch, which is proportional to the length of the branch.

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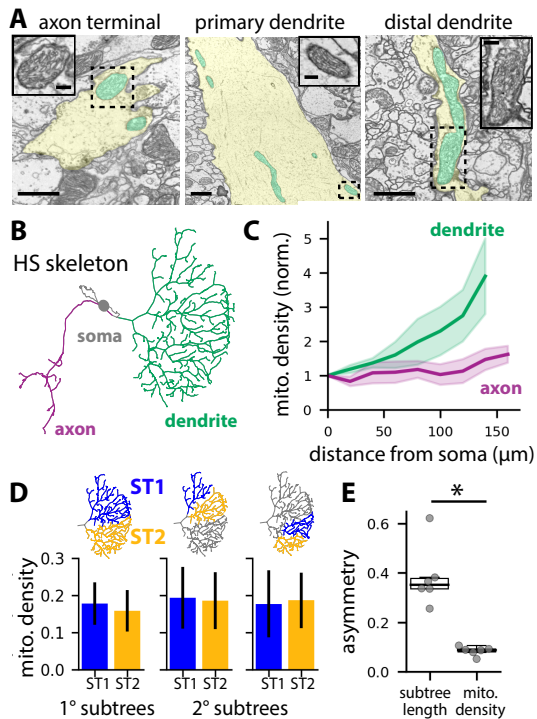


Figure 1: Mitochondrial localization patterns in HS dendrites. A: TEM images of mitochondria (cyan) in different compartments of an HS neuron (yellow); scale bars are 1 μm . Dashed boxes indicate the regions enlarged in the inset images (inset scale bars are 200 nm). B: Skeleton of an HS neuron traced through ssTEM images. C: Average mitochondrial densities plotted versus distance from the soma in the dendrite (green) and axon (magenta). N = 6 neurons; shaded regions indicate the standard error of the mean. For each cell, mitochondrial densities are normalized to the density in the primary dendrite near the soma. D: Mitochondrial densities in sister subtree pairs. Error bars are bootstrap 95% confidence intervals. E: Sister subtree asymmetries in subtree length and mitochondrial density (asymmetry = $((\text{ST1}-\text{ST2})^2/(\text{ST1}+\text{ST2})^2)^{1/2}$, averaged over all sister subtree pairs per cell); $p < 0.01$ (T-test).

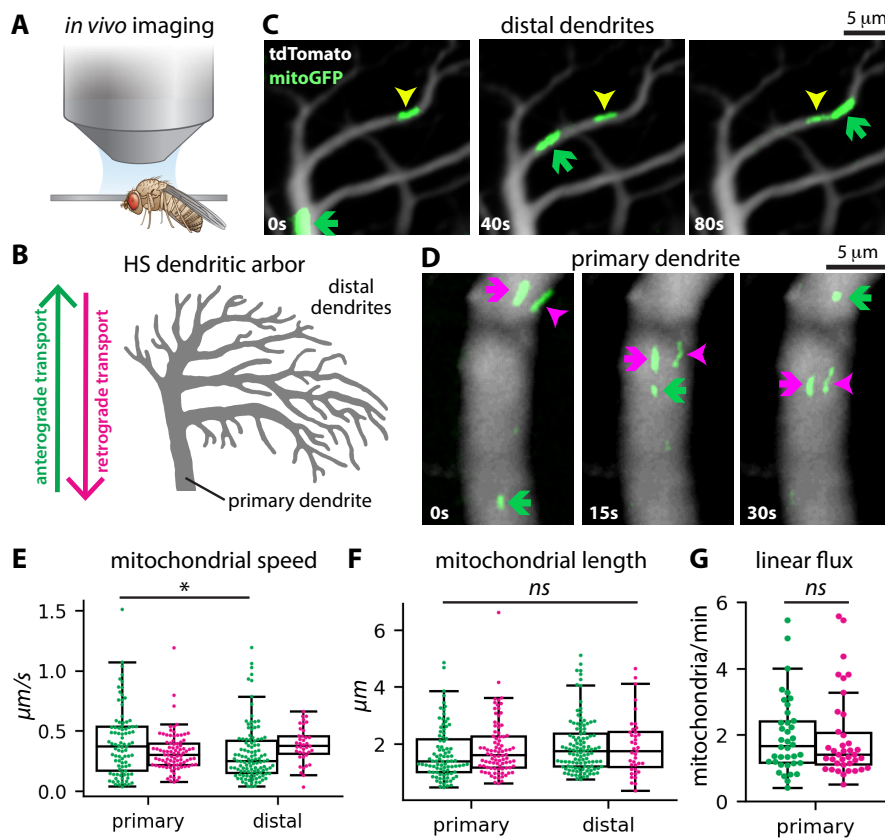


Figure 2: Mitochondrial transport in HS dendrites. A: Experimental setup with confocal microscope positioned above head-fixed fly. B: Schematic depicting an HS dendritic arbor, including primary and distal dendrites and the direction of anterograde versus retrograde transport. C-D: Image time series showing mitochondrial transport in distal (C) and primary (D) dendrites. Arrows indicate mitochondria moving in the anterograde (green) and retrograde (magenta) directions, along with an arrested mitochondrion (yellow). E-F: Average speed (E) and length (F) of mitochondria moving through primary or distal mitochondria in the anterograde (green) or retrograde (magenta) directions. Each dot represents an individual mitochondrion. The asterisk indicates a significant difference (one way ANOVA, post-hoc Tukey's test). G: Mitochondrial linear flux rates (number of mitochondria per minute) in primary dendrites in the anterograde (green) and retrograde (magenta) directions. Dots represent individual primary dendrites. There is no significant difference in anterograde versus retrograde linear flux (T-test).

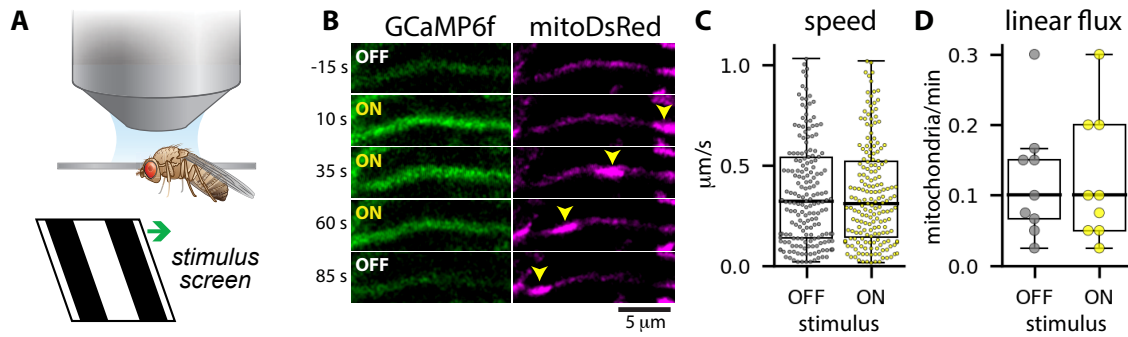


Figure 3: Stimulus-evoked calcium signals do not affect mitochondrial motility in HS dendrites. A: Experimental setup. Neurons are imaged by confocal microscopy while a visual stimulus is presented on a screen in front of the fly. The visual stimulus is square wave gratings moving in the preferred direction (front-to-back across one eye) for HS neurons. B: Calcium responses to the motion stimulus (GCaMP6f, left) and mitochondria (mitoDsRed, right) in an HS dendritic branch. Yellow arrows indicate a moving mitochondrion. C-D: Mitochondrial speeds (C) and linear flux rates (D) when the visual stimulus was OFF (gray) versus ON (yellow). Dots indicate instantaneous speeds measured between successive frames (C) or average linear flux rates for individual flies (D).

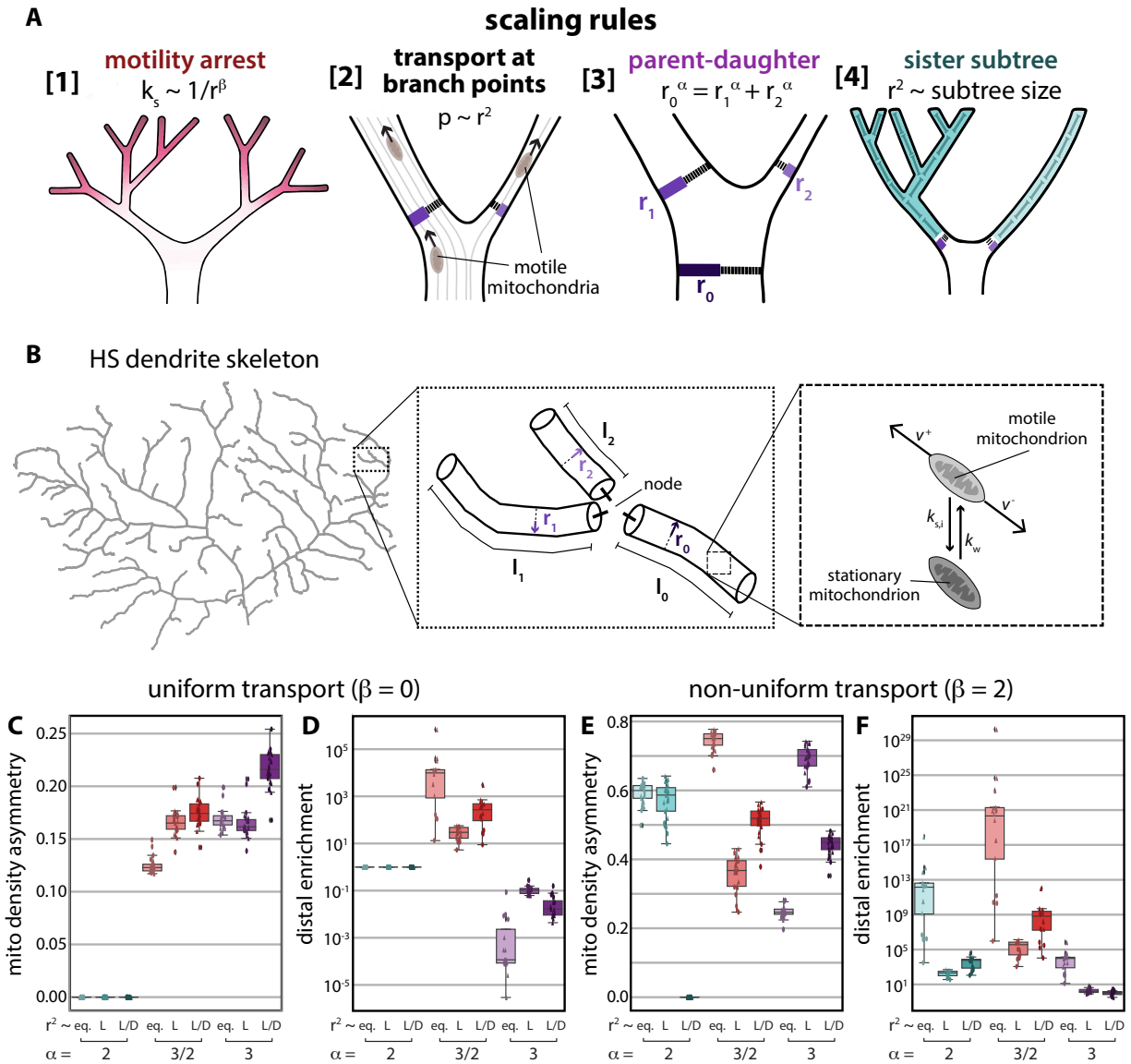


Figure 4: A mean field model for mitochondrial distributions recapitulates experimental measurements when dendrites obey specific scaling rules. A: Scaling rules included in the model. Rule 1: Mitochondrial arrest rates scale with dendrite radius according to $k_s \sim 1/r^\beta$. Rule 2: Mitochondria split at branch points according to the cross-sectional area of each daughter branch. Rule 3: Parent (r_0) and daughter (r_1 and r_2) radii scale according to $r_0^\alpha = r_1^\alpha + r_2^\alpha$. Rule 4: Sister subtrees scale such that thicker trunks support proportionally larger subtrees. Subtree size can be subtree volume, length, or bushiness, where bushiness is length/depth. B: In the model, dendrites are binary trees in which each junction node connects a parent edge to two daughter edges (center panel). Each edge is a cylinder with fixed radius (r) along its entire length (l). The connectivity and length of each edge are extracted from real HS dendrite skeletons (left panel); radii are set according to various forms of parent-daughter and sister-subtree scaling rules (Rules 3 and 4). Within this dendritic structure, mitochondria can move persistently in either the anterograde (v^+) or retrograde (v^-) direction, as well as arresting and initiating motility (right panel). For simplicity, we assume a constant motility initiation rate (k_w) and constant velocities v^+ and v^- ; arrest rates vary according to $k_s \sim 1/r^\beta$ (Rule 1). At branch points, motile mitochondria move into daughter branches according to their cross-sectional areas (Rule 2). C-F: Model results. Dendrite topologies (edge length and connectivity for each dendritic branch) were extracted from HS skeletons traced through ssTEM images (circles, $N = 6$ dendrites, Michael Reiser, unpublished results) or previously published HS dendrite reconstructions (squares, $N = 20$ dendrites, Cuntz et al 2013). Mitochondrial density asymmetry across sister subtrees (C,E) and distal mitochondrial enrichment (D,F) were calculated for $\beta = 0$ (C,D) or $\beta = 2$ (E,F), $\alpha = 2$ (cyan), $3/2$ (red), or 3 (purple), and sister subtree scaling with subtree trunks splitting according to $r_1=r_2$ (eq.), $r^2 \sim L$, or $r^2 \sim L/D$. The mitochondrial density asymmetry is the root-mean squared asymmetry across sister subtrees ST1 and ST2, where asymmetry = $(ST1-ST2)/(ST1+ST2)$. Distal enrichment is mitochondrial density in the distal-most dendritic branches, defined as branches with path distance from the root node $\geq 75\%$ of the maximum path distance, divided by the mitochondrial density in the primary dendrite. Box plots show the median, interquartile range, and $1.5x$ the interquartile range.

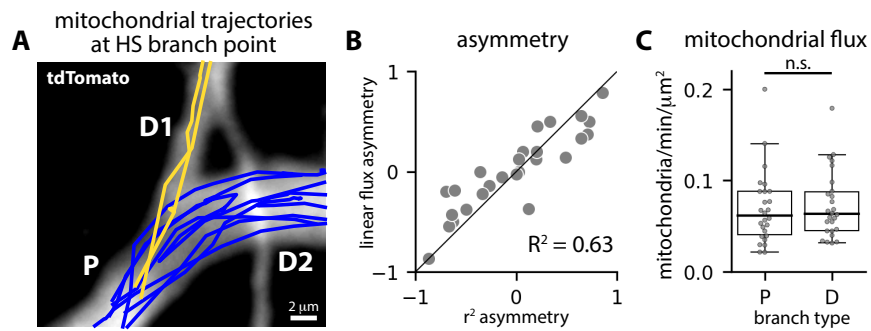


Figure 5: Motile mitochondria split according to dendrite thickness at asymmetric branch points. A: Trajectories of mitochondria moving in the anterograde direction from a parent branch (P) into one of two daughter branches (D1 and D2). Trajectory colors indicate mitochondria that moved into daughter one (D1, yellow) or daughter two (D2, blue). B: Asymmetry in daughter branch radii squared plotted versus asymmetry in mitochondrial linear flux rates; asymmetry = $(D1-D2)/(D1+D2)$, $N = 26$ branch points. C: Mitochondrial flux rates — linear flux rates (mitochondria/minute) divided by dendrite cross-sectional area — in parent (P) and daughter (D) branches; n.s. indicates no significant difference (T test).

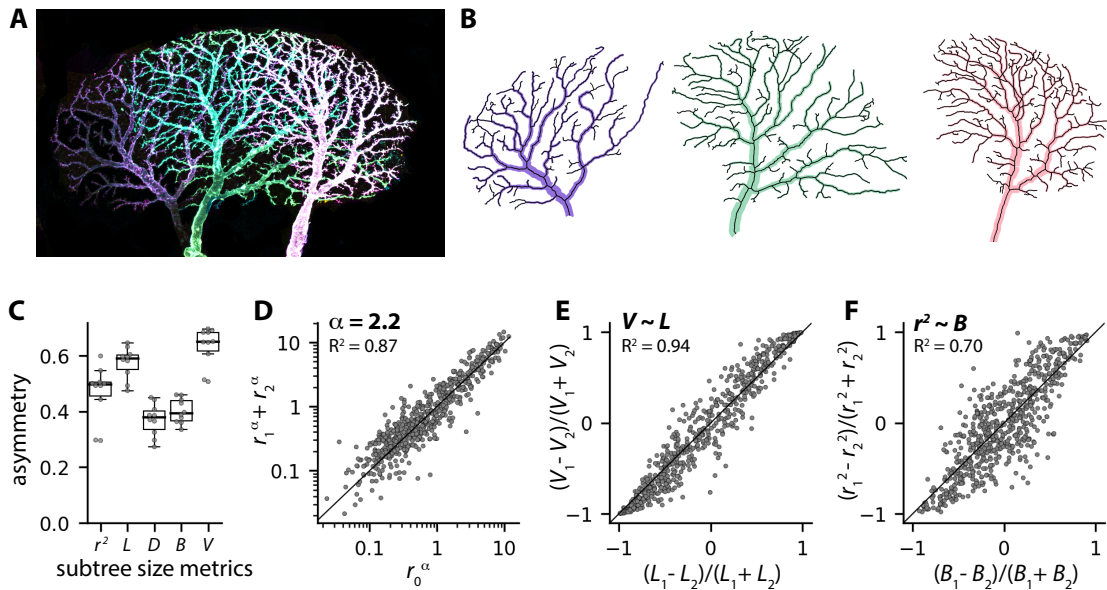


Figure 6: HS dendrites obey dendritic scaling rules. A-B: MCFO image of three HS dendrites (A) and extracted skeletons and radii (B). C: Box plots showing sister subtree asymmetries in trunk thickness (r^2), length (L), depth (D), bushiness (B), and volume (V). Black lines, boxes, and whiskers indicate the median, interquartile range, and 1.5 times the interquartile range, respectively, and each gray dot indicates the average value for a single cell ($N = 10$ HS dendrites). D-F: HS dendrites follow parent-daughter scaling $r_0^\alpha = r_1^\alpha + r_2^\alpha$, with $\alpha = 2.2$ (D), and sister subtree scaling with subtree volume proportional to subtree length (E) and trunk thickness (r^2) proportional to subtree bushiness (F). $N = 649$ branch points from 10 dendrites.

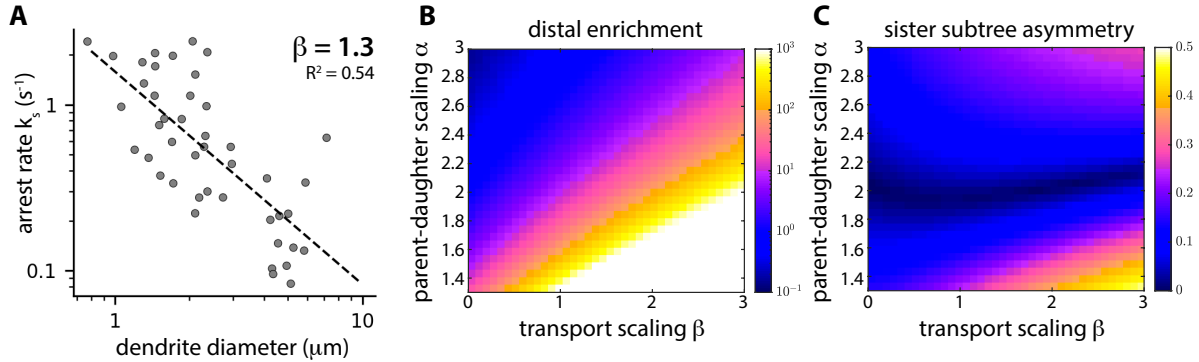


Figure 7: Equitable mitochondrial distributions are robust to changes in transport scaling.

A: Dendrite diameters plotted versus mitochondrial arrest rate k_s . The dashed line indicates the best fit, with $k_s \sim 1/r^{1.3}$. B-C: Model results showing average distal mitochondrial enrichment (B) and mitochondrial density asymmetry across sister subtrees (C) calculated as a function of α (parent-daughter scaling) and β (transport scaling). HS dendrite topologies were extracted from MCFO images ($N = 10$ cells) and dendrite radii were set according to sister subtree scaling rule $r^2 \sim L/D$, as well as power law parent-daughter scaling with the indicated range of values for α .

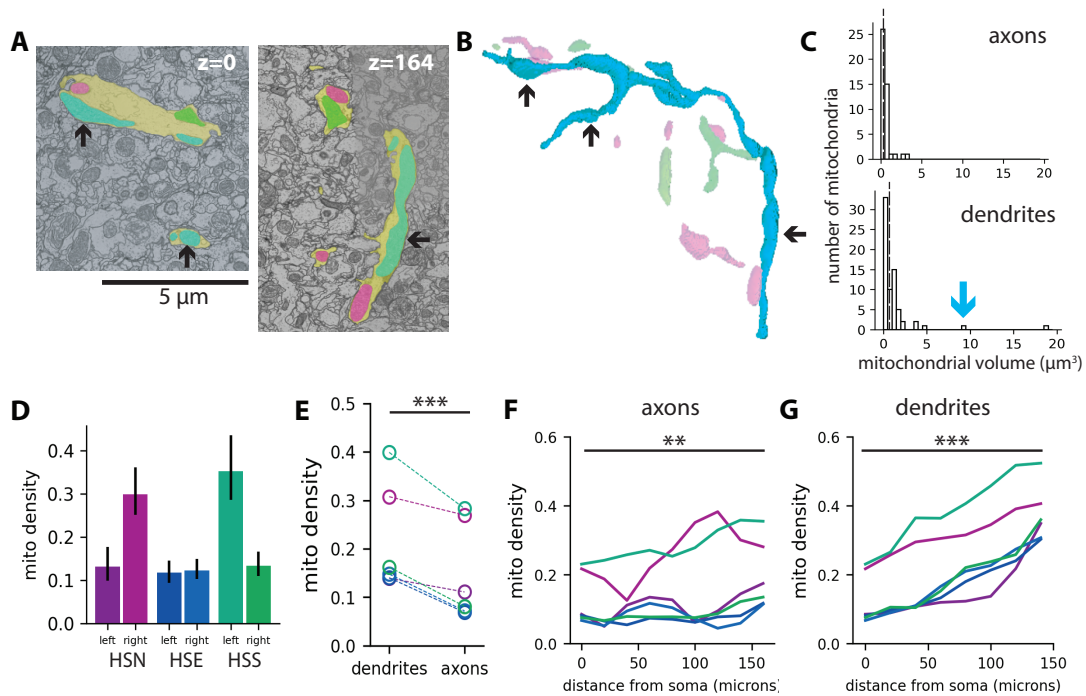


Figure S1: Mitochondrial morphologies and localization patterns in HS neurons.

A: TEM images showing mitochondria (green, cyan, magenta) in an HS dendrite (yellow). B: 3D reconstruction of mitochondria based on ssTEM images. Black arrowheads point to different portions of a single branched mitochondrion. C: Mitochondrial volumes in axons (top, N = 53 mitochondria from 5 cells) and dendrites (bottom, N = 48 mitochondria from 5 cells). The blue arrow indicates the volume of the large mitochondrion shown in B. Dashed lines indicate median mitochondrial volumes ($0.40\mu\text{m}^3$ in axons; $0.54\mu\text{m}^3$ in dendrites). D: Total mitochondrial densities for six HS neurons; error bars are 95% bootstrap confidence intervals. E: Mitochondrial densities in axons versus dendrites. F-G: Mitochondrial density plotted versus distance from the cell soma in axons (F) and dendrites (G). Colors indicate HS subclasses (magenta = HSN; blue = HSE; and green = HSS). Asterisks indicate significant differences (paired t-test;

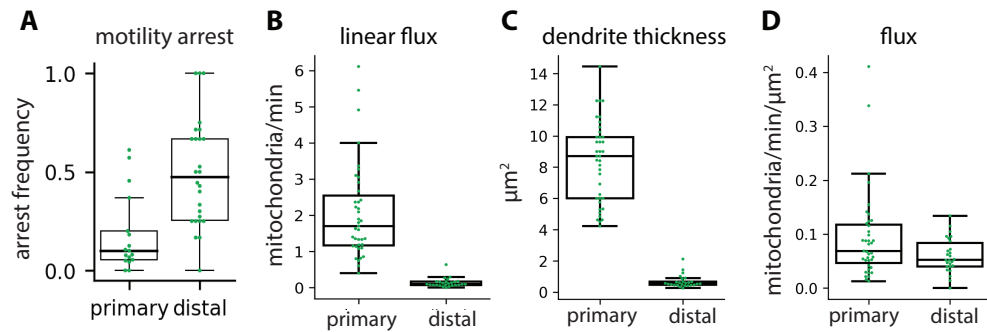


Figure S2: Anterograde mitochondrial motility in primary and distal HS dendrites. Mitochondrial arrest rates (A), linear flux (B), dendrite thickness (C), and flux (D) in primary versus distal HS dendrites. Mitochondrial arrest rate is the fraction of mitochondria that arrested motility per branch. Each dot represents average metric per fly, N = 39 (primary dendrites) or 26 (distal dendrites) flies.

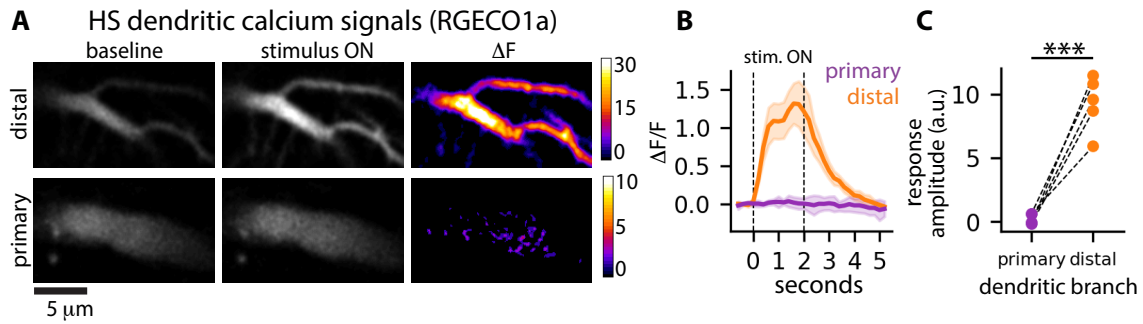


Figure S3: Visual stimulus-evoked calcium responses in HS primary and distal dendrites.

A: Two-photon microscopy images of calcium (RGECO1a) signals in HS distal (top row) and primary (bottom row) dendrites before (baseline, left column) and during (stimulus ON, center column) visual stimulus presentation. The images on the right show the difference between the left and center images ($\Delta F = \text{stimulus ON} - \text{baseline}$). The visual stimulus was a square wave grating moving in the preferred direction for HS neurons. B: RGECO1a responses to visual stimulation in primary (purple) and distal (orange) dendrites, plotted over time. The dashed lines indicate when the stimulus was on. C: Stimulus-evoked RGECO1a response amplitudes in primary and distal dendrites. Individual dots indicate average response amplitudes in one fly; dashed lines connect measurements in the primary and distal dendrites in the same fly. Asterisks indicate a significant difference ($p < 0.001$, paired T test).

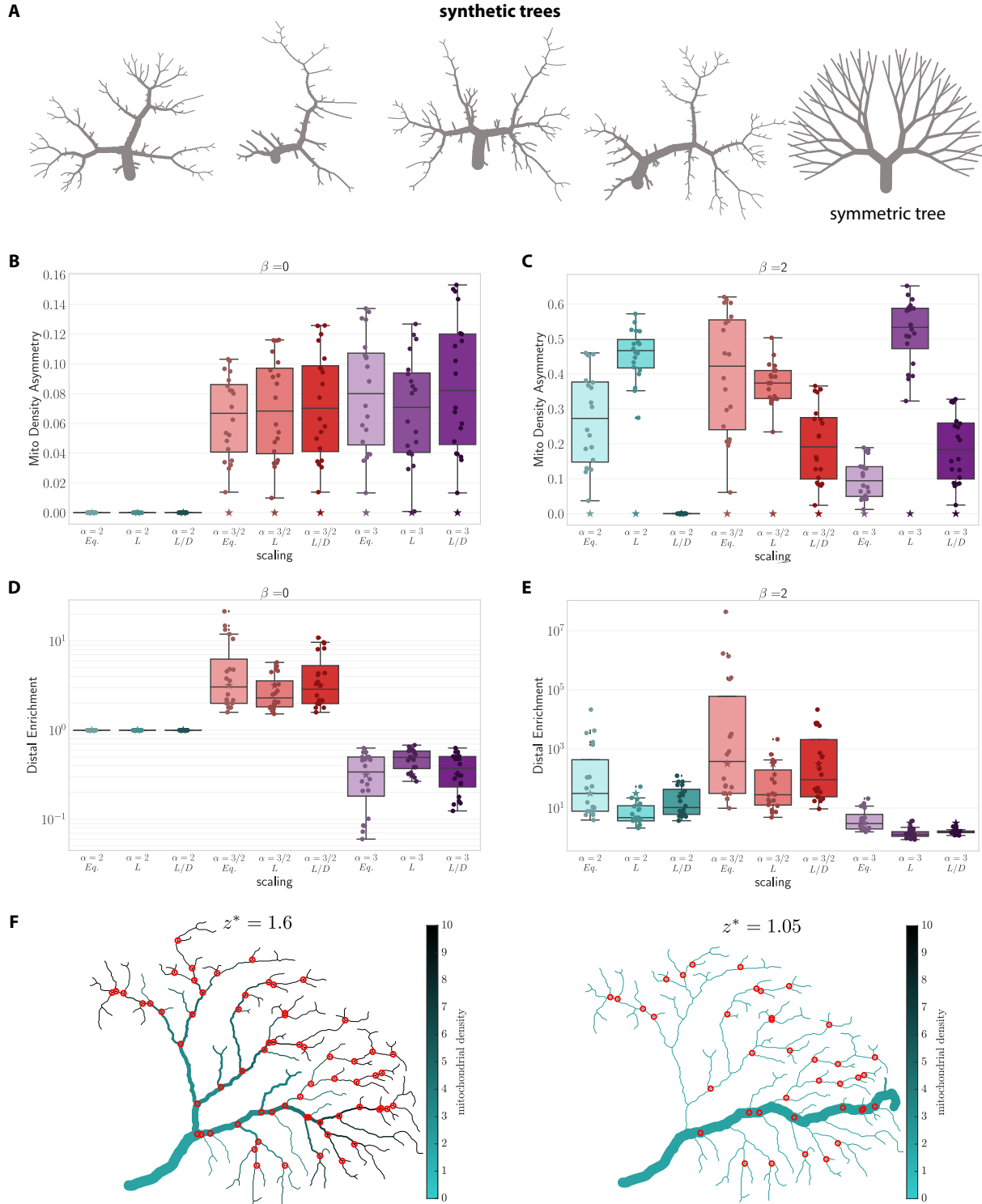


Figure 4: Model mitochondrial distributions in synthetic dendritic trees. A: Representative examples of synthetic trees with random (four left images) or symmetric (right) topologies. B-E: Model results. Mitochondrial density asymmetry across sister subtrees (B,C) and distal mitochondrial enrichment (D,E) were calculated for $\beta = 0$ (B,D) or $\beta = 2$ (C,E), $\alpha = 2$ (cyan), $3/2$ (red), or 3 (purple), and sister subtree scaling with subtree trunks splitting according to $r_1=r_2$ (eq.), $r^2\sim L$, or $r^2\sim L/D$. $N = 19$ synthetic arbors (circles) and 1 symmetric arbor (stars). F: HS dendrites with radii that obey Rall's parent-daughter scaling ($\alpha = 3/2$); $\beta = 0$. The parameter z^* determines the ratio of sister subtree trunk thickness (r_1 and r_2) at each branch point (see Supplemental Methods). Red circles indicate branch points for which there is no solution for equal sister subtree densities. For high values of z^* (left), there are many junctions with no solution. As z^* approaches 1 (right), there are fewer junctions with no solution. However, arbor morphologies become unrealistic, with many thin dendrites branching from a single thick branch.

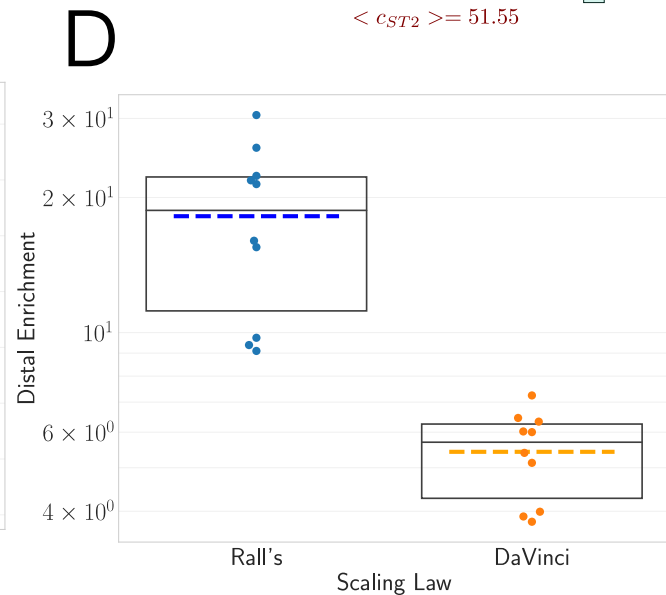
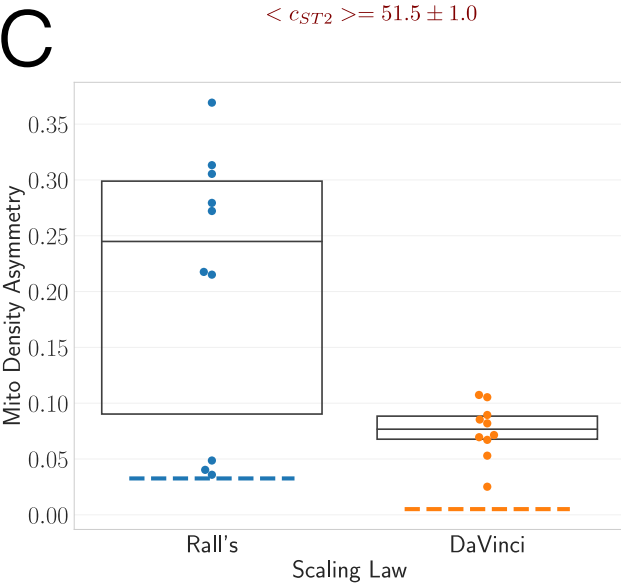
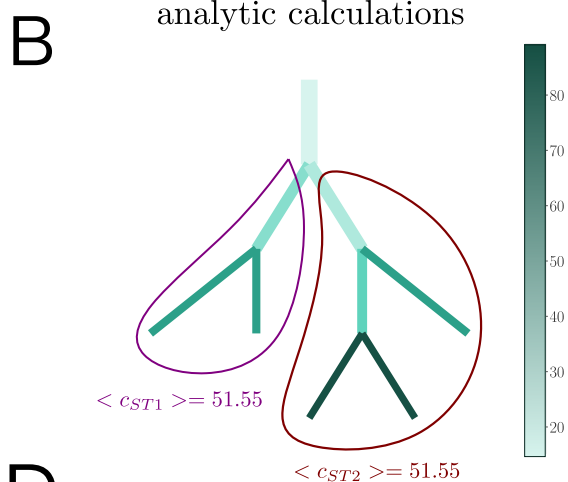
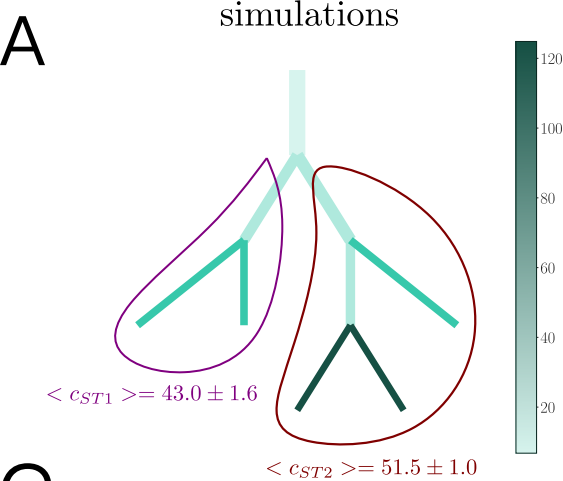


Figure S5: Numerical simulations of mitochondrial distributions. A: Simulated mitochondrial densities in a small synthetic arbor scaled according to Da Vinci's rule ($\alpha = 2$), sister subtree splitting with $L \sim V$, and transport scaling with $\beta = 2$. Total mitochondrial densities in the two largest subtrees ($\langle c_{ST1} \rangle$ and $\langle c_{ST2} \rangle$) are indicated on the plot. B: Analytical calculations of mitochondrial densities for the same arbor as in A. Note that whereas mitochondria are equitably distributed across ST1 and ST2 in the analytical solution, stochastic effects result in asymmetry in the simulated results. C-D: Mitochondrial densities were simulated for 10 synthetic arbors, and mitochondrial density asymmetry (C) and distal enrichment (D) were measured for two parent-daughter scaling rules: Rall's law ($\zeta = 3/2$) and Da Vinci's rule ($\zeta = 2$). Sister subtrees split with $L \sim V$, and transport scaled with $\beta = 2$. Box plots indicate the mean and interquartile range for simulation results; dashed lines indicate average values for numerical solutions for the same arbors.

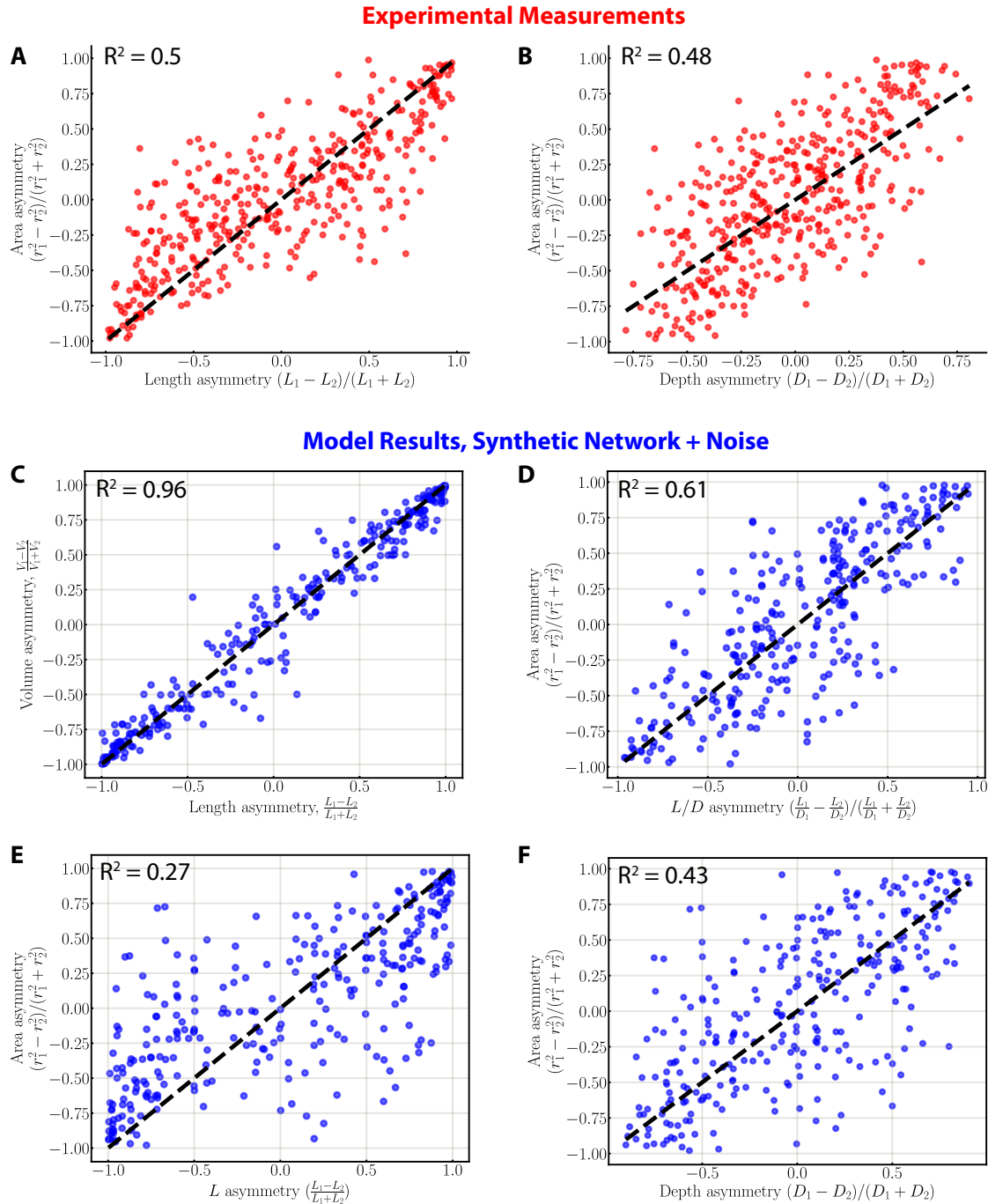


Figure S6: HS dendrites do not obey sister subtree scaling with $r^2 \sim$ subtree length or depth. A-B: Measurements of HS dendrites sister subtree asymmetries (N = 649 branch points from 10 HS dendrites). Trunk thickness (r^2) asymmetry is more weakly correlated with length (A) or depth (B) asymmetry than with bushiness asymmetry (Figure 6F). C-F: Sister subtree correlations in a synthetic tree. Branch radii were set according to parent-daughter scaling with $\alpha = 2$ and sister subtree scaling with $L \sim V$, plus a gaussian noise term (see Methods). The noise has a small effect on measurements of $L \sim V$ scaling (C), and a larger effect on $r^2 \sim L/D$ scaling (D). Subtree length (E) and depth (F) asymmetries show weaker correlations with r^2 asymmetry, as in the experimental results (A-B).

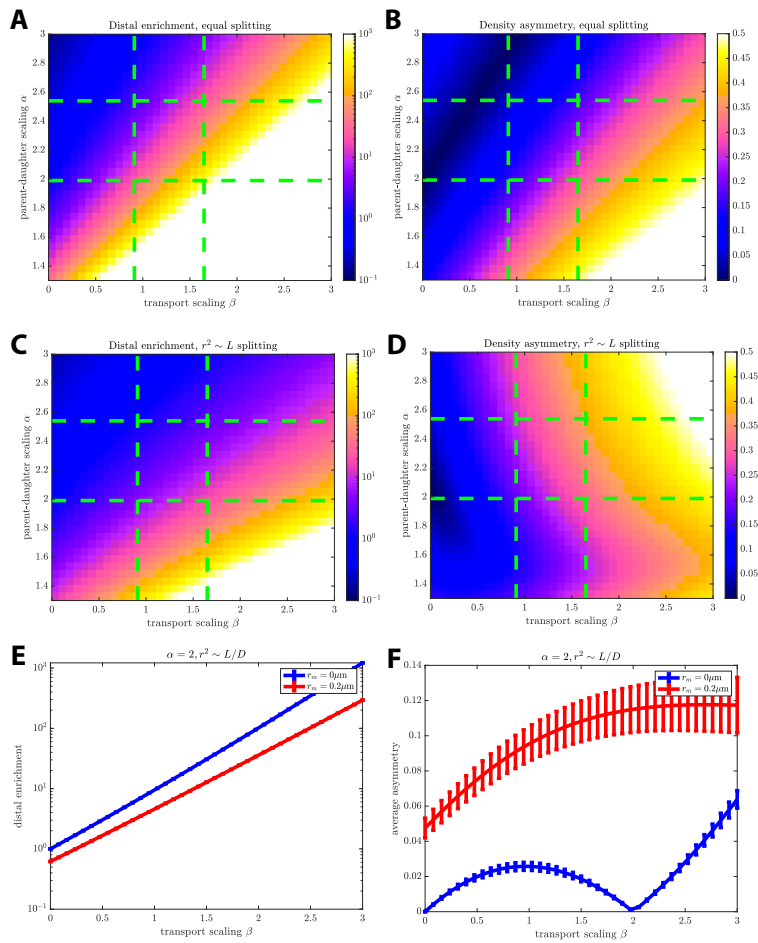


Figure S7: Model mitochondrial distributions in dendrites that obey different scaling rules. A-D: Average mitochondrial distal enrichment (A,C) and density asymmetry (B,D) calculated as a function of α (parent-daughter scaling) and β (transport scaling) for dendrites that obey sister subtree scaling with $r_1=r_2$ (equitable splitting; A-B) or $r^2 \sim L$ (C-D). Green dashed lines indicate 95% confidence intervals for experimental measurements of α and β . E-F: Average distal enrichment (E) and density asymmetry (F) calculated as a function of β , for $r^2 \sim L/D$ and parent-daughter scaling with (red) or without (blue) a minimum radius (r_m): $r_0^2 + r_m^2 = r_1^2 + r_2^2$. Including the minimum radius leads to less distal enrichment (E) and greater density asymmetry (F).