Extended Discussion

Past and current connectomics

The nervous system employs a variety of adaptive mechanisms to maintain certain robust behaviors from birth to adulthood, acquire new behaviors at the appropriate age, and learn and adapt to changing environments and experiences. In the *Drosophila* nerve cord, synaptic density of mechanosensory neurons scales to body size from first to third instar larvae [1]. In the spinal cord of the zebrafish larva, descending neurons lay down tracks chronologically, coinciding with the maturation of swimming behaviours [2]. In the mouse visual circuit, postnatal synaptic remodelling is shaped by intrinsic activity as well as visual stimuli [3]. These and other studies raise the possibility that anatomical changes occur from the level of individual synapses to the global organization of brain networks [4]. An assortment of genetic and cellular factors have been found that affect the morphological and functional maturation of individual synapses [5, 6]. Synaptic changes underlie system-level modifications. However, developmental principles for the collective synaptic changes that shape the adult brain are unknown.

Interrogating whole-brain maturation at synapse resolution is difficult. High-resolution electron microscopy (EM) reconstruction is needed to capture structural changes at individual synapses over large volumes [7]. To uncover brain-wide principles of maturation, these methods must be applied to the entire brain, and to brains at different developmental time points. Moreover, multiple animals need to be analyzed to assess structural and behavioural heterogeneity. With recent advances in automation and throughput of EM, this has become uniquely possible using the nematode *C. elegans*, the first animal that allowed the assembly of a complete connectome by serial section EM reconstruction [8, 9].

Serial-section EM has now been used to reconstruct neural circuits with synapse resolution across species [10–16]. But in larger animals, low throughput makes it difficult to acquire whole brain samples and comprehensively assess plasticity. EM has been applied to assess wiring differences between individuals, for example, comparing the pharyngeal circuits of two nematode species [17], comparing the *C. elegans* male and hermaphrodite connectomes [18], the effect of genotype or age on the *Drosophila* larval somatosensory [19] and mechanosensory [1] circuits, as well as the effect of developmental age on wiring in the mouse cerebellum [20]. These studies examined partial circuits or few samples. The original *C. elegans* connectome was compiled from the EM reconstruction of partially overlapping regions of four adults and an L4 larva. A revisit of this connectome expanded the wiring diagram by re-annotation of original EM micrographs and filled remaining gaps by interpolation [18], making it more difficult to assess differences between animals.

Developmental rules for wiring maturation

Principles of brain maturation should be learned from synaptic changes of an entire brain across maturation. We reconstructed and compared eight isogenic *C. elegans* beginning

with the earliest larva stage and ending with the adult. Previous lineage studies revealed that the vast majority of post-embryonic neurogenesis and differentiation occurs during the L1 and L2 stages [21]. We reconstructed three L1, two L2, and one L3 animals at six different developmental time points to afford the temporal resolution. In contrast to discrete stages of larval development, we found continuous connectomic changes during the period of most rapid growth.

We reconstructed two adults to make direct comparisons between animals of the same age and with the original published connectome. While it took more than a decade to assemble the first *C. elegans* connectome [8], the advent of automation in sample sectioning, image acquisition, and data processing sped up the process, allowing our complete brain reconstructions of multiple animals in less time.

We discovered that synaptic changes across development are organized by several principles that profoundly shape how the brain's network changes. Rules that we uncovered are illustrated in **Fig. 4**. At one level, we observed patterns of synaptic remodeling that differentially alter the number and strength of connections, applied to every neuron. At a second level, we observed patterns of synaptic remodeling that differ between cell types (i.e., sensory neurons, modulatory neurons, interneurons, and motor neurons). At the third level, we observed network-level changes that alter the directionality of information flow and the segregation of information processing throughout the brain. We propose that these three levels of synaptic remodeling might contribute to the behavioral maturation of the growing animal.

Wiring plasticity among isogenic individuals

We found considerable variability in chemical synaptic connectivity among isogenic animals. About 43% of all cell-cell connections, which account for 16% of total number of chemical synapses, are not conserved between animals. This degree of variability contrasts with the view that the *C. elegans* connectome is hardwired. An assumption that individual neurons have identical connectivities probably stemmed from the finding that the same *C. elegans* neuron is identifiable in each animal by virtue of its mostly stereotyped lineage and morphology [8, 21, 22]. The original connectome, which consisted of compiled annotations from two complete nerve rings - JSH (L4 larva) and N2U (adult), and one partial nerve ring N2T (adult) - did not address variability [8].

Stereotypy of cell morphology implies that many properties of the *C. elegans* brain are genetically determined. Our finding supports this notion. In contrast to considerable wiring variability, the morphology of each neurite, and extent of their physical contacts were nearly invariant after birth. Wiring plasticity is developed on a stable scaffold set at birth. Even with such strong genetic regulation, an isogenic population diversifies its wiring.

Synaptic variability between animals is not uniform among cell types. For example, modulatory neurons have considerable more variability in their output connections whereas motor neurons have little variability in their outputs. This contrast suggests that variability is in some way regulated between cell types, and may therefore be

genetically determined and functionally important.

Behavioural variability can confer a fitness advantage to an animal population [23]. Synaptic variability may be a source of such behavioural variability, e.g., in the *Drosophila* visual system, variability among neurite morphologies has been linked to behavioural individuality [24]. Despite of being isogenic, *C. elegans* exhibits individual variability in its behaviors [25], which could be related to the synaptic variability we describe. One mechanism that might give rise to synaptic variability may be differences in gene expression [26]. Stochastic variability of expression levels has been observed even in the housekeeping genes in *C. elegans* embryos [27].

Neuronal activity can also be a driving force for synaptic remodeling. Individuals from an isogenic population reared in similar conditions will still experience differences in their local environments throughout life, a source of differences in neuronal activity that may translate into wiring variability in the fruitfly [28]. In *C. elegans*, L1 and adult animals have been shown to have differences in their olfactory behaviors [29]. Adult olfactory behaviors can also be modified by the early olfactory experiences of the L1 larva [30].

Comparative connectomics across species

Circuit and connectome comparisons across species have revealed instances of wiring plasticity caused by development or genetics. In the *Drosophila* larva, the mechanosensory circuit at two different larval stages is maintained by scaled synapse growth [1]. In the mouse, activity-driven connectivity changes have been uncovered in the cerebellum [20]. Differences in the pharyngeal circuits of different nematode species point to genetic specification of wiring patterns [17]. Comparison of the *C. elegans* male and hermaphrodite reveals sexual dimorphism in their nervous systems with different numbers of neurons and shared and divergent connections [18].

Variability in the placement of individual synapses in largely stereotyped nervous systems has been observed in small invertebrates. EM reconstruction of isogenic *Daphnia maga* revealed both stereotyped and variable synapses in their optic lobes [31]. EM reconstruction of the visual systems of two closely related *Platyneris* larvae also revealed both stereotyped and variable synapses [14]. When the original connectome of *C. elegans* was examined for inter-animal variability by comparing the JSH L4 and N2U adult nerve rings [32], they noted that the numbers of synapses between connected neurons were more variable between animals than between the left and right sides of the same animal, and connections between neurons with fewer than three synapses could also be variable. With eight new datasets, we have been able to quantify the patterns of variable and stereotyped synapses and synaptic connections across cell types and across development. Variable connections on average contained fewer synapses than non-variable connections. Interestingly, partial reconstructions of the mammalian thalamus also suggests that weaker connections may correspond to incidental wiring [13].

Intrinsic variability in the number of synapses between neuron pairs may partly explain observations by light microscopy studies. In the motor circuit, for example, the numbers

of fluorescent puncta corresponding to pre- and postsynaptic markers differed across life stage and between animals [33]. Some of this variability may be due to animal-to-animal variability in synapse formation that we and others observed using serial section EM.

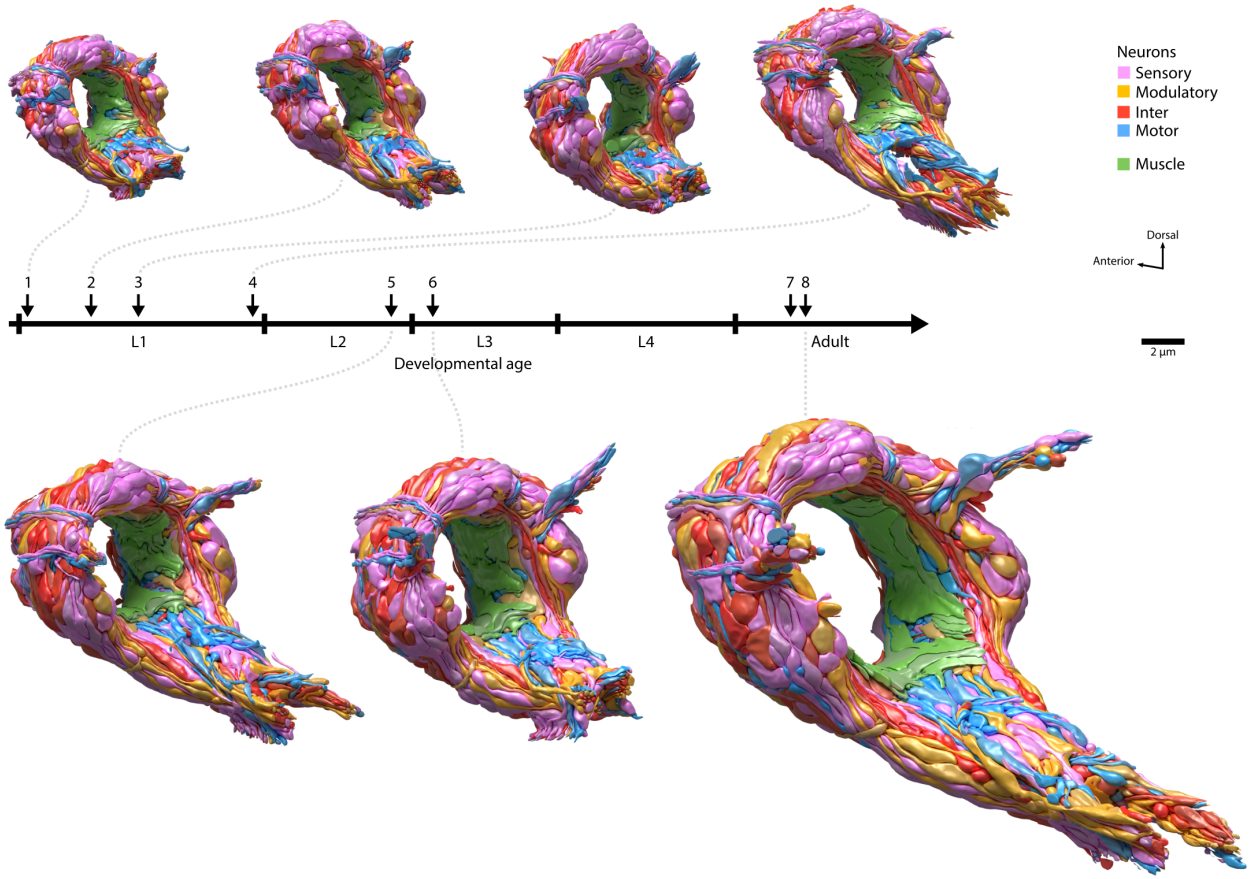
Future work

In larger animals that mature more slowly, maturation involves extensive changes in the nervous system. Apoptosis, neurite degeneration, and synapse pruning remove unwanted circuitry [34]. Neurogenesis, neurite growth, and synapse formation create new circuitry [35]. For the short-lived *C. elegans*, maturation must be fast and efficient. In its small nervous system, each cell is unique, thus each may be characterized by an intrinsic propensity for synaptic remodeling. These changes occur in the context of its stable morphology and fixed amount of physical contact between neighbouring neurites. With these constraints, the nematode has evolved a set of principles for synaptic maturation to build its adult brain.

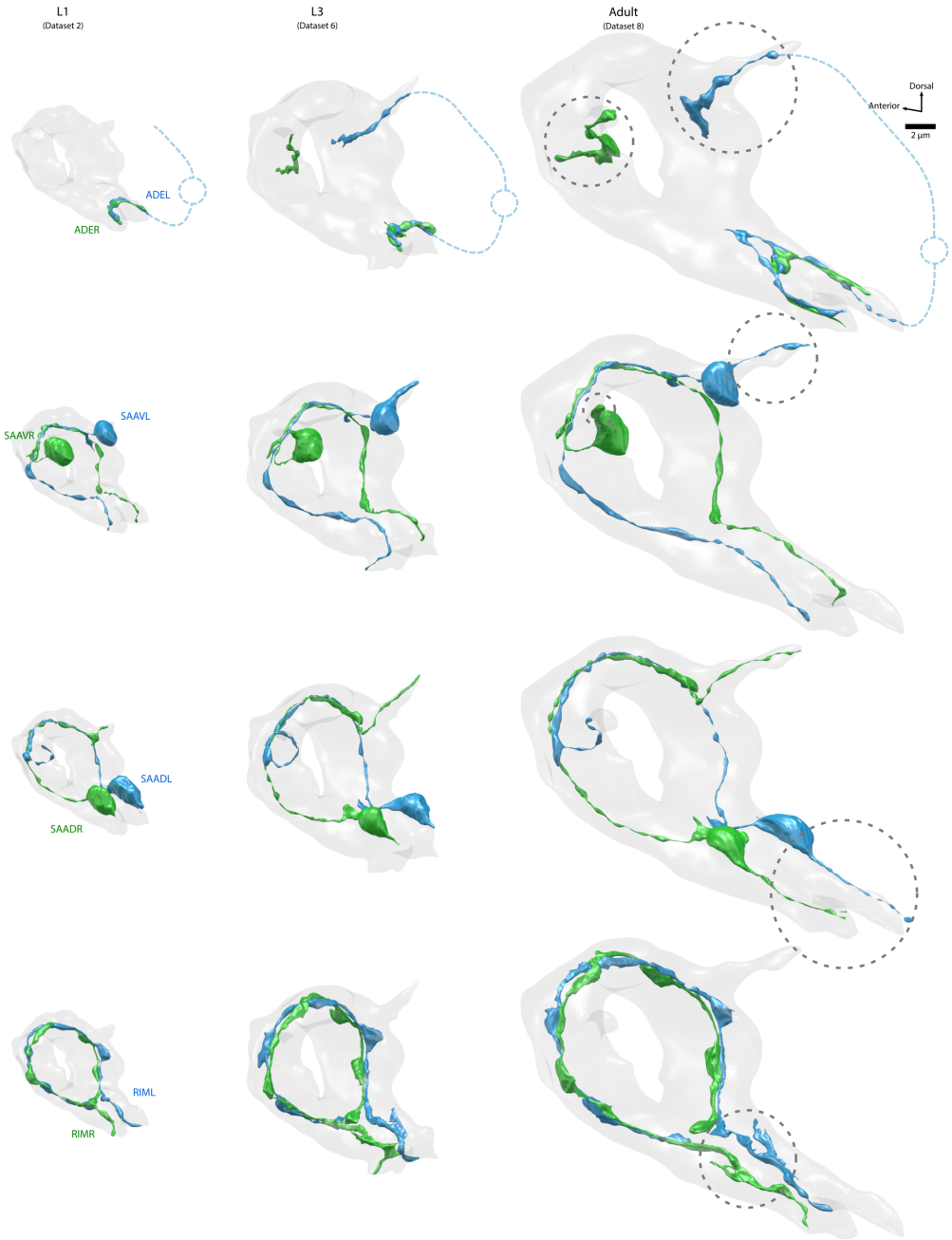
Future work will extend the study of the development of the *C. elegans* connectome. First, we have not included gap junctions, critical components of the nervous system, in our analysis. Our online connectome database (*www.nemanode.org*) includes electrical synapses where gap junctions were most visible. But improvements in sample preparation and analysis are needed to reach the same level of confidence and throughput as we reached for chemical synaptic networks throughout development. Second, we have analyzed only one connectome at most time points. This allowed us to compare stable, variable, and developmentally dynamic synaptic networks across development but not to assess animal-to-animal variability at each age. Increased throughput and the analysis of many animals at each age will allow analysis of the statistical properties of synaptic connectivity.

In the *C. elegans* brain, synaptic remodeling leads to changes from the cell to network level, with likely functional consequences on behaviour. Most investigations of flexibility in neural circuits and behaviours focus on functional modulations of connectomes that are assumed to be anatomically static [36, 37]. Our comparison of connectomes argues that the maturation and variability of brain and behaviour are not separated from wiring changes. Moreover, comparative connectomics is needed to understand the origin of similarities and differences in structure and behaviour, within and across species. High-throughput and high-resolution electron microscopy are necessary to establish the foundation for understanding how genes, experience, and evolution create the behaving adult.

Supplemental Figure S1. Volumetric models for seven *C. elegans* brains at respective developmental stages. All models include the complete neuropil and muscle fibers of the brain, consisting of the nerve ring and ventral ganglion. Glia processes are not included. Cells are colored by type.



Supplemental Figure S2. Three neuron classes grow new neurites after birth. Volumetric models of ADE, SAAV, SAAD, and RIM in L1 (dataset 2), L3 (dataset 6), and adult (dataset 8). These neurons pairs grow new major branches, highlighted by dotted gray circles. ADE's new branches sprout outside the brain; regions not volumetrically reconstructed are denoted by a dotted blue line.



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