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Brain wiring: Love the one you're with

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Recent electron microscopy-based connectomes of the *Caenorhabditis elegans* nervous system provide a new opportunity to test classic models for the development of brain wiring. Statistical analyses now reveal that neuronal adjacencies (the contactome) can partly predict synaptic connectivity (the connectome).

How does genetically encoded development ensure the large number and specificity of synaptic connections observed in brains prior to learning? Approaches and possible answers to this question have a long history of polarized viewpoints in a debate that continues to this day. Roger Sperry, the founding father of the school of thought positing that molecular 'tags' determine connectivity between neurons, was more concerned with countering the opposing school of thought at the time that favored learning and plasticity than with the intricacies of developmental mechanisms[1]. In proposing the chemoaffinity theory, Sperry effectively brought brain wiring into the realm of developmental biology. His views on the rigid determination of connectivity, however, had to be augmented by models that incorporated more flexible developmental mechanisms to account for plastic changes during development as well as variability in the outcome. For example, the recent comparative analysis of several developmental connectomes of the nematode *Caenorhabditis elegans* found that around 43% of all cell–cell connections (16% of all chemical synapses) differed between isogenic individuals[2]. Together with other recent connectomes, these electron microscopy-based descriptions of a nervous system with only 302 neurons also provide a unique opportunity to reassess classic ideas about the developmental mechanisms of synaptic specificity.

In a study published in a recent issue of *Current Biology*[3], Cook *et al.* embarked on such a quantitative analysis of ten recently generated *C. elegans* connectomes[2,4,5], highlighting modern fault lines between current schools of thought. The authors contrast the Sperry model of synaptic specification by 'key-and-lock' mechanisms with a model in which promiscuous synapse formation is allowed to the degree to which developmental adjacency of neurons is sufficient to specify partnerships[6]. The latter idea is often referred to as 'Peters' rule' based on a descriptive study of neuronal overlap as a predictor for connectivity by Peters and Feldman in 1976[7,8]. This dichotomy of two alternative hypotheses does not reflect the quantitative nature of developmental contributors to synaptic specificity that include more than just these two factors[9]. However, the dichotomy is well represented (and thus testable) by connectome data, which provide both quantitative data on synaptic contacts, but also adjacencies between neurons, the 'contactome'. Cook *et al.* reasoned that an analysis of the extent to which the contactome predicts the connectome can provide evidence for the roles of adjacencies versus key-and-lock-type mechanisms. And indeed, the key finding of the work is exactly that: a remarkable, statistical predictability of synaptic connectivity based on neuronal adjacencies. While this finding can be stated in simple terms, the actual analyses and their implications contribute to a more nuanced and integrative view of brain wiring.

The first EM-based connectome of *C. elegans* was published in 1985 by John White who already highlighted restricted 'neighborhoods' in which "*neurones make synaptic contacts with many of these potential partners however. There is some evidence that neurones will still behave in this way regardless of what neighborhood they happen to be in.*"[10] Yet, Peters' rule has subsequently been critically assessed as a general principle[11]. For example, a 2015 analysis of an EM-reconstructed brain region in *Drosophila* found that Peters' rule performed poorly based on a significant decorrelation of contact area and synapses in an adult connectome[12]. However, to test the extent to which adjacency predicts synapse formation, one arguably needs to analyze the contact area during the developmental period of synapse formation, not the adult contact area. The recently published *C. elegans* connectomes span postembryonic development from the first larval stage to adult worms, providing an opportunity for longitudinal assessment (Figure 1A). However, the core circuitry of the *C. elegans* nerve ring already develops during embryonic development[13], which is not covered by these connectomes and thus excluded from the new analyses. During the subsequent larval stages, 80% of newly added synapses are estimated to strengthen existing connections, while the remaining 20% of added synapses establish a set of connections that is highly variable between different animals[13]. Furthermore, synapses in the somatic nerve ring are predominantly of the '*en passant*' type which are progressively added along neurites to accommodate growth during larval stages. As these observations raise questions about the generality of findings in the nerve ring, Cook *et al.* developed a machine-learning approach that can be applied to other neural circuits. Application of this model to the pharyngeal circuit confirmed overall adjacency as a statistical predictor of connectivity. Tests of other circuits in *C. elegans* and beyond will be important to probe limits of generality.

The study by Cook *et al.* makes use of the multiple connectomes by pooling 'aggregate' datasets that allow for statistical analyses. However, outliers can be informative, especially cases where two neurons are hardly connected despite high adjacency or where two neurons connect strongly despite low adjacency. Such cases highlight the possible range in which the quantitative contribution of adjacency must be complemented by other mechanisms to achieve specificity in the

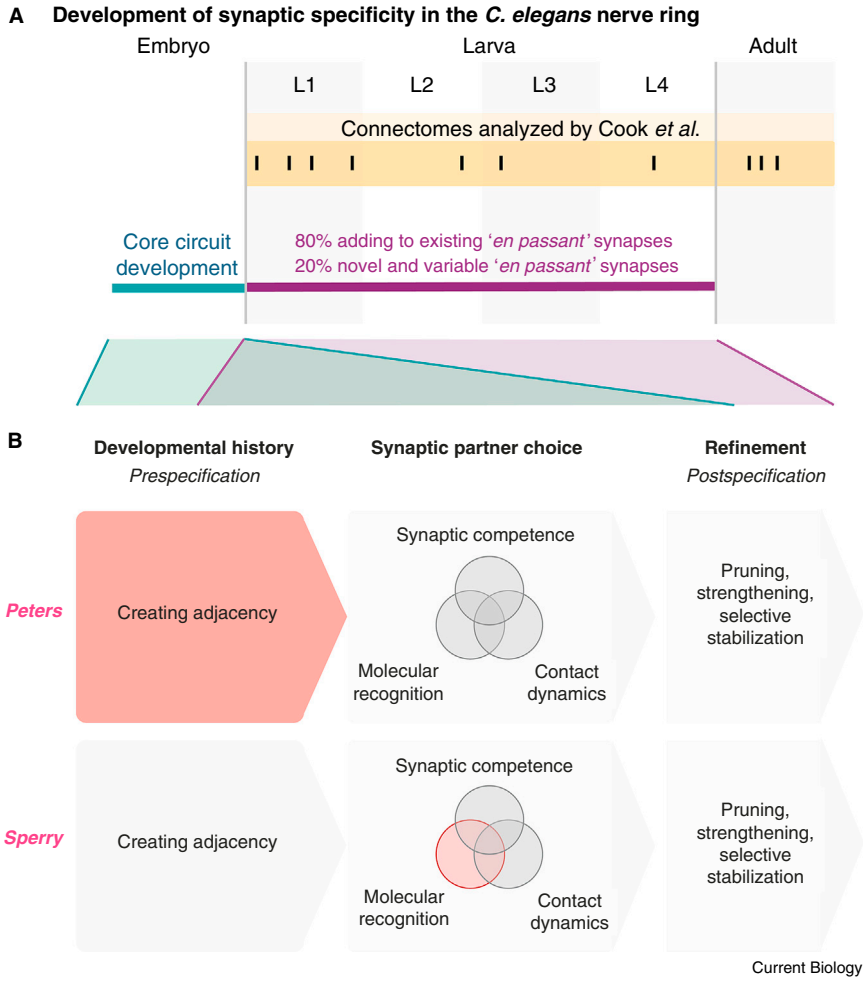

Figure 1. *C. elegans* connectomes and classic concepts for the development of brain wiring.
(A) Ten recent developmental connectomes cover larval and adult stages, but not the development of core circuitry during embryogenesis. (B) A series of developmental steps prior to, during and after synaptic partner choice contribute to synaptic specificity in the outcome — both during embryogenesis and larval development. Peters' rule represents the sum of developmental processes that create adjacency prior to synapse formation. Synaptic partner choice is a composite of any two partners' synaptic competence, contact dynamics and molecular recognition.

outcome. In one example, the authors describe an uncoupling of adjacency and connectivity strength in the pharyngeal circuit where several strong connections are found despite low adjacency, and one neuron (I6) that only synapses with less than half of its available neighbors. It remains unclear whether these deviations from Peters' rule can be explained by key-and-lock-type molecular interactions. In the nerve ring, an enrichment of matching cell surface molecules at larval stage 4 in connected compared to unconnected direct neighbors has been described for specific neurons[14], but again, the quantitative contribution of this mechanism for specificity in the outcome remains unknown. Finally, some factors are left entirely out of the model, including directionality of synapses, which is another parameter that may require some molecular specification mechanisms. Pre- or postsynaptic specializations require specific molecular machinery that may only be available in certain neurite regions; such compartmentalization has been described for single neurites in the *C. elegans* nerve ring[15]. In addition, even the angle of axo-dendritic interactions, i.e., the type of adjacency, can affect synapse formation[16]. Hence, it is not necessarily obvious from the contactome alone what subcellular regions are synaptically competent. These and other factors that differentially contribute to specificity are likely to decrease the effect of adjacency on connectivity in pooled data. In light of this, the observed predictive power of the contactome for the connectome suggests that simple adjacency is clearly a relevant quantitative contributor in *C. elegans*, while the precise amount of that contribution likely differs substantially for individual synapses.

So, is the Cook *et al.* study a decisive test between two competing hypotheses, and does Peters' rule beat Sperry's chemoaffinity? The study convincingly reveals adjacency as a significant quantitative contributor to specificity in the outcome, but leaves ample space for other mechanisms along the way. The key to bridging the two hypotheses may lie in appreciating how they can work together. In fact, less simplistic views of Sperry and Peters are better understood as limiting cases of the same process, especially if the hypotheses are viewed as maximally selective synapse formation independent of adjacency versus promiscuous synapse formation determined by adjacency. The limiting case of 'total promiscuity', i.e. the ability of any neuron to form synapses with any other neuron, is unlikely given known molecular interactions that can bias synaptic partner interactions[17]. At the other end of the spectrum, precise molecular key-and-lock mechanisms for all synapses are equally unlikely, given the known ability, and often developmental necessity, to form synapses with variable partners[6]. Synaptic specificity is the outcome of a growth process that must be based on mechanisms that settle somewhere between these two limiting cases.

A more fundamental issue with the dichotomy is that even a combination of both still misses factors that quantitatively contribute to specificity in the outcome[9]. In particular, adjacency is not only a static vicinity as measured in EM connectomes, but a dynamic variable that can depend on the kinetics of neuronal interactions. In flies, neurons have been shown to increase both synapse numbers and the pool of possible partners by slowing down these interaction kinetics, thereby providing more time and stability for interactions that would be prevented by faster dynamics[18,19]. Furthermore, both partners must be synaptically competent at the time of interaction and molecular recognition, implicating a number of molecular components pre- and postsynaptically to allow synapse formation[17]. All three

factors, molecular recognition, interaction dynamics and synaptic competency, contribute at the moment of synaptic partner choice (Figure 1B)[9,20]. By contrast, Peters' rule can be understood as the sum of all of the preceding development that brought the partners into vicinity prior to synapse formation[6,8]. And finally, initial partner choices ore often modified by pruning or selective stabilization (Figure 1B)[20]. Both Sperry and Peters highlighted important contributors to the development of synaptic specificity. But to understand the outcome, the mechanisms that came to be associated with their names might be better thought of as collaborators that each quantitatively contribute to the beauty of brain development[9], which neither alone could achieve.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Sleep: Hemispheres fight for dominance

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A new study shows that bearded dragons have a peculiar way to coordinate sleep state changes between brain hemispheres. The hemisphere that acts first imposes its activity on the other during their REM sleep-like state.

How do winners win? For an athlete, let's imagine a boxer training for a match, where success could be measured by the hours spent working-out or by maximizing muscle mass, endurance, and speed. However, when there is a second boxer in the ring, then what matters most is being stronger, and faster, than your opponent. A new study published in *Nature* by Fenk *et al.*[1] reveals that, not unlike these athletes locked in competition, there is a night-long fight for dominance between