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Neuronal strategies for meeting the right partner during brain wiring

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Two neurons can only form a synapse if their axonal and dendritic projections meet at the same time and place. While spatiotemporal proximity is necessary for synapse formation, it remains unclear to what extent the underlying positional strategies are sufficient to ensure synapse formation between the right partners. Many neurons readily form synapses with wrong partners if they find themselves at the wrong place or time. Minimally, restricting spatiotemporal proximity can prevent incorrect synapses. Maximally, restricting encounters in time and space could be sufficient to ensure correct partnerships between neurons that can form synapses promiscuously. In this review we explore recent findings on positional strategies during developmental growth that contribute to precise outcomes in brain wiring.

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Introduction

Brain wiring is a developmental growth process. As in any other growth process, cellular interactions are restricted in space and time. Correspondingly, surface molecular interactions are restricted to those that 'get to see' each other during development. Many of these interactions contribute to such positional effects during brain wiring, others directly ensure synaptic partnerships [1–5]. Disruption of either of these two types of molecular functions is likely to lead to brain wiring defects.

Since the late 1980s, the quest for molecular mechanisms that contribute to brain wiring focused on attractive and repulsive molecular signals that specify where to grow an axon or to make a synapse. This approach has blossomed over the years and is considered in excellent recent reviews [1,5]. In addition, recent years have seen an increasing number of remarkable molecular and cellular mechanisms that contribute in less expected ways to developmental growth, and ultimately specificity in brain wiring. Processes like axon pre-sorting, branch self-avoidance and tiling, or approach angles and speed are all essential for the right neuronal partners to meet each other prior to synapse formation [6,7••,8•]. As the brain grows, every neuron runs a cell-intrinsic growth program and responds to environmental cues. The sum of all the mechanisms that restrict encounters between potential partners during the time when both are able to form synapses is a significant contributor to synaptic specificity.

The study of dynamically changing processes during development, as opposed to a focus on final outcomes, is key to the recognition of the developmental steps that bring the right partners together. Mutations in many genes that encode cell surface molecules known to engage in interactions with other cell surface molecules, result in adult brain wiring defects. However, the interpretation of an 'attractive' or 'repulsive' guidance mechanism is only one aspect of the remarkable variety of molecular mechanisms that contribute to spatiotemporal positioning during development [6,9]. The adult phenotype may reveal little about the transient or dynamic nature of developmental events that occurred at a specific time and place. In addition, earlier defects can change the developmental growth program dramatically and mask later functions.

Among developmental growth processes, the identification of neuronal partners during brain wiring is particularly challenging to study in an *in vivo* context. This difficulty is further exacerbated if the molecular and cellular mechanisms are dynamic and transient and only observable in living brain tissue. As a consequence, it remains largely unknown to what extent spatiotemporal positioning facilitates or determines synaptic partnerships *in vivo*. In the following sections, we review recent progress in our understanding of the remarkable, and often surprising, molecular and cellular mechanisms that demonstrate positional strategies during developmental growth of synaptic connectivity.

Pre-specification, post-specification and synaptic promiscuity

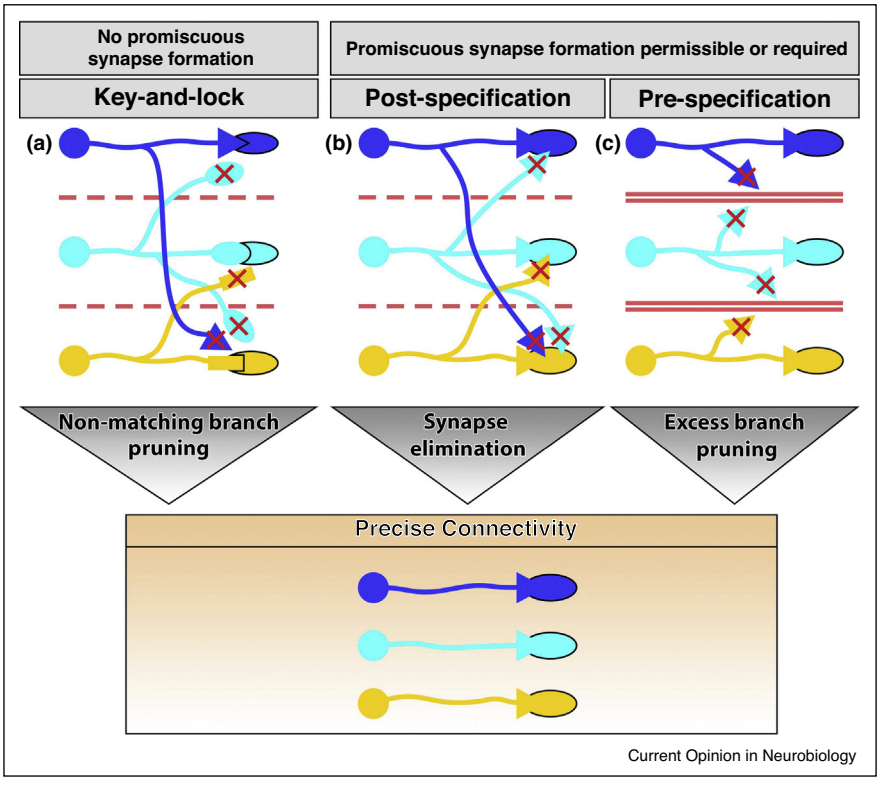
Early proposals for molecular mechanisms of neuronal connectivity focused on precise matchmaking, that is, key-and-lock mechanisms for neuronal connections based

on Sperry's idea of chemical tags of specificity: *'a kind of chemical code with matching values between the retinal and tectal maps.'* [10]. In its strict form, molecular matchmaking pre-determines synaptic partners and thus prevents the neuron from forming synapses promiscuously with incorrect partners. (Figure 1a). However, many neurons have the ability to form synapses with incorrect partners, including themselves [9,11], as also shown in several recent examples below. Notably, activity-dependent synaptic pruning initially requires excessive and therefore somewhat promiscuous synapse formation for the development of precise connectivity. We call this *post-specification* of connectivity, which is can occur through synapse elimination in an activity-dependent or activity-independent manner [1,12,13] (Figure 1b). By contrast, synaptic *pre-specification* occurs when only certain neurons get to contact each other at the same time and place during the developmental period when they are competent to form synaptic connections (Figure 1c). A theoretical neuron that is only exposed to correct partners at the time it is competent to make synapses, could do so promiscuously without sacrificing specificity.

Birth order

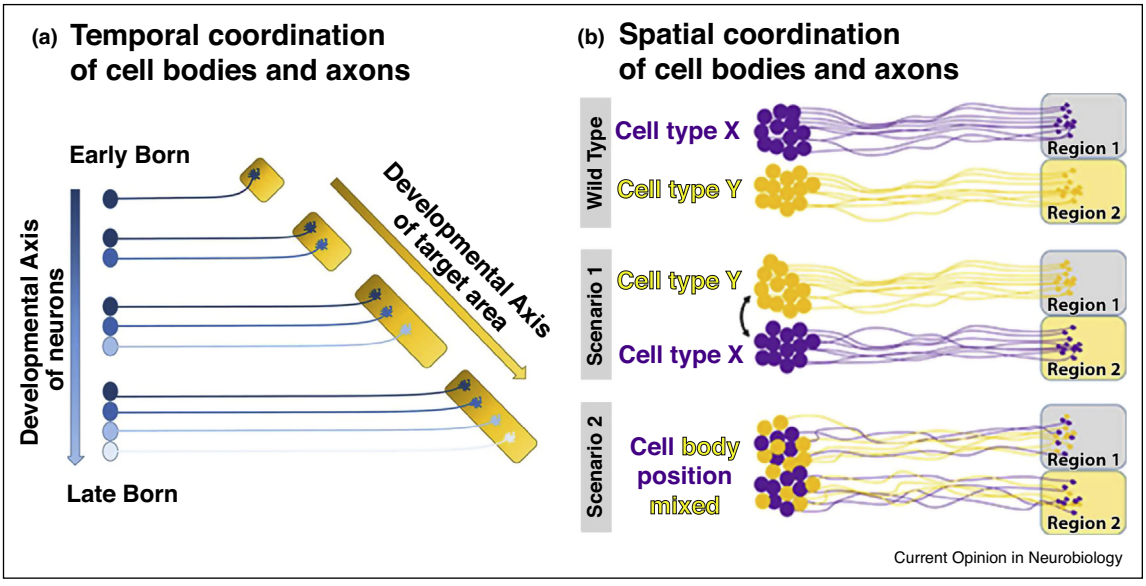
Brain development requires the coordinated neuronal differentiation in a temporally regulated manner [14,15]. Furthermore, development of neural circuits is intricately linked to the timing of the neuronal birth order [16•,17•,18•,19]. The birth order can contribute to the temporal organization of development by enabling successive neurite outgrowth, leading to successive target area innervation and thereby spatial segregation of subsequent synapse formation (Figure 2a). For example, during motor circuit development in mice, the timing of neuronal birth leads to spatial segregation of antagonistic extensor-flexor pre-motor neurons and their synaptic partners [20]. In that study, the change in the spatial positioning was shown to change the synaptic partner choice without changing the cell-fate or expression of known cell-type specific cell surface receptors. In the vertebrate visual system, the difference in the neuronal birth order can lead to differential occupancy of the target field and therefore altered target selection [21]. Here, early born neurons extended axons over a large area, while late born neurons are constrained to occupy a significantly

Figure 1



Mechanisms that contribute to correct synaptic partnerships.
The following processes can contribute to precise connectivity during developmental growth. **(a)** Key-and-lock recognition, or molecular matchmaking, determines precise partners and precludes synaptic promiscuity. **(b)** Post-specification: neurons initially form exuberant or promiscuous synapses. Activity-dependent or activity-independent fine-tuning eliminates incorrect synapses in a typically competitive developmental process. **(c)** Pre-specification: neurons that have the capacity to form unspecific synapses can be restricted in their encounters of potential partners in time and space, ensuring precise connectivity.

Figure 2



Temporal and spatial coordination of cell body and axon positioning.
(a) The temporal order of cell differentiation leads to differences in the temporal and spatial positioning of cell bodies. Such temporal differences are reflected in spatial segregation of axons in the target field. **(b)** Cell body positioning can play crucial roles for axon targeting. In some cases, depicted as Scenario 1 and Scenario 2, swapping cell body positions can pre-determine altered connectivity. If synapse formation is sufficiently promiscuous, synapses may form in the incorrect target areas. If synapse formation requires a specific molecular match, then mis-targeted axon terminals should not form synapses in incorrect target areas.

smaller target field with a reduced need for synaptic pruning. These studies demonstrate that temporal coordination of neuronal specification can affect the regulation of synaptic partner choice, by either spatial segregation of afferent axons with their appropriate partner cell neurites or by making promiscuous synapses to be refined later (pre-specification and post-specification, respectively; Figure 1b,c).

In the *Drosophila* visual system, a birth order-dependent temporal sequence of axon growth in the brain leads to positional segregation of two types of photoreceptor axons, called R8 and R7. This early segregation of positions is sufficient to bring R7 axons in significant overlap with neurites of their main post-synaptic partner cells, called Dm8 [22]. The study showed that changing the position of R8 axons, without affecting their cell fate, is sufficient to bring R8 axon terminals in sufficient proximity with the R7 target cells Dm8 to induce synapse formation between these incorrect partners. Similarly, the highly stereotypic connections of T4/T5 (major neurons in the motion vision circuit in *Drosophila*) are formed in a temporal sequence that corresponds to the birth order and coordinated differentiation program of these neurons [16•,17•,23•].

Finally, in the *Drosophila* navigation circuit, the temporal sequence of neurogenesis has recently been shown to regulate axon targeting of columnar neurons [18•]. This study showed that each of the four classes of columnar neurons in the *Drosophila* central complex differentiate during a tight temporal window. Neurons born at the same time connect to the same region in the central complex, whereas neurons born at different times target to different regions. These recent examples from both vertebrates and invertebrates highlight how genetically identical neurons that are born at different times, can encounter different partner neurons during development, leading to different connectivity.

Cell body position

Closely related to the time of birth is the resulting cell body position. No two neurons can occupy the exact same space, and their relative positions can pre-determine restrictions for connectivity (Figure 2b). Following birth, many neurons migrate to different positions that set up zones required for the spatiotemporal matching of neurogenesis and connectivity, as elegantly shown in the fly visual system [24].

In mouse cortex the correct migration of cortical neurons is a prerequisite for structural cortex folding [25]. Recent work revealed direct coupling of the migratory routes of developing cortical interneurons to the axon targeting program in a dynamic fashion [26]. In this study, the migratory routes of interneurons were cell-intrinsically altered through conditional deletion of the Mafb-a gene. The mutant cells with altered migratory routes exhibited

significantly altered axonal arborizations compared to mutant cells that had migrated normally. In a separate study, knock-down of the microtubule binding protein Dcx was utilized to characterize the positional effect following altered migration of cortical neurons [27]. Interestingly, neurons that are ectopically positioned due to Dcx knock-down form synapses in ectopic positions, most likely with non-cognate synaptic partners. Hence, here as elsewhere, neurons reveal a principal capacity to establish synaptic contacts with available partners at the time they are competent to form synapses.

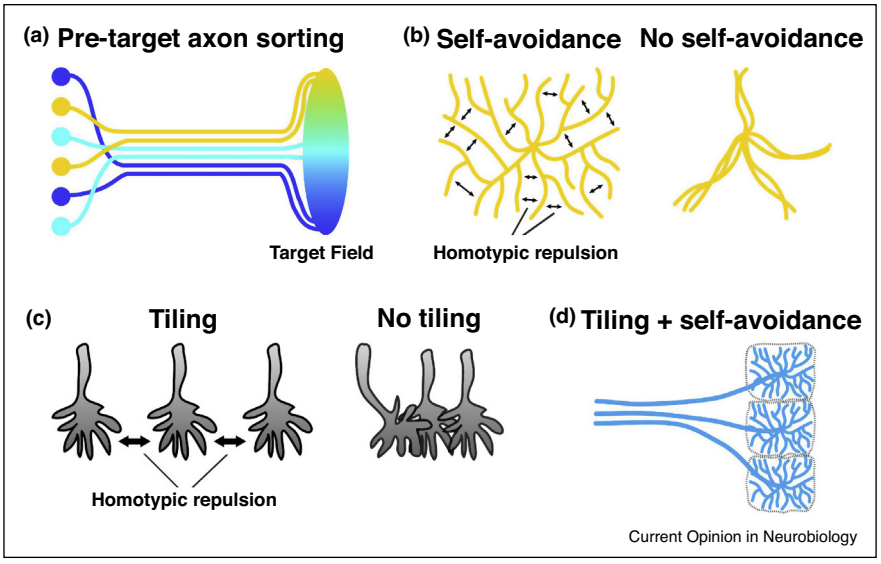
Cellular positioning, which is a prerequisite for proper connectivity, has been studied in quantitative detail for motor neuron cell bodies that are organized into clusters called 'pools' in the spinal cord. Several type 1 and type 2 classical cadherins function in the spatiotemporally precise arrangement of these motor pools [28,29•]. Interestingly, these studies did not reveal an obvious 'Cadherin code' for motor pool organization, but suggested that partially redundant Cadherin functions temporally separate segregation phases. In all cases highlighted here, the positions of cell bodies altered the subsequent growth processes, leading to ultimate wiring defects.

Pre-target axon–axon interactions

Historically, the visual system holds a special place in the study of neuronal connectivity. Retinal output neurons map neighboring points in visual space as neighboring connections in both the vertebrate and invertebrate brains, a principle known as retinotopy [30]. Remarkably, the spatial organizations of axons along their length, that is, their 'neighborliness' (Figure 3a), was shown early on to be preserved in flies [31] and cichlid fishes [32], and more recently, based on whole brain connectomics, in zebrafish [33]. In the fly visual system, a complicated wiring principle called 'neural superposition' further pools only those retinal axons in distinct synaptic units that carry the same visual information [34]. In the vertebrate olfactory system, olfactory sensory neuron axons pre-sort before reaching their corresponding glomeruli and establish topographic order in the anterior-posterior axis of the olfactory bulb [35]. During retino-collicular wiring in the mouse visual system, nasal retinal ganglion cells project to the caudal part of the superior colliculus, whereas temporal retinal ganglion cells project to the rostral side; this leads to the formation of a precise topographic map and target-independent, inter–axonal interactions are a necessary part of this process [36]. A more recent study utilized elegant live imaging experiments to characterize axon pre-sorting in Xenopus explants [37••]. This study characterized how axons from the same (homotypic) side fasciculate, while heterotypic interactions with axons from the other side result in 'tip-toe-tracking'. Both processes can be directly related to filopodial dynamics that are defective in the *cyfip2* mutant. Finally, axon–axon interactions have also recently been shown to regulate midline binary choices of retinal ganglion cells in mice [38].

In addition to axon sorting through inter–axonal interactions, topographic order among axons can be established by

Figure 3



Axon–axon and dendrite–dendrite interactions create patterns that restrict neuronal encounters in time and space.
(a) Inter–axonal interactions facilitate selective fasciculation and topographic sorting among axons before they reach their target field. **(b)** Homotypic repulsion between sister processes causes arborizations to spread (left). Loss of self-avoidance results in crossing and clumping of arborizations (right). **(c)** Homotypic repulsion between processes results in equal spacing (tiling). Loss of repulsion causes axons to overlap and clump together. **(d)** Concurrent utilization of these self and non-self repulsive mechanisms can create patterns that restrict and facilitate specific neuronal encounters.

intermediate secondary structures before axons reach their final target regions. For example, representation of mouse facial whiskers is transferred to the neocortex by thalamocortical axons. During development, these axons are topographically pre-ordered as they pass through the basal ganglia primordium, and before reaching the neocortex [39]. These examples highlight how early axonal patterning serves as important input for downstream developmental processes that lead to correct connectivity.

Branch patterning in the target region

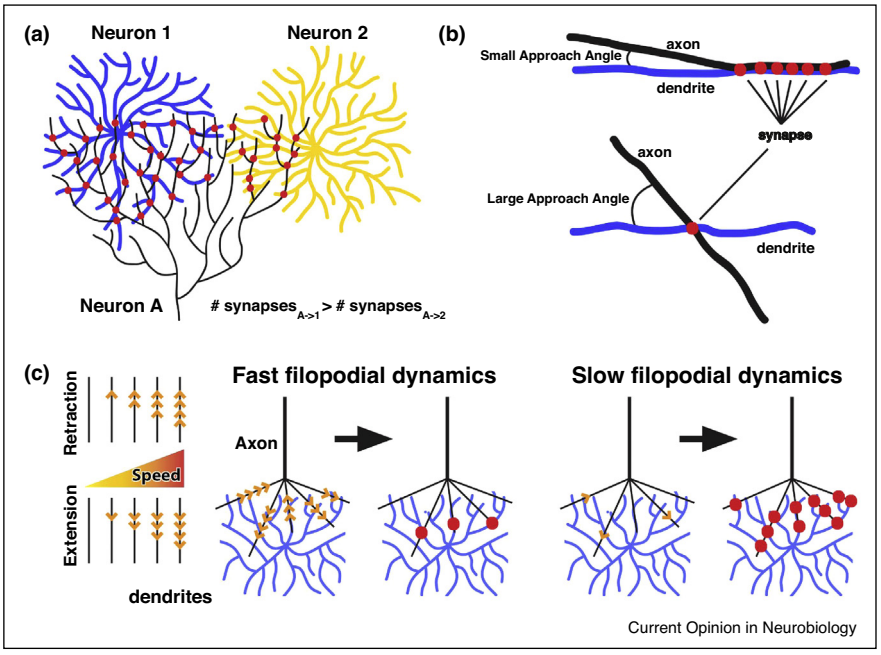
Axonal and dendritic projections of many neurons branch out in their respective target areas in search of neuronal partners. A key discovery was the role of self-avoidance for the spreading of branches, as reviewed previously [40,41]. In the absence of self-avoidance, branches clump together and thereby reduce the target area for synaptic partnerships (Figure 3b). In *Drosophila*, the Down syndrome cell adhesion molecule 1 (Dscam1) gene can produce 38016 transmembrane proteins through non-deterministic alternative splicing and this variability is required as random discrimination marks for 'self' versus 'non-self'. Similar to *Drosophila* Dscam1, the vertebrate clustered proto-cadherin (Pcdh) cell surface proteins are required for self-avoidance, with an analogous function for self/non-self-discrimination in mouse retinal starburst amacrine cells [41,42]. In a recent study, it has been shown that Pcdh diversity is also required for the mouse olfactory neural circuit assembly [43•]. In the complete loss of all Pcdh genes, olfactory sensory neuron axons were clumped and distorted which led to the formation of abnormal proto-glomeruli and thus mis-wiring.

While self-avoidance requires recognition of 'self' and blindness with respect to 'non-self', tiling is based on repulsion between 'non-self' branches (Figure 3c). The same classes of molecules mediating repulsion have been implicated in self-avoidance and tiling [6,44,45]. In addition, repulsion through mutual inhibition has previously been shown to pattern the spatial positioning of axons [46]. In a recent study, it has been shown that regular tiling of radial glial cells in the neocortex is essential for the laminar and columnar organization of neurons in cerebral cortex [47]. Once axons find themselves in an incorrect position of a laminar structure, synapse formation may happen between incorrect partners, as shown in the *Drosophila* central complex [48]. Repulsion-based mechanisms can thereby pre-pattern axonal and dendritic positions for presumptive synaptic partnerships (Figure 3d), and changes of these patterns have downstream effects on synaptic connectivity.

Synaptic partner selection strategies

What happens when presynaptic and postsynaptic branches meet? Molecular interactions precede synapse formation [1]. The interactions may impose specificity

Figure 4



Positional and dynamic properties of axo-dendritic contacts restrict synapse formation.
(a) Overlap between axonal and dendritic arbors influences the number of synapses between neurons based on Peter's rule. If no other mechanisms contributed to synaptic specificity, neuron A (black) should form more synapses with neuron 1 (blue) than neuron 2 (yellow) due to larger overlap. **(b)** Approach angles of axons and dendrites were recently shown to be a determinant of synaptic specificity. **(c)** Kinetics of axonal filopodia can restrict synaptic partner choice by modulating number of synapses. In this case, fast and destabilized filopodia more often fail to establish contacts, while slower, stable filopodia may increase the probability for synapse formation.

in order to exclude incorrect partnerships, or they may allow synapse formation to occur with some degree of promiscuity. It has long been known that the number of synapses can be a function of overlap between dendritic and axonal arbors of two potential synaptic partners, a principle known as 'Peter's rule' [49] (Figure 4a). Specificity can be 'sharpened' several-fold above predictions from Peter's rule through adhesive biasing, as shown in the vertebrate visual system for a specific synaptic amacrine cell type and a retinal ganglion cell type that both express the homophilic adhesion molecule sidekick2 [50]. Another type of retinal ganglion cells, the 'ONα' type, are in contact with several bipolar cell types, yet 70% of its synapses are with B6 bipolar cells. When B6 cells are ablated, synapses form with other bipolar cell types that normally have few or no synapses with the ONα retinal ganglion cells [51]. Finally, in the hippocampus, Schaffer collateral synapses with parvalbumin-positive interneurons match predictions by Peter 's rule, while Schaffer collateral synapses with pyramidal cells exhibit increased specificity [52]. Hence, axon-dendritic overlap can critically contribute to synapse formation without being by itself sufficient for synaptic specificity.

Being at the right time and place based on axon-dendritic overlap may not be enough to ensure that neurons actually meet each other. A recent study in the spinal sensory-motor reflex circuit found that axon-dendritic overlap (akin to Peter's rule) was insufficient to explain the connection specificity [7••]. Instead, the authors found that, remarkably, specific approach angles of axons and dendrites served as a determinant of connection specificity (Figure 4b).

The idea of axon-dendritic overlap as a determinant for synapse-specific contacts has mostly been studied in fixed preparations. Recent evidence suggests that the kinetics, that is, speed and stability, of axon-dendritic interactions may serve to further restrict synaptic partner choice. In the *Drosophila* visual system, photoreceptor axon terminal dynamics can be dialed up and down through modulation of autophagy, leading to more synapses for slower, stable filopodia and fewer synapses for faster, destabilized filopodia [8•,53]. Remarkably, the increased synapse formation also leads to the recruitment of incorrect synaptic partners whose dendritic arborizations are available in the target area. Hence, both interaction angles and speed can quantitatively restrict to what extent neurons meet each other.

Concluding remarks

Neurons want to make synapses. If the correct partners are not available, many neurons have the capacity to make promiscuous synapses with incorrect partners, including with themselves. Developmental growth brings partners together in a slow process during which earlier steps serve as necessary basis for subsequent developmental steps. Ever more precise analyses of molecular and cellular mechanisms in this process reveal developmental steps that would not have been knowable from analyses of developmental outcomes alone. More developmental steps increase the opportunities for the growth program to restrict what neurons encounter each other at the time they express specific surface molecules required to turn encounters into synapses. The recent discoveries of an increasing number of positional strategies are a direct consequence of spatiotemporally higher-resolved analyses. These highlights illuminate only a few of the ways in which the growth program controls neuronal encounters. Each highlight reflects an incomplete snapshot of a process that must occur in the context of cell-intrinsic properties, tissue properties and molecular interactions. Only together, these properties and mechanisms create positional effects and constitute the 'composite instruction' for directional growth and synapse formation.

Conflict of interest statement

Nothing declared.

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