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Fine-scale topography in sensory systems: insights from *Drosophila* and vertebrates

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Abstract

To encode the positions of sensory stimuli, sensory circuits form topographic maps in the central nervous system through specific point-to-point connections between pre- and post-synaptic neurons. In vertebrate visual systems, the establishment of topographic maps involves the formation of a coarse topography followed by that of fine-scale topography that distinguishes the axon terminals of neighboring neurons. It is known that intrinsic differences in the form of broad gradients of guidance molecules instruct coarse topography while neuronal activity is required for fine-scale topography. On the other hand, studies in the *Drosophila* visual system have shown that intrinsic differences in cell adhesion among the axon terminals of neighboring neurons instruct the fine-scale topography. Recent studies on activity-dependent topography in the *Drosophila* somatosensory system have revealed a role of neuronal activity in creating molecular differences among sensory neurons for establishing fine-scale topography, implicating a conserved principle. Here we review the findings in both *Drosophila* and vertebrates and propose an integrated model for fine-scale topography.

Keywords

Fine-scale topography; Sensory systems; *Drosophila*; Activity-dependent projections; Synaptic connectivity

Introduction

The neural circuits of sensory systems are typically organized into two-dimensional neural maps to encode features of sensory stimuli. Depending on what features are encoded, each neural map can be either discrete or continuous (Luo and Flanagan 2007). For example, the olfactory maps encoding odorant types are discrete. In a discrete map, neurons detecting different types of stimuli are anatomically well separated. On the other hand, the retinotopic maps encoding the positions of visual stimuli are continuous. A continuous map in the nervous system usually reconstructs quantitative features of sensory stimuli, such as the locations of visual or somatosensory stimuli and the frequencies of sound; thus, these maps are also called topographic maps (Luo and Flanagan 2007). Two features of topographic

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maps make them distinct from discrete neural maps. First, neurons in a topographic map are of the same type. This is true not only for the specific sensory features they detect but also for the fact that the cell-fate of individual neurons in the map is determined by the same genetic program. Second, the axon terminals of neighboring afferent neurons in a topographic map are adjacent to each other, forming a “continuous” map. A unique issue in continuous maps is the establishment of the synaptic connections that are specific enough to distinguish adjacent presynaptic terminals, which may initially be intermingled with each other and may be separated by only a few microns once development is completed. Studies in the vertebrate visual system have revealed that the mechanisms underlying regional topography (“coarse”) and the mechanisms specifying the topographic locations of neighboring neurons (“fine-scale”) are different. Research in both vertebrate and *Drosophila* topographic maps has provided insights into the mechanism underlying the formation of fine-scale topography.

Three lines of studies have shaped our current understanding of the establishment of fine-scale topographic maps. First, studies carried out in the frog visual system by Roger Sperry in the 1940s laid out the initial conceptual framework for understanding the mechanisms underlying the establishment of synaptic specificity during the development of retinotopic maps (Sperry 1943, 1947). Sperry demonstrated that the axons of retinal ganglion cells (RGCs) in a surgically inverted eye have the ability to find their original targets even though the connections are no longer functionally appropriate. He proposed that each neuron possesses a distinct molecular tag on its surface that helps that neuron to find its target. Later studies identified the concentration of membrane ligands (ephrins) and receptors (Ephs) as molecular tags for each pair of pre- and post-synaptic neurons to form coarse topographic maps (Flanagan and Vanderhaeghen 1998). However, whether molecular tags, including gradients of surface molecules, are used for establishing fine-scale topography remains to be determined. Second, Constantine-Paton, Cline, and other investigators demonstrated that neural activity is essential for fine-scale topography in vertebrate retinotopic maps (Cline 1991). Thus, gradients of Ephs and ephrins direct a coarse retinotopic map while neural activity is required for establishing the point-to-point topography. Third, Zipursky, Clandinin, and colleagues demonstrated in the *Drosophila* visual system that fine-scale topography can be achieved by intricate regulation of cell-cell adhesion, independent of neural activity (Clandinin and Zipursky 2002; Schwabe et al. 2013). These landmark discoveries have provided us with several cornerstones for understanding how the exquisite synaptic specificity in fine-scale topography is established. Nevertheless, major gaps remain in our knowledge on this important issue in developmental neuroscience. For example, based on studies in different organisms, there appears to be more than one mechanism for establishing fine-scale topography. Are these mechanisms mutually exclusive or do they operate together in one sensory system? Here, we review these studies and attempt to propose an integrated model of fine-scale topography.

Fine-scale topography established through intrinsic differences between neighboring neurons: the *Drosophila* visual system

Fine-scale topographic maps in the *Drosophila* visual system

The compound eye in *Drosophila* consists of approximately 750 ommatidia, each of which contains 8 photoreceptors, known as R1-R8 (Clandinin and Zipursky 2002; Hadjieconomou et al. 2011). These R cells are categorized into three groups based on the second-order neuropils in which the axons of these neurons terminate (Harris et al. 1976). The six outer photoreceptors (R1-R6), which detect green light, project their axons to the lamina. R7 and R8 sense ultraviolet and blue light, respectively, and terminate in two distinct layers in the medulla. Topographic maps representing the visual world are formed in each of these three target regions.

The retinotopic map formed by R1-R6 cells in the lamina has been extensively studied for fine-scale specificity in synaptic connections. In the lamina, the terminals of R1-R6 cells form an array of anatomical units called cartridges, each of which is responsible for passing along the visual excitation elicited by a light point in the visual space (Clandinin and Zipursky 2002; Hadjieconomou et al. 2011). The array of the cartridges reconstructs the visual space by forming a topographic map; neighboring cartridges are responsible for neighboring points in space. Because R1-R6 photoreceptors in the same ommatidium see distinct light points, the six axons terminate in different cartridges. At the same time, the light from a point is received by six photoreceptors (R1-R6) that are distributed in six neighboring ommatidia, and the axons of these six neurons converge on the same laminar cartridge. As a result, every cartridge possesses the terminals of R1-R6 cells from six different ommatidia. The R1-R6 photoreceptors in the eye each form a topographic map (Fig. 1a). For instance, R4 photoreceptors from adjacent ommatidia select cartridges that are next to each other. The formation of these topographic maps exhibits remarkably high fidelity with less than two photoreceptors terminating at inappropriate cartridges in each eye (Schwabe et al. 2013).

The R1-R6 topographic maps are established through two steps. First, the photoreceptor axons from the same ommatidium are bundled together prior to reaching the lamina (Clandinin and Zipursky 2000). The bundles do not intermingle with each other and thus preserve the spatial arrangement of the ommatidia, contributing to the coarse topography. Second, once the axon bundles enter the lamina, R1-R6 axons segregate away from the bundles. The extensions of the six axons out of the bundles are stereotypic in such a way that their terminals reconstruct the visual space. R4 axons, for example, exhibit stereotypic extensions with a consistent angle from the bundles, and hence different R4 axons from neighboring bundles do not mix with one another (Fig. 1a). Thus, such precise projections out of the axon bundles ensure the fine-scale connectivity in the fly visual system.

Genetically hardwired fine-scale topography in the *Drosophila* visual system

The molecular mechanisms underlying the fine-scale topography in the fly visual system has been studied at single-neuron resolution. One approach employed by Clandinin and

colleagues is to label a single ommatidium with the fluorescent lipophilic dye DiI, so that the locations of the R1-R6 axon terminals from the ommatidium can be assessed (Clandinin and Zipursky 2000). Through this technique, they found that the extension of the photoreceptor axons out of the bundle is defective in mutants lacking particular subsets of R1-R6 cells. For instance, in mutants lacking R3 and R4, the axons of the remaining photoreceptors successfully defasciculate from the axon bundle, but fail to select the proper cartridges. This indicates that interactions among axons in the same ommatidium play a role in their fine-scale projections from the bundle.

Screening of candidate molecules responsible for the axon-axon interactions was facilitated by genetic techniques for eye-specific gene manipulation (Lee et al. 2001; Lee et al. 2003). Moreover, fly genetic mosaic techniques, such as flip-out and mosaic analysis with a repressible cell marker (MARCM) (Lee and Luo 1999), made it possible to visualize and genetically manipulate single photoreceptors within an ommatidium (Lee and Luo 1999; Lee et al. 2001; Clandinin and Zipursky 2002). Using these techniques, Clandinin, Zipursky, and colleagues identified two cadherin superfamily proteins, Flamingo (Fmi) and N-cadherin (Ncad), as responsible for the axon-axon interactions underlying fine-scale topography (Lee et al. 2001; Lee et al. 2003). When all photoreceptors are homozygous for Fmi or Ncad mutation, these neurons fail to connect with the appropriate targets.

Later studies revealed that Fmi and Ncad act redundantly in individual photoreceptor axons. Inhibition of either Fmi or Ncad (through mutation or RNAi) in single photoreceptors does not cause any error in the target selection of the mutants (Chen and Clandinin 2008; Schwabe et al. 2013). The projections become defective only when Fmi and Ncad genes are simultaneously knocked down in R1, R2, R5 and R6 through RNAi expression (Schwabe et al. 2013). The axons frequently choose inappropriate targets in spite of the successful segregation from their original bundles. Most of the defective axons terminate close to their proper cartridges, suggesting that Fmi and Ncad regulate fine-scale topography, but not coarse topography.

Several lines of evidence suggest that the axon-axon interactions underlying fine-scale topography rely on the relative, rather than absolute, levels of cadherin proteins among neighboring axons. First, R4-specific overexpression of Fmi causes targeting errors in neighboring neurons despite the normal expression of Fmi in these neighbors (Chen and Clandinin 2008). Defective targeting of wild-type photoreceptors also appears when Ncad is overexpressed in R4 specifically (Schwabe et al. 2013). The R4-specific overexpression of Ncad involves a targeting error of the axons from adjacent ommatidia that have a direct contact with the R4 axons. Second, Fmi is differentially expressed in the axons of the six different photoreceptors in a lamina cartridge (Lee et al. 2003; Schwabe et al. 2013). Its protein level is high in R2 and R5, intermediate in R1 and R6, and low in R3 and R4. Therefore, the axon-axon interactions are precisely regulated by the intrinsic differences in the activity of cell-adhesion proteins.

The axon-axon interactions direct the fine-scale projections of photoreceptors by polarizing the growth cones of extending axons (Schwabe et al. 2013). The distribution of filopodia in

the growth cones (i.e. growth cone polarity) correlates with the direction of axon terminal extension, which in turn points toward the specific targets. Thus, it is likely that the polarity of a photoreceptor growth cone directs the axon terminal to the topographically correct target. The regulation of the growth cone polarity requires the cadherin-mediated axon-axon interactions. When both *Fmi* and *Ncad* are knocked down in R1, R2, R5 and R6, the growth cones exhibit variable polarity and do not point toward their correct target cartridges. This abnormal polarity directs the axon toward improper direction, disrupting topographic orientation of neighboring axon terminals.

Together, these findings suggest that the axon-axon interactions, which are based on differentially regulated intrinsic cadherin activity, instruct the growth cone polarity of individual photoreceptors in an ommatidium, which then directs the axon terminals to their correct targets for establishing fine-scale topography.

In addition to axon-axon interactions, axon-target interactions are necessary for the establishment of fine-scale topography of photoreceptors, although their roles are likely to be permissive. While *Fmi* is specifically expressed in photoreceptors (Lee et al. 2003), *Ncad* is expressed in both photoreceptors and their target neurons (Lee et al. 2001). When *Ncad* expression is removed from some of the target neurons in the lamina, photoreceptor axons show defective projections (Prakash et al. 2005). Consequently, cartridges that harbor *Ncad* mutant postsynaptic neurons do not contain six photoreceptor presynaptic terminals. This result suggests that *Ncad* expression in the target neurons is essential for photoreceptor cells to terminate at the appropriate cartridges. Therefore, *Ncad* mediates attractive interactions between pre- and post-synaptic terminals. This is different from *Fmi*, which is neither expressed nor required in the target neurons (Chen and Clandinin 2008). These results point to distinct roles for adhesion molecules, despite the redundancy in the presynaptic terminals as mentioned above.

Remarkably, the topographic maps in the fly visual system are formed independently of neuronal activity (Hiesinger et al. 2006). Electron microscopy of the lamina shows no morphological abnormality of cartridges in several mutants that have defective neural activity. The cellular composition of each cartridge is normal despite a defect in the generation of electrical potentials or in the release of neurotransmitter. Moreover, *DiI* injection of single ommatidia in *trp;trpl* mutants, which are defective in light-elicited response, shows normal projections of photoreceptor axons. R4-specific labeling of the mutants further confirms the distribution of a single R4 terminal to each cartridge. Therefore, genetically programmed mechanisms are sufficient to instruct individual photoreceptors to their own targets by differentially regulating the intrinsic levels of cadherin activity between neighboring neurons.

Fine-scale topography established through Hebbian synaptic strengthening: the vertebrate visual systems

Fine-scale topography in the vertebrate visual system

A large number of studies on the development of vertebrate retinotopic maps have provided profound insights into the development, cell biology, and plasticity of neural circuits. In the vertebrate visual system, the retinal ganglion cells (RGCs) project their axons to the midbrain visual centers: the optic tectum in amphibians and birds, and the lateral geniculate nucleus and superior colliculus in mammals (Ruthazer and Cline 2004; Huberman et al. 2008; Cang and Feldheim 2013). RGC axon terminals in these target structures form topographic maps that represent the two-dimensional arrangements of RGC cell bodies in the retina. For example, in frogs, RGCs in the nasal part of retina project their axons to the posterior side of the tectum whereas temporal RGCs terminate at the anterior side (Fig. 1b) (Ruthazer and Cline 2004). In these maps, neighboring RGCs project their axons to neighboring synaptic targets without intermingling with each other. This sorting of the terminals establishes point-to-point specificity in the synaptic connections, or fine-scale topography. Here, the establishment of fine-scale retinotopic maps requires neural activity (Debski and Cline 2002; Ruthazer and Cline 2004; Ackman and Crair 2014).

Neural activity-dependent regulation of fine-scale retinotopy

Sperry proposed that each RGC possesses a distinct molecular tag, allowing it to find the topographically correct target. As it is hard to conceive the existence of such molecular tags for thousands of RGCs in each retina, he later revised this model by suggesting that concentration gradients of a few molecules may serve as such molecular tags (Sperry 1963). Studies by Bonhoeffer and Flanagan groups subsequently discovered that the concentration gradients of cell-surface proteins Ephs and ephrins in RGCs and their targets direct the formation of topographic maps (McLaughlin and O'Leary 2005; Flanagan 2006). Thus, the concentration of membrane proteins (ligands and receptors in this case) serves as a unique molecular tag for each pair of pre- and post-synaptic neurons. Although it is conceivable that fine-scale topography might be established with the same mechanism, a number of studies have demonstrated that there are important differences between the mechanism underlying fine-scale topography and that underlying coarse topography.

One important aspect of fine-scale topography in vertebrates is its requirement of neural activity during development. For example, in chick embryos, blocking neural activity in the eye by the injections of tetrodotoxin (TTX) results in defective retinotopic maps that contain enlarged synaptic terminals. As a result, neighboring RGCs have overlapping terminals, abolishing topography despite the presence of Ephs and ephrins (Fig. 1b) (Kobayashi et al. 1990). Neural activity during development is also essential for the segregation of the axons from the two eyes (Ruthazer and Cline 2004). However, because synaptic connections in retinotopy involve point-to-point accuracy while eye-specific segregation does not, the molecular mechanisms underlying these two activity-dependent processes in development are likely to be different.

A number of studies support the “co-activity” hypothesis for establishing the retinotopic maps and eye-specific segregation (Cline 1991). This hypothesis states that neighboring RGCs exhibit similar activity (i.e. are co-active), and are thus connected to the same target neurons. This model is in principle a special case of Hebb’s postulate of synaptic connection, which states that correlated pre- and post-synaptic activity results in the selective strengthening of synapses (Hebb 1949). There are two key tenets of the co-activity hypothesis: 1) neighboring presynaptic neurons are co-active. As discussed below, patterned retinal RGC activity supports this in both topographic maps and eye-specific segregation (Ruthazer and Cline 2004); 2) co-active presynaptic terminals form synapses with postsynaptic targets that exhibit the same co-activity (e.g. same postsynaptic neurons or different neurons with the same activity pattern). While this is supported in eye-specific segregation (Zhang et al. 2012), its involvement in the establishment of topographic maps remains hypothetical.

An important line of evidence supporting the co-activity hypothesis is the presence of coordinated spontaneous activity among neighboring RGCs that sweeps across the entire retina in waves prior to the eye-opening (Meister et al. 1991; Wong et al. 1993; Feller et al. 1996; Ackman et al. 2012). In mice lacking the $\beta 2$ subunit of the nicotinic acetylcholine receptor (i.e. $\beta 2$ knockout mice, or $\beta 2$ KO) (Grubb et al. 2003; McLaughlin et al. 2003; Mrsic-Flogel et al. 2005; Chandrasekaran et al. 2005; Pfeiffenberger et al. 2006; Willshaw et al. 2014), normal patterns of RGC activity are disrupted, which consequently affects the co-activity of neighboring RGCs. In these mutants, both topography and eye-specific segregation are defective. The RGCs in the mutant mice possess enlarged terminals and, as a result, defects in fine-scale connectivity in retinotopic maps.

The role of retinal waves in establishing topography has been controversial as a result of inconsistent phenotypes observed in $\beta 2$ KO mice (Sun et al. 2008; Chalupa 2009; Feller 2009; Stafford et al. 2009), which might be due to the fact that $\beta 2$ deletion is not specific to the retina in $\beta 2$ KO mice. To address this issue, $\beta 2$ gene was expressed in the ganglion cell layer, leading to a restoration of retinal waves in $\beta 2$ KO mice (Xu et al. 2011). These waves are smaller in size than wild-type, and individual waves occur locally without propagating across the entire retina. Despite this abnormality, RGCs project the axons to the topographically appropriate locations. The abnormally enlarged terminals in $\beta 2$ KO are rescued in these mice, implicating the successful establishment of fine-scale topography. The smaller waves only produce co-activity in local areas, and hence, co-activity in local areas is sufficient for establishing point-to-point connections. These findings suggest an instructive role of the retinal waves in fine-scale topography.

How might the co-activity of neighboring presynaptic neurons contribute to fine-scale topography? In regard to the original Hebbian hypothesis, the co-activity model is based on the assumption that the activity of the target neurons synchronizes with the patterned retinal RGC activity. The synchronous activity between pre- and post-synaptic neurons would produce stable synapses through Hebbian plasticity. An in-vivo calcium imaging study revealed the presence of waves in the postsynaptic neurons in mouse (Ackman et al. 2012). The postsynaptic waves emerge at the same frequency and at the same speed as the

presynaptic RGC waves. Moreover, the postsynaptic wave disappears upon the inhibition of the waves in the retina, indicating that the postsynaptic wave is driven by the activity of RGC presynaptic terminals. This would ensure that the afferents and target neurons are co-active, which might lead to specific connections between the afferents and their appropriate targets. Indeed, coincidence in the activities of RGCs and their target neurons strengthens the synaptic connections between these neurons in mouse (Butts et al. 2007; Shah and Crair 2008). Thus, “neurons that fire together, wire together” to establish synaptic specificity in the establishment of fine-scale topographic maps.

The connections between co-active pre- and post-synaptic neurons rely on NMDA-type glutamate receptors (Constantine-Paton et al. 1990; Cline 1991). RGCs are glutamatergic neurons, and NMDA receptors are localized on the dendrites of the postsynaptic neurons of RGCs. It is postulated that co-activation of multiple neighboring RGCs leads to sufficient depolarization to activate postsynaptic NMDA receptors (Cline 1991). As a result, the synaptic connections between these RGCs and the postsynaptic partner(s) are strengthened. Therefore, NMDA receptors serve as a detector of co-activity of neighboring afferents. The molecular mechanism downstream of NMDA receptors in establishing topography remains to be determined.

The co-activity model explains how neighboring afferents converge on shared targets, but does not explain how different neighboring afferents might connect with different postsynaptic targets. Recent studies on retinal waves suggest a role of the sequence of RGC activation for synaptic segregation among different neighboring afferents (i.e. the point-to-point connectivity). Interestingly, the propagation of retinal waves exhibits a directional bias (Stafford et al. 2009; Ackman et al. 2012). The waves frequently start from the ventral-temporal retina and sweep toward the dorsal-nasal pole. The propagation at a fixed direction provides two neighboring neurons along the propagation axis with less correlated activity than those perpendicular to the axis (Stafford et al. 2009). Importantly, RGC terminals with defective retinal waves (as in $\beta 2$ KO mice) exhibit enlargement along the axis of wave propagation (Grubb et al. 2003; Mrcic-Flogel et al. 2005; Ackman and Crair 2014), indicating that the topography along this axis is particularly dependent on the retinal wave. This finding highlights the importance of sequential activation among adjacent RGCs in establishing fine-scale topography.

The sequential activation in the form of a wave might cause intrinsic differences, such as biochemical activities, in neighboring neurons along the axis of wave propagation. This possibility is supported by the findings that neural activity dynamically regulates the activity of cell adhesion molecules. For example, in a cultured hippocampal neuron, N-Cadherin stabilizes and localizes to the cell surface in response to neural activity, enhancing its adhesive activity (Itoh et al. 1997; Tanaka et al. 2000; Brigidi et al. 2014). The retinal wave is also known to produce oscillations in the intrinsic cAMP level of individual RGCs (Dunn et al. 2006). The cAMP oscillations in RGCs then modify the intrinsic ephrin signaling to control its repellent action in vitro (Nicol et al. 2007). The molecular differences among neighboring afferents along the axis of wave propagation may modulate the axon-axon interactions to establish the point-to-point topography.

Interactions between neural activity and intrinsic differences: the nociceptive system in *Drosophila* larva

The topographic map in the nociceptive system in *Drosophila* larva

Accumulating evidence supports that the regulation of nervous system development by neural activity exists in invertebrates as well. For example, in *Drosophila*, neural activity regulates synapse formation at the neuromuscular junction (Menon et al. 2013). An activity-dependent topographic map in *Drosophila* would be instrumental for analyzing the molecular mechanisms underlying fine-scale topography. A recent study identified such a system in the larval somatosensory system (Yang et al. 2014).

The dendrites of the larval nociceptive neurons, which are termed class IV dendritic arborization (C4da) neurons based on a morphological categorization (Grueber et al. 2002), cover the body wall in a complete but non-overlapping fashion (Grueber et al. 2002; Grueber et al. 2003). Yang et al. reported that the synaptic terminals of the three C4da neurons in each hemi-segment form a dorsal-to-ventral topographic arrangement in the ventral nerve cord (VNC), which corresponds to the dorsal-to-ventral distribution of their dendritic fields on the body wall (Yang et al. 2014). This topographic system is fine-scale because it distinguishes the locations of neighboring neurons. Unlike the dendritic tiling on the body wall, which is large in size, the fine-scale topography formed by the C4da synaptic terminals in the VNC is confined along a distance of roughly 6 microns (Yang et al. 2014). Moreover, the synaptic terminals are intimately adjacent to each other, leaving little space in between. These features allow for direct assessment of fine-scale topography, but also call for new techniques for efficient analysis.

Yang et al. took advantage of the C4da-specific promoter from the *pickpocket* gene (Grueber et al. 2003) to generate a reference of the C4da synaptic area (“C4da neuropil”) by marking these neurons with a membrane marker, e.g. the red fluorescence protein tdTomato tagged with the membrane protein CD4. They then generated single C4da mutant neurons, labeled by GFP, in the background of this reference. Automated image analysis was used to deduce the relative position of the synaptic terminal of the GFP-labeled mutant in the C4da neuropil. An advantage of the C4da system is that randomly labeled single neurons can be reliably identified based on the position of the cell bodies on the body wall. As a consequence, the differences among the three C4da neurons can be systematically studied.

Although it consists of only three neurons, the C4da topography is composed of two aspects, which are likely to be established through different mechanisms. The three C4da neurons in each hemi-segment are termed the dorsal (D), middle (M), and ventral (V) neurons based on the locations of their cell bodies and dendritic fields in the body wall (Yang et al. 2014). After the axons of the three neurons reach the VNC, the D axon immediately defasciculates from the M and V axons. The D axon projects dorsally while the M and V axons project ventrally. Both then converge on the C4da neuropil situated in the most medioventral portion of the general synaptic area in the VNC. Once inside the C4da neuropil, the terminal of the D axon stays dorsally to those of the M and V axons. The M and V axons, which

reach the C4da neuropil as a single nerve, separate from each other gradually during development so that the M terminal locates dorsally to the V terminal. Consequently, the presynaptic terminals of the three C4da neurons occupy distinct areas in the C4da neuropil, establishing fine-scale topography (Fig. 1c).

Neural activity-dependent regulation of fine-scale topography in the larval nociceptive circuit

The mechanism separating the D terminal from the M/V terminals is different from that separating the M and V terminals; neural activity specifically regulates the projections of M and V neurons. When the activity of M neurons is blocked by the expression of Kir2.1, an inward rectifier potassium channel that blocks both spontaneous and evoked activity, M terminals end in the ventral portion of C4da neuropil where V neurons normally terminate (Fig. 1c). The effect of Kir2.1 is specific to M neurons, and its expression in D or V neurons has no effect on the topographic locations of their terminals. Conversely, activation of V neurons through dTrpA1 (at 30°C) shifts their terminals to the middle portion of the C4da neuropil. Expression of dTrpA1 in either D or M neurons does not affect the topography. These results suggest that the levels of neuronal activity in M and V neurons direct the separation of their terminals. The separation of the D terminals from the M/V terminals is independent of neuronal activity, though the underlying mechanism remains unknown.

In the C4da system, it is likely that neural activity instructs fine-scale topography by setting up different levels of intracellular signaling activities in M and V neurons. In other words, neuronal activity creates an intrinsic signaling gradient among neighboring afferents. First, although C4da neurons are almost genetically identical, they exhibit different levels of the *Drosophila* ortholog of the tripartite motif protein Trim9 (dTrim9), a putative E3 ubiquitin ligase of the TRIM family (Reymond et al. 2001). The protein level of dTrim9 is higher in V neurons than M neurons (Morikawa et al. 2011). Second, the dTrim9 protein level is regulated by neuronal activity. When the activity of M neurons is blocked by Kir2.1, dTrim9 levels in these neurons increase to levels comparable to V neurons. Conversely, activating V neurons by dTrpA1 decreases dTrim9 level and consequently eliminates the difference between M and V neurons. Third, the gradient in dTrim9 protein level is essential for the topographic projections. When dTrim9 is overexpressed specifically in M neurons to abolish the M-V difference, the M axons terminate in the ventral portion of the C4da neuropil, which is a similar phenotype to Kir2.1 expression. Conversely, the removal of dTrim9 gene from V neurons recapitulates the phenotype of those with activation by dTrpA1. Finally, restoring the dTrim9 gradient rescues the topographic defects caused by activity manipulation. Therefore, genetic manipulation to establish the gradient of dTrim9 is sufficient to specify the projections of M and V neurons regardless of their activity levels.

Altogether, these results suggest that neuronal activity regulates the levels of dTrim9, a cell-intrinsic molecule that is differentially expressed in individual neurons, to separate the M and V terminals. How might neural activity establish such a molecular difference among neighboring afferents? One possibility is that the activity levels of M and V neurons are different. Although this could be caused by genetic programs, it is equally possible that M

and V neurons in developing larvae, which are behaving animals, might be activated at different levels. Another possibility is that the responses to neuronal activity differ between M and V neurons so that the same level of neuronal activity leads to different levels of dTrim9 proteins. This would be caused by a genetically programmed difference, and neuronal activity would just be a factor linking an intrinsic difference to differential expressions of signaling molecules. Future studies are needed to provide insights into how the nervous system combines genetically encoded programs and activity-dependent mechanisms for establishing fine-scale neuronal wiring.

Concluding remarks and future perspectives

Topographic maps are unique in that, although neurons in the map are of the same type, the axon terminals of neighboring neurons are continuously aligned to form point-to-point connection specificity. In the fly visual system, genetic programs sufficiently establish fine-scale topographic maps, whereas both vertebrate visual system and fly nociceptive system require the activity of afferent neurons for their fine-scale topographic alignments. Despite these findings, many important questions remain to be answered in order to understand the mechanisms underlying activity-dependent fine-scale topography.

Genetic code versus neural activity in specifying synaptic connections

Why is the genetic code insufficient for establishing fine-scale topography in the vertebrate visual system and fly nociceptive system but sufficient for the fly visual system? The six photoreceptor axons from each ommatidium are bundled together in a fashion that preserves the spatial arrangement of the cell bodies (Clandinin and Zipursky 2002), which may require Eph gradients in the targets (Dearborn et al. 2002). Intrinsic differences in cell adhesion then precisely instruct the projections of the six axons to form specific connections with their respective targets. These two steps prevent adjacent presynaptic terminals from overlapping with each other at any stage of the development, resulting in a continuous map composed of a large number of neurons of the same type.

The vertebrate visual systems take a different strategy. Morphogens such as BMP construct broad gradients of Ephs and ephrins over large populations of pre- and post-synaptic neurons (Peters 2002). These broad gradients are insufficient for endowing distinct identities to neighboring axon terminals, producing overlapping synaptic terminals among the neighbors. In order to refine the coarse topography, neuronal activity is used as an additional cue to distinguish adjacent axons for their fine-scale separation. The sorting of the larger number of axons in vertebrates might be beyond the capacity of genetic programs, and the difference in the strategies might be attributed to differences in the brain size across species.

The recent finding of activity-dependent topography in the fly nociceptive system demonstrates cross-species conservation of the activity-dependent fine-scale sorting of sensory afferents. Among the three nociceptive neurons forming topography in one hemisegment, two project their axons as a single nerve (Yang et al. 2014). The axons of the two neurons are not patterned topographically in the nerve, and their presynaptic terminals overlap initially. Despite the small number of afferents, the sorting of the adjacent terminals

relies on the relative levels of neuronal activity. Elucidating how neuronal activity generates molecular differences among neighboring neurons will shed light on how the nervous system uses neuronal activity for its development.

The link between neural activity and synaptic specificity in fine-scale topography

Despite remarkable progresses in understanding activity-dependent fine-scale topography, how neural activity leads to specific synaptic connections in fine-scale topography remains undetermined. Studies of the fly visual system revealed that fine-scale topography requires a network of cell-cell adhesion, which is based on differential regulation of adhesion properties between neighboring neurons (Fig. 2). In this system, interactions among axons with different levels of cadherin activity direct the axons to their correct targets.

Axon-axon interactions appear to be a conserved mechanism underlying the formation of neural maps. In the vertebrate olfactory system, receptor neurons detecting different odors project their axons to anatomically separated glomeruli. The formation of such a discrete map relies on axon-axon interactions of olfactory receptor neurons, which are partly regulated by stimulus-induced activity of these neurons (Sakano 2010; Nishizumi and Sakano 2015). Odor-driven neuronal activity produces a difference in the expression profile of cell surface molecules among neurons with different receptors (Serizawa et al. 2006). The activity-dependent membrane molecules include adhesive molecules, such as kirrel2/3, and repulsive molecules, such as ephrinA/EphA. Interactions between afferents with different levels of these proteins contribute to the receptor-type specific axonal segregation in the olfactory system.

In the vertebrate visual system, it is possible that the retinal waves dynamically control adhesive characteristics of individual RGC axons (Fig. 2). For example, the waves might produce transient gradients of adhesion activity among neighboring axons along the axis of its propagation. Such local gradients can instruct individual synaptic connections through a network of cell-cell interactions. In this regard, the activity-dependent regulation of Trim9 levels might act as a mediator between neural activity and cell adhesion. It is interesting to note that Trim9 protein, which is specifically expressed in the nervous system in mammals (Berti et al. 2002; Tanji et al. 2010), exhibits a gradient along the dorsal-to-ventral axis in mouse spinal cord (Berti et al. 2002). This raises the possibility that Trim9 gradient instructs fine-scale topography in vertebrates.

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List of Abbreviations

C4da neurons class IV dendritic arborization neurons

RGC	retinal ganglion cell
VNC	ventral nerve cord
Fmi	Flamingo
Ncad	N-cadherin
β2KO	Mouse knockout of β2 subunit of the nicotinic acetylcholine receptor

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Figure 1. Fine-scale topography in *Drosophila* and vertebrates

(a) In the *Drosophila* visual system, R1-R6 photoreceptors form a retinotopic map. R4 photoreceptors from neighboring three ommatidia (labeled in blue, red, or green) terminate at adjacent cartridges in the lamina. Gray: R2 and R5 photoreceptors. Brown: R7 and R8 photoreceptors. Modified from Clandinin and Zipursky 2000. (b) In the vertebrate visual system, the fine-scale topographic projections of RGCs require neuronal activity. Upon the inhibition of neuronal activity, RGCs exhibit enlarged presynaptic terminals which extensively overlap with adjacent RGC terminals. The square in the tectum is magnified at the bottom. N: nasal. T: temporal. A: anterior. P: posterior. (c) In the nociceptive system in *Drosophila* larva, the topographic projections of M (in red) and V (in green) neurons depend on the relative levels of neuronal activity. The activity inhibition of M neurons shifts their axon terminals ventrally. VNC: ventral nerve cord. For more detail, see Yang et al. 2014.

Figure 2. Mechanisms underlying fine-scale topography

In *Drosophila*, local gradients of intrinsic molecules (e.g. cell adhesion molecules in the visual system and Trim9 in the somatosensory system) instruct fine-scale topography. In the vertebrate visual system, broad gradients of guidance molecules are not sufficient to distinguish neighboring afferents. The retinal wave of neuronal activity might transiently provide a local gradient of intrinsic molecules, which in turn specifies individual connections.