Chapter 14
Axonal Growth and Targeting

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Abstract The growth and guidance of axons is an undertaking of both great complexity and great precision, involving processes at a range of length and time scales. Correct axonal guidance involves directing the tips of individual axons and their branches, interactions between branches of a single axon, and interactions between axons of different neurons. In this chapter, we describe examples of models operating at and between each of these scales.

14.1 Introduction

The modeling of information processing by neural networks has had a long and fruitful history (see, for example, Chap. 10). In contrast, relatively little is understood about the computational principles involved in initially wiring such networks. In the developing human brain, hundreds of billions of neurons form hundreds of trillions of connections by extending their axons over sometimes vast distances (on the cellular scale). How do these axons "know" where to grow? This is the axon guidance problem. In this chapter, we describe various mathematical modeling approaches that have been taken, and how they have informed our understanding of this crucial process.

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Understanding axon guidance requires studying processes that occur on a variety of time and length scales. The tips of axons, growth cones, are semi-autonomous structures, capable of responding to multiple cues in their environment, and have hence been the target of focused research. However, on a larger scale, guidance involves the entire axon; for example it may involve the selective pruning or promotion of axonal branches to achieve a specific aim. On a larger scale again, wiring up the nervous system is a collective problem, as axons interact with each other and with other cells. At this level, growth cones, axons and their substrates cooperate to shape guidance. There may also be a role for electrical activity in guiding this stage of nervous system wiring, as well as its more established role in refining and patterning established neuronal architecture.

A wide variety of modeling approaches have been used to tackle axon guidance, ranging from models taking a detailed simulation perspective, attempting to reflect the biophysical basis of axon guidance in as much detail as possible, through to highly abstract models focussing on how particular strategies for specifying guidance routes might allow for the formation of a complex nervous system. (For other reviews, see van Ooyen 2003; Maskery and Shinbrot 2005; Graham and van Ooyen 2006; Simpson et al. 2009.)

In this chapter, we examine models that operate at each of the scales previously identified: ranging from the behavior of individual growth cones, through the dynamics of entire axons and their branches, to the dynamics of populations of axons. We first review models of the growth cone—both how it moves, and how it is guided by external cues. We then consider the axon as a functional guidance unit, looking at models that study how resources (e.g. cytoskeletal proteins) are allocated between different branches of the same axon, or between the cell body and growth cones. Finally, we look at the issues encountered when considering the guidance of populations of axons, such as how interactions between axons can act to improve the robustness and specificity of projections.

14.2 Guidance for the Tip: Models of the Axonal Growth Cone

The embryonic environment is awash with chemical signals that direct the intricacies of nervous system development. In order to guide axons, these chemical signals are detected by special structures at the tips of growing axons, known as growth cones (Gordon-Weeks 2000) (Fig. 14.1). First identified and described by Ramon ý Cajal in the late nineteenth century, these complex, motile “battering rams” read the information provided by the environment, and transduce it into decisions about the direction in which the axon should grow. They are micromachines with sensory and motor capabilities, tasked with wiring the nervous system. Thus, to understand axon guidance, it is of key importance to understand the growth cone, and how it senses and responds to the chemical signals that provide the map for axonal pathfinding.

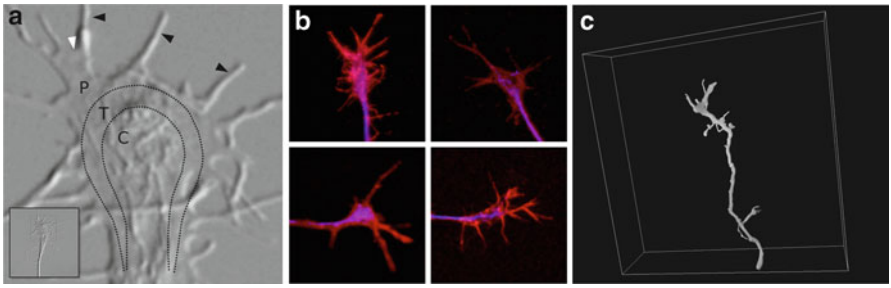

Fig. 14.1 Growth cones are complex structures on the tips of developing neurites: (**a**) Anatomically, the growth cone can be roughly partitioned into three sections: the central zone (*C*), transitional zone (*T*) and peripheral zone (*P*). Magnified region marked on inset in *red*. The *arrowheads* indicate filopodia (*black*) and a lamellipodium (*white*) (**b**) Growth cones display widely varying morphology, even when grown on the same substrate, from the same tissue source (in this case, rat dorsal root ganglion neurons, grown on laminin and stained for actin (*red*) and tubulin (*blue*)). (**c**) In three dimensions, growth cones tend to take on a more "streamlined", filopodially dominated morphology (All images courtesy of Z. Pujic, Goodhill lab)

Although growth cones can show great variability in their morphology, three distinct regions can be defined, as illustrated in Fig. 14.1a: a central region containing organelles and rich in microtubules, a thinner peripheral domain predominantly consisting of a network of actin filaments, and a narrow transitional domain between the previous two regions.

As with many other motile biological structures, actin dynamics are crucial for growth cone motility and morphology. Actin filaments tend to be oriented with their "barbed" ends—the ends at which unpolymerized actin monomers (G-actin) are most easily incorporated into the filament—towards the outside of the growth cone. Actin polymerization thus tends to push against and expand the outer membrane. On two dimensional substrates (where all modeling work so far has occurred), these dynamics lead to the formation of two distinct classes of structure at the growth cone leading edge: filopodia and lamellipodia (Gordon-Weeks 2000) (Fig. 14.1b,c).

14.2.1 Models of Growth Cone Motility

14.2.1.1 Descriptive Models

A large part of modeling work has been devoted to simply describing how growth cones behave, and to extracting rules about their behavior which can be incorporated into more explanatory models. A number of attempts to capture the dynamics of growth cones were made throughout the 1990s by Buettner and colleagues (e.g. Buettner et al. 1994; Buettner 1996; Odde et al. 1996). Growth cones were filmed while growing on different substrates, and the results analyzed to extract statistical regularities. Buettner et al. (1994), described the growth cone using a

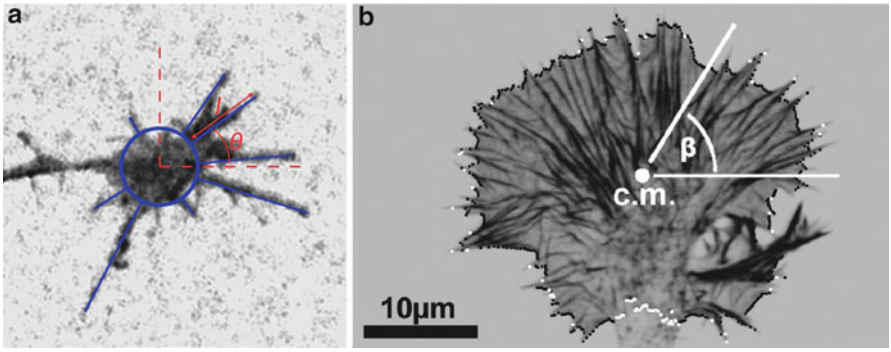

Fig. 14.2 Example methods for describing growth cones: (**a**) Buettner (1996) modeled filopodia searching for target tissue. In this case, modeling the growth cone as a circular central region, with radially extending filopodia was found to be sufficient. (**b**) Betz et al. (2006, 2009) studied the dynamics of protrusion and retraction of elements of the growth cone boundary, and how these related to actin polymerization and retraction within the growth cone itself. They used radial profiles of actin staining intensity, and radial displacement of the growth cone boundary to quantify these phenomena. *c.m.* = center of mass (**Panel b** from Betz et al. 2006)

hybrid system: a contour detailing the shape of the lamellipodia, and a series of "sticks" extending radially from the centroid of the growth cone representing the filopodia (Fig. 14.2a). These models were fit by hand to movies, and the statistics of the resulting parameters examined. For example, it was observed that filopodia tended to extend and retract with a constant rate, switching between the extension and retraction phases according to a gamma-distribution. The statistical models of growth cone behavior obtained in this manner informed estimates of how easily a growth cone could cross a gap between two permissive substrates, and also the likelihood of it contacting a locally expressed guidance cue.

Advances in experimental technique allowed the motion of the growth cone as a whole to be compared to the underlying dynamics of the cytoskeleton. For example, Odde et al. (1996) analyzed the joint statistics of growth cone motion and microtubule dynamics, finding that the two were coupled with a slight delay, giving credence to the idea that growth cone advance involves the active coordination of actin and microtubule dynamics.

Betz et al. (2006, 2009) measured the extension of the membrane from the central region as a function of time (Fig. 14.2b), and then fit the motion of a small section of membrane with a one-dimensional random walk in a potential field (Fig. 14.3). They found that the membrane dynamics both of growth cones, and of cell-lines made to express growth-cone-like characteristics, could be well described if the field consisted of two shallow wells (Fig. 14.3b). Intriguingly, however, real growth cones showed a form of "stochastic resonance", in which the noise properties of the effective random walk were tuned to the potential field, such that small modulations in the steepness of the underlying potential surface had a strong effect on the resulting dynamics.

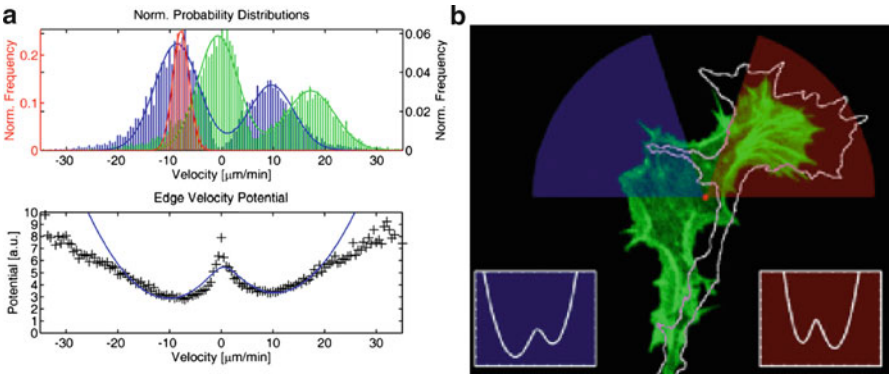

Fig. 14.3 Growth cone membrane dynamics tend to hop stochastically between extension and retraction: In Betz et al. (2006, 2009), the extension or retraction rates of small patches around the growth cone periphery were measured by calculating the change in distance from the *center* of the growth cone to the periphery in subsequent video frames. (**a**) In the *top panel*, the distribution of rates of rearward actin flow is shown in *red*, the distribution of edge velocity in *blue*, and the inferred distribution of the rate of actin polymerization in *green*. The distribution of, and temporal correlation in, the edge velocities can be captured as a random walk in a bimodal potential field, shown in the *lower panel*. The "hump" between the two dips in the *lower panel* controls the rate at which protrusion switches to retraction, and vice-versa. (**b**) In a turning growth cone, the potential field is biased towards protrusion on one side, and retraction on the other (Images from Betz et al. 2009)

14.2.1.2 Regulation of the Cytoskeleton, and Growth Cone Morphology

Though not specific to growth cones, a series of models have studied how the interaction between the plasma membrane and actin cytoskeleton (mediated or tuned by membrane-associated regulatory proteins) lead to the formation, and control the dynamics, of filopodia and lamellipodia. Gov and Gopinathan (2006) studied the linkage between actin and membrane dynamics caused by the preferential localization of membrane-associated actin regulatory proteins with regions of specific membrane curvature. The authors developed a partial-differential equation model describing the coupling between the curvature-dependent diffusion of membrane-bound molecules, and feedback onto membrane curvature through the modulation of actin dynamics by those molecules. Two distinct behaviors were observed, depending on whether the regulatory proteins favoured regions of positive (i.e. outward-bulging) or negative (i.e. inward) curvature. For positive-curvature-preferring regulatory proteins, small positive curvature fluctuations in the cell membrane tended to be amplified by the attraction of regulatory proteins to those regions (Fig. 14.4a), and the subsequent local promotion of actin polymerization. Depending on the relationships between the rate of diffusion of the regulatory proteins, the rate at which they promote actin polymerization, and the membrane tension, this amplification effect could lead to dynamic instabilities, in which the bump would continue to grow, suggesting a possible mechanism for the formation

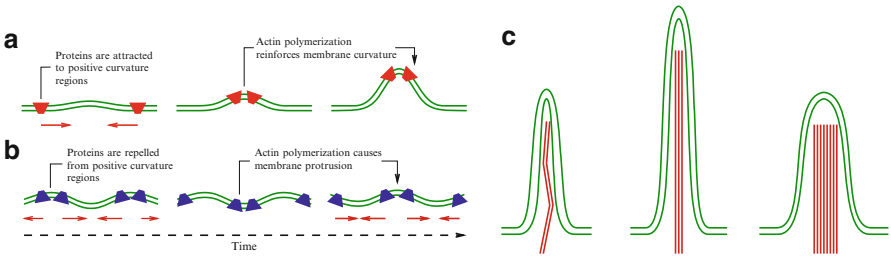

Fig. 14.4 Formation and growth limits to filopodia: (**a**) Gov and Gopinathan (2006) suggest that a positive feedback loop involving the accumulation of membrane-bound molecules (here shown in *red*) that prefer positive curvature and promote actin polymerization in local pockets of positive curvature might lead to the formation of filopodia. (**b**) In contrast, traveling waves may occur when these membrane-bound proteins (now in *blue*) prefer regions of negative curvature. (**c**) When there are too few actin filaments, tension causes a filopodium to buckle and collapse. In constrast, when there are too many, g-actin cannot be delivered to the filopodium tip fast enough to overcome depolymerization (Figures **a** and **b** adapted from Gov and Gopinathan 2006)

of filopodia. In contrast, when the regulatory proteins prefer regions of negative curvature, traveling waves of actin polymerization resulted, reminiscent of dynamics observed in lamellipodial structures (Fig. 14.4b).

Given that a filopodium has begun to form, it is of interest to know how far and how fast it can grow (thus potentially limiting the sensory range of a growth cone), and how it might interact with other nearby filopodia. Atilgan et al. (2006) used energy-minimisation arguments to demonstrate that at least two bundled actin filaments are required to overcome membrane elasticity and initiate filopodium growth, and that membrane deformation induces an effective attractive force between nearby filopodia. Mogilner and Rubinstein (2005) showed that filopodial length is limited on the one hand by the number of filaments in the cross-section of the actin fibre bundle (i.e. its strength), and on the other by the rate at which unpolymerized actin can be delivered to the filopodium tip by diffusion. If the bundle contains too few filaments, it buckles under strain from the membrane (Fig 14.4c, left). However, if it contains too many, then the rate at which new actin must be delivered to the tip is higher than can be achieved by diffusion, limiting the ultimate length of the filopodium (Fig. 14.4c, right).

14.2.1.3 The Generation of Traction Force/Growth Cone Advance

Concurrently with actin polymerization at the growth cone periphery, the actin cytoskeleton is withdrawn towards the central region of the growth cone, probably through the combination of myosin action and pressure from the membrane, where it depolymerizes (Medeiros et al. 2006). Free G-actin moves to the leading edge of the growth cone either through diffusion or active transport, where it is again incorporated into the F-actin cytoskeleton. This cycle of actin polymerization and cell membrane extension at the leading edge, retrograde actin flow, and actin depolymerization in the central region acts as an engine that can be harnessed to

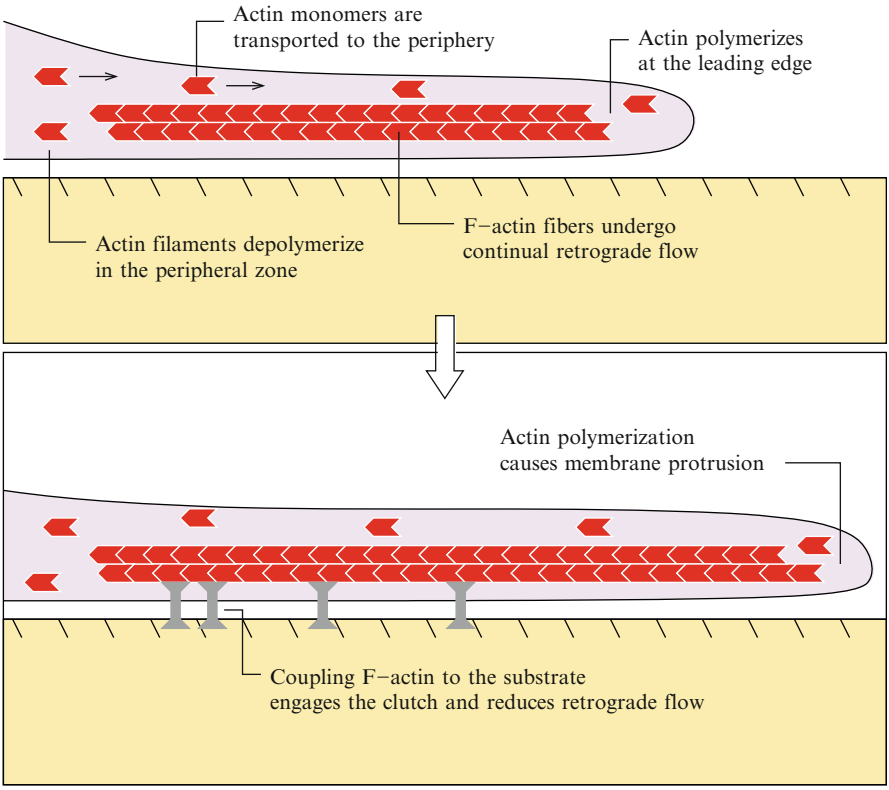

Fig. 14.5 The actin treadmill and molecular clutch: In the *top panel*, actin fibers within the growth cone are uncoupled from the substrate. They undergo continual retrograde flow, depolymerization in the peripheral zone (*left* of the figure), and polymerization at the leading edge. Coupling the F-actin to the substrate (*lower panel*) reduces the rate of retrograde flow, allowing polymerization at the leading edge to push the membrane forward

provide motility (Suter and Forscher 2000). However, in order to provide forward movement, and not to simply cycle on the spot, the actin cytoskeleton must be coupled to a permissive substrate. The idea that growth cones can regulate their motion by modulating this coupling has been called a "clutch mechanism", and provides a useful conceptual framework for analysing growth cone motility (Fig. 14.5). For an excellent review of models of actin-treadmill-driven motility (not specifically focussed on growth cones), see Mogilner (2009).

Microtubules also play a crucial role in growth cone motility (Gordon-Weeks 2004). They form a thick bundle in the neurite shaft that extends from the cell body to the growth cone. This bundle provides stability for the growing neurite, and acts as a scaffold along which materials from the cell body may be transported to the growth cone. Microtubules penetrate into the central region of the growth cone, sometimes remaining bundled, at other times splaying out to explore the boundary between the central and peripheral domains. Within the

growth cone, the microtubules demonstrate "dynamic instability" (Mitchison and Kirschner 1984), rapidly growing and shrinking, probing the peripheral region. A key event in growth cone motility is the capture and subsequent stabilization of microtubules by bundles of F-actin, which typically correlates with a reduction in F-actin flow in the capturing filopodium. The rest of the growth cone tends to shrink around the stabilized microtubule/F-actin core; ultimately, the invaded filopodium then forms another growth cone.

Hely and Willshaw (1998) developed a pair of models, one addressing the role of interactions between microtubules in governing their dynamics, and the other examining microtubule invasion of the peripheral zone. Isolated, individual microtubules tend to switch stochastically between phases of rapid growth or rapid shrinkage. Hely and Willshaw (1998) argued that the rate at which these switches occur in bundled microtubules in the growth cone is modulated by two effects: a backward pressure from the actin cytoskeleton which tends to promote shrinkage, and crosslinking between neighboring microtubules which tends to enhance stability and thus promote growth. The authors accounted for these effects phenomenologically by assuming that microtubules near the rear of the bundle were both "shielded" from actin pressure by, and stabilized by cross-linking with, the longer microtubules. Simulations showed that when these effects were sufficiently strong, a microtubule bundle could grow even under conditions when individual microtubule filaments should display net shrinkage, thus demonstrating the important role of interactions between microtubules in governing their behavior.

Permissive contact between a growth cone and a target cell leads to a reduction in the rate of retrograde actin flow along the axis connecting the growth cone to the cell (Lin and Forscher 1995). Based on this observation, Hely and Willshaw (1998) analyzed the degree to which such a reduction was sufficient to enhance microtubule invasion into the region of the peripheral zone closest to the target cell. In this model, the authors assumed that microtubules within the growth cone extended in random directions, undergoing dynamic instability with the probability of switching depending on the local rate of actin flow and the proximity to other microtubules. However, they found that even under unrealistically favourable conditions, very few microtubules invaded the target region of the peripheral zone, suggesting that microtubules are actively directed within the growth cone, rather than relying solely on random search.

14.2.2 Models of Guidance

The growth cone has a sensory task in addition to, and closely associated with, its motor task of extending the axon: detecting and responding to chemical and mechanical cues in its environment in order to guide axon growth. Our understanding of axon guidance has undergone an explosion in the last few decades, thanks to the discovery and cloning of many of the proteins involved; in particular, the "guidance cue" molecules (Tessier-Lavigne and Goodman 1996). Growth cones

detect these cues through specialized chemical receptors expressed on their surface. Interactions between these receptors and the guidance cues lead to changes in conformation of the receptors, and subsequent downstream signalling (Lauffenburger and Linderman 1993). If the cue is expressed in a graded manner, then this downstream signalling is in turn asymmetric across the growth cone's spatial extent, with the potential to regulate asymmetric remodeling of the cytoskeleton.

Often, guidance cues are highly expressed on the surface of individual cells. In this case, the contact of a single filopodium with such a cell can completely reorient the growth cone (O'Connor et al. 1990). The problem the growth cone faces in this situation is thus that of searching out such targets. A few models have addressed how this search might be undertaken efficiently. Buettner (1996) studied how the parameters governing filopodial initiation, growth and eventual collapse influenced the probability of contacting a single such cell. Taking a more abstract approach, Maskery et al. (2004) investigated the interplay between deterministic and random growth cone behavior in searching for, and responding to, a guidance cue expressing cell. They found that there was an optimal balance between the two types of behavior, in which the growth cone could both efficiently search (requiring a degree of random wandering), and effectively respond (requiring a deterministic component) to such localized cues.

Guidance cues can also be present in long-range gradients, potentially produced by the diffusion of secreted molecules from a localized source or graded expression in the substrate (Dickson 2002). One challenge we face in understanding how growth cones respond to such gradients is that the growth cone's sensory system is inherently noisy: receptor-ligand interactions are stochastic events (Bialek and Setayeshgar 2005; Mortimer et al. 2009; 2010a), receptor signalling involves the addition of further noise (Ueda and Shibata 2007; Mortimer et al. 2010a), and the gradient itself will be subject to thermal fluctuations further degrading the signal (Bialek and Setayeshgar 2005). Furthermore, if the gradient is to provide guidance over an appreciable range, it cannot be too steep as the growth cone can only effectively respond to the graded signal within a reasonably narrow range of concentrations (Mortimer et al. 2009).

How can growth cones detect and respond to shallow gradients reliably given their noisy sensory apparatus? In addition to models applied directly to growth cones, models of other gradient sensing cells are also likely to be of use in understanding this phenomenon. In the interests of focus and brevity, we will constrain ourselves to models dealing specifically with growth cones, but provide further references to related modeling work in other systems (see e.g. Bialek and Setayeshgar 2005; Ueda and Shibata 2007; Herzmark et al. 2007; Endres and Wingreen 2008). Two methods by which this problem has been attacked are: directly modeling the molecules or mechanisms thought to be involved in the growth cone response; and, seeking to understand the limits to gradient sensing imposed by noise in the growth cone's sensory systems. We give examples of each of these model classes in the following sections.

14.2.2.1 Mechanistic Models

Many molecular mechanisms are involved in growth cone guidance. A variety of guidance cues and their cognate receptors have been identified and these in turn act through a range of internal signalling pathways. These pathways include calcium signalling (both from intracellular and extracellular sources), active redistribution of receptors, cyclic AMP and cyclic GMP pathways, the phosphatidylinositol pathway, MAP Kinases, the rho-GTPase pathways, the directed transport of vesicles for subsequent exocytosis (and potential autocrine signalling) and even asymmetrically localized protein synthesis within the growth cone (reviewed in Zheng and Poo 2007; Mortimer et al. 2008; Lowery and van Vactor 2009). Although no models currently exist incorporating all of these mechanisms, models have been developed which attempt to capture some subset of them. More recently, advances in experimental techniques allowing for visualization and quantification of protein levels in different regions of the growth cone have provided inspiration for progressively more complete models (see Chap. 3 for a discussion of modelling signalling pathways in general).

An early model in this vein was developed by Aeschlimann and Tettoni (2001). Their aim was to obtain a biophysically plausible model of growth cone movement and neurite extension that could at least roughly mimic experimentally observed behavior. In their model, filopodia took on a central role, as both the sensory organs and primary motor units of the growth cone. Each filopodium was assumed to produce a small pulling force in the direction in which it extended. Depending on the size of the net force generated in this manner, the distal axon segment was assumed to stretch or, if the force was above some threshold, lengthen through inelastic extension. The growth cone was able to respond to external guidance cues via calcium signals produced at the bases of filopodia: through an unspecified mechanism, contact of a filopodium with an external cue would lead to the opening of calcium channels at base of the filopodium, thus producing an influx of calcium. Calcium dynamics were modeled through standard diffusion equations. Modeling calcium concentration provided a link between the sensory and motor systems, as the probability of initiating a filopodium at a given angular location was determined by the calcium concentration at that location. With this model, the authors were able to qualitatively reproduce the kinds of trajectories traced out by growth cones in the presence of a gradient formed by diffusion from a point source.

In Goodhill et al. (2004), filopodia again played a central role in both the sensory and motor behavior of the modeled growth cone. As with Aeschlimann and Tettoni (2001), filopodia were assumed to be the driving force of growth cone motility, though the relationship between filopodial force and growth cone movement was modeled implicitly. At each timestep, the growth direction was determined by taking a weighted average of the direction of net filopodial force and the current direction of growth. Again, in this model, the direction of filopodial initiation was assumed to be biased by external cues. A key issue that this model tackled was that direct proportionality between external concentration and the probability of filopodial initiation is not sufficient to produce a turning response to shallow

external gradients. Rather, some degree of internal amplification of the external gradient must be performed, sharpening the distribution of filopodial initiation. The performance of the growth cone in responding to the gradient was found to depend on the strength of this amplification. The model also predicted that the dependence of gradient sensing performance on concentration would be different for attractive, as opposed to repulsive, gradients—a prediction that has not yet been tested.

Xu et al. (2005) presented a related model, in which at each timestep, the growth cone's movement was calculated by averaging its current heading with an estimate of the gradient direction determined by input from surface receptors. In contrast to Goodhill et al. (2004), these authors focussed on the role of temporal and spatial averaging of receptor generated signals, rather than on force transduction through filopodia. They directly simulated a random-walk based model, in which growth cone response to a gradient is mediated through the production of a second messenger by bound receptors. Temporal and spatial averaging occurred through the dynamics of decay and diffusion of the second messenger. The authors fitted their model to a corpus of experimental data (Rosoff et al. 2004), finding that a good fit could be obtained when the diffusion rate and decay time of the second messenger were such that signals diffused over roughly a third of the growth cone, over a lifetime of about 3 min.

The observation that, preceding growth cone turning, bound GABA receptors are actively trafficked to the up-gradient side of a growth cone exposed to a gradient of GABA (Bouzigues et al. 2007) has inspired two recent models. Causin and Facchetti's (2009) assumed that a similar mechanism might play a role in growth cone response to netrin, through the DCC receptor. Netrin-DCC binding was assumed to drive the activation of L-type calcium channels (LCCs); these activated LCCs then indirectly recruited bound DCC receptors through an unspecified mechanism, modelled as a convective force proportional to the vector gradient of calcium channel activation. The authors incorporated these mechanisms into a partial differential equation model, with the aim of showing that they were sufficient to produce an asymmetric distribution of receptors in the presence of an external gradient. A crucial parameter of their model turned out to be the strength of the coupling between the gradient of activated LCCs and the transport of DCC receptors: a stable asymmetric distribution of DCC was only achieved when the coupling strength exceeded a threshold dependent on the diffusion rate of the receptors, and the degree of amplification in the signalling cascade leading from netrin-DCC binding to channel activation. Bouzigues et al. (2010) developed an explicit stochastic partial differential equation model linking the transport of bound GABA receptors to microtubule growth. The tip of each microtubule was assumed to exert a pull, modelled as a localised reduction in a potential energy function, on GABA receptors diffusing on the surface of the growth cone. The microtubules themselves were assumed to be biased in their growth by the distribution of activated GABA receptors: as with Causin and Facchetti's (2009) model, this leads to a positive feedback loop by which localised receptor activation leads to receptor recruitment. The receptor diffusion coefficient again emerged as an important

parameter in this model: large values tended to reduce the time taken for an asymmetry to form, but tended also to reduce the strength of the asymmetry for a given gradient strength.

14.2.2.2 Abstract Models

Goodhill and Urbach (1999) and Mortimer et al. (2009) sought to understand the limitations imposed by noise to a growth cone's gradient sensing abilities. Following the seminal work of Berg and Purcell (1977), these studies argued that the perception of a guidance cue gradient by a growth cone is essentially a problem of signal estimation: on the basis of noisy measurements from its receptors, the growth cone must estimate the direction of the external gradient (for a more general discussion of the role of noise in nervous system function see Chap. 8). The reliability with which such estimates can be made is ultimately limited by the steepness of the gradient, the time the growth cone has to make its decision and the amount of noise associated with measuring concentration via the binding of receptors.

Goodhill and Urbach (1999) directly applied the results of Berg and Purcell (1977) to analyze two methods by which a growth cone might detect and respond to a gradient: "temporal" and "spatial" gradient sensing. Under a temporal gradient sensing strategy, the growth cone is assumed to measure the concentration at a given point in space, then move to a nearby point and again measure the concentration. Comparing these two measurements then gives the growth cone some idea of whether it is tending to move up, or down, the gradient. In contrast, under a spatial strategy, concentration measurements at two different spatially-separated points on the growth cone itself are compared. In each of these cases, the ability of the growth cone to detect the gradient is limited by the difference in concentration ΔC between the two measurement points (determined by the distance between the points, and the steepness of the gradient), and the noise associated with concentration measurement σ. This latter value is determined by quantities such as the diffusivity of the guidance cue and the number of receptors involved in making the measurement. In the case of a temporal sensing mechanism, all receptors on the growth cone can be used to measure the concentration at both of the measurement points. However, for the spatial sensing strategy, at most only half of the receptors can be used at each of the measurement locations.

Mortimer et al. (2009, 2010b, 2011) extended these ideas, and directly compared the results with experimental data on growth cone chemotaxis. In this study, it was assumed that growth cones implement some form of spatial strategy, and techniques from statistical decision theory were used to determine the optimal form for this strategy. Mortimer et al. (2009) constructed a statistical model of the probability of observing a particular pattern of receptor binding under any given gradient conditions. They then applied Bayes' theorem to invert this model in order to obtain the gradient conditions with the highest probability of causing an observed pattern of receptor binding. Mathematical analysis of this strategy revealed that a growth cone's performance in a gradient sensing task should be proportional to the gradient steepness, and depend in a defined way on the background concentration. The

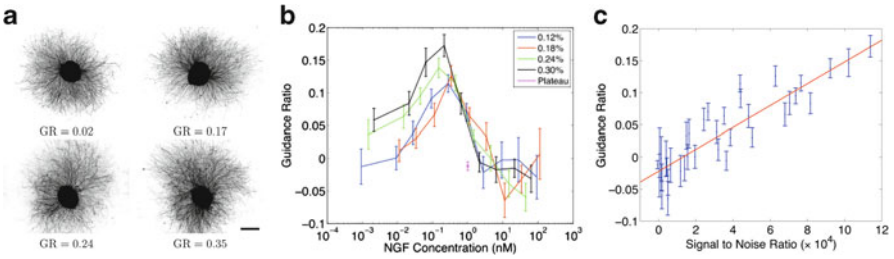

Fig. 14.6 A Bayesian model of axon guidance is consistent with guided growth of rat dorsal root ganglion neurites: (**a**) Example images of rat dorsal root ganglion explants, illustrating the "guidance ratio" (GR), which quantifies the degree of asymmetric growth. In these examples, explants were exposed to an upwards-pointing gradient of nerve growth factor. (**b**) The magnitude of the guidance response depends on the steepness of the gradient (expressed as the percentage change in concentration across 10 μm) and average background concentration. Guided growth tends to increase with increasing steepness, and to be biphasic in concentration. The model in Mortimer et al. (2009) predicts a linear relationship between the guidance ratio, and a "signal-to-noise ratio" which depends on the background concentration and gradient steepness. This relationship is demonstrated in (**c**), based on the data in (**b**). Error bars are SEMs

response of rat dorsal root ganglion neurons to gradients of nerve growth factor (NGF) displayed performance consistent with this prediction over a large range of gradient conditions (Fig. 14.6). Surprisingly, however, the authors were unable to detect any tendency for neurites to correct their direction of growth by turning in the direction of the gradient (Mortimer et al. 2010b). Rather, the results were consistent with a model in which neurites modulate their growth rate in response to the gradient, speeding up when heading toward higher concentrations of NGF, and slowing down when heading toward lower concentrations. A possible benefit of this kind of strategy when responding to shallow gradients is that concentration comparisons might be made over (for example) the length of the axon, rather than being confined to either side of the growth cone, thus increasing any observed differences in concentration.

14.3 Models of the Entire Axon

Axons often display not one, but multiple growing branches each with its own growth cone. This raises the possibility that axon guidance should rightly be considered a behavior of the entire axon, rather than of the growth cone. For example, it might be that axon branches that are detected to be growing in a good direction are favoured in their growth at the expense of other, less optimal branches. This idea along with early observations that implicated a form of competition between branches of the same neurite (reviewed by Smalheiser and Crain 1984) has influenced several modeling studies, which investigated the potential effects of such competition, along with the mechanism by which it might be mediated.

Li et al. (1992, 1995) demonstrated that competition between branches can lead to a sharpened response to a weak environmental signal. They modeled the growth of branching neurites from a single neuron in the presence of either a smooth, linear gradient of an external factor, or placed on a corridor of a high concentration of factor, surrounded by an environment of low concentrations. In both of these models, the growth rate of a neurite was determined in part by the concentration of factor local to the growth cone, and in part by inhibition from the other neurites. The degree to which two neurites inhibited one another was assumed to depend on the number of branch points separating them, so that neurites which are more distant had less influence than neurites that are closer. As a result, neurites at lower concentrations of growth factor tended to face more inhibition (as well as slower intrinsic growth) than neurites at high concentrations. Thus, the relative effect of any asymmetry in the environment on neurite growth rate was amplified.

How might competition between neurite branches be mediated? Neurite elongation tends to occur at the growth cone. However, the raw materials to support this growth are largely manufactured in the cell body, and must be transported to the growth cone to be incorporated into the growing axon as it extends. This suggests one way in which such competition might occur: through competition of individual axon branches for resources to support their continued growth.

Tubulin is a strong candidate for such a resource. Microtubules (polymerized tubulin) form the backbone of neurites. Tubulin polymerizes and is incorporated into the cytoskeleton predominantly at the growth cone, but it is manufactured in the cell body. Several models have thus focused on the dynamics of tubulin transport and assembly, and the role of competition between different neurites for tubulin. Van Veen and Van Pelt (1994) developed an ODE model of tubulin transport and polymerization in neurite growth, examining the results for an unbranched neurite, a neurite with one branch, and for neurons with complex arbors. They assumed that tubulin was synthesized in the soma at a constant rate, was transported to the neurite tips through diffusion (active transport was judged to be insignificant), and that the rate of neurite growth was directly proportional to the rate of tubulin polymerization at the growth cone. For a single neurite, they found that tubulin concentration in the growth cone reached a constant value, while the neurite length and the tubulin concentration in the soma increased linearly with time. In contrast when multiple neurites were involved, they found that competition occurred depending on the local rates of tubulin polymerization in the growth cones. Despite the simplicity of this model, it was able to reproduce a number of experimental results: that growing neurites tend to extend at a constant rate, that individual neurites in an arbor occasionally retracted in favour of other growing neurites, and that dormant growth cones would occasionally activate and begin growing some time after their formation.

Van Veen and Van Pelt (1994) made the simplification of directly modeling tubulin concentrations only at branch points, the soma and in the growth cones, treating diffusion along neurite segments at steady-state. Clearing the way for further studies, Graham and van Ooyen (2001) extended the compartmental models commonly used to study the propagation of electrical activity in mature neurons to

the case of axonal development, in which neuronal morphology can change over time. They highlighted the challenges associated with developing such models: in particular, that large artificial transients can arise depending on choices made in how the system is "recompartmentalized" as it grows (see also Kiddie et al. 2005).

McLean et al. (2004) and Graham et al. (2006) examined the dynamics of tubulin transport and polymerization in more detail for the growth of a single neurite. They employed a partial differential-equation approach, allowing them to model how tubulin concentration varies along the length of the axon. This model included both active and diffusive tubulin transport, along with tubulin degradation, and simulations demonstrated non-trivial interactions between the three mechanisms. When the rate of tubulin degradation was set to zero, the results of Van Veen and Van Pelt (1994) were reproduced: namely, the neurite grew at a constant rate due to a constant tubulin concentration in the growth cone, with tubulin concentration in the soma increasing linearly with time. These results were independent of whether active transport, diffusion or a combination of both transport mechanisms were included (though the shape of the tubulin concentration gradient along the length of the neurite was affected by the choice of transport mechanism). However, when tubulin degradation was included, the neurite no longer grew indefinitely: rather, it eventually reached an equilibrium length which depended on the rates of transport, degradation and tubulin production. Graham et al. (2006) studied the sensitivity of this final equilibrium length to other parameters of the model, with the particular aim of understanding how easily neurite length could be regulated by a cell. They found that for short neurites, the length was essentially insensitive to variations in the rate of active transport, while long neurites showed insensitivity to the diffusion rate.

Despite the theoretical advantages of competition between neurites from the same neuron, there is experimental evidence suggesting that such competition does not, in fact, occur (Lamoureux et al. 1998). This suggests that the cell must actively regulate the production of key cytoskeletal molecules (such as tubulin) in order to support uniform growth independent of the number of neurite tips. First steps towards understanding self-regulation of tubulin production were taken by Graham et al. (2006), though only in the context of a single growing neurite; future extensions of this model to include multiple branches may shed light on how competition between branches is avoided.

14.4 Guidance at the Systems Level

Modeling the guidance of neuronal projections involving sometimes large numbers of axons adds another layer of complexity to considerations for individual growth cones and/or axons. Within projections, axons may provide scaffolds for each other, compete with each other, and interact in other more complex ways. A key step in understanding the behavior and targeting of neural projections is to focus on the interactions between growing axons and/or growth cones. Insights gained from this can then be combined with knowledge of individual axon and growth cone guidance.

Modeling the targeting of large numbers of axons has been undertaken within a number of biological contexts; such as within specific parts of the spinal cord and brain, or more abstractly, such as the development of sheets of neurons or neural networks. One of the most useful paradigms for studying this problem is the development of retinotopic maps; the primary examples being retinotectal/retinocollicular maps (from retina to midbrain) or retinogeniculocortical maps (maps from retina to thalamus to visual cortex). Of these two, the influences on neuronal guidance have been better established for retinotectal maps, an area which has also received a large amount of theoretical attention. As such, focusing on the retinotectal map gives us a broad spectrum of modeling to discuss with a minimum of background.

14.4.1 Background: The Retinotectal Map

Neural connections between the retina in the eye and the visual centers of the brain are referred to as visual maps. They are described as topographic, or retinotopic, if the spatial relationships between cells in the retina are preserved in their pattern of termination elsewhere in the brain. The retinotectal (also retinocollicular) map is the neural representation of visual space formed by projections arising in the retina (from the retinal ganglion cells or RGCs) and terminating in the midbrain (more specifically, the target is the optic tectum in fish, frogs and other lower vertebrates; and the superior colliculus/SC in mammals) (Fig. 14.7).

A number of different mechanisms have been postulated to play roles in retinotectal map development. The most important of these are as follows:

- Chemoaffinity: The idea, proposed by Sperry (1963), that gradients of molecular markers can be used to specify axial positions, and thus one or more in each of two areas is sufficient to specify a topographic map between these areas.
- Competition and other axon–axon interactions: Competition between axons is usually for a limiting resource, such as target space, neurotrophins, or synaptic input. Axons can also exert a range of other influences on each other in addition to competition.
- Branching: Multiple interacting agents are generated by branching processes, and the interactions of branches with each other and with molecular cues can effect different forms of guidance. Types of branching include:
  - Growth cone splitting: bifurcation of the leading edge of a growing axon.
  - Backbranching: Branching, usually associated with retraction of the primary axon, that occurs just proximal to the growth cone.
  - Interstitial branching: branching that may occur anywhere along the entire length of the axon, usually at right angles (or nearly so) to the main axon shaft.

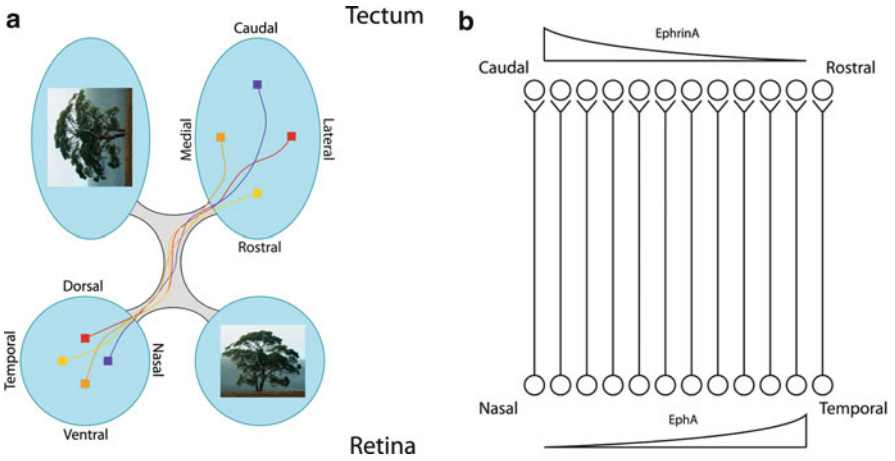

Fig. 14.7 The retinotectal map: a paradigm example of topographic map development. During development, the retinal ganglion cells (RGCs)—the output layer of the retina—send out axonal projections to the midbrain tectum. If two cells are close to each other in the retina, their terminations are similarly close in the tectum. In this way, an image of visual space is transmitted faithfully to the brain. (**a**) Nasal retina maps to caudal tectum, and temporal retina maps to rostral tectum. Similarly, dorsal retina maps to lateral tectum, and ventral retina maps to medial tectum. (**b**) The patterns of terminations are in part achieved by the use of chemoaffinity gradients; i.e. gradients of molecules that can specify unique positions along the gradient axis by unique levels of that molecule. In this example we show one axis of the retinotectal map: that of nasotemporal retina to caudorostral tectum. A row of cells is depicted in the retina, with positions identified with EphA receptor level. Similarly a row of tectal postsynaptic cells is shown marked by ephrinA ligands. EphA-bearing cells are typically repelled by ephrinA ligands, so that here high EphA maps to low ephrinA and vice versa. (Note this is a simplified example, in that gradients of EphA receptor are also present in the tectum, and similarly gradients of ephrinA ligand are present in the retina.) The dorsoventral to mediolateral axis is controlled in an analogous way by EphB-ephrinB interactions, although this interaction is attractive rather than repulsive (not pictured)

- Marker induction/regulation: It has been hypothesized that ingrowing axons can upregulate various molecular cues in the target which can in turn influence the guidance of the ingrowing axons (Willshaw and von der Malsburg 1979).
- Neural activity/synaptic modulation: Neural activity can change the strength of synaptic connections between neurons, and hence can have a range of effects on reforming neuronal projections; but plays more of a refining rather than defining role in retinotectal mapping by increasing the precision of maps generated by the above mechanisms. We do not consider models of neural activity in this section.

A large amount of data has been gathered over more then 50 years on retinotectal map development, which can be summarized as follows:

- Systems manipulations: In the 1940s–1980s, before roles for specific molecules were identified, gross anatomical manipulations of the retina and/or tectum were performed, including ablations and surgical grafting (Udin and Fawcett 1988).

- Eph/ephrins: Ephrin ligands and their receptors, the Eph receptors, were implicated in retinotectal maps by a number of studies performed in the 1990s (McLaughlin and O'Leary 2005). Distributed in appropriate gradients in retina and tectum/SC in many model systems, they are the best-known candidates for Sperry's chemoaffinity gradients (Fig. 14.7) although other molecules are known to also play roles.
- Molecular-genetic manipulations: disruptions of Eph/ephrin gradients have been performed and display stereotypical and largely understood patterns of map abnormalities, further implicating them in mapping.

Many of the above mechanisms and molecules are not unique to the retinotectal system, and have also been implicated in the development of other brain regions (e.g. Wilkinson 2001; Poliakov et al. 2004). Hence many concepts from retinotectal map development may be generalizable not only to other topographic maps in the brain, but also to brain wiring in general. Elucidating how these relatively simple patterns form can thus substantially contribute to our understanding of strategies that the CNS uses to wire itself up.

14.4.2 Approaches to Modeling the Guidance of Multiple Axons

The retinotectal system has been a target for computational models since the 1970s, and generations of models of map development have generally followed generations of experiments. Instead of analyzing one of the many models in great detail, or all models only briefly, we will focus on specific illuminating examples of problems that arise in modeling the retinotectal system, and the methods used to approach these problems.

Terminology used to describe topographic map models and development varies from model to model, and also over time. We refer to 'projections' of neurons and their axons as arising from an origin and projecting to a target. Origin and target can be either discrete or continuous, but usually the origin is a discrete array and the target is a discrete lattice or continuous sheet. We refer to individual members of an array as growth cones, axons, or branches, or generally as 'agents'.

14.4.2.1 Competition in the Development of Targeted Neural Populations

Competition is a central concept in developmental neuroscience, as it is in biology in general (see van Ooyen 2001 for a review). Competition describes an interaction whereby multiple agents seek to exclusively control a limiting resource. In axonal growth and targeting the most common applications of this idea are competition for physical space, for neurotrophins/growth factors, or for synaptic input/activity.

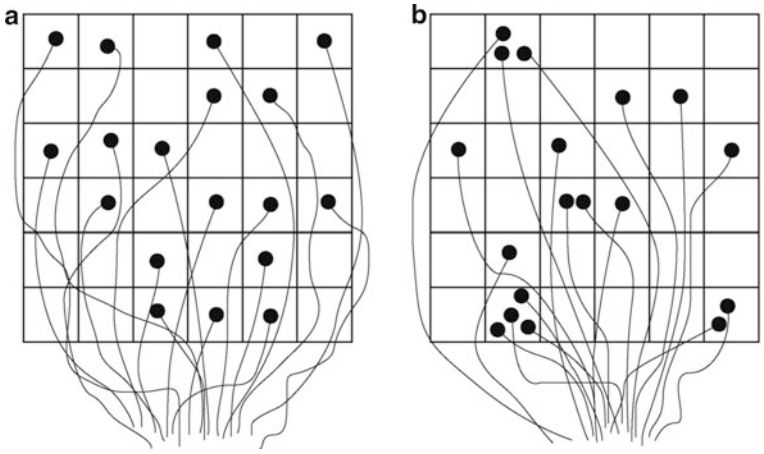

Fig. 14.8 Competition through synaptic normalization. Two 6×6 arrays representing schematized postsynaptic sites on the target are shown. Eighteen RGCs, half the number of available lattice points, are depicted projecting to each array. Axons are shown as projecting from bottom of picture and their terminations are represented by *black discs* on the target. (**a**) A one-to-one mapping is enforced, so that axons compete for space, and as a result, spread out over more of the target. (**b**) The number of contacts that can be made with each lattice point is now not fixed, and there is no competition, and in this case, less coverage of the target

A straightforward way to include competition in models is to use a discrete mapping model where contacts between the projecting array and the targeted array are one-to-one (Fig. 14.8). This models a competition for space or synaptic contacts, as only one axon can occupy each lattice point at a given time. The term synaptic normalization is often applied to models that limit pre- and post-synaptic cells to one synaptic contact each, although it can also be used to refer to cases where each can make a small fixed number of contacts, greater than one. Examples of this type of model are Hope et al. (1976) and Koulakov and Tsigankov (2004). Although this is simple to implement in models, it may also oversimplify (it neglects the potential of multiple inputs/outputs to single agents), and that the discrete nature of the target array may lead to unrealistic axon/growth cone motion, as axons hop from lattice point to lattice point (though this latter issue can be mitigated by increasing the density of lattice points).

A more realistic approach is to allow a small fixed number of contacts (greater than one) to be made with each lattice point by axons. An early exploration of this idea was carried out by Prestige and Willshaw (1975). These authors considered a mapping between discrete arrays and showed that when the number of contacts between arrays was unlimited, a more rigid type of chemoaffinity assumption needed to be made, whereas if the number of contacts was fixed, simpler chemoaffinity assumptions could be made. This model demonstrated how different forms of chemoaffinity could lead to topography depending on competitive constraints, and has been influential in our understanding of how these mechanisms

interact in map formation. A number of models have subsequently used similar normalization constraints (for contacts or synapses), e.g. Willshaw and von der Malsburg (1979), Weber et al. (1997), van Ooyen and Willshaw (2000), and Willshaw (2006).

In a continuous domain, competition for space can be enacted by considering growth cones to behave like similarly charged particles, such that two nearby growth cones experience increasing mutual repulsion as they get closer together. This approach was taken in the XBAM (eXtended Branch Arrow Model) model of Overton and Arbib (1982), and in an updated version of this model by Simpson and Goodhill (2011). In these models, the repulsive interaction was limited to a small area around the growth cone within which it could reasonably explore (and hence interact with other growth cones).

Alternatively competition can be considered to be a type of smoothing mechanism for a population of interacting neurons. Honda (2003) added this type of constraint to his model, whereby initially a map was set up using chemoaffinity rules, and then a smoothing process was undertaken in which axons were moved from areas of higher density to areas of lower density. This kind of algorithm promotes uniformity or smoothness of the map.

14.4.2.2 Axon–Axon Interactions as a Sorting Process

An appealing strategy is to consider the ordering of topographic maps as a sorting process. An example of this is the work of Hope et al. (1976), who considered the movements of discrete axons on a lattice. It was assumed that a pair of interacting axons could compare a scalar attribute common to all axons (e.g.: some molecular label). Given the axons' relative positions and relative molecular label, the axons could opt to exchange places according to a particular rule. Although this is a relatively strong assumption from a biological point of view, it is an effective algorithm in that it can reproduce several phenomena important in the field of retinotectal mapping. This was demonstrated in simulations of normal and surgically altered development.

Overton and Arbib (1982) developed this approach further by moving to a continuous domain. In this model the positions of axons are continuously-valued, and hence more specific assumptions about the algorithm need to be made. Instead of switching places, axons experience a vector push towards or away from each other (again depending in some way on relative position and molecular label). This model produced more realistic axon growth and targeting, while maintaining the sorting process as a key feature. More recently, Simpson and Goodhill (2011) updated this model to 2D, and modified the sorting process to reflect a more realistic type of axon–axon interaction based on repulsive guidance receptor signaling.

Koulakov and Tsigankov (2004) used a lattice sorting approach similar to that of Hope et al. (1976). In this model, pairs of axons were considered iteratively, and an exchange probability calculated which depended proportionally on differences in molecular labels between the axons. This model was updated by the same

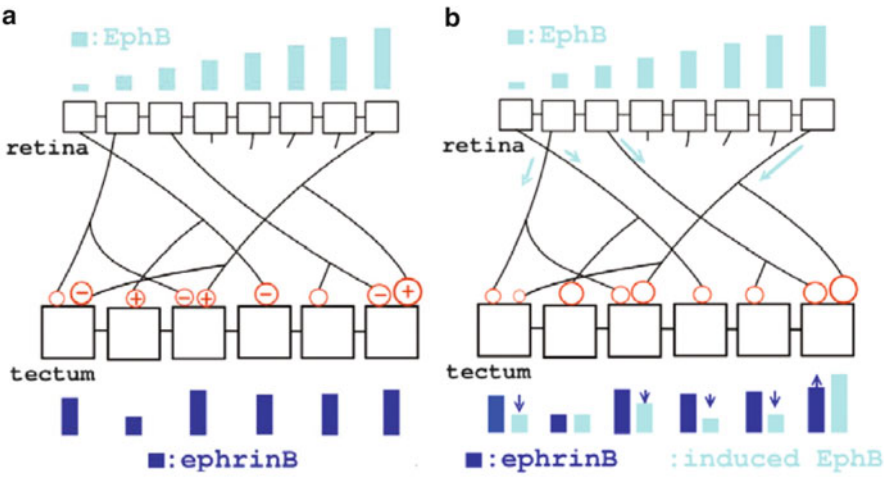

Fig. 14.9 The retinal induction mechanism. A one-dimensional mapping is depicted from retina to tectum. The dorsoventral to mediolateral axis is pictured, which is governed by EphB-ephrinB interactions. A row of cells in the retina bearing EphB receptors projects to a row of cells in the tectum bearing ephrinB ligands. One or more synapses are made by each RGC (shown in *red*). (**a**) Updating the synaptic strengths. Synapses are strengthened or weakened according to how similar the amount of EphB in the presynaptic cell is to the amount of ephrinB is in the postsynaptic cell. If the amounts are more similar, the connection is strengthened, whereas if the amounts are quite dissimilar, the connections are weakened. (**b**) Induction/regulation. Levels of EphB are up- or down-regulated in postsynaptic cells in proportion to the amount of EphB in presynaptic contacts and the strength of their respective synapses. Single postsynaptic cells may receive contributions from more than one presynaptic cell. Levels of ephrinB in the postsynaptic cells are constantly adjusted to be closer to the level of induced EphB in that cell (Figure from Willshaw (2006))

authors by adding an additional constraint involving correlated activity. This was combined with differences in molecular labels to calculate an energy for the entire map (Tsigankov and Koulakov 2006). Depending on whether this energy would be increased or decreased by the swap, a probability of the swap occurring was again calculated.

14.4.2.3 Axonal Regulatory Effects

Willshaw and von der Malsburg (1979) proposed that ingrowing axons can up- or down-regulate certain molecular markers (e.g. axon guidance molecules) in cells of the target array (Fig. 14.9). These molecules in turn influence axonal behavior, and hence axons exert indirect influence on each other through this regulatory process. Allowing axons to influence their guidance environment in this manner gave this model a rich dynamics and some unique characteristics; most notably, a 'neighbor matching' property that may be compared with models of correlated neural activity (Willshaw and Price 2003). A consequence of this property is that maps generated

by this type of scheme tend to be piece-wise continuous, but globally discontinuous. Initial conditions that specify polarity with some weak initial gradients are required for global continuity and correct overall map polarity. These ideas were explored from a theoretical point of view by Häussler and von der Malsburg (1983) and have also formed the basis for the more recent model of Willshaw (2006).

Similar indirect regulatory effects of axons were also considered by Gierer (1983) in order to explain observations that maps could expand or compress under certain circumstances and still remain topographic. Gierer (1983) proposed that ingrowing axons seek to minimize the generation of a substance p, which was generated in growth cones in response to retinal and tectal gradients of guidance cues. The default behavior for these axons was a gradient descent mechanism, towards a minimum of p; i.e. $\frac{\partial p}{\partial x} = 0$. This part of the model allowed for guidance, without axon–axon interactions. Regulation between axons was included in the following manner: each axon contributed extra substance p in a neighborhood around its current position, which other axons tended to avoid (in keeping with the assumption that axons seek to minimize this p). As functions of the tectal x-axis position and retinal u-axis position, and time t, Gierer modeled this extra contribution as $r(x,t)$, and the contribution from gradients as $g(x,u)$ (which could be chosen largely arbitrarily), so that the total p was:

$$p = g(x,u) + r(x,t) \tag{14.1}$$

$$\frac{\partial r}{\partial t} = \varepsilon\rho(x,t) \tag{14.2}$$

Here $\rho(x,t)$ is the local density of fiber terminals, and ε is a constant, so that increased densities of axons causes increased contributions to the local level of p, and hence a greater tendency to move away from this local area. This mechanism tended to smooth the mapping out and give it a target filling property.

14.4.2.4 Branching

So far our discussion has been limited to problems involving only one growth cone per axon, however mature biological neural networks are complex interconnected structures made up of highly branched nerve fibers. Branches can interact with each other intra-axonally and inter-axonally, so that branching can be considered a generator of multiple interacting agents. Systems of branching neurons can display their own unique behaviors, including pathfinding and dynamic branch regulation. The former idea has been explored by representing the addition of new branches as new contacts or synapses (Willshaw and von der Malsburg 1979; Willshaw 2006). By making the generation of new contacts more probable in areas where there are existing (successful) synapses, a kind of pathfinding or guidance is achieved. This mechanism alone might simply lead to clustering of contacts, but combined with other mechanisms (retinal induction in this case), branching of this type can help order the map.

More detailed mechanisms for patterns of branching in retinotectal axon pathfinding were considered by Gierer (1987). Growth cone splitting, back-branching, and random interstitial branching were employed, and their effect on pathfinding was examined. The main differences noted between these three cases were variations in trajectories and axonal arbor morphology. The pathfinding potential of interstitial branching was also investigated in the model of Yates et al. (2004), based on previous work showing that interstitial branching was biased by gradients of chemoaffinity-type markers/molecules (Fig. 14.10). It was shown by these authors that differential branching according to chemoaffinity-type gradients can generate topography similarly to axonal guidance models. Each axonal arbor has its own internal dynamics, in which branching depends in part on the rest of the arbor, and as such represents intra-axonal interactions between branches. Inter-axon interactions were also included by assuming that as branching occurs and arbors grow, the markers that they carry contribute to the gradients of markers already on the tectum. This added marker has additional more complex effects on arbor refinement and targeting. A similar biased branching approach was taken by Godfrey et al. (2009), but this time a different form of gradient-based chemoaffinity was employed, which was also influenced by growth factors. An additional 'resource' component was added to the model to constrain growth of axonal arbors (also influenced by growth factors), introducing a more explicit form of intra-axonal interaction to the model (See Sect. 14.3).

14.4.2.5 Molecular Mechanism Models

Some models of how growth cones use their receptors to navigate in ligand gradients also give rise to ways in which axons can interact based on similar processes. For example, the servomechanism model of Honda (1998, 2003) (based on the observations of Nakamoto et al. 1996) proposed that ingrowing axons seek a specific level of 'signal'. This signal, S, was produced in an axon i as a function of its receptor level, R_i, and local ligand level $L(\mathbf{x})$ (where $\mathbf{x}$ is a position vector). Using first order mass action kinetics this becomes $S_i(\mathbf{x}) = R_i L(\mathbf{x})$. A 'servomechanism' was proposed along with this idea such that an axon seeks to minimize the difference $\Delta S = S_{0,i} - S_i(\mathbf{x})$, where $S_{o,i}$ is a reference signal. In the first stage of the model this was simply used to move individual growth cones, but later during a smoothing stage of the model ΔS was used to compare growth cones in locally dense areas, and move ones which had higher values of ΔS. In this way growth cones interacted indirectly through this relative affinity (cf. Prestige and Willshaw 1975, which goes into more detail on types of affinity; also discussed in Goodhill and Xu 2005).

Models utilizing gradient-based chemoaffinity rules have tended to focus on interactions between guidance receptors on ingrowing RGCs and ligands in the target. However, both these sets of receptors and ligands are also present on RGC growth cones, and efforts have been made to quantify how growth cones may interact with each other based on these molecules. Such a model was suggested by Reber et al. (2004) following on from the work of Brown et al. (2000). Here it was

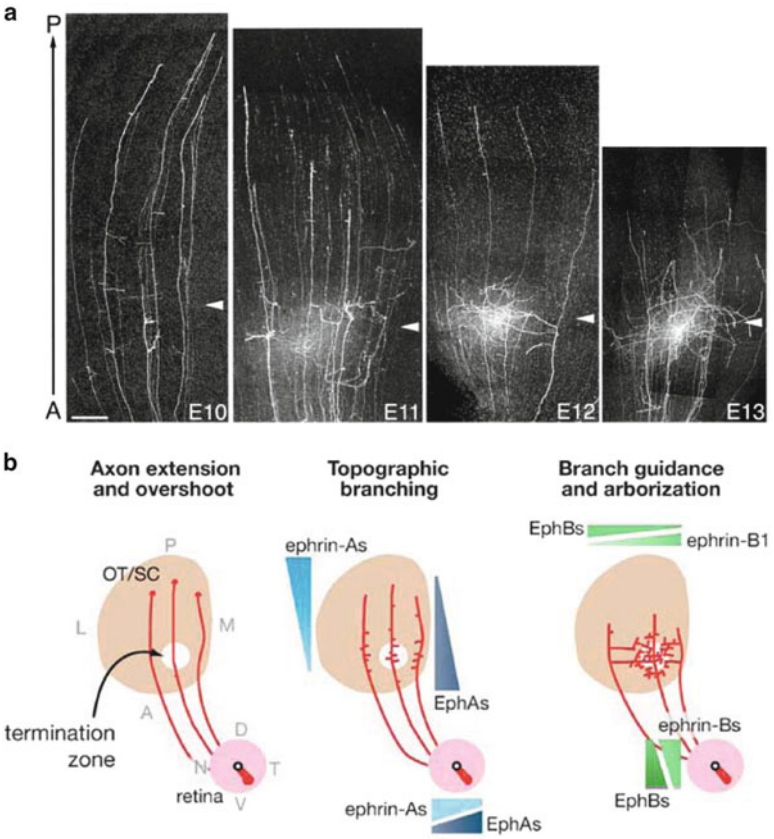

Fig. 14.10 Mechanisms of interstitial branching in retinotopic map development. (**a**) The retinotectal projection in chick optic tectum from embryonic day 10 to day 13. Vertical axis of the picture corresponds to anterior (A, or rostral) to posterior (P, or caudal) tectal axis. *Arrowhead* indicates appropriate termination zone of these RGC axons which have been labeled with a single DiI injection into temporal retina. RGC axons initially overshoot their target, then send out interstitial branches roughly at *right* angles to the primary axon centred on their target. Topographic specificity increases with time until a topographically correct termination zone is present at E13. Figure from Yates et al. (2001); scale bar 250 μm. (**b**) This process is modeled conceptually in the following manner. RGC axons initially overshoot their desired termination zone in a non-guided manner. Then interstitial branching is controlled by opposing inhibitory gradients of EphA and ephrinA in the optic tectum/superior colliculus (OT/SC), which interact with the same molecules present in the retina to determine an optimal region for branching. Then opposing gradients of EphB/ephrinB molecules control medial/lateral guidance of branches towards the termination zone. Note that in contrast to some previous examples, this model relies on there being dual gradients (of Eph receptors and ephrin ligands) present in both retina and tectum/superior colliculus (Figure from McLaughlin and O'Leary (2005))

noted that specific relationships appeared to exist between RGCs based solely on comparisons of EphA receptor level between axons. When, under the conditions of certain genetic manipulations, the receptor ratios between nearby axons fell below a certain threshold, receptor-based topographic organizing behavior was significantly altered. It remains unclear what the exact mechanisms are behind these receptor ratio comparisons but two recent studies present suggestions (Tsigankov and Koulakov 2010; Simpson and Goodhill 2011).

14.4.2.6 Integrating Multiple Constraints

A problem common to most models of retinotectal mapping that involve axon–axon interactions is how to combine information from interactions with other axons with information from environmental cues (e.g. physical cues, guidance cues, neural activity, etc.). The integration of this information may occur within the growth cone, the axon, or the whole cell including the cell body. However, the individual signal transduction pathways are often not well described, nor how each individual pathway is integrated with the others. (There are exceptions to this however, with some pathways/systems representing good candidates for modeling signal integration, such as the Rho GTPases; for example see Giniger 2002; Sakumura et al. 2005.) As a result, most of the approaches have been more abstract or phenomenological in nature, attempting to model the effects of signal integration rather than specific biophysical events in how it occurs.

A straightforward way to integrate multiple signals is the approach taken by Overton and Arbib (1982) and Simpson and Goodhill (2011). In these models, independent influences on growth cone motion are modeled as vector contributions, which are combined in a linear sum. This is obviously a simplification of growth cone signal transduction machinery, nonetheless both studies demonstrated the ability of this model to display complex behavior, including the reproduction of specific experimental observations.

Fraser and Perkel (1990) took another approach, motivated by the idea that adhesion between projecting axons and their targets may be central in regulating targeting of projections. These authors defined an ‘adhesive free energy’ which was an additive combination of contributions from other axons, from target-based adhesive markers, competition for physical space, and effects of correlated neural activity. The model considered the movement of terminal arbors subject to the above energetic constraints. At each step, arbors randomly moved to new nearby positions. The new positions were either accepted or rejected with probability dependent on the adhesive energy of the new configuration. A similar approach was employed by Tsigankov and Koulakov (2006). Independent contributions to an energy function from chemoaffinity and neural activity were combined linearly for each axon, and then these were summed to calculate a total map energy. Similar to the switching/sorting algorithm of Hope et al. (1976), pairs of axons were chosen at random, and their positions exchanged with a probability that was a function of the map energy.

Some models use the more familiar concept of synaptic modulation as a common pathway for fusing various mechanisms of axonal guidance. These models use the creation and modulation of synapses to achieve targeting and mostly do not specifically consider the movement or targeting of growth cones. A prominent example of this type of model is the series of models based on Cowan's model. The first major model in this series (Whitelaw and Cowan 1981) used an ODE to update synaptic strengths in proportion to the product of two axons' chemospecific affinity and their coincident activity. The most recent version of the model (Weber et al. 1997) takes a more typical synaptic modulation scheme; where the additive influences of gradient-based chemoaffinity cues, chemical affinity for other axons, and neural activity combine to alter synaptic strengths. In contrast, models such as Willshaw (2006) also use a form of synaptic modulation, but instead assume that it is dependent on the similarity of molecular label on the presynaptic and postsynaptic cells.

14.5 Summary

Throughout this chapter, we have discussed many of the aspects involved in the correct wiring of the axons of the nervous system. In particular, we have illustrated that axon guidance is a field of study with facets that give rise to problems on a variety of spatial and temporal scales.

- At a sub-cellular scale, the growth cone integrates sensory information, and directs axon growth. Models at this level have provided insight into the biochemical events involved in axon extension, and the processes by which cues in the embryonic environment are interpreted.
- At the cellular scale, the processes of axon extension and branching determine the neuron morphology. Models at this level have suggested a functional role for interaction between multiple branches of the same axon, and have begun to explore the machinery regulating the manufacture of the raw materials required for axon extension.
- Finally, at the systems scale, interactions between axons play an important role. Through the example of retinotectal map formation, we have discussed models incorporating such interactions. These models have demonstrated that axon–axon interactions help to make the topographic structure of the retinotectal projection robust to a range of experimental manipulations. Future work in other systems may yield similar insights.

An ultimate understanding of axon guidance would incorporate elements at each scale, and would enable us to link defects in (for example) growth cone cytoskeletal remodeling to gross defects in wiring between brain regions. Developing such an understanding is a grand challenge for the future.

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