CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

1

Introduction

What are growth cones?

Of all animal cells, neurons have the most diverse and remarkable shapes. They vary in appearance from the simple, unipolar cells of invertebrate ganglia to the highly complex pyramidal cells of the human cerebral cortex with their profusion of processes and thousands of synapses. The basis for this diversity of shape is the ability of developing neurons to produce long and branching cellular processess, of which two distinct kinds are recognised: axons and dendrites. In mature neurons, axons convey action potentials away from the neuronal cell body, or soma, to the axon terminals, whereas dendrites transmit information toward the soma. Primary sensory neurons form an exception to this general rule since they have an axon which conveys action potentials *towards* the cell body. Axons and dendrites, collectively referred to as neurites when they are growing, are necessary for neurons to carry out their primary function, in which they have no equal, that of intercellular communication. In the central nervous system (brain and spinal cord), neurons usually communicate with each other, whereas in the peripheral nervous system they also communicate with a variety of effector cells such as muscle and secretory cells. In the adult, the distance over which the neuron must sustain its cellular processes to communicate with another cell may be considerable; it is many metres in the case of pyramidal motoneurons in large mammals such as the whale.

From an evolutionary standpoint, the diversity of neuronal shape has increased as the basic function of neurons to communicate with each other and with effector cells has become more sophisticated. Diversity of shape in neurons is essentially a matter of the distribution of axons and dendrites and their branches in three-dimensional space. How do developing neurons achieve

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

this diversity? What mechanisms determine the final, adult morphology and the pattern of synaptic connections between cells? These questions are currently at the forefront of research in the neurosciences, not only because of their intrinsic interest but also because of the hope that answering them may help us to repair the damaged nervous system in adult humans, where the capacity to regenerate lost connections and damaged processes is impaired.

In the majority of animals, neurons are generated (neurogenesis) during embryogenesis or larval stages and the process is largely complete shortly after hatching, eclosion, or birth. In vertebrates, neurons originate during embryogenesis from cells that are produced in specialised regions of the ectoderm: the outer, epithelial cell layer of the three germ-layers of the vertebrate embryo. The origin of neurons in invertebrates is more complicated. In flies, such as *Drosophila*, neurons derive from the ectoderm, but in nematodes, such as *Caenorhabditis elegans*, neurons originate in a fragmented pattern throughout the worm, whereas in the leech all of the five embryonic stem cells (teloblasts) that derive from the egg, give rise to neurons (see Shankland & Macagno, 1992). In *Hydra*, neurons are generated throughout the life of the animal from multipotential interstitial stem cells.

In the embryo, neurons begin their postmitotic lives as unremarkable, non-process-bearing, rounded cells and must, therefore, extend neurites (Fig. 1.1). The extension of neurites, *neuritogenesis*, begins at the surface of the neuron as a localised, highly active membrane protrusion that develops into an entity called the growth cone (Fig. 1.1). In the simplest sense, these are motile enlargements at the tip of the extending neurite (Fig. 1.2) that were discovered and named over one hundred years ago by one of the founders of modern neurobiology, Ramón y Cajal (1890). Growth cones are present at the tips of both growing axons and dendrites. Growth cones are also formed by regenerating mature axons and dendrites when they are damaged by trauma such as crushing or cutting.

What do growth cones do and why have they attracted so much attention? Broadly speaking, growth cones have three crucial functions in the developing nervous system. First, they guide the growing neurite through the developing embryo to locate the cell with which the neuron will form a synapse. Secondly, they build the extending neurite behind them as they advance, like a spider spinning a web or the laying of a telephone cable by a cable-laying ship. Lastly, they form the pre- or postsynaptic element of the synapse. Their importance thus lies in the fact that they establish the process-bearing morphology characteristic of the neuron and that they form the correct connections between cells. In many cases, growth cones are required to navigate complicated routes through a variety of terrains – both hostile and friendly – to arrive at their destinations. Once they reach their target, their task is not over, for they must select an appropriate synaptic partner and make the transformation into a synapse.

Although growth cones have attracted considerable attention since their discovery, it is only in recent years that the appropriate technologies have

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

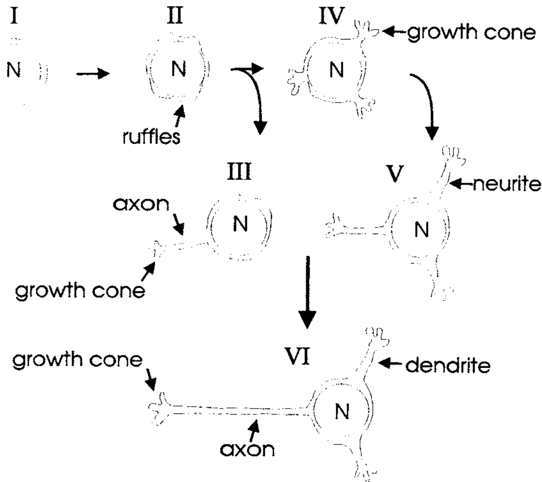

Figure 1.1 Drawings illustrating the development of a neuron. Immature neurons are small, unremarkable cells with a large nucleus (N) and a small amount of cytoplasm (I). After a period of surface membrane ruffling (II), neurons extend processes that end in growth cones (III, IV). *In vivo*, the first process to emerge is usually destined to become the axon (III) and later emerging processes become dentrites (VI), whereas *in vitro* the processes are bi-potential (neurites) and may develop into either an axon or a dendrite (V, VI).

become available to study them in detail and at a molecular level. When I first began my own research on growth cones in 1982 the number of original papers on the subject was relatively small. Now, little more than a decade and a half later, there are as many *reviews* on growth cones as those original research papers and the numbers of research papers are increasing at an astonishing rate, so much so that keeping abreast of the literature is a major undertaking.

This book attempts to bring together all that is currently known about growth cones that may help us to explain how they achieve these remarkable feats. Our ultimate aim is to describe growth cone behaviour completely. When we have achieved this, we shall have made a significant advance in understanding how the most important part of our bodies, our nervous systems, are properly formed. In addition, we may have clues as to how we can repair the damaged nervous system.

Growth cone morphology and behaviour

What do growth cones look like and how do they behave? Answering these questions has provided us with clues as to how growth cones function.

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

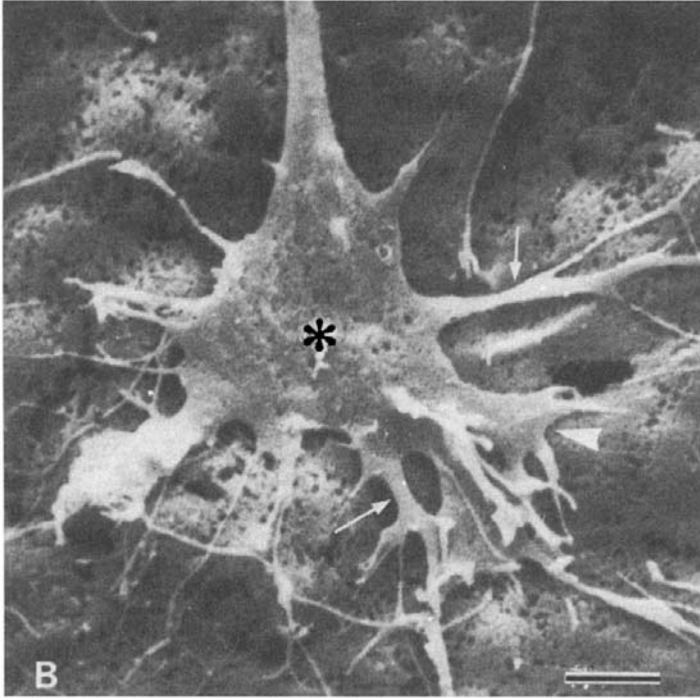

Figure 1.2 A scanning electron micrograph of a growth cone from a sensory neuron growing in the skin of a tadpole. The neurite, in this case an axon, enlarges at the growth cone (asterisk), which extends at its margins filopodia (arrows) and lamellipodia (arrowhead). In this location, the growth cone is largely two-dimensional, as growth cones in culture. The bar marker is 2 μm. (From Roberts, 1976, with permission of Company of Biologists Ltd.)

Appearance and behaviour under the light microscope

Growth cones have been observed with the light microscope in fixed and stained tissue sections of developing and regenerating neural tissue for over one hundred years. In the living state they have been observed in developing animals and in culture. The first observations of growth cones were made by Ramón y Cajal in sections of the embryonic chick spinal cord stained with the silver chromate method of Camillo Golgi, which entirely impregnates a small (about 5%) proportion of the cells in a tissue with a dense, black precipitate (Fig. 1.3; Ramón y Cajal, 1890). Although more detailed descriptions using more powerful techniques have been made since, the salient features of Ramón y Cajal's observations remain valid today. Growth cones are highly motile enlargements at the ends of growing neurites and are capable of extending two principal types of processes at their margins: fine, finger-like extensions called filopodia, or microspikes, and flat, sheet-like expansions called lamellipodia (literally sheet-foot), or veils, which are usually extended between adjacent filopodia (Figs 1.2 and 1.5). Although Ramón y Cajal only saw growth

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

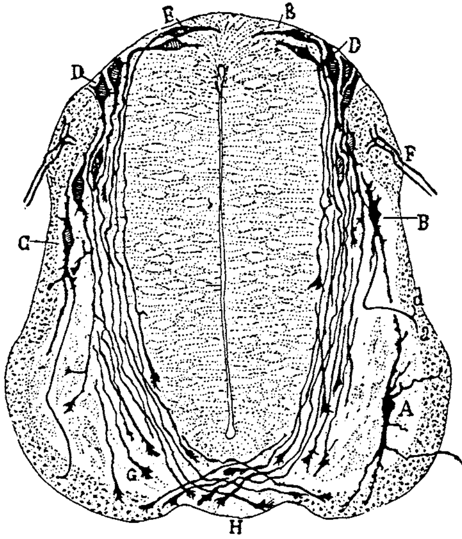

Figure 1.3 The first published picture of a growth cone. A drawing of a transverse section from the spinal cord of a 4-day-old chick embryo stained with the Golgi method. Commissural interneurons (D) have extended axons towards the floor-plate (H) and are tipped with growth cones (G). (From Ramón y Cajal, 1890.)

cones in static images, he recognised their significance and imagined them, with characteristic insight, to be highly motile – rapidly changing their shape as they insinuated or battered their way through the neuropil to their destinations or crawled through pre-existing spaces between cells (Ramón y Cajal, 1990, p.149). Ramón y Cajal's curiosity about growth cones led him to pose many questions about how they grow, how they navigate a path to their appropriate destinations, and how they then select a suitable synaptic partner. These questions, which are the central theme of this book, therefore have a long history. For many of these events he proposed molecular mechanisms, some of which, for instance chemotropism (see Chapter 3), are finding experimental support today.

Proof of the motile activity of growth cones was first demonstrated by Ross G. Harrison (1907, 1910), who developed tissue culture techniques for the express purpose of seeing them in the living state for the first time. In his first experiments he cultured explants of frog embryonic neural tissue in lymph clots and remarked on the rapid, amoeboid-like movements of the growth cones, which extended and retracted many fine, 'filamentous' processes, or pseudopodia as he called them, at their periphery (Fig. 1.4). Growth cones in these cultures could move with speeds of up to about 1 μm/min, although these were not sustained for long. Harrison observed growth cones bifurcating to form two new neurites and the retraction of side-branches (Fig. 1.4). He suggested that growth cones required a substratum in order to extend; they could not grow through a fluid medium (Harrison, 1914). This was an impor-

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

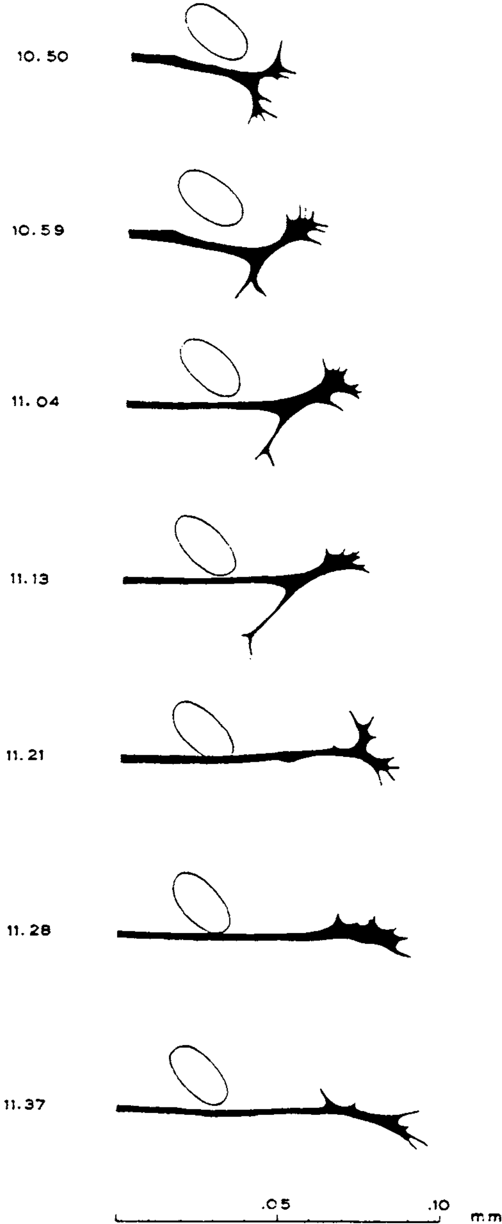

Figure 1.4 Harrison's (1910) camera lucida drawings of a neurite and its growth cone growing in a lymph clot from an explant of frog branchial ectoderm. The time of day is indicated on the left. The erythrocyte, shown in outline, marks a fixed point. Note how the morphology of the growth cone changes rapidly on a time-scale of minutes. The sequence also shows the extension and retraction of a side-branch.

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

tant idea from the point of view of understanding how growth cones advance since it implied that growth was not simply the extrusion of material from the neuronal cell body, like toothpaste from a tube, but that purchase on a substratum was necessary. It so happens that growth cones can develop pull or traction by attaching to a substratum, a property that has been measured directly (Lamoureux, Buxbaum & Heidemann, 1989). Harrison saw neurites arising directly from isolated neuronal cell bodies and proved, therefore, that neurites were not derived from the fusion of other cell processes, an idea prevalent at the time (for reviews, see Harrison, 1935; Hughes, 1968; Jacobson, 1991).

By using suitably shaped lymph clots as bridging-implants, Harrison induced growth cones, unaccompanied by other cell processes, to grow across a surgical gap in the frog embryonic spinal cord, and thereby demonstrated the motile independence of growth cones (Harrison, 1907).

Harrison's pioneering observations of growth cones in culture (Harrison, 1907, 1910) were quickly confirmed and extended by others (Burrows, 1911; Lewis & Lewis, 1912; Levi, 1917, 1926, 1934; Matsumoto, 1920; Lumsden, 1951; Hughes, 1953; Nakai, 1956; Nakai & Kawasaki, 1959). Burrows (1911), working in Harrison's laboratory, developed the culture technique further by growing neural tube from a warm-blooded animal (chick) in chicken plasma, which was more convenient to work with than lymph, and confirmed Harrison's main findings. Burrows obtained the first permanent histological preparations of neuronal cultures, staining them so that neurofilaments could be seen. He also mechanically dissociated neural tube fragments into small clumps of cells, so that neurons could be viewed in their entirety and confirmed that neurites originate directly from neuronal cell bodies (Burrows, 1911). He observed that elongation or growth of the neurite was not of uniform rate but was interspersed with frequent quiescent periods and that growth cones and neurites could retract as well as advance. Lewis and Lewis (1912) substituted simple salt solutions for plasma clots, one advantage of which is that neurites grow directly on the substratum, usually a glass coverslip, and therefore in one plane, rather than at different levels, as in a clot. This meant that neurites and growth cones could be followed continuously in the same focal plane of the microscope. Levi (1917) described in detail the behaviour of the growth cone during continuous observation. He, and others (Matsumoto, 1920; Lewis, 1950; Hughes, 1953; Nakai, 1956), noticed translucent vacuoles of up to 1 μm in diameter in growth cones. Hughes (1953) thought that the vacuoles formed by pinocytosis at the distal margin of the growth cone and that this allowed an intake of water for growth. Whilst we now know that biological membranes are generally freely permeable to water and our views on the mechanism of axon growth have changed somewhat (see Chapter 2), there is ample evidence for endocytotic uptake of extracellular fluid at the growth cone (Birks, Mackey & Weldon, 1972; Wessells & Ludueña, 1974; Weldon, 1975; Bunge, 1977; Cheng & Reese, 1987; Dailey & Bridgman, 1993). However, to what extent endocytosis at the growth cone underlies membrane recycling or

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

pinocytosis, i.e. the bulk uptake of extracellular fluid, is not clear (Gordon-Weeks, 1988a; see Chapter 2).

Hughes found that some vacuoles were retrogradely transported and he measured their rate as 3.8–17 μm/min (see also Nakai, 1956). This is within the range of the rates of retrograde movement of vacuoles and actin filaments reported more recently (e.g. 1–6 μm/min; Forscher & Smith, 1988; Dailey & Bridgman, 1993). It would be interesting to know more about these organelles, especially their relation to microtubules or actin filaments. The ultrastructural correlate of the vacuoles has been established and they are a common feature of electron micrographs of growth cones (e.g. Tennyson, 1970; Dailey & Bridgman, 1993; and see below).

Hughes also noted that growth cones could retract on contacting other growth cones or neurites; this was the first observation of contact inhibition. This finding presaged an important topic of current research: the role of growth cone inhibition and collapse in pathfinding (see below and Chapter 3). Earlier, Levi had shown that neurites severed from their cell bodies in explant cultures continue to elongate (Levi, 1926; Levi & Meyer, 1945). Hughes (1953) also experimented on the effects of severing neurites in explants of chick brain and spinal cord using fine metal needles and found, remarkably, that the distal neurite and its growth cone survived for over 3 hours, elongating at a speed indistinguishable from the unsevered neurite. These observations suggested that growth cones possess considerable autonomy from the cell body and also, possibly, that the addition of new material for growth of the neurite occurred at the growth cone (Hughes, 1953).

The technique of isolating growth cones in culture from their parent neurite, and hence the cell body, has been used on a number of occasions in order to answer a variety of questions about growth cones (Shaw & Bray, 1977; Haydon, McCobb & Kater, 1984; Baas, White & Heidemann, 1987; Guthrie, Lee & Kater, 1989; Martenson *et al.*, 1993). The technique has also been used *in vivo* to isolate retinal ganglion cell growth cones from their cell bodies, by removing the retina after the axons of the retinal ganglion cells had left the retina and entered the optic nerve (Harris, Holt & Bonhoeffer, 1987). They labelled growth cones with a fluorescent dye and filmed them, using video microscopy, growing in the optic tract and tectum in *Xenopus* embryos. Severed growth cones continued to grow for up to 3 hours and their behaviour was essentially indistinguishable from unsevered growth cones, demonstrating again that growth cones have considerable autonomy from their cell bodies.

Nakai and Kawasaki (1959) studied filopodial behaviour and in particular the consequence of filopodia contacting various cell types (Schwann, fibroblast and macrophage) and inanimate objects such as glass particles. They established that filopodia were indiscriminate in what they touched, but that they showed differential adhesion to structures and were possibly contractile. Filopodia can extend anywhere along the growing neurite in culture, but this is not true of lamellipodia (Lewis & Lewis, 1912; Nakai, 1956; Pomerat *et al.*, 1967). Filopodia are extended and retracted regardless of whether the neurite itself is extending, retracting or not growing. Watching growth cones in culture

© Cambridge University Press www.cambridge.org

Cambridge

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

one gains a sense of the independence of their highly motile behaviour from the overall stately advance of the neurite. The rate of extension of filopodia and their length vary between different neuronal types (Table 1.1). In a detailed study of growth cone filopodial extension in rat superior cervical ganglion neurons, Argiro, Bunge and Johnson (1985) found that filopodia could extend from the neurite shaft, the growth cone margin or from each other and that extension was preceded by the formation of a phase-dense nodule of cytoplasm at their origin. When branched filopodia extended, the position of the branch point remained fixed with respect to the base of the filopodium, suggesting that growth is by addition of material distally. The rate of extension declined as the filopodium increased in length and the initial rate of extension (maximally 0.12 $\pm$ 0.4 μm/sec) was positively correlated with the final length of the filopodium. Bray and Chapman (1985) also made a detailed analysis of filopodial behaviour of growth cones in culture, comparing growth on plain glass with growth on glass coated with polylysine, a highly adhesive substratum. Neurite extension rates were considerably slower on the more adhesive coated-glass and filopodial length and life-times increased (Table 1.1). Filopodia were extended at the leading edge of the growth cones, moved laterally and then shortened or became lateral filopodia on the axon shaft. Bray and Chapman (1985) interpreted the behaviour of filopodia in their cultured cells as supporting a role for filopodia in growth cone advance. This important topic, the function of filopodia, is discussed in detail elsewhere (see Chapters 2 and 3).

Growth cones were first observed in the living animal by Speidel (1933). Taking advantage of the transparency of the tail fin of the frog tadpole, Speidel was able to observe the growth cones of cutaneous sensory neurons, continuously for hours or intermittently over a period of weeks, establishing axonal arbors in the skin of anaesthetised animals. He established that the appearance and behaviour of growth cones in culture, as reported by Harrison (1907, 1910) and others (see above), was representative of their appearance and behaviour *in vivo* and thereby gave credibility to tissue culture work. However, culture systems do not completely mimic *in vivo* conditions and, therefore, individual growth cones may not display the full behavioural repertoire *in vitro* that they possess in the animal. Furthermore, cultures tend to be two dimensional, whereas most *in vivo* situations are three dimensional. In the skin of the tail fin, growth cones had a central light region, usually showing within it darker regions, which he called granules, and a peripheral region with up to eight filopodia. The rate of translocation of the growth cone changed continuously from a maximum of about 1 μm/min, or approximately one growth cone length every 10 minutes, which was similar to that seen previously in tissue culture, to an average of about 200 μm in 24 hours. Often the growth cone stopped temporarily, for no apparent reason, or retracted, and although this last behaviour may have been a consequence of morbidity, it had been seen previously in culture (e.g. Burrows, 1911). Growth cones, on encountering objects in their path, such as fibroblasts, formed an enlargement or varicosity, which was left behind at the obstruction. Larger obstructions caused "giant" growth cones to form, and often a change in the direction of growth. Growth

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521018544 - Neuronal Growth Cones
Phillip R. Gordon-Weeks
Excerpt
More information

Table 1.1. *Growth cone characteristics*

Source	Substrate	Speed (μm/hr)	Diameter (μm)	Filopodia: Length (μm)	Filopodia: Extension rate (μm/sec)	Reference
Aplysia	Poly L-lysine	5–25	50	5–15		Goldberg & Burmeister, 1986
PC12/SCG	Collagen	20 ± 2 (PC12) 18 ± 4 (SCG)		10–25 Longest 34 Average 16 ± 7		Aletta & Greene, 1988
Ti1 pioneers	Grasshopper limb bud	10 ± 2 (filopodial) 4 ± 1 (lamellipodial)		59 ± 3 (all forms)		O'Connor *et al.*, 1990
Q1 commissural neuron	Grasshopper CNS			21.2 ± 7.7 (turning) 20.7 ± 7.1 (turned)	0.025 ± 0.012 (turning) 0.028 ± 0.017 (turned)	Myers & Bastiani, 1993
SCG	Collagen	8–22 (embryonic) 4–13 (adult)				Argiro *et al.*, 1984
RGC (*Xenopus*)	*In vivo*	16 (filopodial) 52 (lamellipodial)				Harris *et al.*, 1987
RGC (zebrafish)	*In vivo*	10.7 (tectum) 17.8 (optic tract)				Kaethner & Stuermer, 1992
RGC (chick)	Poly L-lysine L1 N-Cadherin Laminin	10 (23)[a] 59 (122) 90 (151) 123 (188)				Lemmon *et al.*, 1992

© Cambridge University Press