

## Axon guidance in the insect periphery

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The central theme of this essay is the importance of considering neural development, including axonal pathfinding, as but one of a multitude of simultaneously occurring developmental processes. It is very tempting to suppose that the observed regularity of axonal outgrowth is the consequence of mechanisms dedicated to axon guidance, for example, that specific paths are laid down whose exclusive function is to guide axons. I propose to argue from two case studies in insects that regularity does not imply such exclusiveness.

The first example is the leg of the embryonic grasshopper in which neural development has been studied by both the Goodman (Ho & Goodman, 1982) and the Bentley (Bentley & Caudy, 1983) laboratories. I will illustrate my thesis using the recent observations of Caudy & Bentley (1986*a,b*). They find that as the pioneer sensory axons grow from their peripherally situated cell bodies towards the base of the leg, their growth cones change shape markedly and in a characteristic sequence. In some regions they are relatively slender, with filopodia oriented predominantly in the direction of growth; in other regions they flatten and spread out, and their filopodia extend for long distances to the side as well as ahead. These transitions look very much like those seen under culture conditions when growth cones pass from a surface of low to one of high adhesivity. Therefore, Caudy and Bentley argue, the shape of the growth cone *in vivo* can be used as a monitor of neurone–substrate affinity (where affinity may or may not be synonymous with adhesiveness). If this argument is accepted, the observed shape changes provide us with a road map of affinity from the cell body of a sensory neurone all the way to the base of the leg. This road map shows regions of especially high affinity associated with the leg segment boundaries and intervening regions of relatively low affinity in the distal reaches of each segment. The spatial resolution of the map is not good enough to reveal whether there is a continuous, gradient-like increase

in affinity from the distal to the proximal border of each segment, or whether there is a sudden, step-like increase forming a ring of high affinity at the proximal end of the segment.

Now, what has all this to do with axon guidance? It is profoundly important, for we know from *in vitro* studies that, when they are confronted with a heterogeneous substrate, axons regularly follow the path of greatest adhesiveness (Letourneau, 1982). Thus, an axon growing in the leg should be drawn in the proximal direction because affinity is greater there than it is distally. When it encounters the segment border, however, it should either stop or be re-directed onto a circumferential trajectory, because at the border it faces an affinity cliff which it will not cross. The outcome should be a series of aborted axons or a ring of axons at each segment border, and no connections to the CNS. This would obviously be disastrous for neural function and, in fact, it does not happen. Why not?

It could be, of course, that the inference of an affinity cliff at each segment border is wrong. However, two more interesting explanations are available. First, the affinity landscape of the distal leg apparently does not mature until the sensory axons have grown past the site of the first affinity cliff at the border between the tibia and the femur; thus, the problem is circumvented due to the timing of specific developmental events. Second, at the major proximal boundary, between the femur and the coxa, the pioneer growth cones are given a helping hand by a pair of neurones located just on the proximal side of the cliff. Neurone–neurone affinity is supposed to exceed even the greatest neurone–epithelium affinity, so that the exploring pioneer filopodia find an effective target and the growth cone follows them over the cliff. On this interpretation the coxal neurones should be critical for the successful navigation of the pioneers. This is nearly the case, as ablating them results in much delayed and highly variable crossing of the boundary by the pioneers.

These observations pose an interesting interpretive challenge. What is the role in axon guidance of the proximal increase in affinity inferred to be present in each segment? Given the history of inquiry in this field (e.g. Nardi, 1983), we might be tempted to say that it provides polarity information for the axons – it draws them in a proximal direction. But, as we have seen, it also creates a serious obstacle to axon growth beyond the segment boundary. It would seem quite inappropriate to describe the segmental affinity distribution as an axon guidance mechanism if that implies that its ‘purpose’ is to guide axons. Rather, I would argue (in agreement with Caudy and Bentley) that it is far more likely that this affinity distribution has some quite general role in development, perhaps related to the creation of the leg segments themselves. It has a profound influence on axonal navigation, but that is far from being its only, or perhaps even its primary, role in development.

A second example comes from observations made in our laboratory on the navigation of pioneer sensory axons in the wing of *Drosophila*. Like the grasshopper leg pioneers, these axons originate in peripheral sensory cell bodies and grow towards the base of the appendage and thence to the CNS. It has been possible in this system to probe the response of the sensory growth cones to the non-neural substrate in a novel way. Seth Blair (Blair & Palka, 1985; Blair, Schubiger & Palka, 1986; Blair, Murray & Palka, in preparation) has devised a technique for implanting fragments of neurone-bearing tissue into developing wings that are themselves devoid of neurones because of the mutation *scute*. Axons emerging from the implants grow over the host tissue and their growth pattern (uninfluenced by any other neurones, since the host is genetically aneural) reveals the influence of wing epidermal cells on nerve cells. The axons grow in a rather regular way: no matter where the implant lodges, the emerging axon bundle(s) have a very high probability of growing towards and along a path down the ‘midrib’ of the wing (seemingly colocalized with the future third longitudinal vein) that would have been followed by the wing’s own axons had the *scute* mutation not prevented them from developing. As in the grasshopper leg, we do not have the resolution to distinguish whether there is a discrete band of high affinity along the midrib, or some graded distribution with a peak along the path that the axons follow. Either way, this looks like a good example of an axon guidance mechanism.

But is it? Do the axons respond to a cue whose sole function is to guide axons or to some feature that has multiple roles in development? Again, there are

indications that the latter is the case. Margrit Schubiger (Blair, Schubiger & Palka, 1986; Schubiger & Palka, in preparation) has found that the cells along this path step out of the cell cycle conspicuously early. Kuhn and collaborators (1983) have shown that a band of cells in a similar location can be recognized in the unverted wing disc (before there are any neurones) on the basis of elevated activity of a number of histochemically detectable enzymes. This region shares both cell cycle and enzymic characteristics with another band of cells, the future wing margin – and only the anterior half of this marginal band will ever carry axons. Thus, axons grow along metabolically distinct cells, but not all of these distinct cells have axons growing on their surfaces. It seems likely that wing tissue, like leg tissue, shows regional specializations that influence the growth of axons, but it would be inappropriate to regard these specializations as having a unique axon-guiding function.

I have argued from two examples that it is important to regard pioneer axons, navigating through previously uninervated peripheral tissue, as components of larger systems that are developing in their entirety. The hypothesis for discussion, perhaps slightly overstated, is ‘Guidance cues yes, exclusive guidance mechanisms no’.

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