Neuronal Connectivity: Bis Repetita Placent

Alain Ghysen

The mechanism that allows a sensory neuron to extend its terminal branches along the appropriate fascicle within the CNS turns out to be the same as that which positioned the fascicle earlier on, and the gene that controls this position is the same as that which determined the neuron's identity.

The establishment of a reproducible pattern of connectivity is as essential to a neuron's function as its physiological properties. Yet our understanding of neuronal connectivity is much less advanced than that of neuronal physiology, perhaps because connectivity has to be apprehended within the complexity of the entire central nervous system (CNS). Taking advantage of the relative simplicity of the fly sensory system, Zlatic *et al.* [1] have now elucidated the molecular mechanism that drives a given type of sensory neuron to extend its terminal branches along a specific fascicle in the central nervous system of *Drosophila* larvae. They report that, contrary to expectations, the axon does not recognize a given fascicle — rather, it recognizes the position at which this fascicle runs. This is because the axon responds to the same positional cue that directed the formation of the fascicle: as the Romans said, *bis repetita placent* (things twice repeated please). This finding provides a new answer to the old question of how to make an arrow hit its target.

The sense organs of insects are a favorable system for studying neuronal development. This is because they are innervated by a fixed number of neurons — one in the case of the mechanosensory bristles — and because they often occupy stereotyped positions on the body, such that one can deal with identified neurons [2]. Analysis of sensory projections in adult flies revealed that the axons recognize and follow preexisting pathways which differ for different types of sensory neuron [3]. The identification and experimental analysis of these pathways was hindered, however, by the fact that axonogenesis occurs during metamorphosis, when the central nervous system itself is massively remodelled. Recent work on adult sensory neurons has revealed that their axons are at least partly guided towards and within the CNS by the neurites of persistent larval neurons [4]. Ablation of the latter neurons results in definite defects in axonal guidance. Thus, larval neurons seem to provide a preassembled scaffold that guides or at least facilitates the navigation of adult axons, and conversely the adult sensory projection emerges as an ordered expansion

Laboratory of Neurogenetics, INSERM E313, cc103 Univ. Montpellier II, place E. Bataillon, 34095 Montpellier, France. E-mail: aghysen@univ-montp2.fr

of the larval array. The problem then becomes to understand what guides the larval sensory axons.

At the time the larval neurons establish their central projections, the landscape is definitely simpler than during metamorphosis. The entire CNS is conspicuously organized along an orthogonal scaffold made of two connectives that extend longitudinally, and of transversal commissures. The connectives are organized in about twenty distinct fascicles [5]. Individual fascicles are pioneered by identified, segmentally reiterated neurons which recognize each other from segment to segment and thereby establish a continuous tract from head to tail [6]. The longitudinal fascicles that make up the connectives extend at various medio-lateral and dorso-ventral levels within the CNS in a reproducible pattern (Figure 1A). Much has been learned in the past few years about the establishment of this stereotyped pattern. It appears that the medio-lateral position at which a given fascicle extends is defined by the interaction between the midline repellent, Slit, and its receptors, the Robo family of proteins [7,8]. Growth cones expressing various combinations of the Robo receptors are constrained to grow at various distances from the midline, and therefore establish fascicles at various medio-lateral levels.

Identified central neurons extend their axons specifically along one or another of these fascicles [9], leading to the concept of 'substrate pathways': individual fascicles would be labelled by combinations of membrane molecules that would effectively allow incoming axons to select and recognize their appropriate guide. This hypothesis prompted a search for markers present on subsets of fascicles. This search, however, led to disappointing results: the few markers that were identified clearly contribute to the bundling together of the fibers, but do not seem to play any important role in pathway selection [10,11].

The question of pathway recognition has now been reexamined by using as probes the larval sensory axons. Much as in the adult, different neurons extend their branches along different fascicles in a completely reproducible manner [12]. For example, the dbd and ch neurons project to the ipsilateral connective and extend their terminal branches longitudinally. They differ from each other in that ch neurons extend their terminal arbors at an intermediate medio-lateral position, whereas dbd neurons follow a more medial course (Figure 1A,B). In order to understand what it is that the incoming axon recognizes, Zlatic *et al.* [1] undertook to evaluate the role of the Robo system, which measures the distance from the midline. They observed that ch and dbd neurons both express *robo*, but only the ch neurons also express *robo3*. In *robo3* mutants, the dbd projection is unchanged, consistent with the observation that these neurons do not express *robo3*, but the ch projection is shifted more medially, as if the ch now behaves as a dbd neuron (Figure 1C). As the position of the fascicles is also

shifted and somewhat disorganized in *robo3* mutants, it might be that the change in the ch projection merely reflects a displacement of the corresponding fascicle. To settle this matter, the authors re-expressed *robo3* in the *robo3* mutant, but only within the sensory neurons (Figure 1D). They observed that this restores a wild-type ch position, although the fascicles are still abnormally located, demonstrating that the sensory terminal and the fascicles read the distance from the midline independently of each other. The reciprocal experiment was to express *robo3* ectopically in dbd neurons: this resulted in transformation of the dbd projection into a more lateral projection typical of the ch axons (Figure 1E).

When *robo* is mutated, the picture is very different: both projections extend at the appropriate medio-lateral level, but they frequently cross the midline, something they never do in the wild type (Figure 1F). The effect of inactivating *robo* and *robo3* is simply cumulative: ectopic expression in the sensory neurons of the *robo* antagonist *commissureless* (*com*) causes the ch terminal arbors to be shifted to a mediolateral position, as in the *robo3⁻* mutant, and the axon to cross the midline, as in the *robo⁻* mutant. This shows that the two Robo receptors control two different reactions to the Slit repelling effect: crossing the midline or not, in the case of Robo, and measuring the distance from it, in the case of Robo3.

Granted that the particular combination of Robo receptors specifies whether or not an axon will cross the midline, and the position where the terminal arbors will extend, what is it then that determines this combination? A good candidate would be the proneural gene that is responsible for the formation of the neuron, as it is known that different proneural genes determine different classes of sensory neurons [13,12]. The proneural gene *atonal* (*ato*) specifies the formation of the ch but not of the dbd neurons, which depend on *amos* [14]. Ectopic expression of *ato* in the cells that will form dbd neurons results in the expression of *robo3*, and in a lateral shift in the position of the terminal branches (Figure 1H).

As mentioned above, the different terminal arbors differ not only in their medio-lateral but also in their dorso-ventral position. As expected from its dedication to measuring the distance from the midline, the Robo–Slit system appears to have no effect on dorso-ventral positioning (Figure 1 C–G). One is led, therefore, to hypothesize a different system for this dimension. Interestingly, the dorso-ventral position of the arbor is also transformed to that typical of ch neurons by ectopic expression of *ato*, suggesting that *ato* controls both medio-lateral and dorso-ventral properties (Figure 1H). This change in the terminal arbor does not merely reflect a complete transformation of dbd into ch neurons, however, as the cell body and dendrites retain their distinctive dbd characteristics.

Coming back now to the question of how to ensure that an arrow hits its target, the simplest-minded view would have been that the target is truly attractive, like the female moth is to its males. This view, however, is inconsistent with a large body of evidence suggesting that what is recognized is not the target itself, but the
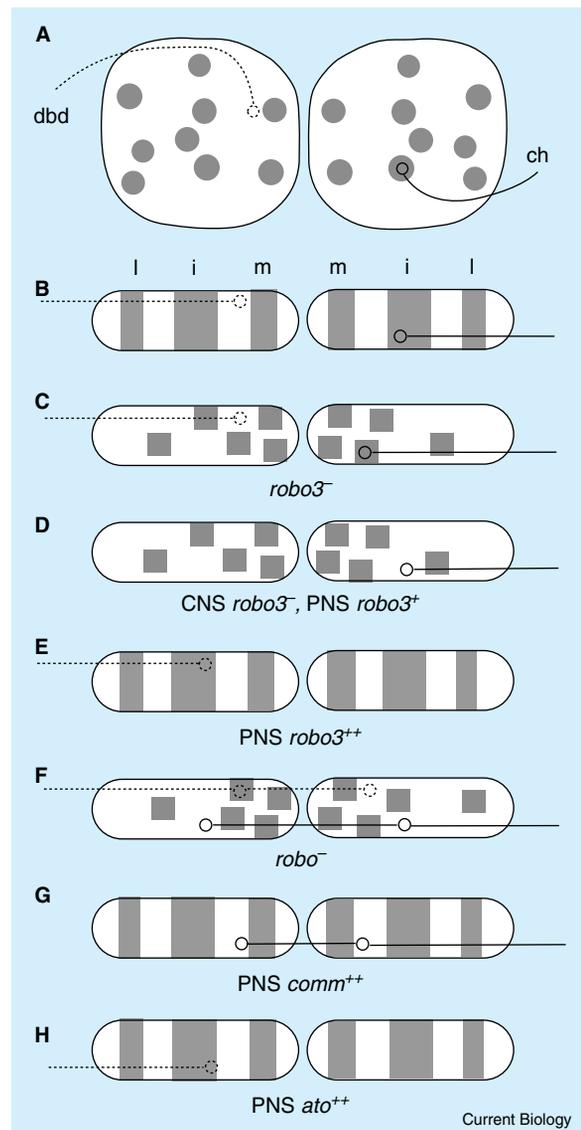

Figure 1. Connective neurons as a scaffold for CNS development in *Drosophila* larvae.

(A) A transverse section of the connectives. The grey circles represent the subset of fascicles labelled by *fas2*. The central projections of two sensory neurons are shown: a dbd neuron on the left (dotted line), and a ch neuron on the right (plain line). The terminal branches extend longitudinally at the positions marked by the open circles. (B) Simplified scheme of the connectives: the nine *fas2* fascicles define three regions, one medial (m), one intermediate (i) and one lateral (l), which can be used to accurately define the position of the terminal branches of sensory axons. (C–G) The various phenotypes discussed in this paper. Note that in (C), (D) and (F), the mutant genotypes affect the distribution of the fascicles such that they can hardly be recognized any more. Note also that in all but the last case, the position of the dbd projection remains dorsal, while the position of the ch projection remains ventral.

path that leads to it, much as in the Japanese tradition of Kyudo the arrows are supposedly guided towards the target along the mental sight of the archer. Yet what appears now is a third scheme, where both the arrow and the target respond to the same surrounding cues, such that the path of the arrow can be precisely

adjusted to hit the center of the target — both in the horizontal and in the vertical axes. As Zlatic *et al.* [1] emphasize in the very last line of their discussion, this general mechanism would ensure a high degree of congruence between the different systems that have to interact — as both the formation of fascicles and their discrimination by incoming axons relies on inter-actions between the same set of molecules — and would in effect provide "a coherent platform on which detailed connectivity could then be established".

References
1. Zlatic, M., Landgraf, M. and Bate, M. (2003). Genetic specification of axonal arbors: atonal regulates robo3 to position terminal branches in the Drosophila nervous system. Neuron *37*, 41–51.
2. Bate, C.M. (1978) Development of sensory systems in arthropods. In Handbook of sensory physiology, M. Jacobson, ed. (Springer-Verlag, Berlin/New York), Vol IX, pp. 1–534.
3. Ghysen, A. (1978). Sensory neurones recognize defined pathways in *Drosophila* central nervous system. Nature *274*, 869–872.
4. Williams, D.W. and Shepherd, D. (2002). Persistent larval sensory neurones are required for the normal development of the adult sensory afferent projections in *Drosophila.* Development *129*, 617–624.
5. Bastiani, M.J., du Lac, S. and Goodman, C.S. (1985) The first neu-ronal growth cones in insect embryos: model system for studying the development of neuronal specificity. In Model neural networks and behavior, A.I. Selverton ed., (Plenum Publishing Corportion), pp. 149–174.
6. Bate, C.M. and Grunewald, E.B. (1981). Embryogenesis of an insect nervous system. II. A second class of neuron precursor cells and the origin of the connectives. J. Embryol. Exp. Morphol. *61*, 317–330.
7. Rajagopalan, S., Vivancos, V., Nicolas, E. and Dickson, B.J. (2000). Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS. Cell *103*, 1033–1045.
8. Simpson, J.H., Bland, K.S., Fetter, R.D. and Goodman, C.S. (2000). Short-range and long-range guidance by Slit and its Robo recep-tors: a combinatorial code of Robo receptors controls lateral posi-tion. Cell *103*, 1019–1032.
9. Raper, J.A., Bastiani, M. and Goodman, C. (1983). Pathfinding by neuronal growth cones in grasshopper embryos. I. Divergent choices made by the growth cones of sibling neurons. J. Neurosci. *3*, 20–30.
10. Elkins, T., Zinn, K., McAllister, L., Hoffmann, F.M. and Goodman, C.S. (1990). Genetic analysis of a *Drosophila* neural cell adhesion molecule: interaction of fasciclin I and Abelson tyrosine kinase mutations. Cell *60*, 565–575.
11. Whitlock, K.E. (1993). Development of *Drosophila* wing sensory neurons in mutants with missing or modified cell surface molecules. Development *117*, 1251–1260.
12. Merritt, D.J. and Whitington, P.M. (1995). Central projections of sensory neurons in the Drosophila embryo correlate with sensory modality, soma position and proneural gene function. J. Neurosci. *15*, 1755–1767.
13. Dambly-Chaudière, C. and Ghysen, A. (1987). Independent subpat-terns of sense organs require independent genes of the *achaete-scute* complex in *Drosophila* larvae. Genes Dev. *1*, 297–306.
14. Huang, M.L., Hsu, C.H. and Chien, C.T. (2000). The proneural gene *amos* promotes multiple dendritic neuron formation in the *Drosophila melanogaster* peripheral nervous system. Neuron *25*, 57–67.