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Making sense of behavior

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ABSTRACT Neurobiological evidence shows that, during the development of the nervous system, inherited behavioral sense is built into the brain with remarkable fidelity. However the way in which the underlying circuitry and its functional characteristics are represented in the genome is not well understood. One response to this is to investigate the machinery of functional development in the nervous system and to set down in principle how genetic control is exerted at this level.

KEY WORDS: *behavior, development, genetics, Drosophila*

"The problem of tracing the emergence of multidimensional behavior from the genes is a challenge that may not become obsolete so soon."
(Benzer 1971)

The impetus for writing about this was a chance remark at a Developmental Genetics Course that I attended twenty years ago in Rome. We were apprenticed to Antonio García Bellido and for two weeks we were completely in his thrall. One evening, after yet another sumptuous meal, the conversation inevitably arrived at the question of how we were going to look for the genes that regulate behavior. Someone said that the obvious thing to do was to look for mutants with behavioral phenotypes. Feeling argumentative, I said that I thought that was not a good idea, because nervous systems were made of cells and that if we wanted to get a grip on behavior we would have to understand what the cells were doing and look for mutations that affect events at this level. Gratifyingly, there was an explosion of agreement from Antonio, who even went to the extent of pumping my hand in his enthusiasm. Ever since, I have wondered when, by investigating the cellular basis of neural development, we would finally begin studying something approaching the assembly of a behavior and start to understand its genetic control.

In 1968, Donald Wilson published one of a series of papers on insect flight. He was interested in the neural machinery that controls the wing movements of a flying locust and, in particular, he wanted to establish the extent to which the rhythmic output of circuits in the CNS is influenced by the inward flow of sensory information. Although his questions and ideas are buried deep in a paper about flight, I am struck, every time that I read them, by their explicit challenge to geneticists and to developmental biologists. Here is what he says. He begins by asking, "How perfect is the motor score that is built into the thoracic ganglia?". Part of the answer to that question is that, "sensory inputs supply only the genetically unanticipatable information such as wind direction and position of the horizon". Therefore he says, "It seems to me that the CNS has programmed into it by the genetic and developmental processes nearly everything that it is possible for it to know before actual flight occurs". If this is so, and here is a neurobiologist telling us that it is indeed so, then how do we explain the remarkable fact that behavioral "sense" of this kind is inherited and built into the nervous system as it develops? The fact that this is detailed, adaptive sense tells us that the level of control is very precise, but the way in which the information in the genome is deployed to achieve this end is still unexplained.

In single-celled organisms the relationship between a gene, a protein and a way of behaving can be very clear. For example, the fact that *pawn* mutants of *Paramecium* are unable to move backwards is perfectly comprehensible once it is appreciated that the necessary reversal of ciliary beat depends on an influx of Ca^{++}. Genes encode proteins not patterns of behavior and in this case *pawn* mutations identify genes encoding proteins required for calcium channel function (Ramanathan *et al.,* 1988). Unfortunately this happy state of affairs disappears completely with the appearance of networks of neurons, brains and the idea of a neural circuit (real or illusory). A mutation in a gene coding for an ion channel in a higher organism may be a useful way of identifying the gene concerned, but it is scarcely going to be informative from the point of view of understanding behavior. Of course this is because the performance of the nervous system not only requires that each of its components be a functional, excitable cell, but also depends absolutely on the myriad interconnectedness of these cells. And the connectivity of the system is not just revealed by the anatomy but depends on a further level of interaction mediated by ion fluxes and intercellular signaling. The question is, how is this complexity represented in the genome? Is it a "global dynamical system with many interactions" or are there "defined subprograms that individual cells can get hold of and execute for themselves" (Brenner, 1974)?

Clearly the nervous system itself functions as a highly dynamical system whose characteristics are determined by innumerable inter-

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actions between its constituent cells. Yet, as Wilson's observations show, selection can operate on the behavior of individuals and perpetuate states of this system that produce biologically advantageous outcomes. Any small change that produces a favorable configuration will tend to be stabilized. The naturally occurring behavioral polymorphism that maintains *rover* and *sitter* characteristics within a single population of *Drosophila* represents two such alternative states (Osborne *et al.,* 1997). On the other hand, searching the genome for genes that regulate behavior by looking for behavioral phenotypes has so far revealed genes that encode proteins necessary for the development or function of neurons, receptors or muscles, or genes required for generalized functions of the nervous system such as learning and rhythmicity (Hall, 1994). Screens of this kind do not seem to reveal genes that are dedicated to the generation or function of a particular circuit. The clear implication is that if there are subprograms that individual cells can get hold of, then these are not the representation in the genome of circuits that underlie behavior.

In fact the evidence suggests that neurons are born and differentiate in ways that are not conditioned by their future functions as elements of neural circuits. The logic, if there is one, is a developmental one. The CNS in *Drosophila* is generated as a series of clonal modules, each produced by a single neuroblast, the fate of individual cells being determined by a combination of cell lineage and interactions with their neighbors (Goodman and Doe, 1993). The axons of these cells then grow out into a framework of pathways that forms the conserved groundplan of a segmental nervous system (Thomas *et al.,* 1984). The reliability of the process that generates this three dimensional framework of neurons and axon pathways makes it feasible to make a cell by cell analysis of the way in which it is constructed. In contrast to the apparent flexibility with which nervous systems can generate individual variations in behavior, early events such as these, that lay out the foundations of the network, appear highly stereotyped and the machinery that underlies them is increasingly well understood at a genetic as well as a cellular level.

To understand how functions such as "motor scores" can emerge from these beginnings, it is worth remembering that fundamental attributes of the nervous system such as the circuitry underlying locomotion or escape behavior are probably also present as a rather stereotyped and evolutionarily conserved set of cells and connections. It is at least possible to envisage that there is a fundamental framework of circuitry just as there is a scaffolding of initial pathways and that, in a similar way, it would be possible to make a systematic analysis of the way in which this circuitry is assembled. As a first step it would be extremely informative to find out the extent to which the basic functional architecture of the nervous system is a reflection of the evolutionarily conserved way in which cells are generated and allocated to different developmental pathways.

A recent clonal analysis of the mushroom bodies gives a hint of what a thoroughgoing investigation of the developmental origins of functional units of the nervous system might reveal (Ito *et al.,* 1997). This analysis, using a lineage tracer that reveals axonal structure as well as cell bodies, shows that there are four neuroblasts producing identical clones that contribute exclusively to the developing mushroom bodies, and that any one of these neuroblasts is sufficient to generate the full range of mushroom body neurons revealed by the tracer. Although it is not excluded that there may be a small number of mushroom body neurons derived from other sources, the implication is that a single neuroblast generates the complete set of neuronal substructures from which the functioning mushroom bodies are constructed. This is extremely interesting because it suggests that the developmental module, the neuroblast clone, is also a functional module, in the sense that it produces all the circuit elements that are intrinsic to the mushroom bodies. Whether there are similar functional groupings among the progenies of different neuroblasts in other parts of the nervous system is not known. Although almost the complete set of lineages generated in the embryonic ventral nervous system, together with their axonal projections, has been described (Bossing *et al.,* 1996; Schmidt *et al.,* 1997), the circuitry involved in simple larval behaviors such as peristaltic crawling or embryonic hatching, to which these cells are likely to contribute, is unknown.

If the early focus of genetic regulation in the embryonic nervous system is a developmental process that generates neurons, guides axons and facilitates target recognition, then there should follow a phase in which the performance of circuitry laid out in this fashion is itself brought under genetic control. Unless we assume an extraordinary degree of precision and inflexibility in the phenotype of individual neurons, it is hard to envisage how the "motor score that is built into the thoracic ganglia" could be anywhere near perfect without a developmental stage in which the functional characteristics of neurons are assessed and adjusted to optimize the performance of the system. At this stage gene expression would no longer be involved in generating connectivity, but in responding to it and adjusting it. One interesting aspect of this is that, since the performance of individual neurons depends not only on intrinsic properties of excitability but on the characteristics of their inputs and outputs, the realm of action of such a regulatory mechanism would be the complete set of connections formed by any neuron. Thus we would expect to find a highly dynamic system of control that reflected actual patterns of connectivity, rather than abstract circuitry.

How could such a regulatory mechanism be explored? Only a functional analysis can reveal the real value of connections and the actual performance of neurons and sets of neurons. There is already a great deal of information to be gained from a combination of genetic analysis with electrophysiological work at a single accessible connection such as the developing neuromuscular junction (e.g., Davis *et al.,* 1996; Keshishian *et al.,* 1996). However, because the integration and adjustment of multiple connections is so important, it might be extremely informative if even the most rudimentary piece of central circuitry could be investigated in this way, as functioning connections are established and refined. The aim would not be to describe in detail the development of a particular behavioral output of the nervous system, but, as with the analysis of earlier development, and in particular the generation of complex three dimensional shapes by growing neurons, to be able to set down in principle how genetic control is exerted at this level.

A lot of things have happened since that evening in Rome and I no longer think that the sort of direct and rather optimistic approach to studying the development of behavior I suggested is necessarily the most promising way of understanding its genetic control. Nonetheless, as an enterprise, it is a tantalizing prospect that becomes steadily more feasible technically. I still think there is room for a realistic attempt to understand how at least one, small, *functioning* part of the nervous system is put together. If we do that, then we will have gone a long way towards answering Wilson's questions about locust flight.

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