THE CORNER TREASURY OF ARCANE AND NEGLECTED PHILOSOPHICAL GEMS (OF MY OWN MAKING)

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Gem #6: "Elementary, my dear Watson", or how the application of Baconian 'strong inference' solved the problem of how afferent nerve fibers find and innervate their correct sensory targets: an exercise in scientific reasoning.

On the need for theoretical rigor in experimental neurobiology

To begin with, I wish to emphasize the need for empirical and theoretical studies to complement one another when analyzing complex, dynamic non-linear interactions in systems such as neural networks. The design of crucial experiments in order to test competing hypotheses (Platt, 1964) can only be accomplished by simulating the consequences of all plausible mechanisms by means of appropriate mathematical models, since only in exceptionally simple situations will deductive logic suffice to generate the required 'truth tables' (see below). Second of all, I feel justified in concentrating on the level of multicellular 'circuitry' to the exclusion of the molecular and behavioral dimensions below and above it, respectively.

The genius of Sherlock Holmes lay primarily in his realization (most instructive even for professional scientists when applying 'the method'!; see Platt, 1964) that truth can be arrived at most reliably by working from *multiple* working hypotheses (as many of them as common sense dictates to be plausible) and most efficiently by looking specifically for those *crucial* bits of empirical evidence which enable some of the hypotheses to be rejected. Indeed, I once actually spared myself years of inherently inconclusive research in just this fashion, by subjecting a well-meaning suggestion for a technically high-powered study of the neurophysiological basis for 'misdirected' tactile reflexes (in skin-grafted frogs: e.g. Baker, 1978) to a simple but rigorous truth-table analysis, using nothing more than simple Aristotelian logic, before committing ourselves to the project. When it became clear that the three most serious hypotheses all predicted the same result from the proposed experiment (viz., intracellular recordings from motoneurons, mapping the monosynaptic spinal reflex connections originating from different sensory locations; see Table I: 'central switch?') we were still in a position to drop it in favor of a design which *could,* in principle, decide among alternative growth mechanisms (Table I; see also Baker and Corner, 1978, 1981).

The approach we eventually came up with (intracellular recording from identified sensory ganglion cells: see Fig. 1), besides providing most of the answers we were looking for, proved to be far less time-consuming and even to require a much less complicated setup. An earlier proposed mechanism ('innervation selectivity': the reestablishment of the original connections following early skin rotation) could be refuted in a more thorough and convincing fashion, while a mechanism not previously considered seriously at all ('neuronal competition': the overproduction of neurons and elimination of the excess) was shown to be, in fact, the only plausible alternative which was consistent with the total data set (Fig. 2; Table II).

The classical alternative to the selective-innervation hypothesis ('end-organ modulation':

the switching of central sensory projections according to the precise nature of the peripheral innervation; e.g. Weiss, 1969) could thus, at long last, be shown to be at best highly unlikely. Attempts have since been made (see Frank et al., 1988) to approach this same question with respect to the ontogeny of proprioceptive rather than cutaneous reflex selectivity but, falling into exactly the same design trap that we managed to avoid (see above), their results have necessarily remained inconclusive as far as developmental mechanisms are concerned. Therefore, despite some specious argumentation to the contrary (e.g., Frank et al., 1988), there are no more grounds for believing that the *end-organ specification* hypothesis is correct for the afferent (primary sensory) side of the motoneuron than there are for taking it seriously on the efferent side (i.e. the neuromuscular junction, where this putative mechanism had long been in disrepute - for review see Landmesser, 1980).

In a truly brilliant article, the physicist J. Platt (1964) reminded us a long time ago that what Baker and I had done was nothing more than to apply the method of 'strong inference', formalized by Francis Bacon already in the 17th century. Yet, how many of us are not, in practise, more like Inspector Lastrade (of Scotland Yard), who incorrigibly pounces upon the first logically sound explanation compatible with the clues available at that moment (so far so good) but then, under the delusion that he is thereby 'supporting his hypothesis', proceeds merely to accumulate additional data consistent with this initial candidate? Inefficient as best, and dangerously prone to 'miscarriages of justice', we can easily afford to ridicule the detective who goes about his business in this fashion .... as long as Holmes is around to remind us how it *should* be done: "elementary, my dear Watson!". Left to our own devices, however, 'horrible examples' abound of how easily we, too, fall into the trap of the single working hypothesis - or worse yet, of having none at all worthy of the name: the empirical *fallacy* in place of inductive *reasoning* (Popper, 1965)!

Even the simplest non-linear systems, I have learned, routinely display counter-intuitive behavior, and the differential equations describing such systems quickly become so complicated that they are incapable of solution using analytic methods, even after simplification into a 'lumped' network model (e.g. Lopes da Silva et al., 1976). To make matters worse, when the interacting neural elements need to be simulated more realistically, only a 'distributed' model (e.g. Kowalski et al., 1992; Van Ooyen et al., 1992) suffices, thus requiring the services of computers with still greater power and speed. Since the essence of the 'scientific method' remains the reasonably accurate prediction of consequences to be expected from a given working hypothesis, thus enabling empirical validation, it is clear that only the most elementary questions about nervous function (see Table I) can be tackled without the use of numerical methods.

Mathematical models also require us to make fully explicit all of the assumptions embodied in our hypotheses, for without such explication the risk of fallacious or tautological deductions will usually be very great indeed. The time may not be far off, as a matter of fact, when neurobiologists will wonder how their predecessors ever managed - especially amazing in an age when powerful computers are so readily available - to muddle through using, at most, old-fashioned deductive logic to guide decisions about how to optimally apply all their sophisticated (and expensive!) technology!

TABLE I

'Truth-table' of predictions deduced from different proposed mechanisms for the ontogeny of sensory reflex connections in skin-grafted frogs

Hypothetical mechanism	Stimulus site?	Central switch?	Peripheral switch?	Cell death?	Selective outgrowth?	Altered survival?
Impulse patterning	NO	No	No	Maybe	Maybe	(NO)
Innervation selectivity	Yes	Yes	YES	Maybe	Yes	(NO)
End-organ modulation	Yes	Yes	No	Maybe	Maybe	NO
Neuronal competition	Yes	Yes	Maybe	Yes	Yes	Yes

The specific prediction which, in the light of discordant experimental findings, falsified the hypothetical mechanism in question is indicated in capitals (see, respectively, Comer and Baker, 1978; Baker et al., 1978, 1981a,b for details). The final listing (i.e. competition among 'redundant' neurons) is the only presently plausible hypothesis which makes sense of all the experimental findings, and is not in contradiction with any of them (but see Fig. 1 for further refinement).

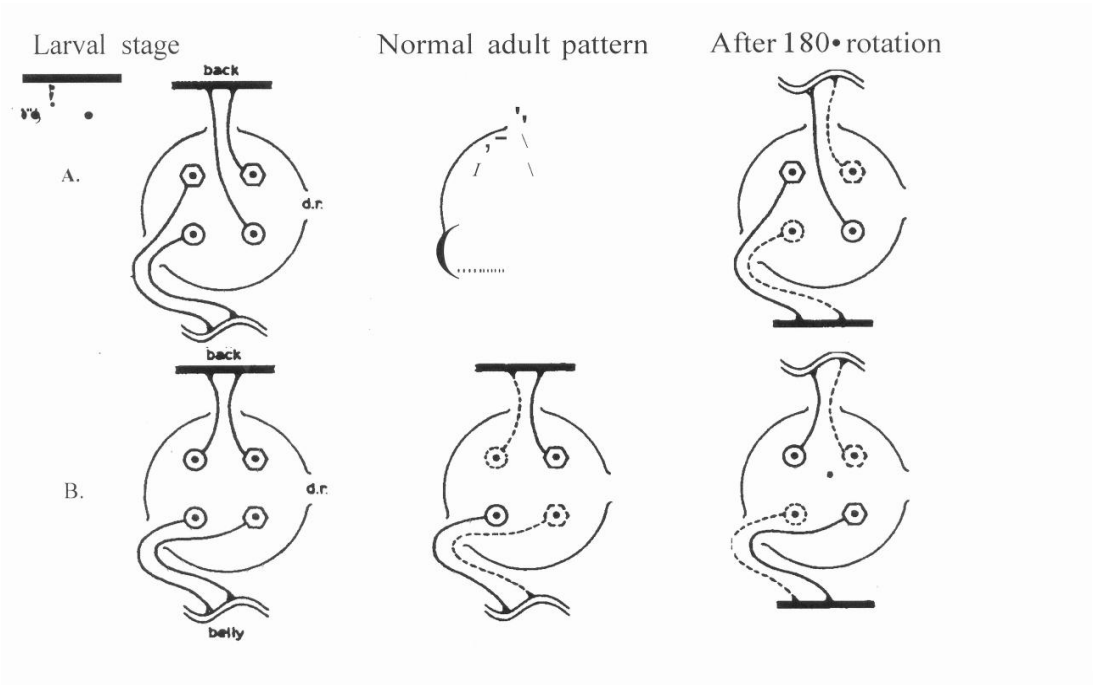

Fig. 1. Two possible mechanisms, in accordance with the redundant sensory ganglion cell hypothesis, for the selective replacement of one cutaneous neuronal population by another. (A) Topographic separation (left) of 'pre-specified back- and belly-skin neurons in, respectively, the dorsal and ventral parts of the ganglion. Stippled cells show the neuron types which would degenerate in normal as compared with skin-rotated animals: the predominant ganglionic projections would thereby become reversed. (B) Admixture of pre-specified 'back' and 'belly' cell types throughout the ganglion but with preferential projections of dorsally and ventrally situated neurons to, respectively, the animal's back and belly: with this model, no readily detectable changes would take place, as indeed turned out to be the case (see Baker and Comer, 1978, 1981).

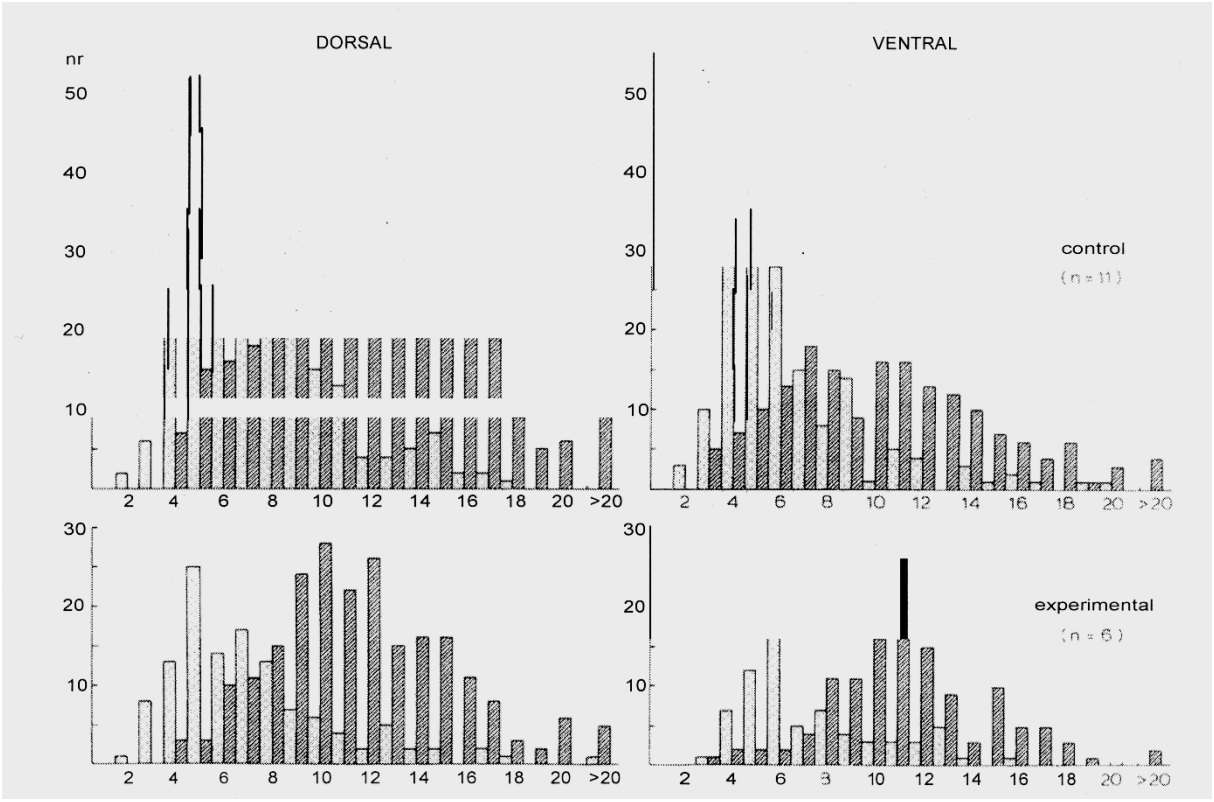

Fig. 2. Size distributions of neuronal soma profiles counted in 20-µ.m thick sections through the dorsal and ventral halves of silver stained thoracic dorsal root ganglia of normal and skin-rotated frogs (see Baker et al.,1981a,b). The number of cells falling in each size class (in microns on the abscissa) is indicated on the ordinate for both the interior (predominantly smaller neurons: see stippled bars) and the 'cortex' ofthe ganglia. The latter group deviates significantly (see Table II) from the normal size distribution, although the total number of neurons per ganglion was unaffected.

TABLE II

Statistically significant DRG neuronal size differences in frogs showing misdirected reflexes versus control frogs

Experimental group	(Mean over N =)	Coeff.-variation		Kurtosis	
		dorsal	ventral	dorsal	ventral
Skin-rotated	(6)	0.27	0.28	1.0	0.8
Un-operated	(11)	0.36	0.39	0.0	-0.1
Skin-rotated	(4)	0.17	0.21	1.5	0.4
Sham-operated	(5)	0.32	0.27	0.1	0.0

Misdirected skin-wiping responses are reproducibly associated with (i) a reduced variance in, and (ii) a non-Gaussian distribution of cell soma sizes (for details see Baker et al., 1981a,b).

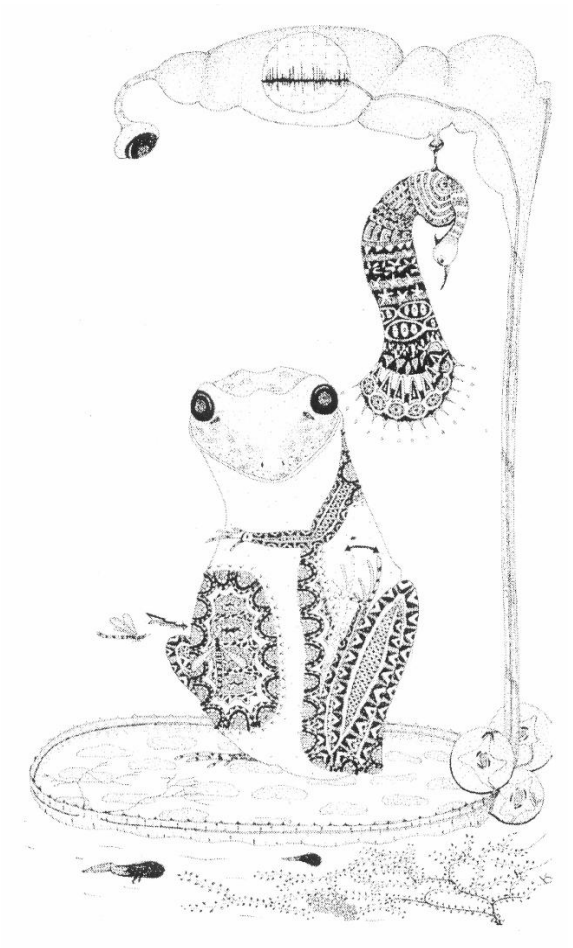

Figure 3. Schematic sketch by Kees Boer of the skin-rotation/ misdirected reflex experiment in frogs. The early operational stage is indicated by the little tadpole at the bottom, while the adult frog brain is drawn schematically at the top.