A ‘neural’ net that can be seen with the naked eye*.

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ABSTRACT

The neurally controlled colour patterns of cephalopod molluscs, and the spatial distribution of the spots in the skin that supply the display elements for these patterns, have been followed during different stages of the life history. The resulting description links physiological with morphogenetic processes of pattern generation and provides a novel contribution to understanding some network properties of this and other systems. Upon denervation of one side of the animal, each of the several generations of spots turns out to be a separate 'neural' net composed mainly of muscles. Waves of colour propagate through these networks with unstable periodicities and in semi-random directions. They are manifestations of the intrinsic connectivity and conductances of the networks. The role of the nervous system is to control the chaos of myogenic origin by modulating either conduction or coupling in the networks.

1. Developmental basis of pattern generation in cephalopods.

All of the well-known species of octopuses, squids and cuttlefish are covered with tiny pigmented spots. Some of these chromatophore organs – so-called because they combine several cell types – are large enough to be seen with the naked eye, especially if they are pulsating. Around them in the skin lie other, slightly smaller, spots in an orderly, regular/irregular, arrangement that extends across the whole surface. Occasionally, waves of colour are seen to wander across this array.

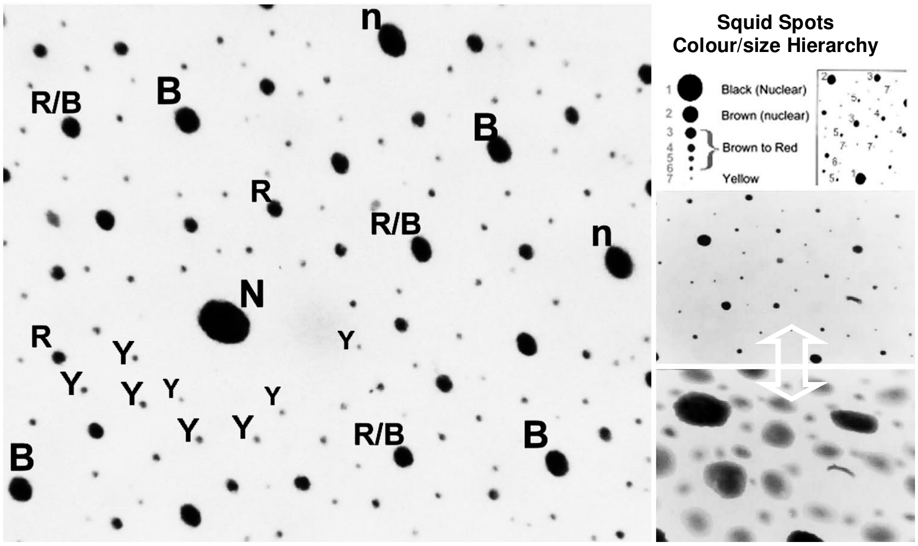

Fig. 1 Chromatophore array (c.3x4mm) in the dorso-lateral surface of an immature squid *Loligo vulgaris*. Spots are fully relaxed. Letters indicate colours (brown, red, yellow), which correlate with size and age class. The large spot N (nuclear) is an early (founder) chromatophore that influences the positioning of all later spots. Scheme of classification shown upper right. Each class is a separate matrix. Middle and lower right: similar array relaxed ⇔ expanded.

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*Published in : Backhaus. W. (ed) 2001 International School of Biocybernetics (Ischia): *Neuronal coding of perceptual systems*: pp. 397-402. World Scientific, Singapore, New Jersey, London, Hong Kong.

Study of the array, of its development, and of the waves running through it, provides insights into the underlying connectivity of the skin and into the principles governing the electrical behaviour of complex surfaces.

The array. The body of octopuses and squids is made up of three fields: mantle, head and arms. In the embryo, and all subsequent stages of development, the distribution of spots is the result of morphogenetic influences combining local and field effects that continue to be exerted throughout life (Fig. 1) (Packard, 1983). In *Loligo* the mantle and arm fields increase in size many hundred-fold and in number of spots from <100 to several 100,000. The 2-dimensional waveform of spot recruitment potentialities (effective potencies) is determined by 1) the main body axes (antero-posterior, dorso-ventral), 2) the positions of the first spot(s), 3) fractal-like iterations of an algorithm that introduces new spots into unoccupied skin and into spaces between existing spots, 4) absolute field size and the accumulated effects of 1) and 3).

The result is a series of harmonics (higher and higher spatial frequencies in each field) out of phase with the original waveform, generally fewer spatial frequencies (numbers of iterations) ventrally than dorsally, and generally larger numbers of spots in later generations than in earlier ones. Spots of any one generation are slightly smaller in size and lighter in colour than the generations preceding them. Successive colour/size classes lie at successively shallower layers in the skin of squids, and successively deeper layers in octopuses. The lateral inhibitory component of the algorithm (Meinhardt, 1982) ensures that spots in superimposed layers never lie directly one above another. They do not die or disappear as the animal grows. Each colour/size class is innervated by a different generation of nerve fibres. (However, the processes of compartmentation of the original motor field, and separate innervation of different spatial frequencies, have been little studied).

With these facts in mind, the life history of the skin and of its separate layers may be read by simple inspection. It is the key to the understanding of its physiology (see Packard, 1995a,b for summaries).

2. Visualisation of the networks

Each spot is an element in a continuous network of spots of the same size/colour/age class that stretches throughout the mantle, arm or head field.

Visualisation of the coloured networks is achieved in its purest form by severing the nerve to the mantle on one side of the squid or octopus and following events over the next few days[1]. As the distal endings of cut nerves degenerate, their inhibitory influence over spontaneous activity of spots wanes[2], and the intrinsic (autonomous) properties of the muscle networks awaken. When the two processes are complete, the networks are under myogenic rather than neurogenic control. One may speak of a myal rather than a neural regime (see below).

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[1] Immediately after surgery – which is extremely simple as the nerve can be seen through the mantle aperture – parts of the mantle supplied by the nerve lose their ability to change colour. They become white (muscle fibres of all spots relaxed) and have a sharp boundary near the midline where operated and intact sides meet. However, during the next few days, patterns of colour – remarkably similar to ones seen on the intact side – gradually return to the operated side also. The nerve is no longer connected to the brain, so these have a life of their own: fading, disappearing and reappearing more or less at random.

[2] The distal processes of nerves continue to exert an inhibitory influence on the muscles of spots for some time after being cut, keeping them relaxed (? hyperpolarised), even after excitatory input from the brain has ceased.

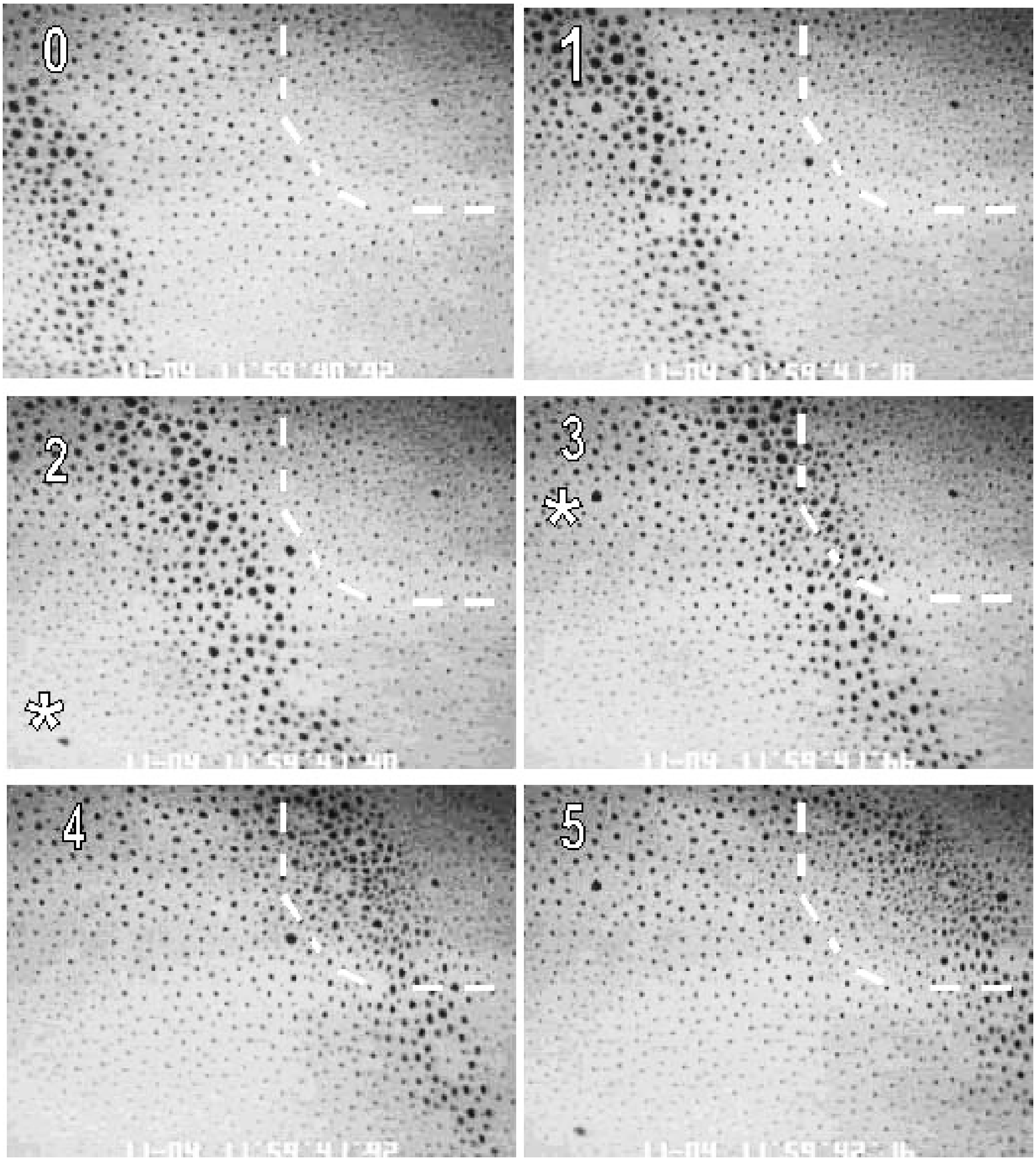

Fig. 2 A wave of twitch contractions (phasic expansion/retraction) seen at 0.25s intervals running through brown and red spots on the denervated flank of a living squid (*Loligo vulgaris*). The wave – generated out of the picture to the left – spares the largest spots, two of which (*) are oscillating independently. Excitation propagates at the wave front with inter-spot delays ~100ms, but near-instantaneously to satellite spots within the wave. Upper right, the wave attenuates (reduced twitch amplitudes) on entry into an area supplied by a few nerve fibres only (see text). (Frame width c. 2cm)

The following properties are exhibited by networks under myogenic control: 1) coupling (connectivity) is strongest between elements of the same size/colour class, weakest between elements furthest apart in the size/colour hierarchy, 2) changes in state of the elements in one part of a network propagate to other parts of the network either as an all-or-none wave or as a gradient, 3) they travel at different speeds, 4) waves are provoked by a) local pacemaker activity, b) external sources of disturbance, c) nerve activity at the edges of the myal territory, 5) wave generation and propagation may also be suppressed by b) and c), 6) patterns of wave generation become chaotic in the absence of external input.

The commonest waves are

- fast all-or-none twitches that propagate separately in brown/red and in yellow networks[3],
- slow and standing waves of graded tonic contractions,
- waves of relaxation.

Waves running through denervated skin (such as the one seen in Figure 2) can be simulated by setting the variables of a simple auto-associative matrix of elements (2-dimensional probabilistic cellular automaton) that can adopt one of two states (equivalent to an expanded or retracted chromatophore) (Plieth, in preparation). It has the following characteristics: open times, closed times (refractory periods), probabilities of spontaneous change of state, cooperativity or connectivity (probability that at the next iteration a spot imitates the change of state of its neighbour(s)).

The wave in Figure 2 changes its characteristics on entering partially innervated skin (upper right of panels 3-5, Fig. 2). Velocity remains the same, but the wave attenuates (reduced twitch amplitude) and extends to class(es) of spots not excited in its path through fully denervated skin (panels 0-2, Fig. 2). Fully innervated skin (out of the picture) completely blocks propagation of the wave (see ref 1*).

Such experimental findings are instances of myogenic/neurogenic interactions.

3. What is the meaning of the waves?

Connectivity (cooperativity) is extremely robust. Long after the death of the squid, its skin continues to generate various kinds of waves. Evidently the observed compliance[4] of chromatophores in the path of a wave is a fundamental property of the coupled ensemble. It persists for as long as the muscle cells show signs of life.

Waves of twitches and contractures are not, however, part of the normal repertoire of physiologically generated patterns in squids. Instead, they appear to be an artefact of the connectivity exposed when nerves are absent or have lost control.

Lack of any specific signalling or communication function for the various waves suggests a simple hypothesis: that the role of the conducting networks is to maintain connectivity and compliance (conformity of behaviour) amongst cells belonging to the same ontogentic class throughout the body[5].

Nerves modulate coupling within the different layers of the skin, either by breaking it in an all-or-none manner (e.g. by closing gap-junctions, which normally clamp cells to a common potential), or establishing gradients of connectivity by

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[3] It is not known by what mechanisms and through what elements the waves propagate. The electrical continua (see note 5) must be of considerable complexity if only because there is little interference between fast brown/red and yellow waves running in layers separated by only a few tens of microns and embedded in connective tissue that receives muscle fibre extensions from different layers.

[4] "Compliance is the linear displacement produced by unit force in a vibrating system" (Chambers Dictionary). I do not know if the word has been previously applied to wave activity in a biological tissue. See note 6.

[5] The inference is that continuity is electrical. The reasoning is as follows: a) changes in transmembrane potential (of the order of a few mV) accompany the contractions, contractures and relaxations of the muscle cells responsible for colour change, b) the colour signs generated by spots are thus indirectly also electrical signs and chromatophores can be regarded as ‘potentiometers’ with analogue output, c) propagated contractile states can be regarded as electrical waves, and d) uniform fields or gradients, etc. of contractile state can be regarded as fields or gradients, etc. of electrical potential. There is, however, a lag in the non-linear ‘potentiometer’ response: the time course of excitation/contraction coupling.

altering conductances. Left to themselves, the networks become chaotic, transmitting random changes of state in one part to all other parts.

4. Conclusions.

Preoccupation with the computational properties of neural nets, tends to lose sight of this basic auto-associative property of a net. My understanding of the dynamics of colour change in squids is that it combines two kinds of control: vertical, information-rich hierarchical control (e.g. by nerves and through pocesses of differentiation), and horizontal continuity of physiological state[6] within layers. The one, the modulator, cannot exist without the other, the medium[7].

In this sense too, the medium is the message. As with other nets, a great deal here is still for the discovering.

Acknowledgements

My thanks to Euan Brown, Gerd Heinz, William Gilly, Isao Inoue, George Mackie, Christoph Plieth and others for illuminating discussions; and to the President, Director and staff of the Stazione Zoologica for precious laboratory facilities.

References

*For further illustrations and examples of wave activity consult:
http://www.gfai.de/www_open/perspg/g_heinz/biomodel/squids/squids.htm

Hutcheon, B. and Y. Yarom (2000) "Resonance, oscillation and the intrinsic frequency preferences of neurons", *Trends Neurosci.*, 23 :216-222.

Meinhardt, H. (1982) *Models of Biological Pattern Formation*, London: Academic.

Packard (1985) "Sizes and distribution of chromatophores during post-embryonic development of cephalopods", *Vie et Milieu*, 35 :285-298.

Packard, A. (1995a) "Organization of cephalopod chromatophore systems: a neuromuscular image-generator", in: *Cephalopod Neurobiology*, N.J.Abbott, R.Williamson and L.Maddock, eds, Oxford University Press, pp. 331-367.

Packard, A. (1995b) "Through the looking-glass of cephalopod colour patterns", in: *Behavioural Brain Research in Naturalistic and Semi-Naturalistic Settings*, E.Alleva, A. Fasolo, H-P. Lipp, L. Nadel and L. Ricceri, eds, Kluwer Academic Publishers, Netherlands, pp.105-130.

Plieth, C. (unpublished) A random activated cellular automaton (RACA) : 150Kb programme supplied to the author.

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[6] Genetic and epigenetic continuity of state within an organism and tissue is embedded explicitly in biological principles as the concept of the genome. There is no similarly explicit principle of continuity of physiological state within a tissue, though it is often implied in special cases described as ‘coupled ensembles’ or ‘functional syncytia’, etc.. Homeostasis (and homeorhesis) are different concepts.

[7] Another way of looking at the same phenomena is to consider them as combining the active and passive properties of a resonant system (Hutcheon & Yarom, 2000), in which the ‘passive’ component is the intrinsic properties of the intercellular network (or continuum) described.