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THROUGH THE LOOKING-GLASS OF CEPHALOPOD COLOUR PATTERNS

A skin-diver's guide to the Octopus brain.

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1. Introduction

Our supporting organisation, NATO, has shown itself ready to revise its military thinking in face of the latest facts. As scientists we should be equally flexible. In the zoological realm, what had long been quoted as the most brilliant example of analogous structures and of convergent evolution - the similarity of the vertebrate and the cephalopod eye, despite their separate phylogenetic histories - is now revealed as having within it the seeds of an ancient homology: perhaps as ancient as animal life itself.

I refer to the recent discovery [38] that the same Homeobox gene family responsible for morphogenesis in the eye primordia of insects and of vertebrates is also contained in the germ cells of squids. It invites us to consider that the rules of Vision operating in the field of evolution - not so very different from the military field - are universal rules that have to do less with the nature of light and optics than with the tasks that can be performed with information extractable from an illuminated world. Emphasis on tasks, in turn suggests that the skin of cephalopods - clearly a successful group of animals - might be a very convenient way of arriving at these rules. Its capacity for colour change and its repertoire of signals and compositions have, over millions of years, been directed at and tuned by the eyes that occupy behaviour space [29].

Moreover, whatever the organisers of "the decade of the brain" had in mind, the plain fact that brains can do nothing without their peripheries might be taken as experimental justification for only studying brains through behaviour. And what better, in the middle of that decade, than to choose a cephalopod animal with its rich and eminently readable periphery - especially as the essays on offer have been little explored.

Here I give an account of some discoveries in five classical areas of neurophysiology - nerve regeneration, motor unit structure, sensori-motor reflexes, muscle tonus, higher motorcontrol - that were made, in keeping with the ethophysiological theme of this meeting, in "semi-natural" conditions not requiring immobilisation or premature sacrifice of the animals. Although still largely descriptive and inferential, the research is at a stage where more traditional physiological and pharmacological analyses can profitably take over.

2. General Considerations and Heuristics

Since cephalopods are still unfamiliar to most people, however, and the method used has not been adopted by any other school, the grammar and semantics needed to read the skin require some introduction (see also [23]).

At the beginning of World War II, Holmes [18] published an illustrated account of the main patterns assumed by the cuttlefish *Sepia officinalis* which he had studied for his PhD thesis during months spent at the Plymouth marine biological Laboratory, England. Here at Acquafredda di Maratea, we have not only this same species, hiding in the sand-beds amongst the sea-grass, but also two other well-known cephalopods: the common octopus (*Octopus vulgaris*), which expert snorkellers somehow manage to discern hunting among the rocks, and the common squid (*Loligo vulgaris*) which local fishermen take with a night-light.

Unlike other polymorphic animals, such as butterflies, birds, sea snails, many fish and reptiles, the variety of patterns documented by Holmes for a single species can all be exhibited by the same individual. They are now known to comprise more than thirty components [17]. Similarly rich repertoires have been described for other cephalopods [16, 23, 37]. The patterns of individual squids, octopuses and cuttlefish differ from those other colour-change artists - flatfish and chameleon - because they are mostly muscular activity driven by the brain: physiologically they are polymorphisms of posture and movement.

Nevertheless these animals behave like vertebrates. I have dealt long ago [26] with the insights to be gained from studying the built-in paradox of a vertebrate life-style developed out of a molluscan body. In the next section I relate some of the heuristic advantages to be gained from in-parallel examination of the colour system of this [for students of vertebrates] ultimate out-group. Readers are referred to the recently published chapter in *Cephalopod Neurobiology* [33] for a summary of the main physiological findings.

3. Methodological Approach

3.1 WHAT CAN BE LEARNT FROM THE COLOUR DISPLAYS?

I have adopted the following three principles throughout these studies:

- 1) let the animal tell its own story
- 2) understand the parts through the whole
- 3) see how it grows

I propose to call the resulting approach, as it applies to brain and behaviour studies, *Ontophysiology* - from Greek *ontos* (present participle) existing, as in ontogenesis.

3.2. ONTOPHYSIOLOGY

A longitudinal, ontophysiological, approach is uniquely suited to the colour-change system of cephalopods (Box 1). Although not amounting to an ethogram, the repertoire of patterns described for the three common species already mentioned, but not for *Sepioteuthis* (which cost hundreds of hours of snorkelling [23]), was established largely by watching them in the semi-natural conditions of aquarium holding facilities: interacting with the observer, with conspecifics, with food items and with potential predators. Several of the patterns, apparently identical with those observed in natural and semi-natural settings, are also spontaneously displayed in the confines of the laboratory wet table, and

under the close-up lens (Fig. 1). As my interest has been the nature of the motor system, I have only been secondarily concerned with the stimulus conditions evoking and modulating these (see later).

The ontophysiological approach requires a certain discipline: that of *not* sacrificing the animal before necessary, and it will be noticed in Fig. 1, that the animal is returned to its experimental tank between experiments - rather than being diverted into the top right-hand corner of the diagram. Many octopus and sepiid species, and one or two squid species survive for many weeks in properly established seawater aquarium systems (see also [44] for reference), as juveniles and as adults. The primary requirement for studying them in the laboratory is a clean, well-aerated, running seawater supply, and this traditionally requires one of the several marine stations scattered round the world.

Basic procedures for the cephalopod brain/behaviour scientist (which can not usually be left to his/her assistants) are:

- check animals and seawater circulation twice daily (mornings and evenings)
- feed animals daily or alternate days (octopuses live crabs, e.g. *Carcinus* for *O. vulgaris*, and occasional anchovies)
- check and feed food organisms (*Carcinus*)
- clean holding and experimental tanks regularly
- allow several days (ideally 2-3 weeks) for animals to adapt to laboratory conditions
- avoid winter temperatures for regeneration experiments

Several species have now been reared from the egg in closed or semi-closed seawater systems.

3.3. EXPERIMENTAL OBSERVATIONS AND DESCRIPTION

Holmes recognised that the patterns of the cuttlefish are stereotypical fixed action patterns. Occurring essentially in the plane of the skin, they are ideal for photographic and video-recording, and this has been the main experimental technique employed throughout this work. As a given pattern always appears in the same place on the surface of the animal and repeatedly under the same circumstances, it is possible, when planning experiments, to return to the same animal, or the same spot on the skin, and record its activity - over seconds or weeks - without any damage to the system being recorded, to do so at magnifications ranging over three orders of magnitude, and, within the limits of resolution and clarity permitted by the transparent epidermis and underlying dermis, to make precise inferences about muscular and central nervous participation in the activities recorded. Box 1 sets out the initial procedures used in this deductive approach.

The set-up is illustrated in Figure 1.

3.3.1. Anaesthesia

Cephalopods tolerate, and recover quickly from, repeated anaesthesia in ethyl alcohol or in nicotine (1-4 parts per million) so that quite radical experimental manipulations can be conducted where required without sacrificing the animal. Repeated anaesthesia with urethane (ethyl carbamate) or magnesium chloride is not recommended. The former appears to be toxic, the latter produces profound relaxation [22], but recovery is slow and may be fatally delayed. For surgery on octopus brains I have recently had excellent results with combined alcohol/urethane anaesthesia: pre-anaesthesia with ethyl alcohol (1-2% depending on size) followed by urethane (0.5-1% and not the 2-4% used in the past).

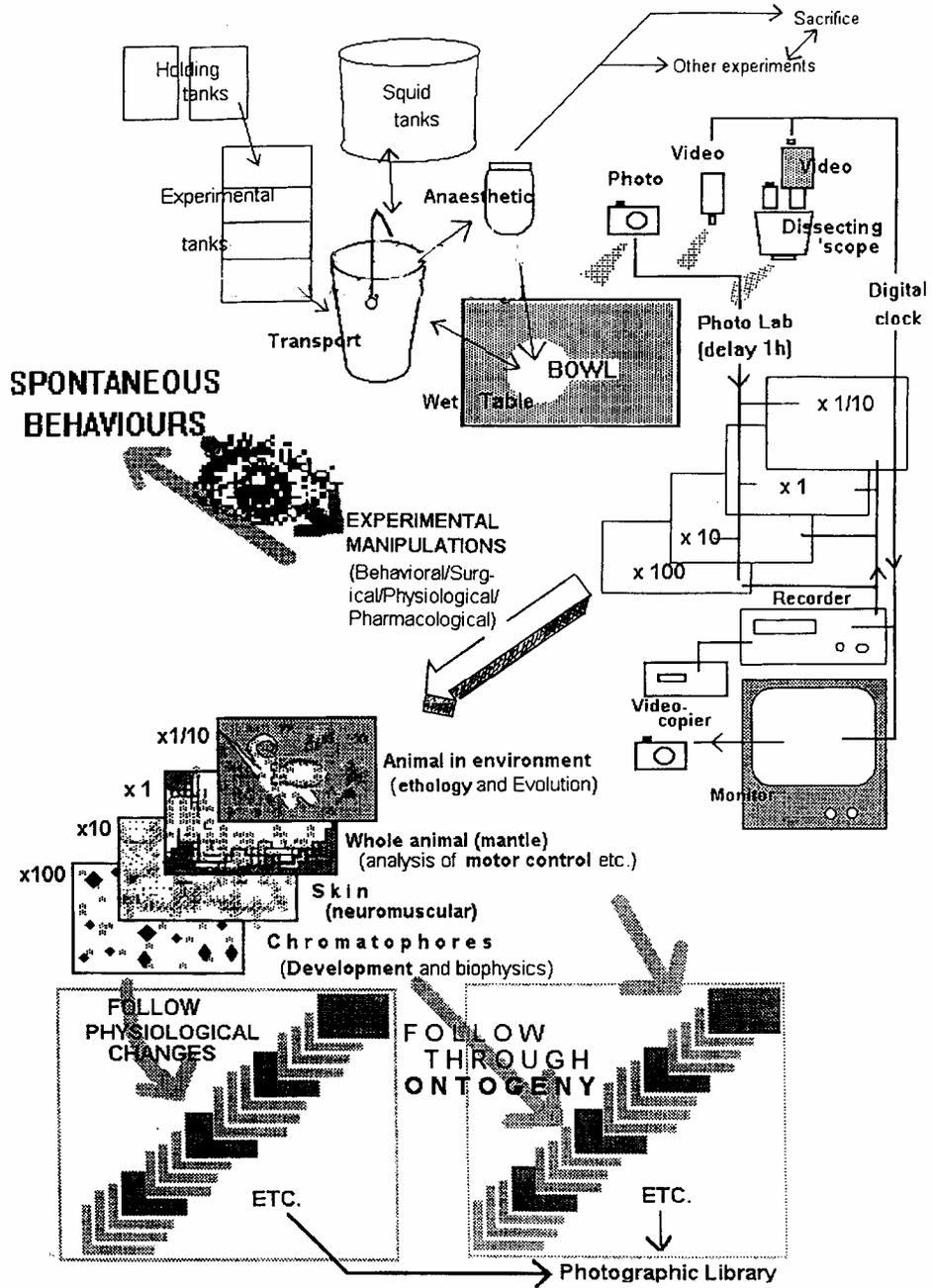


Figure 1. Experimental methods for the collection of photographic data on skin patterns of cephalopods.

BOX 1. ONTOPHYSIOLOGICAL TECHNIQUES (REAL AND POTENTIAL) FOR THE ANALYSIS OF OCTOPUS/SQUID/CUTTLEFISH CHROMATOPHORE PATTERNS

- a) Document (by photo and video)* the patterns of a single individual over as much of its life-cycle as possible, and at various magnifications (from the whole animal, in a social or physical context, down to single groups of chromatophores).
 - b) Adopt a baseline (e.g. complete relaxation) for comparing activity at one stage of an experimental session with activity at another.
 - c) Compare activity in one experiment with activity of the same part in subsequent or previous experiments.
 - d) Follow the effects of selective lesions of the nervous system (e.g. pallial nerve section) to distinguish between peripheral and central control and between different levels of central control (e.g. removal of optic lobe input). Intra-animal control provided by comparison with non-lesioned side.
- * Photo and video supply both in parallel and sequential information. The complexities of in parallel analysis can be avoided by the process of subtraction and by frame-by-frame analysis of phenomena of interest. N.B. The repetition rate of video (40 ms) is higher than the response time and frequency of the system under observation.

3.3.2. Controls

In ontophysiology, the animal is its own control. For instance pre-operatively compared with post-operatively, and operated side against unoperated side. So long as sufficient time is left between treatments, habituation (to drugs and other stimuli) has not proved a problem. Colour patterns are not subject to conditioning [42].

3.3.3. Photographic analysis

Photographic recording and the ontophysiological approach go hand in hand. The great wealth of data present in a photograph or video-frame, require, for analysis, some kind of reduction of the data and conservation only of the transforms. The eye is, on the whole, rather good at this: at spotting the differences between one set of visual data and another. When it encounters two photographs of separate individuals it has no way of knowing whether the differences are meaningless, or trivial, inter-individual differences. To extract the meaning would require, at least, many more samples and complicated statistics. But when the eye encounters differences between two photographs, or video-frames, of the *same* individual it enters a new dimension. They are immediately recognisable as changes taking place within the individual, and physiologically (or ontogenetically) meaningful therefore.

The procedure is capable of revealing truths available in no other way. All of the rules of post-embryonic development of chromatophores [28] were elucidated using this technique.

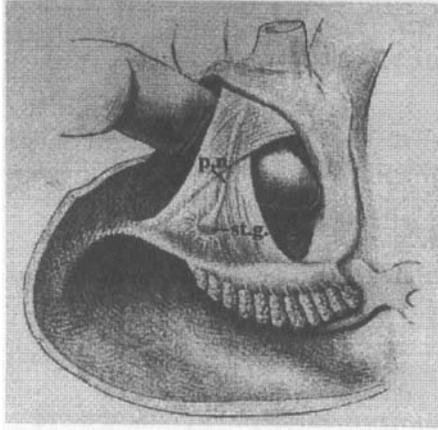


Figure 2. The pallial nerve of an octopus (*Eledone*). For denervation/regeneration studies the nerve (p.n.), running in the mantle connective, is accessed through the mantle aperture and severed just before entry into the stellate ganglion (st.g.). (From [41]).

3.3.4. *Ontogeny*

Questions of chromatophore development, may seem to have little to say about how the brain works. In fact, they supply the single most important key to understanding the relationship between brain and periphery - at least in this system. Cephalopods continue to grow throughout life, so do their brains, allometrically, at higher rates early in life than later [34]. Many of the insights into the role of the brain in pattern generation have come from comparing animals either at slightly different stages of ontogeny, or far apart in age and state of development [17, 37].

3.3.5. *Specificity of findings*

The results obtained from ontophysiological studies on whole animals using photographs are extremely specific. Repeatedly, throughout these studies, I have been forced to recognise that results which one might, through lack of other information, have been prepared to ascribe to chromatophores as a whole, in fact apply to only one particular category of chromatophore in one particular part of the skin.

4. Linking Behaviour with Brains

4.1. REGENERATION OF NERVE SUPPLY TO SKIN IN OCTOPUS

In 1878 the French physiologist, Fredericq [15] cut the pallial nerve (Fig. 2) on one side of an octopus [footnote 1] and demonstrated, through the immediate and dramatic paling of the mantle which resulted (Fig. 3C), that the colours of cephalopod animals are under nervous control. More than a hundred years later this simple operation was employed to make two equally fundamental discoveries: (1) that the nerve can grow back to the skin and restore colour change-ability so completely [39] that only a trained eye can distinguish between the regenerated and the unoperated sides (Fig. 3A and 3B); (2) that the pallor (Fig. 3C) is not just due to passive relaxation of muscles when their nerves are cut, but is an active process.

1. The nerve, with its efferent fibres to the skin from the posterior chromatophore lobes, is easily accessible through the mantle aperture (Fig. 2). By contrast, efferent supply to the skin of the arms, from the anterior chromatophore lobes, runs in the brachial nerve cords and is therefore not easily accessible. The four chromatophore lobes are connected by commissures (left/right) and connectives (anterior/posterior) (Fig. 11) [3].

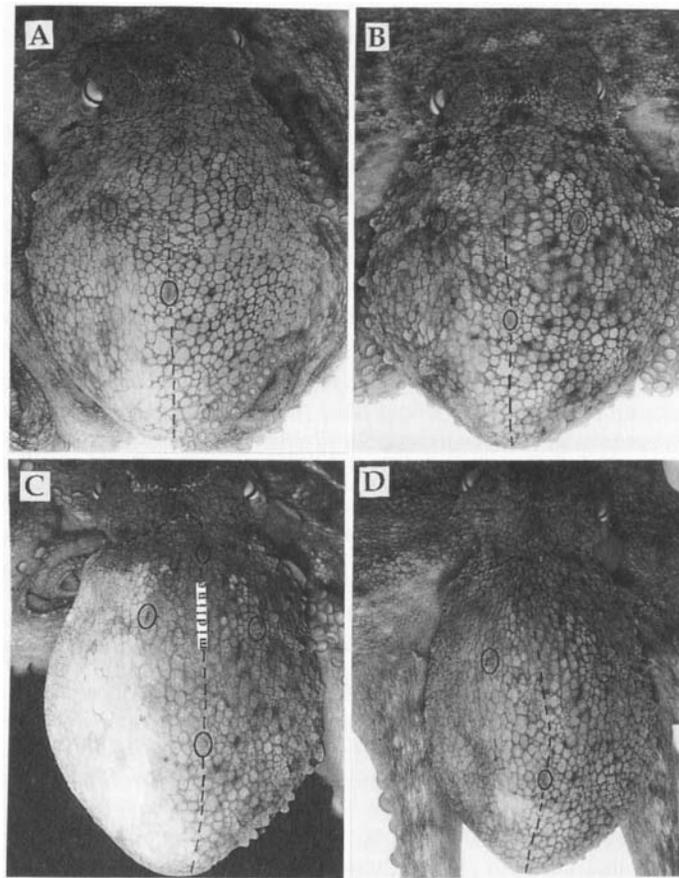


Figure 3. Pattern regeneration and the effects of nerve lesion in *Octopus vulgaris*. In the upper left photograph (A) this octopus was able to generate near-normal patterns of chromatophore control and papillae even though the nerve to the left side of its mantle (LPN) had twice been severed 4 months and 2 months previously (compare operated and intact sides). Upper right (B), same pattern momentarily boosted by stimulation of the eyes (see text) with electronic flash. When the nerve was again cut (lower left (C), 1 h after surgery) areas deprived of central control turned white (pallor). (D) (5 months later, in 0.8% alcoholic seawater), patterning ability again returned to the left side, but was impaired relative to the intact side and preoperative condition. Hatched line bounds normal territory of the LPN (and of pallor following the original LPN section). Note that areas of skin immediately to the left of the midline and at the front of the mantle retain their ability to generate patterns immediately after the third LPN section. This is due to ingrowth of nerves from the right (unoperated) side of the mantle during the course of regeneration over the previous months (mantle length 9-11 cm; encircled, long mantle papillae).

The mantle of an octopus of the size used by Fredericq contains several million chromatophore organs, most of them on the dorsal surface. Each of them has a halo of muscle fibres - 20/30 in red and brown chromatophores - radiating from its central pigment body and lying in the plane of the skin surface. It is these muscles which relax in synchrony when the nerve is cut and which once again come under orderly nervous control if the nerve succeeds in reconnecting with the skin. Orderly control includes the ability to produce a wide range of patterns, or compositions, in which the performance of the operated side of

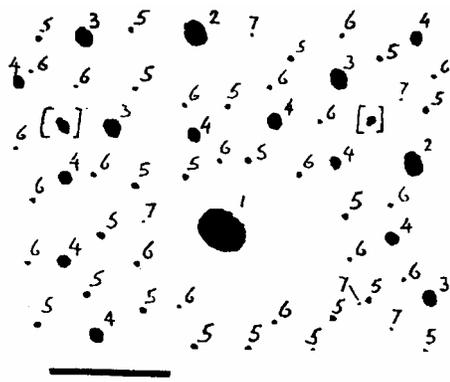


Figure 4. Developmental key to a nested hierarchy. Detail of the chromatophore array on the mantle of a squid (*Loligo vulgaris*) with individual elements (retracted chromatophores) numbered according to size and developmental sequence. 1=largest, oldest, darkest (black) and deepest; 7 = smallest, youngest, lightest (yellow) and most superficial. Note typical spacing. Scale 0.5 mm. (N.B. Similar size/age/colour arrangements hold for octopus skin, but relative positions are different and the youngest are in the deepest layer).

the mantle is almost indistinguishable from that of the mirror-image "control" side whose nerve supply was left intact. Evidently the tens of thousands of nerve fibres growing out from the brain in the pallial nerve "know" which of the many millions of chromatophore muscle fibres are the "right" ones for them to innervate.

How is this apparent miracle achieved?

The mantle that was denervated contained, at hatching, only four chromatophores on its dorsal surface [13, 28], so one way to answer the question is to ask how did the four become four million. Uncovering the rules of development will lead us, as so often in biology, to some further questions about what kind of orderliness the brain possesses. So it is not just a play of words that the term pattern generation is used, in what follows, both for the developmental (epigenetic) processes through which the skin differentiates, and for the neurophysiological (motor) processes governing the multiple compositions appearing on the skin under natural circumstances.

4.2. PATTERN-GENERATION IN THE SKIN

4.2.1. A self-organised hierarchy

The first thing to notice about the octopus skin is that the pattern generating elements - i.e. the chromatophore organs or their undifferentiated precursor cells - are present in the periphery before connection of these elements with the brain [19], and that their spatial and temporal distribution is self-regulated, hierarchical [28] and independent of subsequent innervation. They arise, through episodes of recruitment, as a temporal series that is nested spatially (Fig. 4) and governed by autopoietic rules which, as far as can be gathered, are intrinsic to the skin. Furthermore, although there are great variations in the numbers, sizes and densities of chromatophores from one part of the skin to another - depending on the ontogenetic stage of the animal, whether that part is on the arms, the head or the mantle, and where it lies in the various subdivisions of these fields - there is good reason to believe that the autopoietic rules are the same throughout life (and from one cephalopod to another) and that the variations are due to small variations in the values applying in the algorithms and in the number of iterations undergone at each location. (For instance ventral skin lags behind

dorsal skin during the benthic life of the octopus, though during planktonic life it was well ahead [28]). Everywhere the skin is different yet everywhere the same rules apply.

4.2.2. A chromatophore muscle network

The second thing to notice is that connection between brain and periphery is both direct and indirect. The dark chromatophores of squids all appear to receive direct connections from the brain, and this may be so of the early generations of octopus chromatophores. As the octopus grows and recruitment of chromatophores into the skin continues, however, innervation becomes progressively sparser, fewer and fewer direct connections between nerves and muscles can be found [7]. Nervous coordination of chromatophore organs is largely indirect - almost entirely so on the ventral surface - through a network of muscle-to-muscle contacts [33].

As the network is a dynamic one - a community of coupled ensembles [33] - it may never be possible to work out its structure and extent, but it appears that muscle-to-muscle contacts are preferentially between (a) chromatophores of the same age/size/colour class, (b) chromatophores of the same class in the regional subdivision into which they were born (since dark waves pass less readily between regions or motor fields than within them) [31, 33].

The dramatic waves of colour ("passing cloud") generated by the central nervous system in the Caribbean reef squid (*Sepioteuthis*), and some cuttlefishes and octopuses, when they are hunting, are probably waves of activity spreading through the muscle network. But they have not yet been analysed.

4.2.3. The rules of reconnection

Thus - to return to Fredericq's experiment, and those of Sanders and Young [39] - while the obvious signs of skin patterning disappear when the nerve is severed, the epigenetic pattern (i.e. the pattern that results from epigenesis) remains firmly in place. It remains in the skin's history of differentiation (into separate regional modules) up to the time of operation, and in the nested series of chromatophore generations (discernible in the distinct sizes and colours present at any location within these regions) (Fig. 4). In principle, there needs to be no map in the brain for its nerves to make the right connections, either during the normal course of development or when the nerve regenerates experimentally. The map is already laid out in the skin, in great detail, both in parallel (the regional modules) and in series (the nested generation modules). Theoretically, during normal development, all that the brain needs to "know" is to synchronise the production of motor neurones in its chromatophore lobes with the production of chromatophore muscles in the skin, and to instruct their outgrowing axons to occupy any chromatophore muscle fibres not already innervated.

It is not known whether regenerating nerve fibres return to the skin in the same sequence as in ontogeny. If they do, a small modification of these instructions would ensure a return of normal connectivity. The extensive interconnections existing within the muscle-muscle network - the coupled ensembles already described - must considerably assist the process of reconnection by broadening the target area for an ingrowing nerve fibre: essentially from that of a single chromatophore to that of a whole population of chromatophores².

² Nor is it known whether recruitment of new chromatophores into the skin continues in the absence of a nerve supply to the skin (during the first weeks of regeneration). There is some evidence that it does not, or is drastically reduced. The finest motor units, innervating the smallest (ontogenetically youngest) dark chromatophores, are missing from the photographs of otherwise completely regenerated patterns (Fig. 3A and 3B). There is also some persistent supersensitivity on the operated side, especially posteriorly, suggesting that reinnervation is still incomplete. Moreover, the animal remains "lame" on the operated side, though less obviously so when stimulated (Fig. 3B), presumably because of the surgery's long-term trans-synaptic effects in the posterior chromatophore lobe, and possibly above.

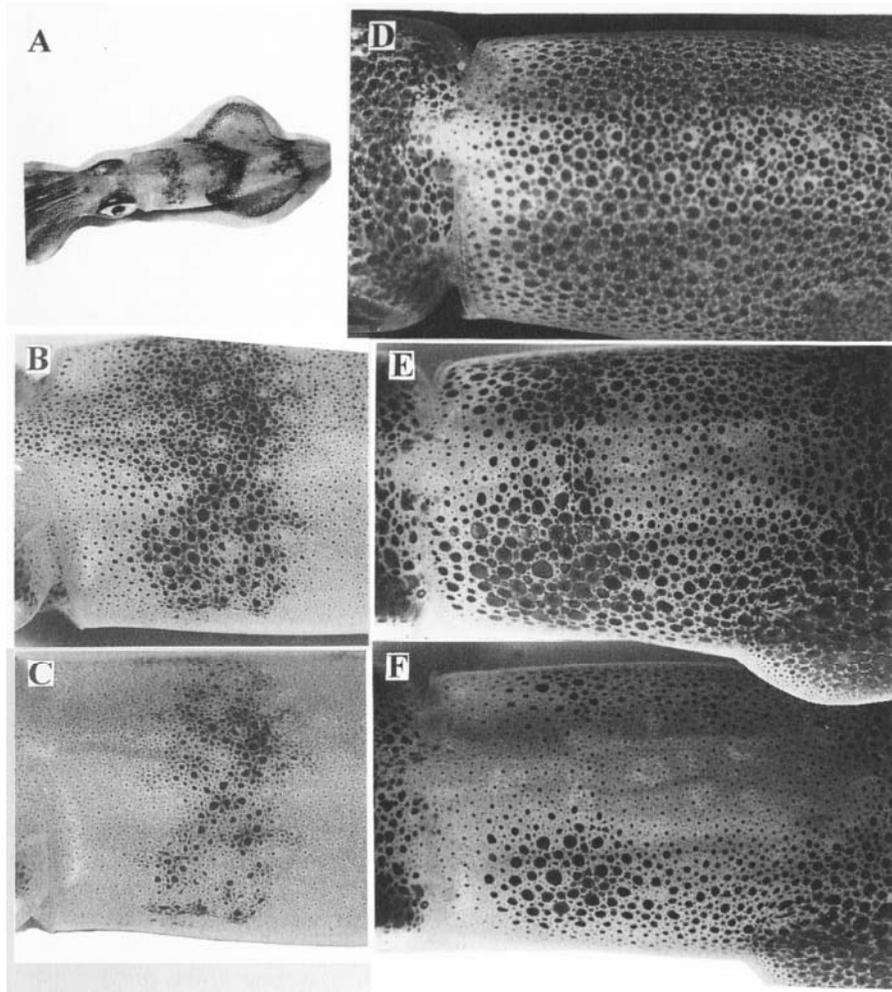


Figure 5. Superposition of components in life. Dorsal view (slightly from the left side) of a sub-adult squid displaying some normal patterns in the confines of the wet table. A) Whole animal exhibiting the cryptic dress "ring" and "fletch" (see Fig. 7), B) and C) same animal showing details of the contribution of individual red and brown chromatophores (black in this print) to the anterior "ring", and of yellow chromatophores (grey in this print) to the "yellow" uniform screen. Note that between B) and C) the amplitude of expression of the components alters (see text). D-F) Details of chromatophore behaviour in the same area of the mantle during some of the other patterns exhibited by this squid: D) "all dark", F) lateral "blush". (Note that the "blush" (see Fig. 7 second from bottom left) is incorporated into "all dark"). E) is an intermediate condition (*Loligo vulgaris*, dorsal mantle length 12 cm, width 3 cm).

4.3. ANALYSIS OF SQUID CRYPTIC PATTERNS DOWN TO MOTOR UNIT LEVEL

The motor unit structure of the patterns displayed by an octopus in life can be explored after its death by electrical stimulation of the skin surface [27].

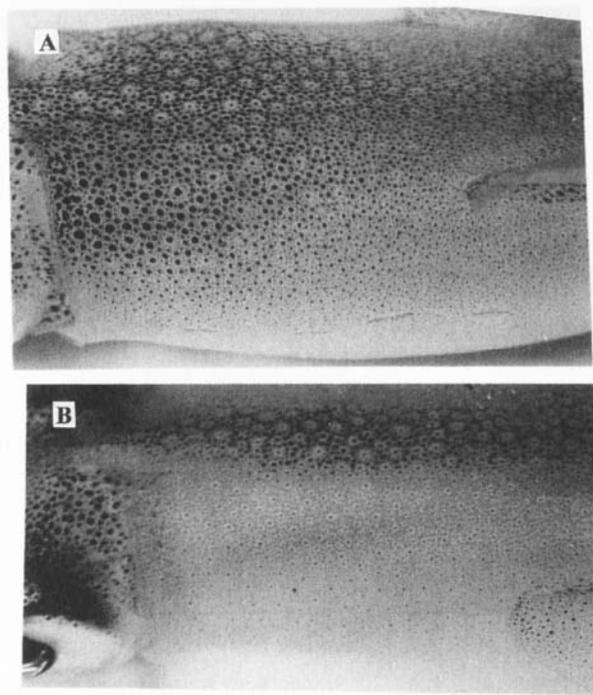


Figure 6. Graded components. Same animal as Fig. 5 (tranquillized in 0.25% alcoholic seawater) exhibiting (A) red and brown gradients, and (B) midline black component dorsally and dorso-lateral gradient, as in countershading. (Black, brown and red chromatophores are black in these prints; yellow chromatophores are grey).

The chromatophores of octopuses are small and crowded and many of them remain tonically expanded at rest, making this kind of ontophysiological analysis more difficult than with squids. Detailed analysis of components - from patterns to components to units down to the chromatophore level, and the changes at each level - was undertaken by Dubas *et al.* [9] for a squid, *Lolliguncula brevis*, with its much larger chromatophores and relatively simple patterns. Here, we shall examine two patterns readily observed in another squid, *Loligo vulgaris*, capturing all of the steps in a single set of photographs without sacrificing the animal and without resorting to electrical stimulation.

The patterns illustrated include the cryptic patterns, "ring" [16] (Fig 5A), and "countershading" (Fig. 6): part of the repertoire of *L. vulgaris* (Fig. 7). They are displayed in captivity when squids retreat to the bottom on being disturbed, and in some individuals even when they are transferred into the coloured bowls (Fig. 1) used to obtain the close-up photographs shown in Fig. 5 and 6.

4.3.1. Amplitude changes and neurophysiological gradients

When fully expressed, the dark rings (three in *L. vulgaris* (Fig. 7, bottom right), more in some other species [16]) characteristic of this pattern go all the way round the mantle, but, when incompletely expressed, they shrink to mere transverse bands confined to the dorsal

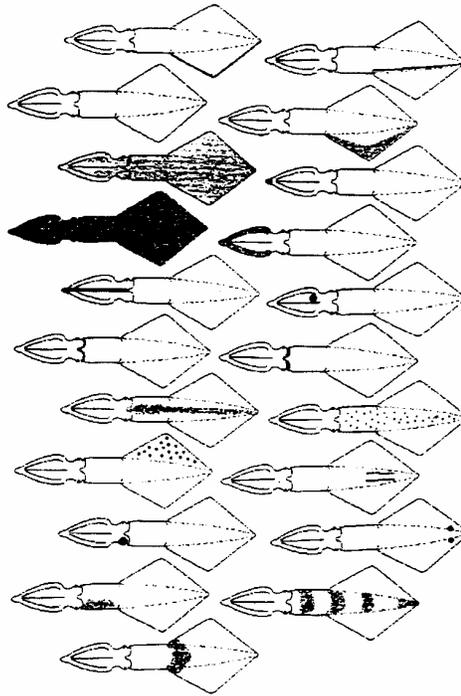


Figure 7. Components listed as icons. Diagrammatic dorsal view of *Loligo vulgaris* showing some of the many chromatic components observed in captivity (after [6]). The "dark fin edge" component is seen upper left, "ring" is bottom right.

surface. In our catalogues of the colour-change repertoire (Fig. 7) of this and other species of cephalopod, we do not attempt to indicate the various degrees of expression of the bands/rings and other such components. They are difficult to describe. Yet the amplitude characteristics of pattern components (for instance eye-bar and extended eye-bar of octopuses [37]) are just as important as their position and colour. How are they achieved? Box 2 summarizes some of the findings discussed up to this point and in the next section.

Several pieces of evidence (see [33, 36, 37] also for reference) show that components are made up of banks of like motor units (of the same class). Differences in degree of expression are due to differences in the activity and number of motor units recruited, much as is envisaged in classical accounts of motor units modulating the forces developed during posture and movement. Such modulation can be achieved either by increases in the force developed within a motor unit (by increasing the firing rate) or by bringing in additional motor units (recruitment) or by both. Ferguson *et al.* [11] succeeded in distinguishing between these two factors by combining electrical stimulation and lesions of adjacent stellar nerves supplying the same chromatophores.

The main factor complicating the interpretation of chromatic responses is that the majority of dark chromatophore organs on the dorsal surface of squids are multiply

innervated [14] (i.e. a number of different nerve fibres, from different motor neurones, supply the many muscle fibres of a single organ)'.

Differential activity of motor units sharing the same chromatophore organ can be seen in its shape when partially expanded, for the shape indicates which muscle fibres are contracting. Of the two main classes of dark chromatophore contributing to "ring" in these photographs, the larger ones (black or brown) tend to be round and the smaller, interspersed (red) ones, may be rectangular or polygonal.

"Spray-gun" effects during feature generation. There are no obvious boundaries, in the close-up photographs of the different manifestations of the bands/rings making up the "ring" pattern of *Loligo* (Fig. 5B and 5C), between chromatophores (spots) that are taking part and those that are not. Nor are there obvious boundaries in the "blush" feature, seen alone (Fig. 5F) and mixed with other components (Fig 5D and 5E) in this same animal. Changes in the intensity of expression of the components - from one photograph to another - are accomplished by a double process: change in the size of the spots, and thus the degree of darkness (mean grey level) of that part of the skin, and changes in the size of the area of skin going dark (the number of expanded spots). One always seems to accompany the other. Similar effects - even though the shapes of the responding areas of skin are quite unlike any seen in life - can be obtained by stimulation of single stellar nerves at different frequencies [11].

Although the photographs allow the possibility that the changes in spatial extent and in grey level of the dark components might be achieved in steps, frame-by-frame analysis of video-records fails to reveal such steps. Over the 40 ms time intervals between frames, all gradations can be found in the two parameters: numbers of chromatophores (spots) and degree of expansion of spots. It is as if the spots were applied with a spray-gun activated at different settings which alter the number and average size of the spots of paint, the smallest of which go furthest. At no setting is a clean edge produced. Even the units responsible for linear components, like the "dark fin-edge" of squids, do not produce a clean edge when stimulated in isolation [10].

Another point of particular interest emerging from these photographs is that the "all-dark" uniform component (Fig. 5D) seems to incorporate the "blush" motor unit(s) (Fig. 5F). Although the expansion of single chromatophores comprising "blush" grades away from the centre, identical expanded chromatophores in "all-dark" and in "blush" have similar expanded shapes.

Uniform and countershading effects. With these facts in mind, we now turn to the much simpler, though in some ways less obvious, condition of the chromatophores in the rest of the skin - upon which the "ring" features are, so to speak, overlaid (Fig. 8). There are red and yellow ones operating under separate control. Both classes produce gradations of colour (notably dorso-ventral gradients), that are employed in countershading and other patterns along the length of the mantle (Fig. 6).

The essence of countershading in a cylindrically shaped animal, like a squid, that adopts a horizontal posture in mid-water is that the colour (or grey level) on the well-illuminated mid-dorsal surface grade off sinusoidally towards the poorly illuminated ventral surface.

Figure 6B shows such a gradient in a yellow component. A slightly more complex gradient of red (and brown) chromatophores over the same area of skin is shown in Fig. 6A.

The positions of these gradients are not fixed. They can occupy the dorsal surface only, grading from well expanded along the midline to zero expansion medio-laterally. Or the gradient may extend from dorsal midline onto the ventral surfaces. The gradient can also be reversed - as in the reverse countershading described [12] for the cuttlefish and observable also in squids and octopuses.

Factors effecting spatially graded chromatophore activity. The phenomena described above can be accounted for by the following operating within motor units and between motor units (Fig. 12):

- anatomical gradients in numbers of chromatophores (§ 4.2.1.)
- variation in firing frequency within a unit
- territorial overlap between units
- gradients in firing frequency across neighbouring units
- increase, with increase in firing frequency, in the numbers of chromatophores incorporated in a response

they depend on well-known properties such as the relationship between nerve firing rate and force of contraction of muscle and on the amount of multiple innervation. The best recent evidence for them in squids is in Ferguson *et al.* [11]. Evidence for gradients of firing frequency (see § 5.1.2. and Fig. 12) across a range of contiguous units responsible for graded expressions of components such as "ring" and "countershading" (Fig. 5 and 6) is so far only indirect. The components go right across the body of squids and are so large that they must consist of several, perhaps very many, motor units. Because of the graded pattern of expansion, the contribution of individual units is masked. Still quite unexplained is the progressive increase in numbers of chromatophores participating in the response that occurs both in squids [11] and in octopuses [8, 33] with increase in firing frequency.

4.4. VISUO-MOTOR AND LIGHT REFLEXES

The question is often asked: what kind of visual information or stimuli are required for particular patterns to be generated. It has been answered by Young and colleagues [47] for one kind of pattern - the photo-generated countershading reflex of oceanic squids - and a similar reflex might modulate the colour gradients employed in the countershading of squids, just described, and the dark screens that modulate octopus camouflage (Fig. 8). Both require estimation of the amount of downwelling light and eventual comparison of this value with the amount of upwelling light. The ratio between the two, for a bottom-dwelling animal, is the albedo. The role of information from the statocysts in a body position--dependent colour reflex has also recently been re-examined [12].

One of the easiest ways to obtain changes in colour displays is by discharge of an electronic flash (Fig. 3B) which causes reflex darkening of the skin [35]. In octopuses, the delay between generalised stimulation of the retina and the onset of the darkening response is 400-600 ms [35]. The increase in grey level occurs without in other ways altering the pattern worn (Fig. 8) and this could be part of the mechanism by which tone-matching [21] is achieved. In squids, the general darkening induced by flash has the effect of abolishing the gradients just described, or, shifts the position of the gradient by extending the region of darkening further round the body. When general darkening occurs, features such as the

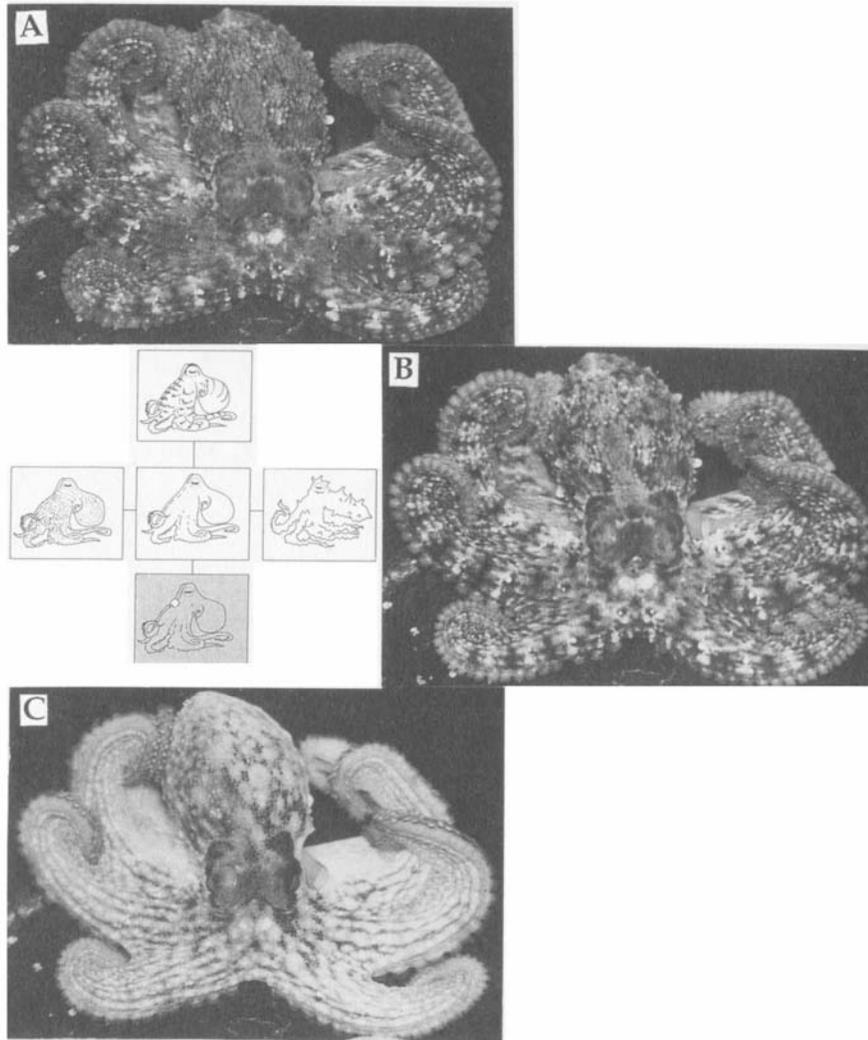


Figure 8. Modular structure of body patterns. Diagram, separate classes of components in the body pattern repertoire of a young octopus: postural (centre), chromatic features (above), screens (left), textural (right), focal "frontal white spot" - exhibited when an octopus is otherwise tone-matched with its environment (bottom). Classes of components are connected and can be superimposed. Photographs, 3-step modification (modulation) of a stereotyped pattern (acute mottle) exhibited by sub-adult *Octopus vulgaris* in black bowl [33]. 1) From B to A, increase in intensity (amplitude) of expression of dark screen without change of pattern, 2) from B to C, dark bars and other transverse component(s) switched off at the approach of a threatening stimulus on the right and 3) addition of new dark component (polka dots).

lateral blush (Fig. 5F) are swamped: the units comprising this component become part of the bigger component "all dark" (Fig. 5D).



Figure 9. Camouflaged Sepia officinalis.

4.5. GENERAL RESEMBLANCE IN OCTOPUSES AND TONIC MUSCLE PROPERTIES

Mention has been made of the fact that many octopus chromatophores remain in a partially expanded condition after the death of the animal. The maintenance of tonus in chromatophore muscles occurs also when the animal is at rest and is perhaps related to the requirements of camouflage in a benthic habitat: octopuses sit on the bottom for long hours wearing the same general dull background colour. A skin musculature possessing tonic properties would reduce the energy costs of an animal continuously matching the albedo.

4.6. RELAXATION OF CHROMATOPHORE MUSCLES: THE IMPORTANCE OF "WHITENESS"

The tonic state is not seen in squids, and can be reversibly abolished in octopuses and cuttlefish by subcutaneous injection of serotonin in the living animal [2], or, post mortem, by serotonin and after electrical stimulation of the skin. Both are well known to relax the "catch" of molluscan muscle. Does this play a role in natural patterning?

As pointed out in [33] chromatophores can only generate visual information by generating brightness-contrast. When all chromatophores are totally relaxed, the body of squids and very young octopuses becomes translucent, or the skin appears white by reflection from underlying tissues. In this condition the animal itself may stand out against the background - or generally resemble the background - but no brightness-contrast is generated within the borders of the animal. The same is true when the dark (black, brown and red) chromatophores are so extensively expanded - "all dark" - that no underlying tissue can be seen between them. Here again, any contrast is limited to the animal as against its background (object/ground).

Feature generation by chromatophores, on the other hand (see above), always involves differential expansion of chromatophores. Other things being equal, the maximum height (amplitude) of the contrast at a location is the same as the ratio between the grey level created by a population of maximally expanded chromatophores and the grey level of the "white" skin in its immediate neighbourhood where chromatophores are absent or maximally retracted. Nervous control over the contraction of chromatophore muscle fibres that cause chromatophores to expand is relatively well documented. Is there also control over chromatophore retraction?

Although debated in the past (see [24] for references) it had lately been generally assumed that the pallor of relaxed skin, and the complete retraction of the pigment bodies of chromatophores into a tight spherical form, was a sign that nerves had ceased firing and that the return to resting length of the radially arranged muscle fibres is accomplished by elastic restoring forces in the cyto-elastic sac enveloping the pigment body. However, it now appears that this is not the full story. One of the first signs of recovery of nerve function in the mantle of an octopus that had been denervated (see above) is the ability to pale⁴: to suppress the tonic darkening characteristic of denervated chromatophores.

Here, then, is another example of an observation, made in semi-natural conditions, forcing a rethinking of physiological facts. It is not known by what mechanism neurogenic relaxation [32] takes place in the intact animal, nor whether, as seems likely it occurs also in squids and cuttlefish. (In the meantime it has been found to be general in vertebrate smooth muscle, mediated by nitric oxide).

4.7. WHAT DOES THE BRAIN DO?

So far I have presented the colour patterns of cephalopods as if all of the action were at the level of the skin. In some ways this is true: as remarked by Young [45] the most interesting patterns are not phasic but static patterns of activity, perhaps requiring more detailed control over muscle fibres than required in the human hand. In final analysis, the details of the complex camouflage patterns of octopus and cuttlefish (Fig. 9) are details inherent in the chromatophore array and its connections at the periphery. These sustained patterns are like a chord of music played upon a keyboard. The single keys are the single components or banks of motor units.

⁴ Even in normal animals, otherwise dark octopuses go pale on the side of the mantle towards a crab when they attack it ("pale on the attack side", Packard and Young, unpublished data). Looking back through the protocol, this phenomenon, in long-term pallial-nerve-sectioned animals, was also the first evidence for neurogenic relaxation [32]. That the ability of nerves to abolish the tonic darkening of denervated chromatophores by actively relaxing their muscle fibres is transmitted through the muscle network and is a normal property, is shown by the fact that even a few days after section of the nerve to one side of the mantle, there is usually a pale strip of skin bounding the midline on the operated side - the paramedian area [30, 331] - due to pattern-generating activity on the normal side.

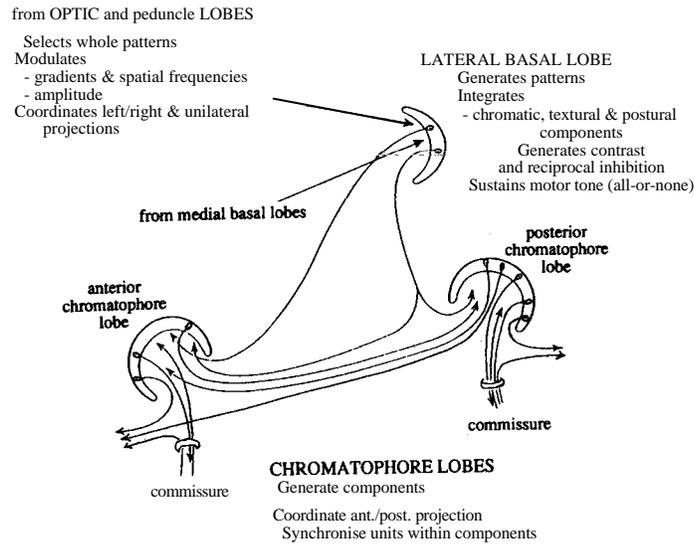


Figure 10. Role of brain in pattern regulation based on behavioural and anatomical studies. The diagram is a modification of Boycott's original drawing (updated [33]).

4.7.1. Lowest motor centres

The lowest and the intermediate brain centres, and their interconnections, responsible for the patterns are shown in Fig. 10.

It is possible to study the patterns of octopuses with most of the brain missing [1, 5]. The optic tracts linking optic lobes with the rest of the brain are severed, the remaining supraoesophageal brain is undercut and removed, with the exception of the superior buccal lobes so that the octopus retains the capacity to eat⁵ (Packard, unpublished data).

The behaviour of the long-term "sub-oesophageal" octopus is extremely limited. The animal is flaccid (motor tonus low), breathing is deep and slow, and the animal is a general grey colour: far from the white pallor, referred to above. Although nerve fibres to the skin and their nerve cell bodies in the chromatophore lobes of the brain are intact, the motor tonus of the skin mediated by the nerves is very low, and little waves of colour, typical of denervated and poorly innervated skin, run about in different directions, especially on the ventral surface. In the absence of higher control, the chromatophore lobes apparently do nothing. However, if stimulated vigorously - mechanically, pharmacologically and even by flash - they can be momentarily revived. Occasionally they revive spontaneously. The little waves are suppressed and coordinated expansion of various populations of chromatophores responsible for single components occurs over the whole body, or the whole of one side of the body, sometimes in exaggerated form. The components appearing in the "sub-oesophageal" octopus are those responsible for the ground colours of patterns, that are difficult to pick out from other, bolder, components during normal pattern generation of the intact animal.

⁵ The survival of such animals during the first days is poor. Especially critical is a transition period (about 48 h at 22° C). Up to this time behaviour and postures are as described in Boycott and Young [5], but then the depth and rate of ventilation drops so low that the animal can die of asphyxia

BOX 2. PERIPHERAL AND CENTRAL NERVOUS ORGANISATION OF CEPHALOPOD CHROMATOPHORE SYSTEMS. FROM EXPERIMENTAL OBSERVATIONS ON WHOLE ANIMALS.

- 1) patterns are stereotypical and hierarchically organized,
- 2) the repertoire of central pattern generators, and the spatial frequencies available, expand during ontogeny in synchrony with the recruitment of chromatophores into the skin,
- 3) chromatophores act as coupled ensembles,
- 4) control is both nervous and myogenic,
- 5) myogenic activity is modulated by nerves through an unknown mechanism,
- 6) nerves can actively relax chromatophore and skin muscles (mechanism unknown),
- 7) motor units can be classified by the locations, and by the colour, size and age (three dependent variables) of the populations of chromatophores they supply,
- 8) motor units of a given class are strongly coupled centrally,
- 9) higher centres modify the coupling within banks (or pools) of motor units producing gradients of activity according to the main axes of the body,
- 10) simultaneous contrast is generated by lateral inhibition,
- 11) pattern generators are in intermediate centres and subject to selection and gain control by the highest centres. □

The following have been observed (dissociated from each other and from other components): yellow, orange, and umber screens, ventral red, "all dark", "orange surrounds", "polka dots". In one "sub-oesophageal" animal, the dorsal trellis - an all-groove reticulated component, perhaps the commonest of all components in the octopus - appeared in the first days following the severe operation, then began to break up and disappeared in subsequent weeks. The bold, repeating, dark bars and white spots are missing.

Similarly pure, and sometimes exaggerated, components of texture and papillae - unassociated with colour, just as pure colour components were unassociated with texture - also occur. Evidently, in the "sub-oesophageal" octopus, the motor neurones, though most of the time switched off, are not only intact, as anatomy would predict, but are also capable of functioning and of being integrated temporally (because synchronous) and spatially (because body-wide) into the components seen in the normal body pattern repertoire. It is their expression in isolation, and lack of integration with other components, that is abnormal in the "sub-oesophageal" preparation.

"Sub-oesophageal" animals are also totally unable to switch from one meaningful pattern to another, or even to switch readily from one component to another, or to modify the expression of these.

Specifications for the lowest lobes of the chromatophore system. Although Boycott [3], from whom Fig. 10 is taken, drew attention to the relatively complex anatomy of the chromatophore lobes of *Octopus vulgaris* and *Sepia officinalis* - both benthic animals -

compared with those of *Argonauta* and of squids with their "simple" colour system, these lobes do not have the kind of cytoarchitecture seen, for instance, in the deep retina of the optic lobes.

Minimum specifications for the chromatophore lobes (anterior and posterior) deduced from the behavioural observations on "suboesophageal" and other animals are that: - they house the motor neurones of the skin,

- the motor neurones possess the appropriate dynamic range (zero/max) (from activity for complete retraction of chromatophores - and relaxation of dermal muscles - to complete expansion - and maximal contraction of dermal musculature),
- they provide connections between motor neurones of the same class supplying different regions of the body (right/left; arms, head, mantle),
- they provide connections between motor neurones of the same class (components),
- the connectivity within banks of motor neurones be modifiable and potentially graded.

Arguments for the last of these (graded and modifiable connectivity) is given in § 5. The other specifications should be obvious from the descriptions given above.

Although the anatomical and physiological mechanisms fulfilling these specifications are still very poorly known - for instance the dynamic range, in terms of firing frequencies, of motor neurones of the chromatophore lobes - there is an established basis, at motor neurone level, for coordination between lobes [9,10,25] (different body parts) and the recent demonstration that some motor neurones are dye-coupled (Ferguson, pers. comm.) provides a basis for the coupled banks of motor neurone generating components.

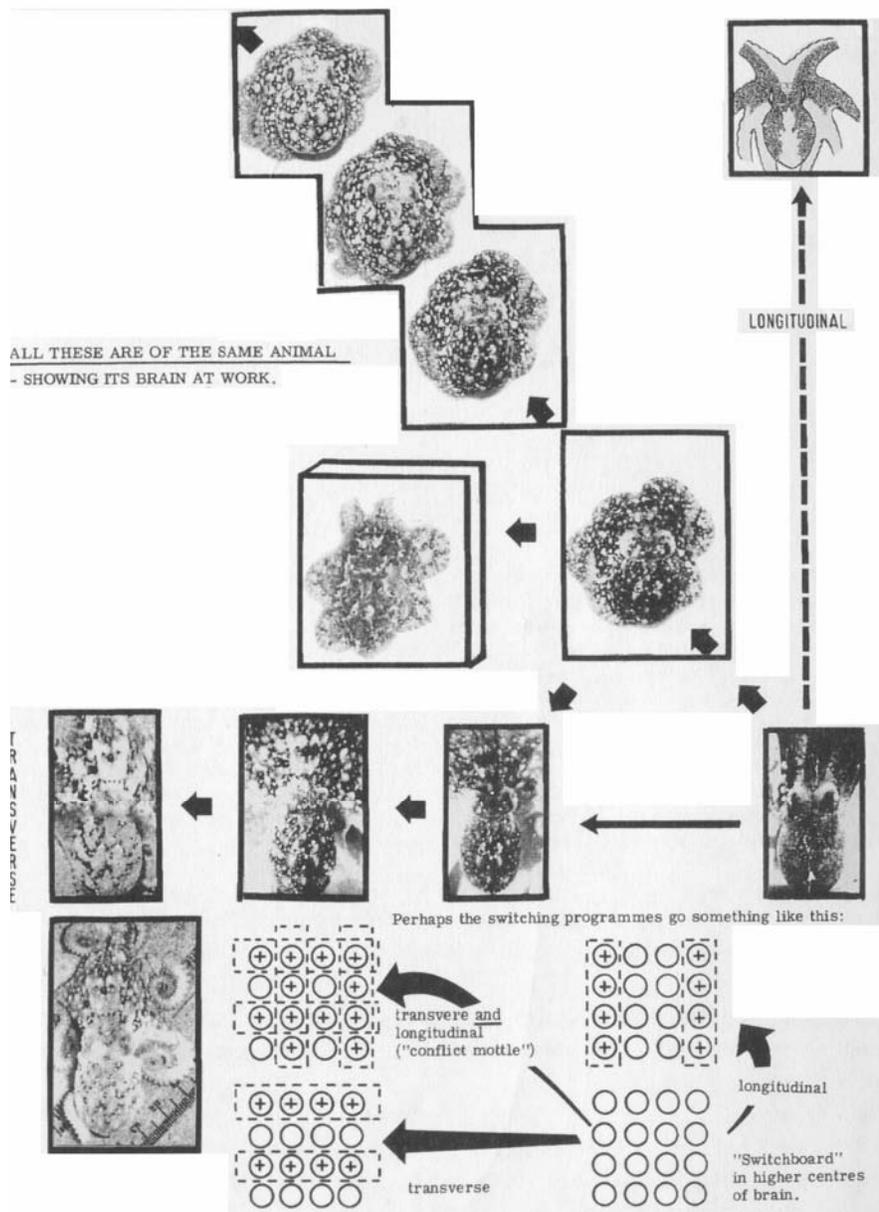
At least one transmitter, FRFamide is known for one of the populations of motor neurones [20]. It is excitatory. L-glutamate appears to be another; its antagonists stain many cell bodies (Messenger, pers. comm.) as well as fine branches at the periphery. Serotonin (5HT) produces a dark mottle ("conflict mottle") in "suboesophageal" octopuses [1]. It is a candidate transmitter for one of the chromatophore interneurone populations, and possibly for certain motor neurones.

4.7.2. Higher motor centres

Similar attempts at task-analysis can, in principle, be pursued for the higher centres of the colour-pattern system. Some of the specifications are indicated in the additions to Figure 10 (taken from [32]) and in Box 2, however, insufficient experiments have been done to distinguish between the roles of optic, peduncle and lateral basal lobes. In principle, the list should not contain entries that duplicate tasks already provided for at lower levels, even though similar mechanisms might be involved.

One of the tasks of the eye and optic lobes is tone-matching the animal with its background. This amplitude control task - to match the albedo (see § 4.4.) - could be performed by two-photometer neurones, one connected with ventral, the other with dorsal retina, that set the appropriate firing frequency (grey level of the skin) of motor neurones already active.

The task of selecting the appropriate spatial frequencies for camouflage patterns of the kind seen in Fig. 8 and 9 is a more refined one. It requires a range of spatial frequency detectors (presumably in the optic lobes) connected to releasers of the appropriate spatial frequency generators - bands, bars, lines, streaks, reticulations and spots - in the skin. These could be in any of the higher lobes.



(N.B. There is also an intensity ("gain") control but this is not shown on the switchboard).

Figure 11. Central control of body patterning. Unpublished poster of some mottles (brightness contrast compositions) worn by a Californian octopus (*Octopus rubescens*) exhibiting body-wide coordination and systematic variations in amplitude and orientation of constituent dark/light (brightness contrast) components. The circles on the "switchboard", arranged in banks (see text), are envisaged as motor units controlling darkening. + = units that are switched "on". Note that amplitude variations in the "transverse" and the "conflict mottle" alter the relative amounts of dark and of light. (Units supplying light areas are switched off.)

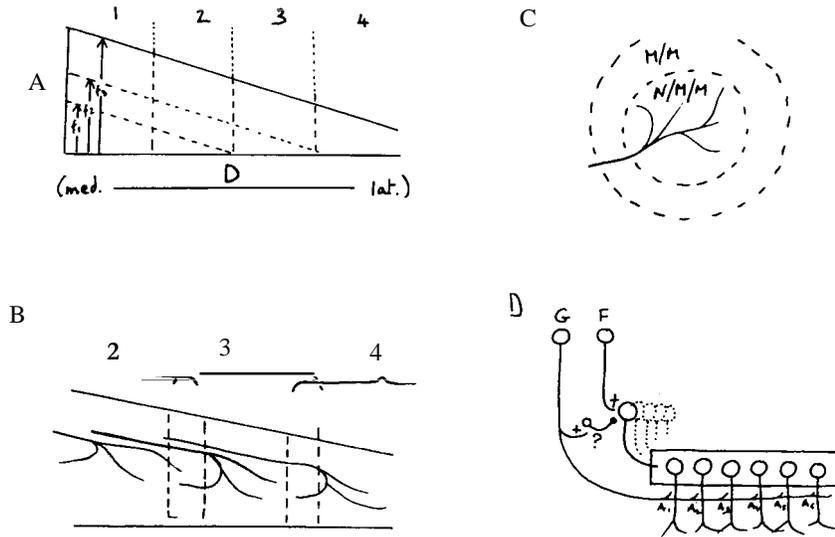


Figure 12. Amplitude control of dark features and colour gradients. A) Illustration of the relationship between the output (D, A) of a bank of motor units 1-4 responsible for generating a feature or a uniform component that alters its amplitude of expression - i.e. both spatial extent (D) and grey level (A) - with firing frequency (f); B) close-up of the terminations of units 2-4 in the bank, illustrating overlap between units (the slope indicates numbers of participating muscle fibres or chromatophores and/or extent of participation); C) terminations of an octopus motor unit in the skin: N/M/M zone of neuro-muscular and of muscle-muscle contacts, M/M zone of myogenic communication only for this unit; D) putative circuitry in the chromatophore lobes and higher centres controlling the output of a bank of motor units (circles linked by a rectangular bar) responsible both for a feature (F) and a gradient (G). The interneurone controlling the gradient (G) activates the members of the bank with serially decreasing strength $A_1 > A_2 > A_3 > A_4 > A_5 > A_6$. (N.B. G and F do not necessarily operate through the same motor units. The "yellow" and "ring" components illustrated in this chapter are different classes of motor units).

But the most obvious of the higher centre tasks are connected with 1) the *synthesis* of several components into stereotypical whole patterns - the chord of music, which is apparently read from a score - that do not require any specific visual input (as the photographs taken on the wet table demonstrate), 2) switching on and off and modifying the intensity of expression (amplitude) of these (Fig. 8), and 3) selection and other phasic aspects of pattern generation.

Patterns comprising several components, both chromatic and textural, can be generated by the lateral basal lobes alone (without optic lobe input) - and for this reason are marked as pattern generating centres in Fig. 10 - but these patterns are not "normal". They are stiff, exaggerated or collapse suddenly, and seem to lack the fine details (high spatial frequency components) of "normal" patterns.

The optic (and/or peduncle) lobes, therefore, can at least be classified as centres of pattern *modulation*.

The need for some kind of control over gradients of activity in the banks of motor neurones responsible for certain components (see above and Fig. 6) is the subject of the next section and could also be furnished by these lobes.

5. Discussion and Conclusions

5.1 INSIGHTS FROM OBSERVATION OF NATURAL PATTERN GENERATION

5.1.1. *Parallel processing, synchrony and coordination*

In the lead up to the "decade of the brain", sequential analysis of brains and behaviour - for instance the various transduction processes on the visual pathway or the different steps in the generation of movement - attained much more sophisticated levels than the analysis of interactions within populations of like elements. The whole of biology seems to have been conditioned by the facility of sequential processing as distinct from parallel processing. Often we remain totally ignorant about the latter, even though a brain cannot learn [40,46], an eye cannot see and a limb cannot move without massive coordination between similar cells at each level of the well-studied pathways for memory, vision and movement. Only recently have we begun to acquire techniques for in-parallel analysis.

Squids, cuttlefish and octopuses are evidently rather good at parallel processing. Common to all the skin patterns, both of octopods and decapods is the *synchrony* and/or *coordination* apparent within populations of similar units. Even though the terminations of the units comprising a component may be many centimetres apart - or, in very large animals such as the Giant Pacific Octopus (*Octopus dofleini*) feet apart - centrally initiated synchrony and coordination extend body-wide (Fig. 11). I have been referring to them here and elsewhere as taking place within *banks* of motor units and also *coupled ensembles* [33]. They are shown diagrammatically in Fig. 11 and 12 as rows of units being depressed by a bar: physical coupling between units being represented by the bar (the single key in the chord of music). Coupling between banks of motor neurones of the same class (components) is so strong that there must be, within the chromatophore lobes (Fig. 12D), interneurones which link banks of the same class together: or else collaterals running between the motor neurones themselves.

This need not detract from the equally important evidence that the coupling can be broken, as in the simple observation that "blush" (Fig. 5F) can be expressed separately from "all dark" (Fig. 5D).

5.1.2. *Amplitude control and physiological gradients*

The "pressure" on the bar is subject to *gain control*, presumably through alterations in firing frequency of interneurones, resulting in alterations in firing frequency of the motor neurones controlled. The "pressure" can also be smoothly *graded* from one end to another of the row of units. Figure 12 illustrates possible mechanisms.

As indicated in Fig. 12D, the necessary connections (the bar itself and the other forms of coupling between units) are an inherent part of the structure of the chromatophore lobes. Control of the bar comes from above this level.

The arrangements suggested in Fig. 12 can explain the observed changes in intensity of expression of a given pattern and variations in recruitment of the units comprising a component. They do not explain the smooth gradients observable with distance *within* the boundaries of a motor unit. The gradients (in numbers of muscle fibres contracting, and/or degree of their contraction) are a frequency-dependent property of the periphery itself. In octopuses (Fig. 12C) there are probably frequency-dependent gradients in the extent of coupling between muscle fibres of the chromatophore muscle fibre network through the process of spatial summation [8, 33].

One should not exclude the possibility that enlargement of the responding area (away from the point of maximal response) that occurs with increase in stimulus frequency is due to invasion of fine branches of a motor unit previously uninvaded by the impulse. Almost nothing is known of the electrical and other properties of these fine branches.

The arrangements also do not explain the famous centrally-controlled waves of colour of hunting cephalopods that pass repetitively over the same area of skin, and that sometimes reverse in direction. They give the impression of waves of electrical activity passing through electrically coupled elements - motor neurones, muscle fibres or both - very much as in spreading depression.

5.2. COUPLED ENSEMBLES AND THEIR MODULATION

The considerations presented here also help to explain the results obtained by extracellular electrical stimulation of the brain. Boycott [4] commented on the consistent finding that while stimulation anywhere in the highest motor centres of the cuttlefish (*Sepia*) brain, produces responses similar to the behaviours seen in life, stimulation of any of the lowest lobes produces total non-modulated responses throughout the system controlled by that lobe. The fins exemplify this very clearly. Stimulation of the optic lobes produces coordinated waves along the length of the fins, stimulation of the fin lobe produces uncoordinated contractions of both sets of muscles (for raising and for lowering the fin) along the whole of its length. Analogous responses are obtained from the chromatophore system: optic lobe stimulation produces coordinated patterning, chromatophore lobe stimulation "produces a total response of all the chromatophores" of one or both sides. These results were not a question of poor localization of the stimulus, and Boycott interpreted them as evidence that the cells responsible for the responses were spread throughout the lobes concerned.

The findings can, instead, be seen as indication of the extent of coupling between neurones responsible for like activities. Stimulation anywhere within a coupled ensemble will activate the whole ensemble if it is strongly coupled. Ensembles existing at higher levels, globally activated by stimulation at any point, will, if that is the activity for which it is responsible, produce modulation of the coupling in the lower ensembles. The sensitivity of the latter to electrical stimulation is very high, suggesting that the modifiable coupling within the ensembles is also electrical. Be that as it may, both in brain and skin, electrical coupling (via modifiable gap junctions?) is an appropriate communication pathway for like elements engaged in the same task.

5.3. VALIDITY OF THE COMPARATIVE APPROACH

Here I have treated octopuses, squids and cuttlefish as one, because of the many behavioural, anatomical and physiological properties that octopods and decapods have in common. But it is worth recalling that the common properties of their colour systems transcend phylogenetic separation of the two groups some 200 million years ago: one third as long as the geological time span separating cephalopods from all other groups of animals (with their very different colour systems).

Cephalopods do not come from Mars. Arguably, the success of these abundant animals has much to do with the uses to which the skin is put. Squids that gave to science the giant axon, extraordinary in its size, share the biophysical properties of other creatures on this

planet. The extraordinariness of the colour-change system is that integrative properties of its neurones and its muscles can be perceived at first hand by the brain/behaviour scientist through the thinnest of transparent coverings. The properties themselves will turn out to be general.

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