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Colour changes in cephalopods after neurotransmitter injection into the cephalic aorta

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[Plate 1]

A method by which small quantities (1–10 μg) of neurotransmitters can be injected into the blood supplying the brain of cephalopods has been used to produce conspicuous and instantaneous colour changes in the skin of the arms, head and body. Of the transmitter substances known to be present in the cephalopod brain, dopamine, noradrenalin and octopamine cause darkening when injected, acetylcholine causes paling and 5-hydroxytryptamine elicits a mottled patterning. Other evidence is presented that these substances are acting centrally to produce these effects, and the findings are related to the known organization of the lobes in the c.n.s. controlling the chromatophores.

A number of substances known to be neurotransmitters elsewhere have been detected pharmacologically in the cephalopod brain (Tansey 1979) and the localization of some of these has been shown histochemically (Matus 1973; Tansey 1980). Such findings say nothing about function, of course, but here we describe evidence that some of these putative transmitters cause specific patterned motor responses when injected (in physiological doses) into the blood vessel supplying the brain of anaesthetized cephalopods.

One would expect that the administration of a transmitter or its agonist would elicit muscle movements that could be recorded conventionally by means of suitable transducers. The motor repertoire of cephalopods includes colour change, however, for their chromatophores are unusual in being muscular organs under direct nervous control. The most striking result of these experiments is that after injection the skin shows an almost instantaneous change in colour (strictly, changes in luminance and wavelength: Messenger 1979), which can simply be photographed.

This finding in itself is not novel: as long ago as 1930 Sereni demonstrated colour changes after administering a variety of drugs into the circulation and quite properly emphasized that the effects that he obtained were mediated by the c.n.s. However, no detailed information was available to Sereni about the pharmacology, histochemistry and connectivity of the lobes in the brain that control the chromatophore system. Since then a great deal of information has been amassed

(see Tansey 1979 for review; Young 1971) and we have been able to take advantage of this to test the effects of injecting very small quantities of the more likely transmitter candidates. This preliminary report includes photographic evidence that 5-hydroxytryptamine and acetylcholine may be important neurotransmitters mediating colour patterning in cephalopods.

Materials and Methods

Full details of the method are given in a separate paper (Andrews & Tansey 1981) and we shall only summarize them here. All the animals used were anaesthetized by immersion in 5 °C sea water. The cephalic aorta was exposed just posterior to the brain and a cannula was inserted to where the vessel forks to the two sides of the brain. During surgery respiration usually stopped, but the systemic and branchial hearts continued beating, although at a much slower rate than that reported for the normal, resting animal (Wells 1978). The integrity of the circulation was shown by the fact that blood would pulse from the aorta if the cannula was inadequately secured. After surgery the animal was placed in sea water at 24 °C and respiration returned in about 5 min. Administration of 'drugs' then commenced: a 1 ml syringe was attached to the cannula and 100 µl portions of filtered sea water (s.w.) or drug dissolved in s.w. were injected at about 4 min intervals in the order: s.w., drug A, s.w., drug B. Preliminary trials showed that in octopuses of between 200–500 g a 10 µg dose in 100 µl of s.w. gave consistent results and this was the dose normally administered. Most of the experiments were carried out with *Octopus vulgaris* (Cuvier), but some specimens of *Sepia officinalis* L., *Eledone cirrhosa* (Lamarck) and *Alloteuthis subulata* (Lamarck) were also used.

Results

Table 1 summarizes the main results of the drug injections. The effects persisted for at least 30 s with 10 µg doses but were shorter-lasting with 1 µg doses.

Table 1. Summary of major effects of administered drugs

drug	colour	arm and mantle muscle tone	respiration	pupil size	inking
DA	darkens	increased	inhibited	no effect	no effect
N-AD	darkens	increased	no effect	no effect	no effect
OCT	darkens	no effect	stimulated	no effect	no effect
GABA	darkens	increased	no effect	no effect	no effect
ACh	pales	decreased	inhibited	dilated	no effect
nicotine	pales	decreased	inhibited	no effect	no effect
5-HT	mottles	increased	no effect	no effect	stimulated

(*a*) *Dopamine* (DA) (10 trials). This usually produced overall darkening, the animal taking on a brownish black appearance. At the same time, small pimples appeared on the skin of the arms and mantle. Respiration was inhibited and the mantle contracted strongly, while the upper (proximal) third of the arms became rigid.

(*b*) *Noradrenalin* (N–AD) (8 trials). This induced an overall red-brown colour and increased motor activity in the arms and mantle.

(*c*) *Octopamine* (OCT) (6 trials). When this was injected, the animal became deep red with pimpling of the skin.

(*d*) δ-*Amino butyric acid* (GABA) (7 trials). This caused a characteristically deep reddish brown colour, and the orange chromatophores on the arms became particularly prominent. Tone in arms and mantle was markedly increased and skin pimpling was conspicuous, so that the mantle skin took on the spikey appearance sometimes seen in normal octopuses (Packard & Hochberg 1977).

(*e*) *Acetylcholine* (ACh) (36 trials). Injection of acetylcholine caused instant loss of chromatophore tone, so that the animal became very pale (figure 2). The skin lost all pimpling and became glassy-smooth, while arms and mantle became flaccid and limp. Respiration was inhibited for up to half a minute. In *Eledone* and *Sepia* (6 trials) identical effects were observed with ACh after 2% ethanol anaesthesia at 11–15 °C.

The darkening effects evoked by dopamine, noradrenalin, octopamine, GABA, and with 5-HT and L-glutamate (below) were all transiently reversed by ACh.

(*f*) *Nicotine* (6 trials). The effects of nicotine were broadly similar to those produced by ACh but they persisted much longer, up to at least 10 min. For this reason nicotine was always administered last. Tubocurarine, incidentally, also produced paling but had somewhat different effects on the body musculature.

(*g*) *5-Hydroxytryptamine* (5-HT) (14 trials). This substance produced a most dramatic effect. When injected, it caused the skin of the mantle and arms to take on a mottled appearance (figure 4) very similar indeed to the 'acute general mottle' of Packard & Sanders (1971). The skin tone was heightened and prominent papillae often appeared over the eyes.

(*h*) *Controls.* None of the transient effects described above was ever obtained when the brain was injected with 100 μl portions of s.w. (figures 1, 3) but as an additional control that the responses were mediated by the c.n.s. and not by the systemic circulation we either denervated flaps of skin on the mantle or sectioned the pallial nerve unilaterally. This latter technique was, in fact, adopted as routine in all the later experiments. In all preparations the denervated area of skin never showed darkening or paling. Similarly, sectioning a brachial nerve uncoupled the chromatophores of that arm from the effects of drug injection; and injection of a local anaesthetic (2% xylocaine) near a brachial nerve abolished all response to ACh in that arm. Further evidence for the effect being centrally mediated was that the latency of colour change was always less than 1 s and that the chromatophores of the arm, head and mantle all responded simultaneously.

(*i*) *Additional data for* L-*glutamate.* We have some additional evidence (gathered separately under different conditions) that L-sodium glutamate, too, can cause colour responses. Specimens of *Sepia* ($N = 3$), *Alloteuthis* ($N = 2$) and *Eledone* ($N = 2$) were anaesthetized in 2% ethanol at ambient temperatures (about 11–15 °C), cannulated and allowed to recover. We used the same concentration as that employed recently by Bone & Howarth (1980), i.e. 5×10^{-4} M, so that we routinely administered 8.3 μg of glutamate. This consistently caused darkening of

the arms, head and intact side of the mantle. In these preparations ACh caused paling, but further glutamate injection caused the chromatophores to expand again. There were complementary effects on mantle and arm muscles.

Discussion

The cephalopod chromatophore comprises pigment granules contained in a cytoelastic sac supplied with a set of radial muscles. These receive excitatory innervation only (Florey 1966; Florey & Kriebel 1969; Cloney & Florey 1968). Nervous excitation causes contraction of the radial muscles and *expansion* of the chromatophore; in isolated skin patches ACh also produces this effect. In the absence of excitation, the cytoelastic sac contracts and the muscles relax, causing chromatophore *retraction*. Expansion renders the animal darker; retraction renders it paler. Since the pigments in different chromatophores range from yellow, through orange and red, to black (Packard & Hochberg 1977) there will be corresponding colour effects. The nerves supplying the radial muscles arise in the suboesophageal brain (in the paired anterior and posterior chromatophore lobes: Young 1971). They probably all run to the peripheral muscles without synapsing. These lobes receive their principal input from the lateral basal lobes and these, in turn, receive a large optic lobe input as well as fibres from the median basal and peduncle lobes (Young 1971).

The following three classes of response were obtained in our experiments.

(i) *Darkening* was caused by dopamine, noradrenalin, octopamine, L-glutamate, and GABA. The first two of these substances have been shown to be present in the cephalopod brain both pharmacologically (Cottrell 1967; Juorio 1971) and histochemically (Matus 1973; Messenger & Tansey 1979; Tansey 1980). They occur in high concentrations in the optic lobes. Octopamine, too, is known to be present and, like dopamine and noradrenalin, it appears to occur in reserpine-sensitive granules (Juorio & Molinoff 1971, 1974). Thus, the three amines could well be neurotransmitters involved in colour change. The evidence for L-glutamate is less good, however (Tansey 1979), although Miledi (1972) showed by iontophoretic application that glutamate was probably affecting at least two types of receptor in the squid stellate ganglion. There is even less evidence for the presence of GABA or of its synthesizing enzyme (for review see Tansey (1979)), so that the effects of these two drugs in the present experiments must be viewed with caution. Sereni (1930) obtained darkening with adrenalin but Juorio (1971) was unable to detect this catecholamine in any part of the cephalopod brain.

(ii) *Paling* was caused by ACh, nicotine and tubocurarine. At first we were surprised by this (1) because of Florey's (1966) finding that ACh applied peripherally caused chromatophore expansion, and (2) because the chromatophore lobes in the

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Description of plate 1

A sequence of photographs taken at about 4 min intervals of the same individual octopus, cannulated for perfusion: 1, control condition; 2, injection of 10 μg of ACh in 100 μl of s.w.; 3, injection of 100 μl of s.w.; 4, injection of 10 μg of 5-HT in 100 μl of s.w. Black and white prints made from Kodachrome 64 transparencies. Mantle length approximately 100 mm.

FIGURES 1–4. For description see opposite.

brain stain strongly for acetylcholinesterase (Barlow 1977; Tansey 1980) and, if it is assumed that the latter indicates the presence of ACh in a neurotransmitter role, this might suggest that the motor neurons causing the chromatophores to expand are cholinergic. These results agree with those of Sereni (1930) and of Chichery & Chanelet (1972), however, and this prompted a re-examination of Young's (1963, 1971) data for the number of cells in these lobes and the number of efferent chromatophore nerve fibres. This revealed such a discrepancy that it is clear there must be large numbers of interneurons in the lobes. One hypothesis that needs testing is that these interneurons are cholinergic and act by switching off the (excitatory) chromatophore motor neurons. This, of course, begs the question: where in the brain are the receptor sites responding to the drugs tested here?

The studies of Boycott (1953), and of Young (1971), suggest that the control system for skin patterning in cephalopods is essentially hierarchical:

optic lobe → lateral basal lobe → chromatophore lobe.

Of course this system is influenced by other regions of the brain, and is undoubtedly responsive to sensory input from the arms and statocyst; on present evidence, however, it seems likely that it is cell systems in the optic lobe, acting of course on visual information, that evoke the various patterns displayed by living octopuses. Any patterning must result from selective excitation and/or inhibition exerted by centres higher in that hierarchy. This leads us to consider our most interesting result, namely:

(iii) *patterning*. This response to 5-HT, which has never been shown before, is remarkable in itself (figure 4) and also because the pattern evoked is very similar to one exhibited by normal, intact octopuses. It is nearest to the 'acute general mottle' (Packard & Hochberg 1977). Patterning must result from the differential expansion and retraction of sets of chromatophores, and if the photographs are examined carefully it will be seen that the resting pattern (figures 1, 3) can either be diminished (by ACh: figure 2) or enhanced (by 5-HT: figure 4). It is particularly clear in colour photographs that this patterning is brought about by inhibition of the orange-brown screening chromatophores. It is interesting to compare this result with the available physiological, pharmacological and histochemical data. In acute preparations Boycott (1961) directly stimulated the three sets of lobes in *Sepia* but only obtained patterned responses when he stimulated in the optic lobe. Chichery & Chanelet (1976) were also able to elicit a specific pattern when stimulating the optic lobe with chronically implanted electrodes. Several workers have reported the presence of 5-HT in the cephalopod c.n.s. but Juorio (1971) was able to show that its concentration in the optic lobe is among the highest for any brain region. Finally, Matus (1973) and Tansey (1980) found the strong fluorescence characteristic of catecholamines and 5-HT in various parts of the cephalopod brain but, of the set of lobes controlling the chromatophore system, only in the optic lobe. Thus the possibility exists that serotonergic cells in the optic lobe may be important 'pattern generators' in cephalopods and that there are 5-HT receptor sites either in the lateral basal lobes or in the chromatophore lobes themselves. This hypothesis could be tested by administering drugs after specific lesions have

been made in the brain. These and other experiments may help us understand the way in which the chromatophores of cephalopods are centrally controlled.

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