

SHORT COMMUNICATION  
NEURAL CORRELATES OF COLOUR CHANGE IN  
CUTTLEFISH

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This paper presents the first direct demonstration in a living cephalopod of neural activity associated with chromatic behaviour. It has long been known that colour change in these molluscs is unique in that it is brought about by chromatophores controlled neurally rather than hormonally (e.g. Hofmann, 1907). The chromatophore muscles are innervated directly by nerves whose cell bodies lie in the brain itself (Sereni & Young, 1932; Boycott, 1953); the muscles respond tetanically when these motoneurons are stimulated above 10–15 Hz (Florey, 1966); electrical stimulation in the chromatophore lobes of the brain causes darkening of the skin (Boycott, 1961); stimulation in the optic lobes can elicit patterning (the expansion of some sets of chromatophores and the simultaneous retraction of others) (Boycott, 1961; Chichery & Chanelet, 1976). Darkening, paling and patterning can also be elicited by introducing specific neurotransmitters into the blood supplying the brain (Andrews, Messenger & Tansey, 1983). But until now no-one has successfully recorded from the nerves supplying a specific set of chromatophores in the living animal and shown changes in activity as the display is switched on and off.

The common cuttlefish, *Sepia officinalis* L., has an especially large repertoire of discrete body patterns (Holmes, 1940; R. T. Hanlon & J. B. Messenger, in preparation). When threatened, the adult displays a bold pattern that in its fullest expression comprises dark fins, expanded pupils, dark rings round the eyes and a pair of large black spots on the otherwise pale mantle (Fig. 1). We term this response the deimatic pattern and the spots, which really characterize it, the deimatic spots (DS). [We prefer deimatic to dymanic (both from the same root meaning 'I terrify') because this term seems to have gained general acceptance for arthropods and vertebrates (Maldonado, 1970; Edmunds, 1974; McFarland, 1981).] Cuttlefish in captivity readily display the DS (bilaterally or unilaterally) to an approaching large object and, because the nerves to the DS lie very superficially under the skin (Kier, Messenger & Miyan, 1985), we have begun to use this system to examine the nature of chromatophore nerve activity before and during a display.

Adult *Sepia* of between 130 and 200 mm were anaesthetized in isotonic MgCl<sub>2</sub> in sea water (Messenger, Nixon & Ryan, 1985). The skin over the left fin nerve foramen

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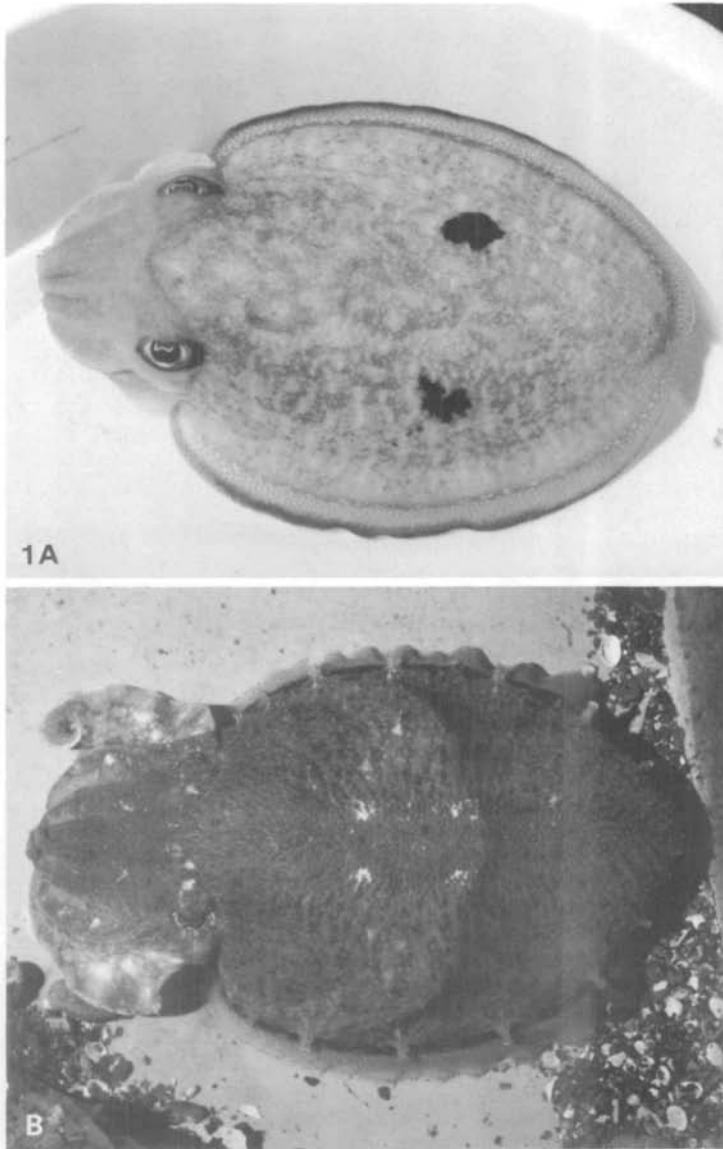


Fig. 1. (A) An adult cuttlefish showing the deimatic spots (DS) and the deimatic pattern. Note the overall paling of the mantle compared with the pattern shown in another animal at rest (B) [mantle lengths 140 (A) and 160 mm].

was cut to expose the fan of nerves running to the fin and dorsal mantle skin. The animal was suspended in a tank of running sea water by a Perspex clamp and allowed to recover: usually this took about 5 min. Little or no bleeding occurs and the animal can be maintained in this condition without evident signs of trauma for periods of up to an hour (subsequently, released animals fed and behaved normally with wound healing apparent within a day or two). A suction electrode connected to conventional recording and stimulating equipment was used to explore branches of the most medial bundle of the fan until stimulation identified the branch innervating the DS chromatophores. Brief, low-voltage stimulation of the nerve (15 Hz, 0.5 ms, 1–5 V) always elicited the DS on the ipsilateral side of the mantle only and, apart from local skin movements, no other effects were ever obtained by DS nerve stimulation. Stimulation at higher voltages and longer durations caused hyperventilation and sometimes jetting. By using a second suction electrode attached to the same nerve we were able to confirm the efferent nature of the activity and to measure the conduction velocity of individual units in the DS nerve; these all fell within the range 1.0 to 1.6 ms<sup>-1</sup>. This agrees with other published values for conduction velocities in cephalopod nerves (Burrows, Campbell, Howe & Young, 1965; Karita & Tasaki, 1973) and is in accord with the small size of the fibres in the DS nerve.

When the electrode was switched to the recording mode, a low level of tonic activity was revealed in a number of units with frequencies of between 3 and 5 Hz (Fig. 2). When the animal was 'threatened' by the approaching hand of an experimenter the spot was immediately displayed and activity in the DS nerve instantly increased (Fig. 2). Single unit activity was raised to levels of between 10 and 60 Hz with initial activity in some units approaching 100 Hz, as shown by the instantaneous rate meter output (displayed in Figs 2C, 3D) and also confirmed by hand analysis of data printed out on a fast time base so that individual units could be identified by their characteristic shapes. These high frequencies contrast with those Florey (1966) found adequate to tetanize the chromatophore muscles in isolated skin preparations and those Dubas, Hanlon, Ferguson & Pinsker (1986) used to drive chromatophores by central cell body stimulation. It is therefore possible that the deimatic pattern, as a startle response, is an unusual chromatophore output. The firing levels observed in this system, however, may reflect the open loop nature of chromatophore motor output, which is controlled without peripheral feedback (Young, 1971).

'Natural' stimulation of the DS *via* the pathway, eye–optic lobe–chromatophore lobe, not only causes darkening of the DS but also a simultaneous paling of the skin around the spot. Recordings taken from nerves innervating these regions show a decrease in firing rate when the DS is displayed (Fig. 3) and suggest there is an inhibitory relationship between the DS and its surround. This is almost certainly brought about centrally as paling was never obtained by stimulating the DS nerve alone. The chromatophore system has been called a 'retina in reverse' (Maynard, 1967) and the similarity between this part of the motor field, with its 'on centre/off surround', and the well-known receptive fields in the vertebrate retina (Hubel & Wiesel, 1962) has not escaped our attention. It is possible to elicit the DS by stimulation in the posterior chromatophore lobe and we have found its motoneurons

to have an especially low threshold for activation and a specific localization within the lobe. It is interesting that the entire DS is always displayed rather than a part of it, suggesting there may be interneurons coupling the final motoneurons for this pattern.

These results show for the first time that the system in the brain controlling the chromatophores is accessible to physiological experimentation and we expect that this preparation will provide important insights into the way this 'reverse retina' is organized.

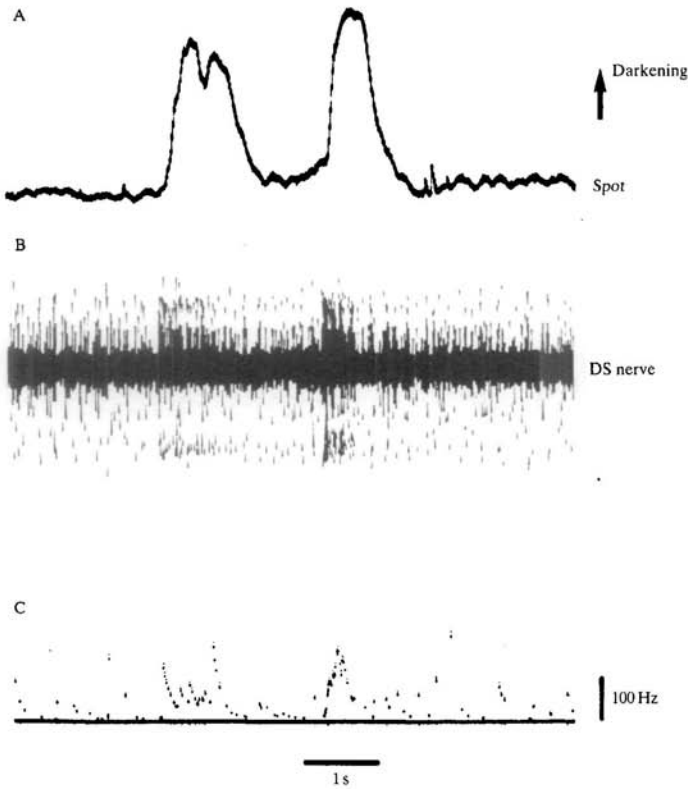


Fig. 2. Changes in DS nerve activity during spot display. Within the portion of trace shown the spot was elicited twice by 'threats' from the experimenter. The trace in (A) (obtained from a U-Matic video tape analysed with a video-densitometer developed by Dr P. Fraser) shows the rapid darkening as the spot is displayed. The traces in (B) and (C) show activity in the DS nerve and the instantaneous frequency of the largest units (single unit activity was measured off a fast pen tracing of the same data) (see text).

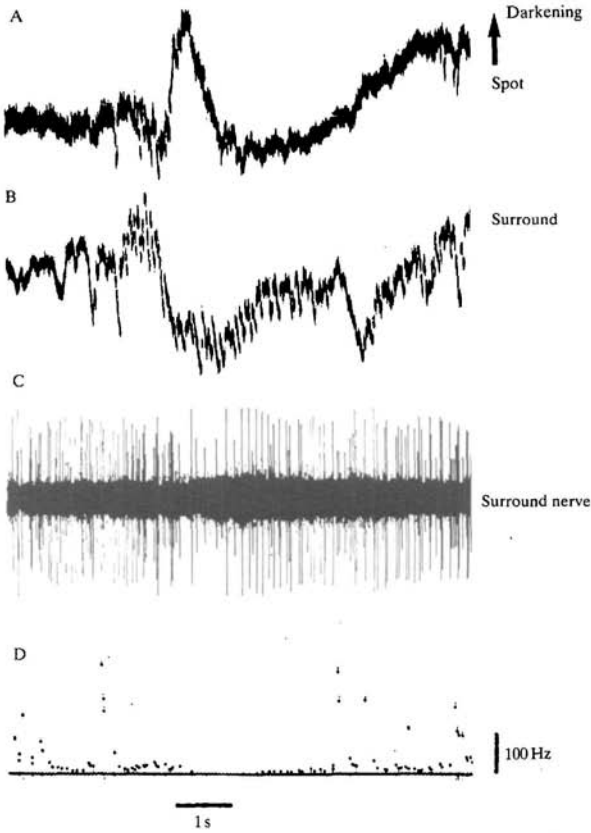


Fig. 3. Comparison of (A) spot intensity and (B) surround intensity. The activity recorded from a nerve innervating part of this surround region is shown in (C) and the instantaneous frequency of the largest units is shown in (D). The paling of the surround (B) is clearly accompanied by a decrease in frequency of surround nerve activity (C,D). Note that the onset of paling in the surround is simultaneous with onset of darkening of the spot.

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