Invertebrate Neuroscience, 2, 95-114 (1996)

Review

Neurotransmitters of cephalopods

J. B. MESSENGER

Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

ABSTRACT

1. ACh, dopamine, noradrenaline, 5-HT, L-glutamate, and GABA are widely distributed in cephalopods and probably all function as neurotransmitters; octopamine also occurs and at one site is known to act as a neuromodulator.
2. Several peptides are also present, as well as nitric oxide synthase.
3. In the brain and sense organs cholinergic, aminergic, serotonergic and glutamatergic systems seem to be the most important.
4. ACh is also active in the gut, vascular system and some body muscles: it is generally inhibitory. The ACh receptors are similar to the vertebrate nicotinic type.
5. The catecholamines are important in the gut and vascular system: they are generally excitatory. The NA receptors are like the α-adrenergic subtype of vertebrates, but the nature of the DA and OA receptors is less certain.
6. 5-HT is important in the gut but is endogenous in some chromatophore nerves and acts on receptors that seem like the vertebrate 5-HT_1 type.
7. L-glutamate is an excitatory transmitter at the chromatophore (and probably at other) nerve–muscle junctions and is an extremely strong candidate for being the excitatory transmitter at the squid giant synapse. There are NMDA receptors on Schwann-cells but the receptors on neurons and muscles are like the vertebrate kainate type.
8. Little is known about the mode of action of cephalopod peptides; nor has it ever been shown that they co-exist with conventional transmitters in these animals.
9. The structure of one (FMRFamide) receptor has been elucidated, but apart from this nothing is known of the molecular biology of receptors in cephalopods.

KEY WORDS: neurotransmitters; neuromodulators; cholinergics; adrenergics; glutamatergics; peptides

Introduction

Although 'modern' cephalopod neuroscience can be said to date from the 1930s, when J. Z. Young rediscovered the squid giant axons (Young, 1939), the senses and nervous system of cephalopods have been studied for well over a century and there is an extensive literature on them. Historically this is because, from the end of the nineteenth century, cephalopods became regularly available to visiting scientists at the world's major marine biological stations: *Sepia*, *Loligo* and *Octopus* at Naples; *Sepia* and *Loligo* at Plymouth; and *Loligo* at Woods Hole.

However, the animals themselves are of obvious interest to neuroscientists. They have the largest brains of any invertebrate and well developed sense organs, the organs of balance (statocysts) being of a complexity comparable to those of vertebrates. Their behaviour is also advanced: these are transformed molluscs, many of them agile predators that hunt by sight and evade their enemies by selecting from a rich repertoire of behaviours (Hanlon and Messenger, 1996). They also have remarkable learning abilities that were explored in great detail by neuroscientists and psychologists during the 1950s and 1960s (see Young, 1965, 1983; Sanders, 1975; Wells, 1966, 1978).

It is not surprising, then, that there is a substantial literature on putative neurotransmitters in cephalopods. Much of the earlier work has been admirably reviewed by Tansey (1979) and will not be considered again here; but many new data have appeared since Tansey's account, including evidence that L-glutamate, GABA and several peptides are present in cephalopods as well as some indication about receptor subtypes. This review attempts to summarise this recent work and to highlight the more obvious gaps in our current knowledge.

In this account we follow the broad classification of neurotransmitters and neuromodulators given by Walker and Holden-Dye (1991). The key references to the cephalopod brain are: Boycott (1961) for *Sepia*; Young (1974, 1976, 1977 and 1979) and Messenger (1979) for *Loligo*; and Young (1971) for *Octopus*.

I. Acetylcholine

Acetylcholine (ACh) and its associated enzymes (ChAT and AChE) are present in high concentrations in the cephalopod CNS. ACh was first described in

Octopus as long ago as 1935 by Bacq and subsequently several workers have reported its occurrence in the brain or optic lobes of several cephalopod species (see Tansey, 1979, for details). One important paper implicating ACh as a neurotransmitter was that of Florey and Winesdorfer (1968), who, in homogenates of *Octopus dofleini* optic lobes, found bound ACh associated with the nerve-ending fraction at levels one hundred times greater than those found in comparable fractions in mammals. Despite this the physiological evidence for its being a neurotransmitter in cephalopods is still meagre and although AChE has been localised histochemically there has been no localisation to date of ChAT, a better, though still imperfect indicator of ACh.

A. ACh in the brain

As can be seen in Table 1, ACh has been extracted only from the optic lobes and from the (gross) supraoesophageal brain lobes. AChE is widespread, however, and *may* indicate the presence in a lobe of ACh acting as a transmitter.

The only physiological evidence for a cholinergic system in the brain comes from the experiments of Chichery and Chanelet (1972), who observed that ACh injected under the cuttlebone of *Sepia* produces pupillary constriction and paling, and from the more extended series of experiments in *Octopus* carried out by Andrews *et al.* (1981, 1983). The latter involved cannulating the cephalic aorta in such a way that drugs in a small (100 μL) volume of fluid could be delivered directly into the cerebral blood supply (Andrews and Tansey, 1981). In octopuses of about 250 g a 10 μg 'pulse' of ACh or nicotine, but not muscarine, causes instant cessation of respiratory movements, pupil dilation and complete relaxation of mantle, arm and skin musculature, including the chromatophore muscles so that the animal pales. All these effects are transient, lasting about 20 s. Carbachol produces a similar but more transient effect but tubocurarine acts first as an ACh agonist and then a partial antagonist. There is evidence that in these experiments the ACh is acting at the lower motor centres in the suboesophageal lobes (Boycott, 1961), including the chromatophore lobes, whose neuropil stains positively for AChE (Tansey, 1978). A consistent feature of all these results is that ACh appears always to be inhibitory.

Biochemical studies on the optic lobe (or 'optic ganglion') of two genera of squid have provided clear evidence for nicotinic type receptors (Demushkin and Kotelevtsev, 1980) and revealed at least two types of binding site, one for ACh that is more sensitive to agonists, the other for α-bungarotoxin (BGT) that is more sensitive to antagonists (Chen *et al.*, 1988). The suggestion that the AChR may not be exactly like that of vertebrates is supported by the finding of Chichery and Chichery (1985), who in *Sepia* optic lobe obtained complex and prolonged motor effects with tubocurarine, atropine and 'flaxedil'.

B. ACh in the peripheral nervous system

The squid stellate ganglion has long been known to contain AChE (Nachmansohn and Meyerhof, 1941) and this has also been shown histologically near the giant synapse (Brzin *et al.*, 1975). ACh and ChAT have also been identified in this region (for a review, see Stanley, 1984). The stellate ganglion is a large and complex structure (Young, 1974), with many thousands of cells apart from the giant fibre system, so none of these findings tells us anything about the nature of the transmitter at the giant synapse (Section VII). Indeed Stanley (1984) concluded that 'the evidence suggests strongly that ACh is not the excitatory transmitter at this synapse', a statement with which we would agree (Section VII). However, there is some *prima facie* evidence for an inhibitory cholinergic input on to the third-order giant fibres, although the details of the circuitry involved remain unknown. Several other peripheral ganglia contain cholinergic systems (Table 2), as do the arm cords in *Octopus*.

Abbreviations used: ACh, acetylcholine; AChE, acetylcholine esterase; AChR, acetylcholine receptor; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid; ANF, atrial natriuretic factor; ATP, adenosine triphosphate; BGT, α-bungarotoxin; ChAT, choline acetyltransferase; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CNS, central nervous system; DA, dopamine; D_1, D_2, dopamine receptor subtypes 1 and 2; DAB, diaminobenzidine; DNQX, 6,7-dinitroquinoxaline-2,3-dione; DPAT, dipropylaminotetralin; EM, electron microscope; ERG, electroretinogram; FaRPs, FMRFamide-related peptides; FLRF, Phe-Leu-Arg-Phe; FMRF, Phe-Met-Arg-Phe; GABA, γ-amino butyric acid; GTP, guanosine triphosphate; HPLC, high performance liquid chromatography; 5-HT, 5-hydroxytryptamine (serotonin); L-DOPA, 3-(3,4 dihydroxy phenyl) alanine; L-GLU, L-glutamate; LM, light microscope; L-NAME, N-ω-nitro-L-arginine methyl ester; NA, noradrenaline; NADPH, nicotinamide adenine dinucleotide phosphate; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; NSV, neurosecretory system of the vena cava; OA, octopamine; ODAP, β-*N*-oxalyl-L-α,β-diamino propionic acid; PAP, peroxidase anti-peroxidase; SRIF, somatostatin; TEA, tetraethylammonium; TTX, tetrodotoxin; VIP, vasoactive intestinal peptide; YGGFMRF, Tyr-Gly-Gly-Phe-Met-Arg-Phe.

	Brain region	ACh	AChE	DA	NA	5-HT	OA	L-GLU	GABA	Sub P	SRIF	FMRF
1	Anterior suboesophageal mass		+	+	+	+	+	+	+		+	
2	Middle suboesophageal mass			+	+		+	+				
	anterior pedal lobe		+	G	G	+			+	+	+	
	anterior chromatophore lobes		+	G	G	+						
	lateral pedal lobes		+	G	G	+			+			
	posterior pedal lobe		+	G	G	+			+			+
	anterior funnel lobe			–	–	+			+			
3	Posterior suboesophageal mass			+	+		+	+				
	palliovisceral lobe		+	G	G	+			+		+	+
	fin lobes (decapods)										–	
	posterior chromatophore lobes		+	+	+	+		+	+		–	
	vasomotor lobes			G	G	+			+			
4	Magnocellular lobes			G	G	+			+		+	+
5	Supraoesophageal lobes	+										
	superior buccal lobe		+	+	+	+	+		+			
	posterior buccal lobe		+	+	+	+	+		+			
	anterior basal lobe		+	G	G	+			+			
	interbasal lobes			G	G	+			+			
	median basal lobe		+	G	G	+	+	+	+		+	
	lateral basal lobes		+	G	G	+		+	+			
	inferior frontal lobe		+	+	+	+	+		+			+
	subfrontal lobe (octopods)		–	G	G	+			–			
	superior frontal lobe		+	+	+	+	+	+	+		+	
	vertical lobe		+	+	+	+	+	+	+		+	
	subvertical lobe		+	G	G	+			+		+	
	precommissural lobe		+	G	G	+						
	dorsal basal lobes		+	G	G	+			+			+
	subpedunculate lobes		+	G	G	+			+			
6	Optic lobes	+		+	+		+	+				+
	cortex		+	G	G	+			+	+	+	
	medulla		+	G	G	+			+	+	+	
	peduncle lobes		+	G	G	+	+		+		+	
	olfactory lobes		+	G	G	+			+		+	+

Table 1. Distribution of possible transmitters/modulators in the brain. +, *present*; –, *reported absent*; G, *green fluorescence (i.e. DA and/or NA)*; *blank, no data available. The data here, which have been pooled from several genera, decapod and octopod, are based on Chichery and Chichery, 1974; Cornwell* et al., *1993; D'Aniello (pers. comm.)., Juorio, 1971; Juorio and Molinoff, 1974, Kime and Messenger, 1990; Loi* et al., *1996; Parr, 1988; Tansey, 1978, 1980; Uemura* et al., *1987.*

C. ACh in the sense organs

In the eye there is some evidence that the photoreceptors may be cholinergic. Lam *et al.* (1974) incubated the retinas of four species of cephalopods with labelled precursors to various transmitters and found considerable quantities of labelled ACh (and dopamine, see Section II) in the retina. Since there is ultrastructural evidence of round, agranular vesicles in the terminals of photoreceptor cells in the optic lobe (Gray, 1970; Cohen, 1973) it seems reasonable to conclude that ACh is a transmitter of these cells. It is curious that Barlow (1977) found AChE in the optic lobe plexiform layer (in which the photoreceptor cells terminate) in *Octopus* but not in decapods.

Better evidence is available for ACh being a transmitter in the statocysts of *Octopus*. Each statocyst con-

GANGLION	ACh	AChE	DA	NA	5-HT	OA	L-GLU	SRIF	FMRF
Stellate	+	+	+	+	+	+	+	+	+
Inferior buccal			+	+	+				
Gastric		+	+	+	+	+			
Cardiac		+	G	G					

Table 2. Distribution of possible transmitter/modulators in the peripheral nervous system. G, *green fluorescence (i.e. DA and/or NA). Based on data in D'Aniello* et al., *1995; Feldman, 1986; Juorio, 1971; Juorio and Molinoff, 1974; Kime and Messenger, 1990; Kling, 1986; Makman* et al., *1987; Nachmansohn and Meyerhof, 1941, Osborne* et al., *1986; Parr, 1988; Suzuki and Tasaki, 1983.*

tains two distinct mechanoreceptor structures, the macula system (concerned with linear acceleration and gravity) and the crista system (concerned with rotational acceleration) (Budelmann, 1990). Auerbach and Budelmann (1986) found specific AChE histologically in the sensory epithelia of both systems, closely associated with axonal membranes; this activity was greatly reduced after section of the statocyst nerves, suggesting that it was the efferent fibres that were cholinergic. These authors also determined that ACh was present in the macula and crista by using radiochemical (bio)assays. These findings agree with ultrastructural evidence about vesicle size and type in the epithelia (Budelmann *et al.*, 1987) and with histological evidence of AChE in cell bodies as well as in the neuropil of the lateral pedal lobe (Tansey, 1980).

Subsequently Williamson (1989), recording from the crista nerves of *Octopus*, showed physiologically that bath application of ACh (or carbachol) depresses the resting activity level (Fig. 1) and that this effect is enhanced by eserine. Tubocurarine and atropine did not block the inhibitory effect obtained by stimulating the efferents, although gallamine did, suggesting that the ACh receptor subtype in this system may be nicotinic, though different from those occurring in vertebrates.

D. ACh in the viscera

In the *Octopus* gut the experiments of Andrews and Tansey (1983a) have shown that the upper gut is sensitive to ACh, nicotine and carbachol (though not, again, to muscarine). The resting tone and level of spontaneous activity of both crop and stomach are reduced *in vitro* by ACh and gastric motility is inhibited. The lower gut is unresponsive to ACh although nicotine, after increasing tension briefly, suppressed all spontaneous contractions. In the oesophagus there were differences in response between the anterior and posterior parts but both appear to contain cholinergic systems. ACh is present in the cephalopod gut (Bacq, 1935) and because Andrews and Tansey (1983a) also demonstrated that AChE is present in the gastric ganglion and nerves their pharmacological experiments strongly suggest that gut motility in cephalopods is regulated by cholinergic pathways (acting antagonistically to catecholamines: see Section II).

The vascular system of cephalopods has long been known to be influenced by ACh (e.g., Ghiretti, 1948; see also reviews by Hill and Welsh, 1966; Wells, 1983). Recently Schipp and his collaborators (see also references to Fiedler, Jakobs, Kling) have investigated in detail the pharmacology of the systemic heart (hereafter the 'heart'), the paired branchial hearts (auxiliary hearts that pump blood into the gills) and the aorta of the cuttlefish, *Sepia*, and shown that all contain an inhibitory cholinergic system.

Kling (1986) showed histochemically that AChE is present in the cardiac nerves and in the heart muscle itself. He also found that ACh inhibits the isolated heart, reducing amplitude and frequency of beat; nicotine, but not muscarine or pilocarpine, mimics this

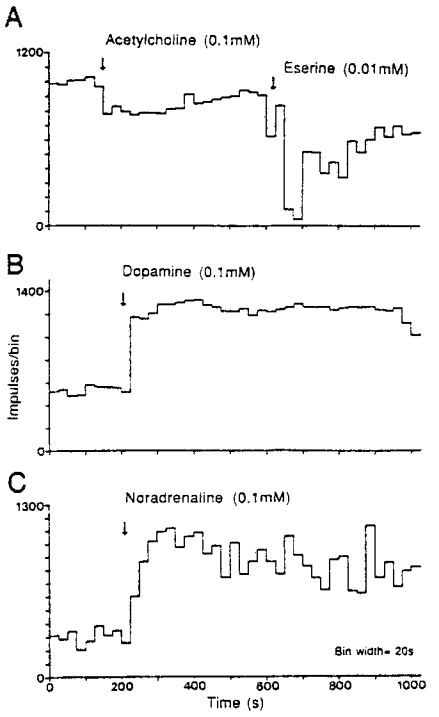

Fig. 1. *ACh and eserine depress the afferent resting activity of* Octopus *statocyst crista nerves (A), while DA and NA enhance it (B & C). (From Williamson, 1989).*

effect. Tubocurarine and BGT reversibly block the response to ACh but atropine and TEA do not (Kling, 1987; Kling and Jakobs, 1987). In the branchial hearts there is high AChE activity especially in the sarcolemma but also in the closely associated cardiac ganglion and nerves, which contain clear vesicles (Schipp *et al.*, 1986). Pharmacological experiments gave results similar to those obtained with the central heart: ACh (or better, nicotine) inhibits activity, and tubocurarine and BGT block this effect. However, atropine has a weak blocking effect and muscarine a weak agonistic effect. Finally, in the cephalic aorta, Schipp and Fiedler (1994) showed that ACh induced concentration-dependent vasodilation in preparations that had been pre-contracted with dopamine. Carbachol was more effective than ACh but muscarine was equally effective. Only TEA exerted any blocking action on ACh. Tubocurarine, BGT, atropine and pirenzepine enhanced or did not block the vasodilatory effect of ACh. These workers also noted that FMRFamide mimics the effects of ACh in this tissue (see Section IX).

These studies of the circulatory system of *Sepia officinalis* are instructive in showing that different tissues in the same species may have different receptor subtypes.

E. ACh in the muscles

In the squid, *Alloteuthis*, Bone *et al.* (1982) found physiological evidence that some groups of muscle fibres have a cholinergic innervation. These are the arm and tentacle retractors, the head retractors, the muscles of the funnel, the radial fibres of the mantle and the superficial longitudinal muscles at the anterior margin of the mantle (Fig. 2). All these muscles are insensitive to L-glutamate, yet it is this that is active at the circular fibres of the mantle, which provide the power stroke for jetting. In cephalopods most muscles act on other muscles rather than on a skeleton, constituting what Kier (1988) terms a 'muscular hydrostat', and this could be the reason for employing different transmitters in functionally different muscle groups whose fibres lie in close proximity, as they do in the mantle (see also Section VII).

II. Catecholamines

Three catecholamines can act as neurotransmitters: dopamine (DA), noradrenaline (NA) and, in certain vertebrates, adrenaline. The first two (but not adrenaline: Juorio, 1971) are widely distributed in the cephalopod CNS, and also occur in the sense organs and viscera. There is ample evidence that DA and NA may act as neurotransmitters in cephalopods, although

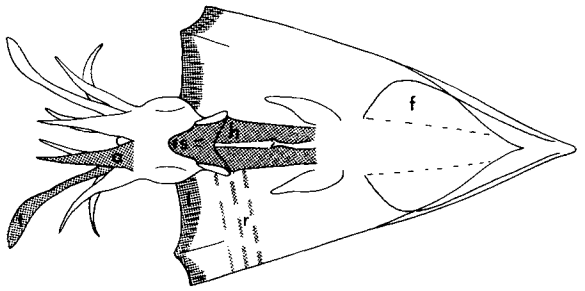

Fig. 2. *At some body muscles of the squid,* Alloteuthis, *the excitatory transmitter appears to be ACh (dark stipple), at others* L-*glutamate. (a, arm; f, fin; h, head retractor; ; l,r, longitudinal, radial mantle muscle; s, funnel; t, tentacle. From Bone* et al., *1982).*

one complication to bear in mind is that DA may be present not as a transmitter itself but as a precursor of NA.

A. Dopamine and noradrenaline in the brain

The starting point for this review must be the important quantitative measurements of Juorio and his colleagues in the 1970s, which were based on thin-layer chromatography, enzymatic and fluorometric analysis of carefully dissected regions of the brains of several genera of cephalopods. In essence Juorio found (1) that DA and NA are present in the cephalopod brain; (2) that they are unevenly though widely distributed across the different brain regions; and (3) that they are both sensitive to reserpine and pargyline (Juorio, 1971; Juorio and Molinoff, 1974). More recently Kime and Messenger (1990) used HPLC to confirm and extend Juorio's analysis, comparing the brains of *Sepia*, *Loligo* and *Octopus*. They too found great variation in the levels of these putative neurotransmitters in the different lobes of the brain and their findings largely agree with those of Juorio.

Fluorescence-histochemical studies by Matus (1973) and by Tansey (1980) provided further evidence for the presence of catecholamines (and 5-HT: Section III) in the cephalopod brain, confirming that these substances are not uniformly distributed as they might be if they had some general metabolic function. Using the glyoxylic acid modification of the Falck–Hillarp technique (De La Torre and Surgeon, 1976) Tansey found the specific, slow-fading, green fluorescence characteristic of DA and/or NA in scattered cell bodies in several brain lobes and in the neuropil of many lobes. There was always good agreement between her data and those of Juorio. In particular it is worth noting the brilliant fluorescence in the inferior buccal ganglion, with its very high level of NA (160.9 ng/mg in *Loligo*: Kime and Messenger, 1990).

Evidence that these catecholamines may have a

neurotransmitter function comes from physiological experiments that injected small (100 μL) pulses of a putative transmitter or one of its agonists into the cephalic aorta of *Octopus* (Andrews *et al.*, 1981, 1983). These workers found that a pulse of DA, NA (or their agonists) produced various motor effects in the mantle and arms, and expansion of the chromatophores so that the animal darkens. DA caused pimpling of the skin and cessation of respiration, while NA enhanced the respiratory rhythm. Subsequently Messenger (unpublished observations) found that a whole battery of α-adrenergic agonists caused darkening when injected and that phentolamine (though not propranolol) paled an already darkened octopus. These effects, which were transient, are entirely consistent with there being α-type adrenergic receptors widely distributed in the *Octopus* motor system, specifically in the chromatophore system. There are appreciable levels of NA in the lateral basal and posterior chromatophore lobes (Kime and Messenger, 1990) and both sets of lobes show strong specific catecholaminergic fluorescence (Di Cosmo and Messenger, in preparation).

The fact that so many injected adrenergics produce colour changes in these rather crude experiments means that they cannot tell us much about receptor subtypes in the chromatophore system. Yet it is clear that the receptors must be similar to the vertebrate α-type. Incidentally the results with dopamine agonists and antagonists were much harder to interpret.

In biochemical experiments, however, using optic lobe homogenates, Capasso *et al.* (1991, 1993) were able to characterise a cephalopod dopamine system. They measured adenylate cyclase activity and showed that there was a dopamine D_2-like receptor, for which OA and DA compete. Moreover they found that the D_1-antagonist SCH-23390 was without effect in this system though the D_2-antagonist YM-09151-2 was inhibitory. In view of results from the retina (Section II C, below) are we to conclude that there are different DA receptors in different parts of the CNS?

In another series of experiments Stefano *et al.* (1981) showed that KCl induced DA release from various parts of the central brain and that this could be inhibited by opioids such as morphine.

B. Dopamine and noradrenaline in the peripheral nervous system

The stellate ganglion of *Sepia*, *Loligo* and *Octopus* contains NA and a little DA (Kime and Messenger, 1990) but there appear to be no fluorescence-histochemical studies of this organ, nor any pharmacological ones. There are catecholamines in most of the other peripheral ganglia (Table 2) but nothing at all is known about their physiology. The inferior and superior buccal ganglia are very accessible and in view of their remarkably high levels of DA/NA they might be suitable targets for future investigators.

C. Dopamine and noradrenaline in the sense organs

In the eye there is very good evidence that DA is an inhibitory transmitter in the efferent fibres from the optic lobe. The incubation experiments of Lam *et al.* (1974) had shown that significant amounts of labelled DA (but not NA) were synthesised in the retina from [^{14}C] tyrosine and later Tasaki and Suzuki (1980) and Silver *et al.* (1983) found green fluorescence in the plexiform layer of the retina, where the efferents terminate. In an elegant series of physiological experiments Suzuki and Tasaki (1983) showed (1) that perfused DA enhanced the intraretinal ERG in an isolated *Octopus* retina preparation, an effect that could also be obtained with apomorphine; (2) that intraretinal injections of DA at different depths localised the site of action to the plexiform zone (Fig. 3); (3) that stimulation of an optic nerve bundle, in which the efferents run, also increased the amplitude of the ERG; and (4) that reserpine abolished the efferent inhibition as well as fluorescence in the plexiform layer. The authors conclude that the dopaminergic inhibitory efferents are concerned with reducing the size of the receptive field. In another study Makman *et al.* (1987) used [3H]-SCH 233390 (a dopamine D1 receptor antagonist) as a radioligand and found binding sites in the plexiform layer of the retina that were diminished following optic nerve lesion.

A recent paper suggests that DA also plays some part in regulating screening-pigment migration during light- and dark-adaptation (Gleadall *et al.*, 1993). In the dark-adapted eye the pigment normally migrates basally but if the efferents are severed by optic nerve section or if the animal is treated with reserpine to

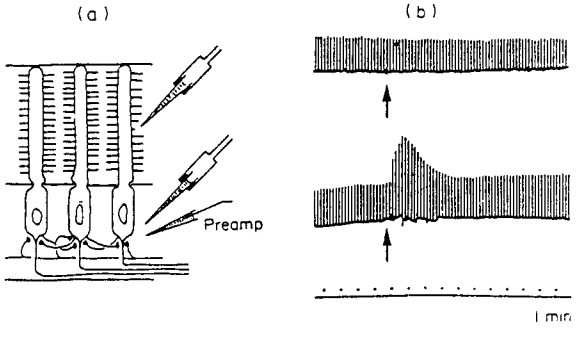

Fig. 3. *(a)* Octopus *retina showing DA injection sites in the photoreceptor region and the plexiform layer (the synaptic zone). (b) DA is ineffective when injected in the photoreceptor region (upper trace) but enhances the ERG when injected into the synaptic zone (lower trace). A similar response occurs when the efferent nerves are stimulated. (Modified from Suzuki and Tasaki, 1983).*

deplete the DA the pigment remains at the apical layer.

In the statocyst Budelmann and Bonn (1982) found specific green catecholamine fluorescence in the sensory epithelia of the macula and the crista systems and suggested the transmitters in the efferent nerves to the statocyst may be DA and/or NA. They also showed that both DA and NA increase the resting activity of the crista nerves. Williamson (1989) extended these findings by showing that DA, apomorphine and NA all increase the resting activity in octopus crista nerves (Fig. 1) and that haloperidol and phentolamine (blockers for DA and α-adrenergics respectively) decrease it. Moreover phentolamine blocks the effects of electrically stimulating the crista nerves, and intracellular recordings have shown that it blocks efferent induced depolarisation (Williamson and Chrachri, 1994).

Once again it has not proved possible to differentiate between DA and NA receptors in this preparation, partly because most pharmacological tools available have been developed for mammals, whose receptors, not surprisingly, are different from those of molluscs (see Discussion).

Another cephalopod sense organ is the olfactory organ, which lies on the head below and behind the eye. In a very interesting series of papers Lucero and her colleagues have shown recently that in the squid, *Loligo opalescens*, the olfactory sensory cells are sensitive to L-DOPA and DA, both of which are present in squid ink (Lucero *et al.*, 1992, 1994; Lucero and Gilly, 1995). The receptor cells contain voltage-gated Na^+, K^+ and Ca^{2+} channels; and K^+ blockers, L-DOPA and DA hyperpolarise the cells and inhibit the action potential (Fig. 4). Incidentally the olfactory nerve and lobe show very strong green fluorescence (Tansey, 1980).

D. Dopamine and noradrenaline in the viscera

In the gut the important study of Andrews and Tansey (1983a) showed, first, that catecholamine fluorescence was present in the sympathetic nerves, gastric ganglion and nerves and the submucosal plexus of the stomach wall; and secondly that DA and NA (though not adrenergic receptor antagonists) were active at different parts of the gut. The effects were mainly excitatory and the cephalopod gut appears to be controlled by a dual cholinergic/adrenergic system as in vertebrates.

In the circulatory system there is specific green fluorescence in the *Sepia* heart (Kling, 1986, 1987), branchial hearts (Fiedler and Schipp, 1991) and vessels (Andrews and Tansey, 1983b). In the heart NA (and to a lesser extent DA, OA and other adrenergics) causes marked increases in amplitude and frequency (Fig. 5), effects that are blocked by phentolamine but not propanolol (Kling and Schipp, 1987). In the branchial hearts DA (and NA and adrenaline) cause an increase in amplitude but not frequency; this effect can be blocked by phentolamine (Fiedler and Schipp, 1990). Some blood vessels are also sensitive to catecholamines but there are important differences between arteries and veins (Schipp, 1987, gives a most useful review). Once again the experimental evidence of responses to DA, NA and other adrenergics (or their antagonists) points to a receptor resembling the α-type of vertebrates.

Finally it is worth noting that according to Wells (1983) the effects of noradrenaline (and adrenaline and tyramine) differ *in vitro* from *in vivo*, where they appear to be inhibitory (Johansen and Huston, 1962; Wells and Mangold, 1980).

III. Indoleamines

Serotonin occurs widely in cephalopods and there is good reason to assume that it functions as a neurotransmitter, although its action on chromatophore muscles is more modulatory.

A. 5-HT in the brain

Many regions of the brain contain 5-HT (Table 1) but the level varies greatly in different lobes (Juorio, 1971; Kime and Messenger, 1990). This is borne out by fluo-

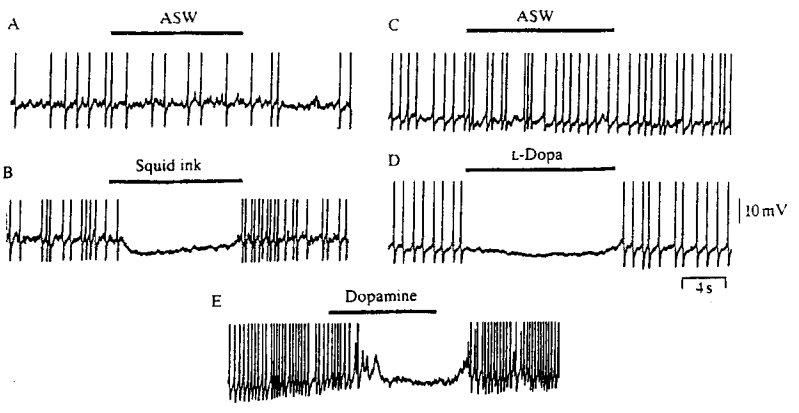

Fig. 4. Loligo opalescens. *Blocking of action potentials in isolated olfactory receptor cells by (B) squid ink (1:20 dilution), (D) L-DOPA (10^{-2}M), and (E) DA (5×10^{-5}M). Artificial seawater (ASW) had no such effect. (From Lucero* et al., *1992).*

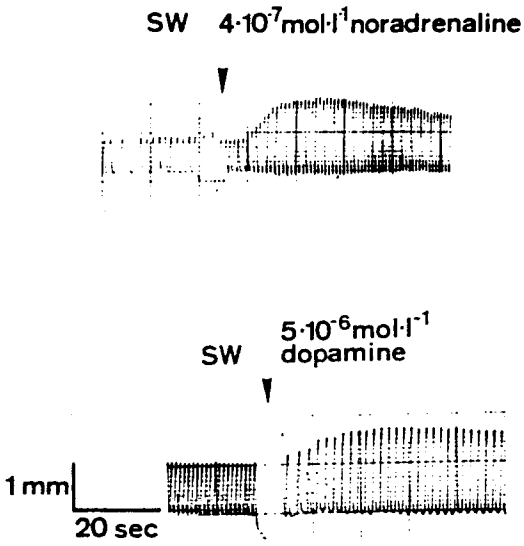

Fig. 5. *NA and DA are both excitatory in the isolated* Sepia *heart. (Modified from Kling and Schipp, 1987).*

rescence histochemical studies (Matus, 1973; Messenger and Tansey, 1979; Tansey, 1980; Di Cosmo and Messenger, in preparation). Fast-fading, specific yellow fluorescence characteristic of 5-HT occurs frequently in the neuropil and can also be seen in cell bodies. The distribution of such staining agrees very well with the chemical extraction data.

However, the best evidence for serotonin in nerve cell bodies and in the neuropil comes from immunohistochemical studies. Using PAP/DAB visualisation both Uemura *et al.* (1987) and Parr (1988) found specific staining in many brain lobes of *Octopus*, both in the sub- and supra-oesophageal regions, including the chromatophore and vasomotor lobes (see below). Kito-Yamashita *et al.* (1990) also demonstrated 5-HT-like staining in the optic lobe (and retina: see below) with immunofluorescence histochemistry.

Using the aortic perfusion technique in *Octopus*, Andrews *et al.* (1981, 1983) obtained clear motor effects with 5-HT, including inking, defaecation and, most spectacularly, a bold chromatophore patterning like that shown by living octopuses in conflict situations and known as 'acute general mottle' (Packard and Sanders, 1971). This chromatic effect, which can be abolished by methysergide, persists after lesions that leave only the sub-oesophageal lobes intact, so that the injected serotonin may be acting at the chromatophore lobes.

Chichery and Chichery (1985) micro-injected 5-HT into the optic lobe of *Sepia* and also obtained wide-ranging postural and motor effects.

B. 5-HT in the peripheral nervous system

Parr (1988) found 5-HT in the neuropils of the stellate, inferior buccal and gastric ganglia though not in their cell bodies. Nothing is known about its function in the first two ganglia; its action in the gut is described below.

C. 5-HT in the sense organs

In the statocyst of *Octopus* no yellow fluorescence indicative of 5-HT was found using the Falck–Hillarp method (Budelmann and Bonn, 1982). In the *Octopus* retina Kime and Messenger (1990) found 5-HT by HPLC analysis; and Kito-Yamashita *et al.* (1990) showed it to be present by immunohistochemistry and immunofluorescence in cell bodies, perhaps in retinal glial cells. Positive staining was also found in the optic nerves and optic lobe. Curiously, in *Loligo* neither Osborne *et al.* (1986) nor Kime and Messenger (1990) could find any 5-HT, suggesting again that there may be differences between the transmitters of octopods and decapods; comparative studies should help resolve this.

D. 5-HT in the viscera

The history of our understanding of serotonin in the cephalopod gut merits a brief comment. Juorio and Killick (1973) could find no trace of 5-HT using chemical extraction, and with the glyoxylic acid fluorescence method Andrews and Tansey (1983a) reported the absence of the yellow fluorescence from gut and gastric ganglion. Yet these same authors demonstrated that 5-HT was active on the lower gut *in vitro*, even though its effects were complex and inconsistent. However, Parr (1988), using the more sensitive immunohistochemical method, found 5-HT-like staining in the neuropil of the gastric ganglion, so that the pharmacological results of Andrews and Tansey (1983a) become readily explicable.

In the vascular system an apparently similar conflict has yet to be resolved. In the systemic heart there appears to be no endogenous 5-HT (Juorio and Killick, 1973; Kling, 1986); nor is there any in the cardiac ganglion (Fiedler and Schipp, 1991). Yet the heart is undoubtedly excited by 5-HT when applied *in vitro*, although Kling and Schipp (1987) speculate that perhaps this effect is mediated by peptidergic receptors. Unfortunately there seem to be no immunohistochemical data for 5-HT in the vascular system. In the isolated aorta of *Sepia*, Schipp *et al.* (1991) have shown that 5-HT can relax previously contracted muscle fibres (Fig. 6), which is interesting in the light of its relaxing effect on chromatophore muscle (below). In the branchial heart 5-HT is not only absent but apparently without effect *in vitro* (Fiedler and Schipp, 1990, 1991).

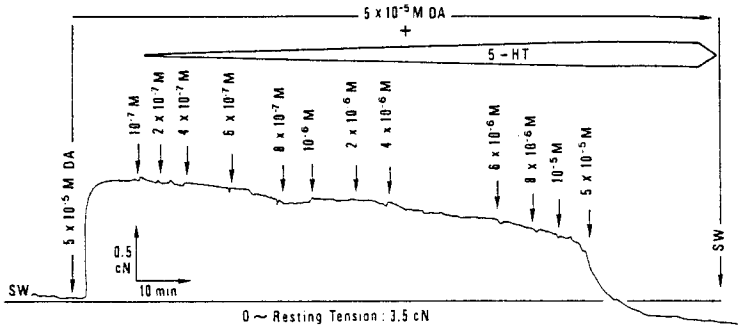

Fig. 6. *A segment of* Sepia *aorta, pre-constricted with DA, progressively dilates as 5-HT is applied in increasing concentrations. (From Schipp* et al., *1991).*

E. 5-HT in the chromatophores

In the skin, topically applied 5-HT induces relaxation of contracted chromatophore muscles and hence paling. In *Sepia* such an effect can also be obtained with 8-hydroxy-DPAT, a 5-HT_1 agonist (Messenger *et al.*, in preparation). There is multiple innervation of the radial muscles (Cloney and Florey, 1968) but no inhibitory nerves (Florey, 1966; Florey and Kriebel, 1969). Recent LM immunohistological evidence has shown that 5-HT is endogenous in some chromatophore nerve fibres (Cornwell and Messenger, 1995). In the EM, as well as fibres that contain L-glutamate (Section VII), there are those that contain only 90 nm diameter electron-dense vesicles. These may contain 5-HT, but they are not organised into synapses (Reed, 1995). Perhaps any 5-HT released acts directly on the calcium-mobilisation mechanism in the muscle; if so its action appears to be modulatory. Without further evidence, however, the role of serotonin in chromatophore control remains unknown.

Another indoleamine, tryptamine, has been found in the CNS of *Octopus dofleini*, and shown to be sensitive to pargyline (Juorio and Philips, 1976). This substance may be a transmitter in the vertebrates CNS but little seems to be known about it in invertebrates (Walker and Holden-Dye, 1991); it occurs in very high concentrations in a starfish, *Pycnopodia* (Robertson and Juorio, 1977).

IV. Histamine

Sereni (1930) found that histamine injected into the circulation of an octopus caused chromatophore expansion but Scuka (1971) showed that it depressed both resting and action potentials in the squid giant fibre. Several regions of the cephalopod CNS contain high levels of this amine (see Tansey, 1979 for references), and since there is evidence that it is a neurotransmitter in gastropods (Walker and Holden-Dye, 1991) it might be worth investigating in cephalopods. On the other hand histamine does not affect the *Sepia* heart *in vitro* (Fiedler and Schipp, 1990).

V. Octopamine

A. Octopamine in the brain

The important study of Juorio and Molinoff (1974) showed (1) that octopamine (OA) is widely, but unevenly, distributed in the cephalopod CNS: in any lobe its concentration is about a third that of NA; (2) that its concentration is decreased by reserpine and increased by pargyline; and (3) that it is found in the same (synaptosome) fraction that contains DA and NA. It must therefore be considered a serious candidate for being a transmitter in the cephalopod CNS.

That the CNS responds to OA was shown by Andrews *et al.* (1981, 1983), who found from their aortic perfusion experiments that it caused transient expansion of the red chromatophores, skin pimpling and increased respiratory movements. Messenger (unpublished data) further showed that a similar response could be obtained from synephrin, and that phentolamine was the most effective antagonist of this response. He also tested a variety of other possible OA agonists and antagonists: the results were equivocal but showed that the OA receptor subtypes of *Octopus* central neurons are certainly different to those of insects (Evans, 1981) and are perhaps more similar to the vertebrate OA_1 type (Nathanson, 1993).

B. Octopamine in the peripheral nervous system

There is OA in the gastric ganglion and in the stellate ganglion (Juorio and Molinoff, 1974: Table 2). The latter finding is of especial interest since Reale *et al.* (1986) have provided good evidence for there being octopamine receptors (possibly of the OA_2 type) on the Schwann cells of the giant fibre of the squid *Sepioteuthis*. They showed that OA (the naturally occurring D(−) isomer) induces long lasting hyperpolarisation of the Schwann cell membrane potential (Fig. 7) and suggest that during violent escape movements (when the giant fibres are firing at high frequency: Villegas, 1984) OA may modulate the giant fibre's Schwann cell activity. The source of the OA may be the small fibres in the stellar nerves but Reale *et al.* (1986) also speculate that it could be delivered to the Schwann cells in the blood (from an unspecified

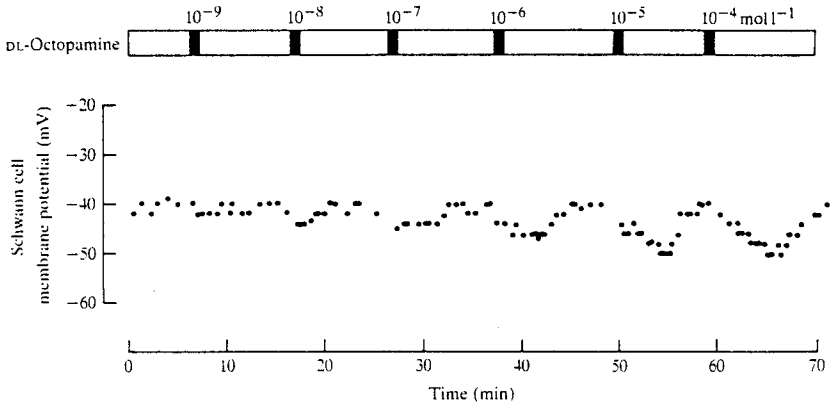

Fig. 7. Sepioteuthis. *Effect of 1 minute pulses of octopamine (black bars) on the Schwann cell membrane potential. Each dot represents a different cell. (From Reale* et al., *1986).*

source). The OA, whatever its source, also appears to potentiate the action of the (nicotinic) cholinergic activation system of the Schwann cell and this extraordinarily complex signalling system will be considered below (Sections VII and IX; Fig. 12).

C. Octopamine in the sense organs

There is evidence for a trace of OA in retina (Juorio and Killick, 1973) but it has not been reported in the statocyst.

D. Octopamine in the viscera

Juorio and Molinoff (1974) found small amounts of OA in various parts of the gut and the circulatory system, notably the branchial hearts, but OA is without effect *in vitro* on the *Octopus* upper gut (Andrews and Tansey, 1983a) or the *Sepia* branchial heart (Fiedler and Schipp, 1990).

Another phenolamine, tyramine, also occurs in the *Octopus* CNS and is sensitive to reserpine (Juorio and Philips, 1976). Nothing is known about its possible transmitter function, however.

VI. Purines

There seem to have been only two studies in cephalopods that have looked at the effects of these substances, now known to be so important in vertebrates (Burnstock *et al.*, 1978). Andrews and Tansey (1983a) report that ATP tested at various concentrations on the crop and stomach of *Octopus* was without effect. Hill and Huddart (1995) tested GTP (guanosine 5′-triphosphate) on the oesophagus, crop and stomach of *Octopus bimaculoides* and on *Eledone* heart. They also obtained no effect with ATP but found that GTP caused strong contractions and twitch activity from the gut (Fig. 8); in the heart the response to GTP was much weaker, and far weaker than the response to FMRFamide.

VII. Amino-acids

A number of amino-acids are known to occur at very high levels in the cephalopod CNS, such as alanine and especially taurine; however, similar levels of free amino-acids are also found in muscle and in other tissues, and it has been suggested that they have an osmoregulatory function in cephalopods (see Tansey, 1979). However, two other amino-acids known elsewhere to have excitatory effects are widely distributed in the cephalopod CNS: L-aspartate and L-glutamate (L-glu) (D'Aniello, personal communication: Table 1). There is also evidence that D-aspartate occurs in the

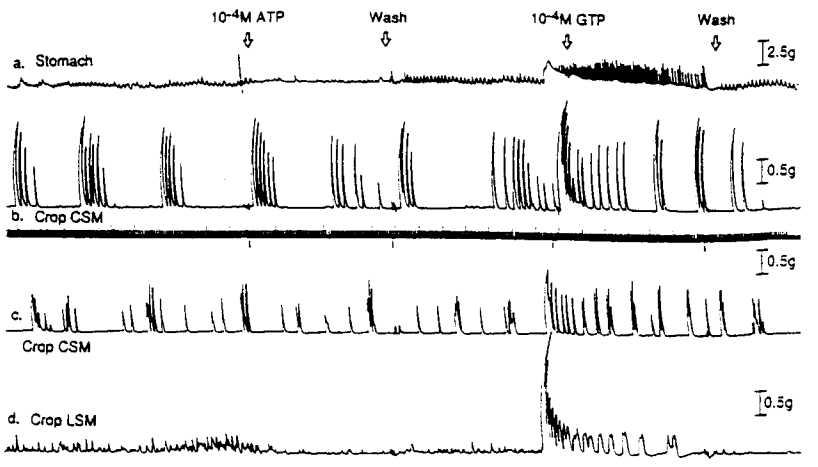

Fig. 8. Octopus bimaculoides. *GTP causes strong contractions at the stomach and the crop circular (CSM) and longitudinal (LSM) muscles. ATP appears to reduce contractions in the stomach and crop LSM. Major time scale marks, 1 min. (From Hill and Huddart, 1995).*

CNS although no function has been ascribed to it (D'Aniello *et al.*, 1995). The evidence for aspartate being a transmitter is not compelling (it is, of course, a precursor of L-glu) but over the last decade a great deal of evidence has accumulated that L-glu is an excitatory transmitter in these animals. Quite recently GABA has been shown to be present in cephalopods.

VIIa: L-glutamate

A. L-glutamate in the brain

In *Octopus* L-glu is distributed widely in the lower, intermediate and higher motor centres, including the chromatophore and lateral basal lobes (D'Aniello and Messenger, unpublished: Table 1). Using the aortic perfusion technique Andrews *et al.* (1981, 1983) found that a pulse of L-glu, kainate or especially quisqualate (Messenger, unpublished) caused strong motor effects in the arms and mantle. L-glu also expanded the chromatophores, especially the black ones, to produce instant and dramatic darkening; as before, such effects were transient.

B. L-glutamate in the peripheral nervous system

The best evidence for L-glu being a transmitter in cephalopods derives from a series of experiments on the stellate ganglion by De Santis, Messenger and their colleagues.

In the squid stellate ganglion the second-order giant fibre branches to make synaptic contact with the 10–12 third-order fibres that originate in the ganglion (Fig. 9a: Young, 1939; Martin and Miledi, 1986). The synapse onto the largest and most medial of the entire set of third-order fibres is known as the 'giant synapse'. This is the largest synapse known in the animal kingdom and its physiology has been the subject of many important papers (see Discussion). The nature of the transmitter(s) at the synapse has always been disputed since the early findings of Miledi (1967, 1969), who first showed that L-glu was active at the synapse but that the depolarisation it evoked had a different reversal potential from that of the EPSP (Miledi, 1969; Llinás *et al.*, 1974). We shall return to this point later.

Nevertheless the only other serious contender, ACh, was effectively eliminated by the experiments of Stanley (1984) and evidence continued to accrue that was consistent with L-glu being the endogenous transmitter (Kelly and Gage, 1969; De Santis *et al.*, 1978; Kawai *et al.*, 1983; Stanley, 1983; Saito *et al.*, 1985; Adams *et al.*, 1985; Adams and Gillespie, 1988). By this time the interest in L-glu as an excitatory transmitter in mammals had produced a battery of specific glutamate agonists and antagonists. De Santis and

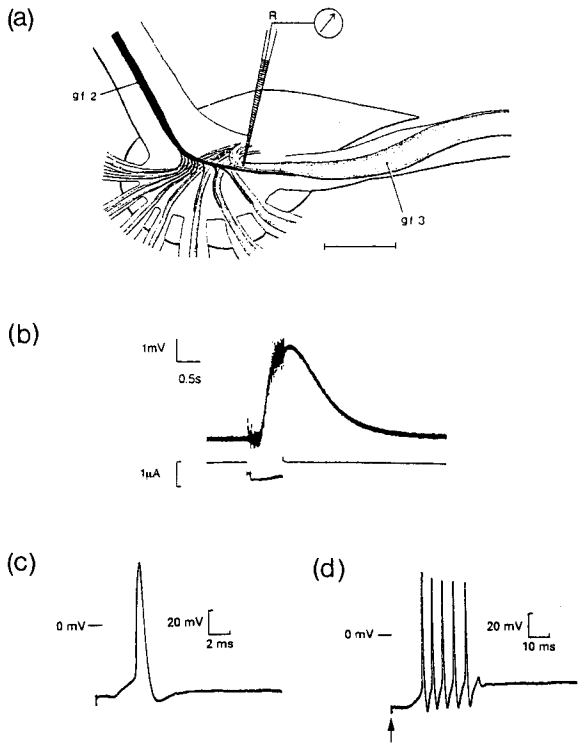

Fig. 9. *(a) Squid stellate ganglion showing the second-order fibre (gf2) synapsing onto 10 third-order fibres. Recording electrode (R) is in the most medial, largest axon (gf3) at the 'giant synapse'. Scale bar 1 mm. (b) Weak depolarisation of postsynaptic membrane by iontophoresed quisqualate (1 × 10^{-2} M). (c) Action potential in gf 3 after presynaptic stimulation. (d) Action potentials evoked by photolytic release of 9 mM L-glu from 33 mM 'caged' glutamate equilibrated at pH 5.5 for 20 min. Flash intensity 100 mJ; 300–350 nm). (From Messenger* et al., *1995 (a,b); Corrie* et al., *1993 (c,d)).*

Messenger (1989) bath-applied many of these substances to the giant synapse and showed (1) that non-NMDA agonists of L-glu (kainate, quisqualate, AMPA, ODAP, bromowillardiine and domoate) reversibly blocked transmission at the synapse, presumably because they desensitize the synapse to the endogenous transmitter(s); and (2) that non-NMDA L-glu receptor antagonists (such as CNQX, DNQX) also cause reversible blocking, demonstrating that the postsynaptic membrane contains glutamate receptors. Argiotoxin$_{636}$ also blocks transmission, though irreversibly (De Santis and Messenger, 1990).

Iontophoretic application of quisqualate, kainate, L-glu and AMPA (in decreasing order) leads only to weak depolarization of the postsynaptic membrane (Messenger *et al.*, 1995) (Fig. 9b) but L-glu released at the synapse by flash-photolysis from 'caged' glutamate depolarises the postsynaptic membrane sufficiently for action potentials to be generated in the third-order fibre (Fig. 9c, d: Corrie *et al.*, 1993). This direct demonstration that L-glu mimics the effect of

the endogenous transmitter at the synapse strongly supports the hypothesis that L-glu is an endogenous transmitter at the giant synapse. Moreover immunofluorescence histochemistry has shown that the second-order fibre stains positively with an L-glu antiserum, whereas the third-order fibres stain positively with an L-glu-receptor antiserum (De Santis *et al.*, unpublished results).

Attempts to show that L-glu is actually released from the second-order fibres have so far failed, however, even in the presence of glutamate uptake blockers. This may be because the synaptic cleft is tortuous (Young, 1973), very narrow (only 12 nm), and extends over a relatively huge area (16 312 μm^2) that contains about 15 000 synaptic contacts (Martin and Miledi, 1986). Diffusion out of such a cleft must be as slow as inward diffusion during the bath-application experiments, where 15–20 minutes are needed to ensure access (De Santis and Messenger, 1989). The unusual morphology of the synapse could also explain the discrepancy concerning the reversal potential. As Llinás *et al.* (1974) put it, 'for geometric reasons it may be quite difficult to mimic the exact timing and distribution of the natural transmitter release with a point source iontophoretic injection'.

Associated with the giant axons are the (glial) Schwann cells, which also have glutamate receptors that are thought to respond to signals from the axon when it fires. It is worth noting that the sensitivity of the Schwann cells to L-glu is quite remarkable (see Fig. 12): it appears to be five orders of magnitude greater than that of neurons or muscles. The general question of neuron–glial interactions is a fascinating one and the squid giant axon/Schwann cell system promises to be an extremely useful model, but with limited space we can only touch upon it here and allude to it subsequently. Two groups have studied the squid Schwann cells: Evans and his associates (Evans *et al.*, 1986, 1991, 1992a,b, 1995; Reale *et al.*, 1986; Evans and Villegas, 1988) and Liebermann and his (Liebermann *et al.*, 1989; Liebermann and Sanzenbacher, 1992). In summary it appears that an activated axon releases L-glu, which acts upon at least three types of glutamate receptor on the Schwann cell, two of the NMDA type and one of the non-NMDA type (Figs 11 and 12). Activation of the latter causes the co-release of ACh and VIP (Section IX), which in turn act back upon the Schwann cell (the former at nicotinic type receptors), leading to long-lasting hyperpolarization.

C. L-glutamate in the sense organs

In the statocyst Tu and Budelmann (1994) have good physiological evidence that L-glu may be a transmitter at the afferent crista fibres of three species of cephalopods. Bath-applied L-glu increases the resting activity of the fibres in a dose-dependent manner (Fig. 10a). Furthermore, by using agonists and antagonists they obtained good evidence that L-glu acts at non-NMDA receptors. With the exception of the Schwann cell this seems to be true of all other glutamatergic systems in cephalopods.

D. L-glutamate in the muscles, including the chromatophores

The first demonstration that L-glu is active at nerve–muscle junctions in cephalopods came from the experiments of Bone and Howarth (1980): they found that strips of mantle and fin muscle responded to L-glu with rapid twitches, sometimes superimposed on slow contractures. Bone *et al.* (1982) confirmed that it is the circular muscles of the mantle that respond to L-glu (see also Section I) but they did not establish whether there are any glutamatergic fibres in the arms or tentacles, for example in the extensors.

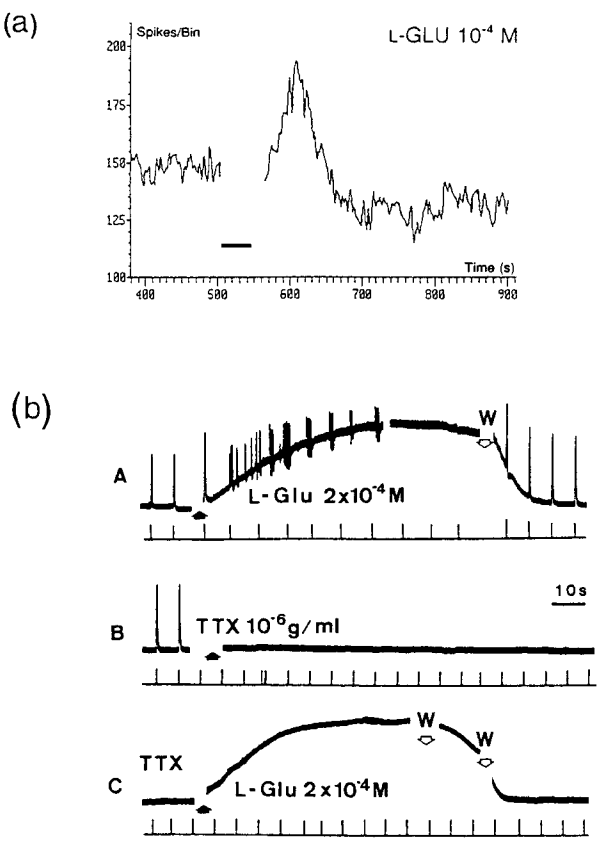

Fig. 10. *(a) L-glutamate (black bar) enhances resting activity of* Sepia *statocyst afferent crista fibres. Bin width 1 s. (From Tu and Budelmann, 1994). (b) L-glu (arrows) generates contracture and repetitive contractions of* Lolliguncula *chromatophore muscles - A ; TTX abolishes response to nerve stimulation - B , but does not alter the response to topically applied L-glu - C. (From Florey* et al., *1985).*

Bone and Howarth (1980) also showed that topical application of L-glu to the gut, the branchial hearts and the ink-sac duct evoked immediate repetitive contractions; none of these findings has been re-investigated. More importantly they reported that topical application of L-glu to cephalopod skin caused immediate expansion of all colour classes of chromatophore. This result was confirmed and extended, first by Florey *et al.* (1985) and later by Messenger and his colleagues (Messenger, 1991; Messenger *et al.*, 1991; Cornwell and Messenger, 1995; Reed, 1995; Messenger *et al.*, in preparation) on three loliginids, *Alloteuthis*, *Loligo* and *Lolliguncula*. Since these results constitute good, if incomplete, evidence that L-glu is an endogenous excitatory transmitter at the chromatophore muscles it is worth summarising the findings of all these workers.

Immunohistochemistry at the light-microscope level shows that the nerves running along the chromatophore radial muscles stain positively with an antiserum to L-glu; at the EM level the staining is seen to be confined to the nerve axons, where 50 nm diameter electron-lucent vesicles are organised into synapses along the radial muscle. Topically applied L-glu or a non-NMDA agonist induces radial muscle contraction and chromatophore expansion. The most effective agonist is domoate. CNQX and DNQX block these effects; TTX abolishes nerve-induced contractions but not contractions resulting from direct application of L-glu (Fig. 10b).

Loi *et al.* (1996) now have evidence in *Sepia* that FMRFamide is also present and excitatory (Section IX) so that it is beginning to look as if there are multiple messengers regulating the chromatophores.

VIIb. GABA

A. GABA in the brain

There has been conflicting evidence about the presence of γ-amino butyric acid (GABA) in the cephalopod CNS (Tansey, 1979), but recently Cornwell *et al.* (1993), using an immunohistochemical technique, demonstrated weak but widespread GABA-like staining in the neuropil and cell layer of many brain lobes of the Northern octopus, *Eledone* (Table 1).

Two results of this study deserve comment. The positive staining in the chromatophore lobes should be seen in conjunction with the finding of Andrews *et al.* (1983) that pulses of GABA injected into the cephalic aorta of *Octopus* lead to colour changes. And the occurrence of GABA-like immunostaining in the lateral pedal lobe, an oculomotor centre, may make more significant the finding (below) that the statocyst is sensitive to GABA.

B. GABA in the sense organs

So far there have been no reports of GABA in the cephalopod retina but in the statocyst Tu and Budelmann (1994) have reported that GABA inhibits the resting activity of crista units in *Sepia*.

There is no information at present about GABA physiology or receptor type.

VIII. Nitric oxide

Chichery and Chichery (1994) were the first to localise histochemically NADPH-diaphorase in cephalopods; this enzyme is an indicator of NOS, and, by implication, of NO. In the *Sepia* brain they found staining in the neuropil of the anterior basal and peduncle lobes, both higher motor centres comparable to the vertebrate cerebellum (Messenger, 1967, 1979; Young, 1977).

Moroz and Gillette (1995 and personal communication) have begun a comparative study of the distribution of NADPH-diaphorase in cephalopods (and other molluscs). In decapods such as *Sepia* and *Rossia* they, too, only detected weak staining in the neuropil, but in *Loligo* they found a few stained somata in the suboesophageal lobes, which contain many motoneurons. In the stellate ganglion, however, staining was restricted to the neuropil, the glia and the capillaries. In *Octopus*, where the staining was much stronger, the neuropil of both suboesophageal and supraoesophageal lobes stained positively but only in the former region were the cell bodies stained. In the stellate ganglion many cell bodies were stained but not the neuropil; several muscle systems were positive for NOS.

It seems clear, in short, that NOS is widespread in the cephalopod CNS, although there appears to be great variation between genera. Direct evidence of a biological role for NO comes from the important experiments of Robertson *et al.* (1994). These workers showed that in *Octopus* intramuscular injections of an NOS inhibitor, L-NAME, completely blocked touch-learning. This result suggests that, as in vertebrates, NO may sometimes be essential for learning, although it does not, of course, show where in the tactile learning system it acts.

IX. Peptides

A. Peptides in the brain

1. Substance P. This was found in the optic lobe and retina of *Sepia* by Osborne *et al.* (1986) on the basis of light-microscopy immunofluorescence. Some cell

bodies and neuropil in the optic lobe were stained but in the retina staining was limited to the plexiform layer. This is where (dopaminergic) efferent fibres from the optic lobe synapse with the photoreceptor cells (Section II); it remains to be established whether substance P is co-localised with DA here. These workers note the virtual absence of substance P-like immunoreactivity from other brain regions and also report the absence from the retina of five other peptides found in vertebrate retinas, including somatostatin.

2. Somatostatin (SRIF). Feldman (1986), using PAP/DAB visualisation, revealed widespread somatostatin-like immunoreactivity throughout the brain of the squid *Loligo*. This important paper showed, amongst other things (1) that SRIF was absent from the optic nerves but present in the optic lobe medulla and cortex (outer granular and plexiform layers) and optic tract; (2) that immunoreactivity was strong in the vertical lobe system, which is associated with establishing visual memories; (3) that SRIF occurs in the stellate ganglion (cell bodies and neuropil) but not in association with the giant synapse; and (4) that it occurs in the suboesophageal lobes but not in the first-order giant cells. The extensive but uneven distribution of this peptide in the squid brain must make it a serious candidate for being a neuromodulator centrally.

3. FMRFamide-related peptides. Recently Chin *et al.* (1994) showed that the optic lobe of *L. pealei* contains FMRFamide receptors, which they characterised using radioligands. They also sequenced a fragment of genomic DNA encoding a precursor of FMRFamide and showed that the optic lobe also contains FLRFamide. Just how widespread FMRFamide is in the cephalopod CNS remains to be established. In *Sepia officinalis* Le Gall *et al.* (1988) found immunohistochemical evidence that FMRFamide occurs in cell bodies in the olfactory and dorsal basal lobes innervating the optic gland, an endocrine organ regulating sexual maturity, and now Loi *et al.* (1996) have reported FaRPs in several lobes of this species, including the posterior chromatophore lobes (Table 1; see below); there are at least four peptides (Loi and Tublitz, 1996).

In *Loligo* a nonapeptide (peptide tyrosine phenylalanine) has been isolated from gross brain extracts (Smart *et al.*, 1992).

B. Peptides in the peripheral nervous system

1. FMRF. Cottrell *et al.* (1992) have shown that the squid stellate ganglion contains FLRFamide, as well as FMRFamide and at least one other peptide. They also found evidence that FLRFamide is active at the giant synapse, potentiating transmission, perhaps by influencing transmitter mobilization. It does not affect the resting membrane potential of either the pre- or post-synaptic cell.

2. Substance P, somatostatin and VIP. In the squid stellate ganglion there is good physiological evidence that these peptides all produce long-lasting hyperpolarization of the Schwann cell of the giant fibre (Evans *et al.*, 1986, 1990). There is also some evidence that a VIP-like peptide may be an endogenous component in the complex signalling system between neuron and glia; specific antagonists of VIP receptors competitively block the effects of VIP in this preparation (Fig. 12) and also reduce the hyperpolarizing effect of the endogenous substance (Evans and Villegas, 1988). VIP can also modulate the Schwann cell response to carbachol and octopamine and substance P can apparently modulate the response to VIP. It may be that a VIP-like peptide is co-localised with ACh in the Schwann cell, for if VIP is blocked its hyperpolarizing effect disappears. Recall too that octopamine, perhaps acting as a neurohormone, may also modulate the Schwann cells. Fig. 11 gives some idea of how complex this signalling system seems to be, as does Fig. 12, which also highlights the extreme sensitivity of the Schwann cell membrane to L-glu and octopamine.

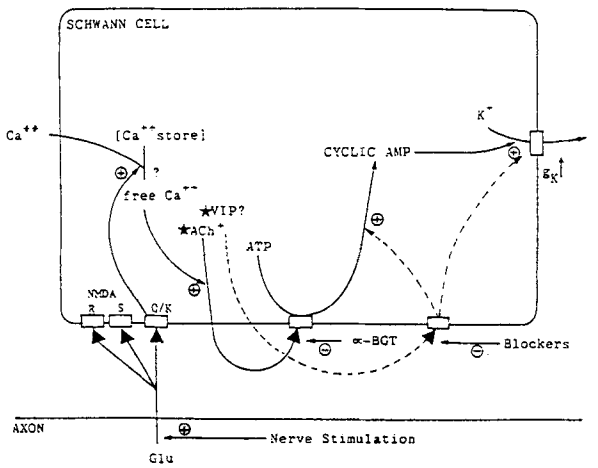

Fig. 11. Sepioteuthis, *the tropical squid. One hypothetical scheme for an axon-Schwann cell signalling system that involves both cholinergic and glutamatergic pathways. (R, S, rapid, slow NMDA depolarizing receptors; Q/K, quisqualate, kainate receptors; +, potentiation, -, inhibition. From Evans* et al., *1991).*

C. Peptides in the vascular system

Another important source of peptides in cephalopods is the neurosecretory system of the vena cava (NSV: Alexandrowicz, 1965; Martin, 1968). This neurohaemal organ comprises about two million small neurons lying in direct contact with the blood and

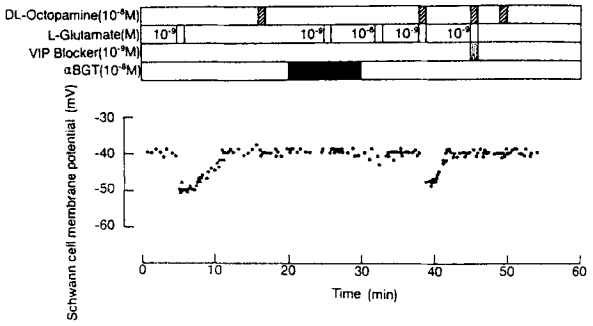

Fig. 12. Sepioteuthis. *Signalling between the axon and Schwann cell is even more complex than the last figure suggests.* L-*glu in the presence of octopamine (after BGT has blocked cholinergic component of response) appears to release a VIP-like peptide that depolarizes the cell for the effect can be blocked by (pCl-*D*-Phe*^{6}*, Leu*^{17}*) VIP. Note the apparently exquisite sensitivity of the Schwann cell membrane to* L-*glu: compare Figs. 9 and 10. (From Evans* et al., *1995).*

situated upstream from the branchial hearts. In *Octopus vulgaris* immunocytochemical investigations have revealed three classes of secretory endings: (1) those that were immunoreactive with antibodies raised against proctolin; (2) those with oxytocin/vasopressin- and neurophysin-like immunoreactivity (see also Reich, 1992); and (3) those with FMRFamide-, α-melanotropin- and atriopeptin-like immunoreactivity (Martin and Voigt, 1987).

Many workers have shown that NSV extracts have marked excitatory effects on the octopod heart and circulation *in vitro* (Martin and Voigt, 1987) and *in vivo* (Wells, 1983) and more recently this has been shown to be true in *Sepia* too (Jakobs, 1991). For example, in the branchial heart both YGGFMRF and oxytocin caused increase in pressure amplitude. In this preparation, however, still larger effects could be obtained with extracts of the NSV itself, suggesting there it may contain other, as yet unidentified, neurohormones (Fiedler, 1992).

In the branchial heart FMRFamide has little effect by itself but counteracts the effects of NA; in the heart FMRFamide and FLRFamide both exert a modulatory effect (Jakobs and Schipp, 1992); in the cephalic aorta FMRFamide, which has been found immunohistochemically in the wall, has a dilatory effect *in vitro* (Schipp *et al.*, 1991).

Finally we should note the experiments of Agnisola *et al.* (1989), who showed that the *Octopus* heart responds to mammalian atrial peptides; the sensitivity to ANF is of the same order of magnitude as that of the rat heart.

D. Peptides in the chromatophores

Recently Loi *et al.* (1996) have shown in *Sepia officinalis* that FMRFamide-related peptides are present in the chromatophore layer of the skin, associated with the chromatophore nerves. Moreover in this species these peptides bring about chromatophore expansion *in vitro* so that they must play some part in the regulation of body patterning in life.

X. Discussion

For reasons of space this review has not considered ion channels in cephalopods; nor has it referred to the elegant and important studies related to transmitter release at the squid giant synapse that have such general importance (see, for example, Llinás and Sugimori, 1995; Augustine *et al.*, 1995). It has looked at the occurrence, distribution and, where possible, the physiology of putative transmitters in this group of molluscs.

It should have become clear that cephalopods use all the 'classical' transmitters, as well as purines, NO and neuroactive peptides. This ought not to surprise us, first because these substances are ancient and widely distributed throughout the animal kingdom (Walker and Holden-Dye, 1989, 1991), and secondly because these are advanced invertebrates (Wells, 1978) whose various activities are likely to require subtle control and regulation. Consider the gut: on top of a basic catecholaminergic-excitatory/cholinergic-inhibitory control system it seems likely that 5-HT and GTP (and perhaps peptides?) are employed when necessary for local regulation. Similarly the vascular system is influenced by a large number of neuroactive substances *in vitro*, including peptides, suggesting that in the free-living animal the circulation can be very finely tuned to meet physiological needs.

The large number of presumed transmitters and modulators in the brain (ACh, DA, NA, 5-HT, L-glu and to a lesser extent OA and GABA) is also evidence of the presence of many control- and meta-control loops (Messenger, 1979). Unfortunately we do not know why some of these systems utilise different transmitters and it is as difficult to make general statements about transmitters in the cephalopod brain as it is elsewhere. The primary sensory neurons, in eye, statocyst and olfactory organ, all appear to use different transmitters (Table 3). At some body muscles the motoneurons use L-glu as the excitatory transmitter at the nerve–muscle junctions; at others they use ACh. In the brain the same transmitters are found in sensory, motor and association areas. Thus the lower, intermediate and higher motor centres (Boycott, 1961) cannot easily be differentiated by their having different transmitters. There also appear to be important differences in the putative transmitters of the two memory systems

Organ	Histochemical and biochemical evidence	Physiological evidence
Eye		
afferents	ACh	none
efferents	DA	DA
also present in retina	5-HT, OA, substance P	none
Statocyst		
afferents	none	L-glu
efferents	DA and/or NA ACh, AChE	DA and/or NA (excitatory); ACh (inhibitory)
Olfactory Organ		
afferents	DA and/or NA	none
efferents	?	none

Table 3. Transmitter candidates in the sense organs. *Based on data in: Auerbach and Budelmann, 1986; Budelmann and Bonn, 1982; Budelmann* et al., *1987; Juorio and Molinoff, 1974; Lam* et al., *1974; Osborn* et al., *1986; Tansey, 1980; Tu and Budelmann, 1994; Williamson, 1989; Williamson and Chrachri, 1994.*

(tactile and visual). On the other hand the parallel fibre regions of two 'cerebellar' lobes, the peduncle and the (posterior) anterior basal lobe, both utilise 5-HT (Messenger, 1967, 1979; Young, 1977; Messenger and Tansey, 1979) and NO (Chichery and Chichery, 1994), whatever that signifies.

Not only do cephalopods use most of the familiar transmitters, but they sometimes use them in the same system. We saw the complexity of signalling between the giant axon and the Schwann cell in the squid (Figs 11, 12) but in the *Sepia* heart a similar situation holds, as can be seen from Fig. 13. This means that the neuropharmacology of cephalopods is not likely to prove an 'easy option' compared to that of mammals. Moreover future workers will surely find yet other neuroactive substances in these animals. Incidentally there is still no evidence in cephalopods for the co-localisation of neuroactive substances in the same neuron: it might be sensible to start looking at some of the peptides.

A much more important task, however, is to localise the transmitters in the cephalopod brain so that physiologists can begin experiments to ascribe function. For this the powerful new immunohistological techniques, with their great sensitivity, offer many advantages. There is also the prospect that it will soon be possible to characterise the receptor subtypes as more pharmacological tools become available, and, more importantly, as the molecular biology of molluscan receptors is developed. What is quite clear, however, is that most cephalopod receptor subtypes (for example ACh, DA, NA, 5-HT, L-glu) are remarkably similar to those of vertebrates, given that the two groups have been separated for over 400 million years (Table 4). It is also clear that they are not identical.

This review must end with a warning. For simplicity, results from different species have been pooled in the tables, perhaps creating the impression that all

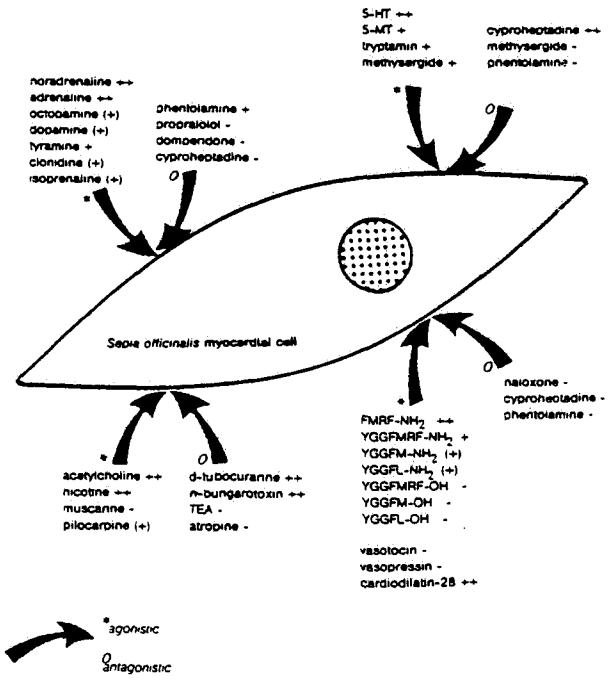

Fig. 13. *The responses of a schematic* Sepia *myocardial cell to various agonists and antagonists. ++, +, (+) decreasing sensitivity, –, no effect. (From Kling and Jacobs, 1987).*

ACh	'nicotinic', α-bungarotoxin
NA	'α-adrenergic'
DA	'DA_1', 'DA_2'
5-HT	'$5\text{-}HT_{1,2}$'
OA	'$OA_{1,2}$'
L-glu	'kainate/quisqualate' (not AMPA)
	NMDA (glia only)
GABA	no data

Table 4. Possible receptor types in cephalopods. *The quotation marks emphasise that these molluscan receptors are similar but not identical to those characterised in mammals. See text.*

cephalopods must be the same pharmacologically. We do not intend to imply this and indeed have noted, for example, the apparent anomalies about the photoreceptor cell endings in the octopod and decapod optic lobe, the seemingly different receptor subtypes in the different parts of the vascular system of one species and the great variation in NOS levels between genera. It will be for future workers to investigate these inconsistencies and build a fuller picture of the comparative pharmacology of these highly evolved molluscs.

Acknowledgements

I am extremely grateful to Linda Noble at the wonderful MBA library at Plymouth for much help during the preparation of this article.

Figure credits

We thank all the authors who gave permission to publish their figures and also the following Journals and their publishers:

Figs 1, 5, 6, 8 & 10b Comparative Biochemistry and Physiology (Elsevier Science Inc).

Fig. 2 Journal of the Marine Biological Association of the UK.

Fig. 3 Vision Research (Elsevier Science Ltd).

Figs 4 & 7 Journal of Experimental Biology (Company of Biologists Ltd).

Figs 9a, b & 11 Oxford University Press.

Fig. 9c, d Journal of Physiology (The Physiological Society).

Fig. 10a Brain Research (Elsevier Science).

Fig. 12 Annals of the New York Academy of Sciences.

Fig. 13 Experientia (Birkhäuser).

References

Adams, D. J. and Gillespie, J. I. (1988) The actions of L-glutamate at the post-synaptic membrane of the squid giant synapse. *J. Exp. Biol.*, **140**, 535-548.

Adams, D. J., Takeda, K. and Umbach, J. A. (1985) Inhibitors of calcium buffering depress evoked transmitter release of the squid giant synapse. *J. Physiol. (Lond.)*, **369**, 145-160.

Agnisola, C., Cariello, L., De Santis, A., Miralto, A. and Tota, B. (1989) Chronotropic and inotropic effects of atrial peptides on the isolated systemic heart of *Octopus vulgaris*. *J. Comp. Physiol. B.*, **158**, 637-641.

Alexandrowicz, J. S. (1965) The neurosecretory system of the vena cava in cephalopoda II. *Sepia officinalis* and *Octopus vulgaris*. *J. Mar. Biol. Assoc. UK*, **45**, 209-228.

Andrews, P. L. R., Messenger, J. B. and Tansey, E. M. (1981) Colour changes in cephalopods after neurotransmitter injection into the cephalic aorta. *Proc. R. Soc. Lond. Biol. Sci.*, **213**, 93-99.

Andrews, P. L. R., Messenger, J. B. and Tansey, E. M. (1983) The chromatic and motor effects of neurotransmitter injection in intact and brain-lesioned *Octopus*. *J. Mar. Biol. Assoc. UK*, **63**, 355-370.

Andrews, P. L. R. and Tansey, E. M. (1981) A technique for central drug administration in *Octopus vulgaris*. *J. Neurosci. Methods*, **4**, 243-247.

Andrews, P. L. R. and Tansey, E. M. (1983a) The digestive tract of *Octopus vulgaris*: the anatomy, physiology and pharmacology of the upper tract. *J. Mar. Biol. Assoc. UK*, **63**, 109-134.

Andrews, P. L. R. and Tansey, E. M. (1983b) Aminergic innervation of the blood vessels of *Octopus vulgaris*. *Cell Tissue Res.*, **230**, 229-232.

Auerbach, B. and Budelmann, B.V. (1986). Evidence for acetylcholine as a neurotransmitter in the statocyst of *Octopus vulgaris*. *Cell Tissue Res.* **243**, 429-436.

Augustine, G. J., Deitmer, J., Hans, M., Swandula, D. and Zipser, K. (1995) Multiple calcium signalling pathways in squid giant presynaptic terminals. In *Cephalopod Neurobiology*, ed. N. J. Abbott, R. Williamson, and L. Maddock, pp. 271-282. Oxford: Oxford University Press.

Bacq, Z. M. (1935) Recherches sur la physiologie et la pharmacologie du système nerveux autonome. XVII. Les esters de la choline dans les extraits de tissus des invertébrés. *Arch. Int. Physiol.*, **42**, 24-42.

Barlow, J. J. (1977) Comparative biochemistry of the central nervous system. In *The Biology of Cephalopods*, ed. M. Nixon and J. B. Messenger, pp. 325-346. *Symp. Zool. Soc. Lond.*, **38**.

Bone, Q. and Howarth, J. V. (1980) The role of L-glutamate in neuromuscular transmission in some molluscs. *J. Mar. Biol. Assoc. UK*, **60**, 619-26.

Bone, Q., Packard, A. and Pulsford, A. L. (1982) Cholinergic innervation of muscle fibres in squid. *J. Mar. Biol. Assoc. UK*, **62**, 193-199.

Boycott, B. B. (1961) The functional organization of the brain of the cuttlefish *Sepia officinalis*. *Proc. R. Soc. Lond. B Biol. Sci.*, **153**, 503-534.

Brzin, M., Tennyson, V. M. and Dettbarn, W. D. (1975) Cytochemical localization of cholinesterase activity at the giant synapse of the squid. *Histochemistry*, **43**, 305-311.

Budelmann, B. U. (1990) The statocysts of squid. In *Squid as Experimental Animals*, ed. D. L. Gilbert, W. J. Adelman and J. M. Arnold, pp. 421-439. New York: Plenum.

Budelmann, B. U. and Bonn, U. (1982) Histochemical evidence for catecholamines as neurotransmitters in the statocyst of *Octopus vulgaris*. *Cell Tissue Res.*, **227**, 475-483.

Budelmann, B. U., Sachse, M. and Staudigl, M. (1987) The angular acceleration receptor system of *Octopus vulgaris*: morphometry, ultrastructure, and neuronal and synaptic organization. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **315**, 305-343.

Burnstock, G., Cocks, T., Kasakov, L. and Wong, H. K. (1978) Direct evidence for ATP release from non-adrenergic, non-cholinergic (purinergic) nerves in guinea-pig taenia coli and bladder. *Eur. J. Pharmacol.*, **49**, 145-149.

Capasso, A., Carginale, V., Madonna, L., Mancaniello, D., Scudiero, R., De Prisco, P. P., De Petrocellis, B. and Parisi, E. (1991) A dopamine- and octopamine- sensitive adenylate cyclase in the nervous system of *Octopus vulgaris*. *Comp. Biochem. Physiol.*, **100B**, 805-803.

Capasso, A., Carginale, V., Borrelli, L. and Parisi, E. (1993) Receptor-mediated inhibition of octopamine-stimulated adenylate cyclase in the optic lobe of *Octopus vulgaris*. *Comp. Biochem. Physiol.*, **106C**, 555-559.

Chen, S-J., Spathis, R. and Schmidt, J. (1988) Binding sites for [^{3}H]-acetylcholine and ^{125}I-α-bungarotoxin in the optic ganglion of *Loligo pealii*. *Comp. Biochem. Physiol.*, **90C**, 317-323.

Chichery, R. and Chanelet, J. (1972) Action de l'acétylcholine et de diverses substances curarisantes sur le système nerveux de la seiche. *Compt. Rend. Séanc. Soc. Biol.*, **166**, 273-276.

Chichery, R. and Chichery, M. P. (1974) Histochemical study of the localization of cholinesterases in the central nervous system of *Sepia officinalis*. *Cell Tissue Res.*, **148**, 551-560.

Chichery, R. and Chichery, M.-P. (1985) Motor and behavioural effects induced by putative neurotransmitter injection into the optic lobe of the cuttlefish, *Sepia officinalis*. *Comp. Biochem. Physiol.*, **80C**, 415-419.

Chichery, R. and Chichery, M. P. (1994) NADPH-diaphorase in a cephalopod brain (*Sepia*): presence in an analogue of the cerebellum. *NeuroReport*, **5**, 1273-1276.

Chin, G. J., Payza, K., Price, D. A., Greenberg, M. J. and Doble, K. E. (1994) Characterization and solubilization of the FMRFamide receptor of squid. *Biol. Bull.*, **187**, 185-199.

Cloney, R. A. and Florey, E. (1968) Ultrastructure of cephalopod chromatophore organs. *Zeit. Zellforsch.*, **89**, 250-280.

Cohen, A. I. (1973) An ultrastructural analysis of the photoreceptors of the squid and their synaptic connections. III Photoreceptor termination in the optic lobes. *J. Comp. Neurol.*, **147**, 399-426.

Cornwell, C. J. and Messenger, J. B. (1995) Neurotransmitters of squid chromatophores. In *Cephalopod Neurobiology*, ed. N. J. Abbott, R. Williamson and L. Maddock, pp. 369-379. Oxford: Oxford University Press.

Cornwell, C. J., Messenger, J. B. and Williamson, R. (1993) Distribution of GABA-like immunoreactivity in the octopus brain. *Brain Res.*, **621**, 353-357.

Corrie, J. E. T., De Santis, A., Katayama, Y., Khodakhah, K., Messenger, J. B., Ogden, D. C. and Trentham, D. R. (1993) Postsynaptic activation at the squid giant synapse by photolytic release of L-glutamate from 'caged' L-glutamate. *J. Physiol. (Lond.)*, **465**, 1-8.

Cottrell, G. A., Lin, J. W., Llinas, R., Price, D. A., Sugimori, M. and Stanley, E. F. (1992) FMRFamide-related peptides potentiate transmission at the squid giant synapse. *Exp. Physiol.*, **77**, 881-889.

D'Aniello, A., Nardi, G., De Santis, A., Vetere, A. Di Cosmo, A., Marchelli, R., Dossena, A. and Fisher, G. (1995) Free L-amino acids and D-aspartate content in the nervous system of Cephalopoda. A comparative study. *Comp. Biochem. Physiol.*, **112B**, 661-666.

De La Torre, J. D. and Surgeon, J. W. (1976) A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique: the SPG method. *Histochemistry*, 49, 81-93.

Demushkin, V. P. and Kotelevtsev, Yu. V. (1980) Properties of membrane-bound acetylcholine receptor from optic ganglion of the squid *Berryteuthis magister*. *Biokhimiya*, **45**, 1773-1779.

De Santis, A., Eusebi, F. and Miledi, R. (1978) Kainic acid and synaptic transmission in the stellate ganglion of the squid. *Proc. R. Soc. Lond. B Biol. Sci.*, **202**, 527-32.

De Santis, A. and Messenger, J. B. (1989) New evidence that L-glutamate is a transmitter at the squid giant synapse. *Q. J. Exp. Physiol.*, **74**, 219-222.

De Santis, A. and Messenger, J. B. (1990) Argiotoxin 636 blocks squid giant synapse. *FEBS Abstracts* P-Mo 065.

Evans, P. D. (1981) Multiple receptor types for octopamine in the locust. *J. Physiol. (Lond.)*, **318**, 99-122.

Evans, P. D., Reale, V., Merzon, R. M. and Villegas, I (1986) Peptidergic modulation of the membrane potential of the Schwann cell of the squid giant nerve fibre. *J. Physiol. (Lond.)*, **379**, 61-82.

Evans, P. D., Reale, V., Merzon, R. M. and Villegas, I. (1990) Substance P modulation of the membrane potential of the Schwann cell of the squid giant nerve fibre. *Glia*, **3**, 393-404.

Evans, P. D., Reale, V., Merzon, R. M. and Villegas, I. (1991) *N*-methyl-D-aspartate (NMDA) and non-NMDA type glutamate receptors are present on squid giant axon Schwann cells. *J. Exp. Biol.*, **157**, 593-600.

Evans, P. D., Reale, V., Merzon, R. M. and Villegas, I. (1992a) *N*-methyl-D-aspartate (NMDA) and non-NMDA (metabotropic) type glutamate receptors modulate the membrane potential of the Schwann cell of the squid giant nerve fibre. *J. Exp. Biol.*, **173**, 229-249.

Evans, P. D., Reale, V., Merzon, R. M. and Villegas, I. (1992b) The effect of a glutamate uptake inhibitor on axon–Schwann cell signalling in the squid giant nerve fibre. *J. Exp. Biol.*, **173**, 251-260.

Evans, P. D., Reale, V., Merzon, R. M. and Villegas, I. (1995) The pharmacology of receptors present on squid giant axon Schwann cells. In *Cephalopod Neurobiology*, ed. N. J. Abbott, R. Williamson and L. Maddock, pp 213-228. Oxford: Oxford University Press.

Evans, P. D. and Villegas, J. (1988) The action of vasoactive intestinal peptide antagonists on peptidergic modulation of the squid Schwann cell. *J. Exp. Biol.*, **138**, 259-269.

Feldman, S. C. (1986) Distribution of immunoreactive somatostatin (ISRIF) in the nervous system of the squid, *Loligo pealei*. *J. Comp. Neurol.*, **245**, 238-257.

Fiedler, A. (1992) The possible role of vena cava peptides in regulation of the branchial hearts of *Sepia officinalis* L. (Cephalopoda). *J. Exp. Zool.*, **264**, 136-143.

Fiedler, A. and Schipp, R. (1990) The effects of biogenic monoamines and related agonists and antagonists on the isolated, perfused branchial heart of *Sepia officinalis* (Cephalopoda). *Comp. Biochem. Physiol.*, **97C**, 71-78.

Fiedler, A. and Schipp, R. (1991) Localization of catecholamine-containing nerve fibres in the branchial heart and cardia ganglion of the common cuttlefish. *Sepia officinalis* L. (Cephalopda). *Tissue Cell*, **23**, 813-819.

Florey, E. (1966) Nervous control and spontaneous activity of the chromatophores of a cephalopod, *Loligo opalescens*. *Comp. Biochem. Physiol.*, **18**, 305-324.

Florey, E., Dubas, F. and Hanlon, R. T. (1985) Evidence for L-glutamate as a transmitter of motoneurons innervating squid chromatophore muscles. *Comp. Biochem. Physiol.*, **82C**, 259-268.

Florey, E. and Kriebel, M. E. (1969) Electrical and mechanical responses of chromatophore muscle fibres of the squid *Loligo opalescens* to nerve stimulation and drugs. *Z. Vergl. Physiol.*, **65**, 98-130.

Florey, E. and Winesdorfer, J. (1968) Cholinergic nerve endings in *Octopus* brain. *J. Neurochem.*, **15**, 169-177.

Ghiretti, F. (1948) Studio comparativo dell'azione della colina e dell'acetilcolina sul cuore isolato dei molluschi gasteropodi e cefalopodi. *Arch. Sci. Biol.*, **32**, 239-251.

Gleadall, I. G., Ohtsu, K., Gleadall, E. and Tsukahara, Y. (1993) Screening-pigment migration in the *Octopus* retina includes control via dopaminergic efferents. *J. Exp. Biol.*, **185**, 1-16.

Gray, E. G. (1970) A note on synaptic structure of the retina of the *Octopus vulgaris*. *J. Cell. Sci.*, **7**, 203-215.

Hanlon, R. T. and Messenger, J. B. (1996) *Cephalopod behaviour*. Cambridge: Cambridge University Press.

Hill, R. B. and Huddart, H. (1995) Actions of GTP on molluscan buccal, cardiac and visceral muscle. *Comp. Biochem. Physiol.*, **111C**, 389-396.

Hill, R. B. and Welsh, J. H. (1966) Heart, circulation and blood cells. In *The Physiology of Mollusca Volume 2*, ed. K. M. Wilbur and C. M. Yonge, pp 125-174. New York: Academic Press.

Jakobs, P. M. (1991) Effects of FMRFamide and some analogues on the isolated systemic heart of *Sepia officinalis* L. in orthograde perfusion. In *First International Symposium on the Cuttlefish Sepia, University of Caen, France, 1989*, ed. E. Boucaud-Camou, pp. 237-247. Caen: Centre Publications, University of Caen.

Jakobs, P. M. and Schipp, R. (1992) The electocardiogram of *Sepia officinalis* L. (Cephalopoda: Coleoidea) and its modulation by neuropeptides of the FMRFamide group. *Comp. Biochem. Physiol.*, **103C**, 399-402.

Johansen, K. and Huston, M. J. (1962) Effects of some drugs on the circulatory system of the intact non-anesthetized cephalopod, *Octopus dofleini*. *Comp. Biochem. Physiol.*, **5**, 177-184.

Juorio, A. V. (1971) Catecholamines and 5-hydroxytryptamine in nervous tissue of cephalopods. *J. Physiol. (Lond.)*, **216**, 213-226.

Juorio, A. V. and Killick, S. W. (1972) Monoamines and their metabolism in some molluscs. *Comp. Gen. Pharmacol.*, **3**, 283-295.

Juorio, A. V. and Killick, S. W. (1973) The distribution of monoamines and some of their acid metabolites in the poste-

rior salivary glands and viscera of some cephalopods. *Comp. Biochem. Physiol.*, **44**, 1059-1067.

Juorio, A. V. and Molinoff, P. B. (1974) The normal occurrence of octopamine in neural tissues of the *Octopus* and other cephalopods. *J. Neurochem.*, **22**, 271-280.

Juorio, A. V. and Philips, S. R. (1976) Arylalkylamines in *Octopus* tissue. *Neurochem. Res.*, **1**, 501-509.

Kawai, N., Yamagishi, S., Saito, M., and Furuya, K. (1983) Blockade of synaptic transmission in the squid giant synapse by a spider toxin (JSTX). *Brain Res.*, **278**, 346-349.

Kelly, J. S. and Gage, P. W. (1969) L-glutamate blockade of transmission at the giant synapse of the squid stellate ganglion. *J. Neurobiol.*, **1**, 209-219.

Kier, W. M. (1988) The arrangement and function of molluscan muscle. In *The Mollusca, Vol. 11. Form and Function*, ed. E. R. Trueman and M. R. Clarke, pp. 211-252. San Diego: Academic Press.

Kime, D. E. and Messenger, J. B. (1990) Monoamines in the cephalopod CNS: an HPLC analysis. *Comp. Biochem. Physiol.*, **96C**, 49-57.

Kito-Yamashita, T., Haga, C., Hirai, K., Uemura, T., Kondo, H. and Kosaka, K. (1990) Localization of serotonin immunoreactivity in cephalopod visual system. *Brain Res.*, **521**, 81-88.

Kling, G. (1986) Histochemical localisation of cholinesterases and monoamines in the central heart of *Sepia officinalis* L. (Cephalopoda). *Histochemistry*, **85**, 241-250.

Kling, G. (1987) The effect of acetylcholine, cholinergic agonists and antagonists on the isolated central heart of *Sepia officinalis* L. *Zool. Jb. Physiol.*, **91**, 13-25.

Kling, G. and Jakobs, P. M. (1987) Cephalopod myocardial receptors: pharmacological studies on the isolated heart of *Sepia* officinalis (L.). *Experientia*, **43**, 511-525.

Kling, G. and Schipp, R. (1987) Effects of biogenic amines and related agonists and antagonists on the isolated heart of the common cuttlefish *Sepia officinalis*. *Comp. Biochem. Physiol.*, **87C**, 251-285.

Lam, P. M. K., Wiesel, T. N. and Kaneko, A. (1974) Neurotransmitter synthesis in cephalopod retina. *Brain Res.*, **82**, 365-368.

Le Gall, S., Féral, C., Van Minnen, J. and Marchand, C. R. (1988) Evidence for peptidergic innervation of the endocrine optic gland in *Sepia* by neurons showing FMRFamide-like immunoreactivity. *Brain Res.*, **462**, 83-88.

Lieberman, E. M., Abbott, N. J. and Hassan, S. (1989) Evidence that glutamate mediates axon-to-Schwann cell signaling in the squid. *Glia*, **2**, 94-102.

Lieberman, E. M. and Sanzenbacher, E. (1992) Mechanisms of glutamate activation of axon-to-Schwann cell signalling in the squid. *Neuroscience*, **47**, 931-939.

Llinás, R., Joyner, R. W. and Nicholson, C. (1974) Equilibrium potential for the post-synaptic response in the squid giant synapse. *J. Gen. Physiol.*, **64**, 519-535.

Llinás, R. and Sugimori, M. (1995) Synaptic transmission in the squid stellate ganglion. In *Cephalopod Neurobiology*, ed. N. J. Abbott, R. Williamson and L. Maddock, pp. 255-270. Oxford: Oxford University Press.

Loi, P. K., Saunders, R. G., Young, D. C. and Tublitz, N. J. (1996) Peptidergic regulation of chromatophore function in the European cuttlefish *Sepia officinalis*. *J. Exp. Biol.*, **199**, 1177-1187.

Loi, P. K. and Tublitz, N. J. (1996) Cloning and sequence of a full length FMRF amide coding cDNA from the brain of the cuttlefish *Sepia officinalis*. *Soc. Neurosci. Abstracts*, **22**, in press.

Lucero, M. T., Farrington, H. and Gilly, W. F. (1994) Quantification of L-DOPA and dopamine in squid ink: implications for chemoreception. *Biol. Bull.*, **187**, 55-63.

Lucero, M. T. and Gilly, W. F. (1995) Physiology of squid olfaction. In *Cephalopod Neurobiology*, ed. N. J. Abbott, R. Williams and L. Maddock, pp 521-534. Oxford: Oxford University Press.

Lucero, M. T., Horrigan, F. T. and Gilly, W. F. (1992) Electrical responses to chemical stimulation of squid olfactory receptor cells. *J. Exp. Biol.*, **162**, 231-249.

Makman, M. H., Berrios, I., Pratt, S., Hanlon, R. T. and Stefano, G. B. (1987) Anatomical localization of dopaminergic systems in *Octopus* retina; evidence for intrinsic dopamine-containing cells and dopamine D_1 receptors. In *Neurobiology: Molluscan models*, ed. H. H. Boer, W. P. M. Geraerts and J. Joose, pp 31-35. Amsterdam: North Holland.

Martin, R. (1968) Fine structure of the neurosecretory system of the vena cava in *Octopus*. *Brain Res.*, **8**, 201-205.

Martin, R. and Miledi, R. (1986) The form and dimensions of the giant synapse of squids. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **312**, 355-377.

Martin, R. and Voigt, K. H. (1987) The neurosecretory system of the octopus vena cava: a neurohemal organ. *Experientia*, **43**, 537-543.

Matus, A. I. (1973) Histochemical localization of biogenic monoamines in the cephalic ganglia of *Octopus vulgaris*. *Tissue Cell*, **5**, 590-601.

Messenger, J. B. (1967) The peduncle lobe: a visuo-motor centre in *Octopus*. *Proc. R. Soc. Lond. B Biol. Sci.*, **167**, 225-251.

Messenger, J. B. (1979) The nervous system of *Loligo*. IV. The peduncle and olfactory lobes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **285**, 275-309.

Messenger, J. B. (1991) Transmitters, toxins and phylogeny. *J. Zool.*, **223**, 687-694.

Messenger, J. B., De Santis, A. and Ogden, D. C. (1995) Chemical transmission at the squid giant synapse. In *Cephalopod Neurobiology*, ed. N. J. Abbott, R. Williamson and L. Maddock, pp. 283-297. Oxford: Oxford University Press.

Messenger, J. B., Katayama, Y., Ogden, D. C., Corrie, J. E. T. and Trentham, D. R. (1991) Photolytic release of glutamate from 'caged' glutamate expands squid chromatophores. *J. Physiol. (Lond.)*, **438**, 293P.

Messenger, J. B. and Tansey, E. M. (1979) Aminergic fluorescence in the cephalopod 'cerebellum'. *J. Physiol. (Lond.)*, **287**, 7-8P.

Miledi, R. (1967) Spontaneous synaptic potentials and quantal release of transmitter in the stellate ganglion of the squid. *J. Physiol. (Lond.)*, **192**, 379-406.

Miledi, R. (1969) Transmitter action in the giant synapse of the squid. *Nature*, **223**, 1284-1286.

Moroz, L. L. and Gillette, R. (1995) From Polyplacophora to Cephalopoda: comparative analysis of nitric oxide signalling in Mollusca. *Acta Biol. Hungarica*, **46**, 169-182.

Nachmansohn, D. and Meyerhof, B. (1941) Electrical changes during nerve activity and relation to concentration of choline esterase. *J. Neurophysiol.*, **4**, 348-361.

Nathanson, J. A. (1993) Identification of octopaminergic agonists with selectivity for octopamine receptor subtypes. *J. Pharmacol. Exp. Ther.*, **265**, 509-515.

Osborne, N. N., Beaton, D. W., Boyd, P. J. and Walker, R. J. (1986) Substance P-like immunoreactivity in the retina and optic lobe of the squid. *Neurosci. Lett.*, **70**, 65-68.

Packard, A. and Sanders, G. D. (1971) Body patterns of *Octopus vulgaris* and maturation of the response to disturbance. *Anim. Behav.*, **19**, 780-790.

Parr, K. L. (1988) The distributuion of 5-HT and other amines in the cephalopod nervous system. Ph.D. Thesis, University of Sheffield.

Reale, V., Evans, P. D. and Villegas, J. (1986) Octopaminergic modulation of the membrane potential of the Schwann cell of the squid giant nerve fibre. *J. Exp. Biol.*, **121**, 421-443

Reed, C. M. (1995) The ultrastructure and innervation of muscles controlling chromatophore expansion in the squid, *Loligo vulgaris*. *Cell Tissue Res.*, **282**, 503-572.

Reich, G. (1992) A new peptide of the oxytocin/vasopressin family isolated from nerves of the cephalopod *Octopus vulgaris*. *Neurosci. Lett.*, **134**, 191-194.

Robertson, H. A. and Juorio, A. V. (1977) Octopamine and some related non-catecholic amines in invertebrate nervous systems. *Int. Rev. Neurobiol.*, **19**, 173-224.

Robertson, J. D., Bonaventura, J. and Kohm, A. P. (1994) Nitric oxide is required for tactile learning in *Octopus vulgaris*. *Proc. R. Soc. Lond. B Biol Sci.*, **256**, 269-273.

Saito, M., Kawai, N., Miwa, A., Yamagishi, S. and Furuya, K. (1985) Evidence for L-glutamate as the neurotransmitter of the squid giant synapse. *Neurosci. Res.*, **2**, 297-307.

Sanders, G. D. (1975) The Cephalapods. In *Invertebrate Learning. Vol. 3: Cephalopods and Echinoderms*, ed. W. C. Corning, J. A. Dyal and A. O. D. Willows, pp. 1-101. New York: Plenum.

Schipp, R. and Fiedler, A. (1994) Cholinergic mechanisms in the neurocontrol of the cephalic aorta of the cephalopod *Sepia officinalis* L. *Comp. Biochem. Physiol.*, **107C**, 149-157.

Schipp, R., Jakobs, P. M. and Fiedler, A. (1991) Monoaminergic-peptidergic interactions in neuroregulatory control of the cephalic aorta in *Sepia officinalis* L. (Cephalopoda). *Comp. Biochem. Physiol.*, **99C**, 421-429.

Schipp, R., Schmidt, H. R. and Fiedler, A. (1986) Comparative cytochemical and pharmacological studies on the cholinergic innervation of the branchial heart of the cephalopod *Sepia officinalis* (L.) *Experientia*, **42**, 23-30.

Scuka, M. (1971) Effects of histamine on resting and action potentials of squid giant axons. *Life Sci.*, **10**, 355-360.

Sereni, E. (1930) The chromatophores of the cephalopods. *Biol. Bull.*, **59**, 247-268.

Silver, S. C., Patterson, J. A. and Mobbs, P. G. (1983) Biogenic amines in cephalopod retina. *Brain Res.*, **273**, 366-368.

Smart, D., Shaw, C., Johnson, C., Thim, L., Halton, D. and Buchanan, K. (1992) Peptide tyrosine phenylalanine: a novel neuropeptide F-related nonapeptide from the brain of the squid, *Loligo vulgaris*. *Biochem. Biophys. Res. Comm.*, **186**, 1616-1623.

Stanley, E. F. (1983) Depolarizing and desensitizing actions of glutaminergic and cholinergic agonists at the squid giant synapse. *Biol. Bull.*, **165**, 533.

Stanley, E. F. (1984) The action of cholinergic agonists on the squid stellate ganglion giant synapse. *J. Neurosci.*, **4**, 1904-1911.

Stefano, G. B., Hall, B., Makman, M. H. and Dvorkin, B. (1981) Opioid inhibition of dopamine release from nervous tissue of *Mytilus edulis* and *Octopus bimaculatus*. *Science*, **213**, 928-930.

Suzuki, H. and Tasaki, K. (1983) Inhibitory retinal efferents from dopaminergic cells in the optic lobe of the octopus. *Vision Res.*, **23**, 451-457.

Tansey, E. M. (1978) A histochemical study of the cephalopod brain. Ph.D. Thesis, University of Sheffield.

Tansey, E. M. (1979) Neurotransmitters in the cephalopod brain. *Comp. Biochem. Physiol.*, **64C**, 173-182.

Tansey, E. M. (1980) Aminergic fluorescence in the cephalopod brain. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **291**, 127-145.

Tasaki, K. and Suzuki, H. (1980) Efferent inhibitory system in the octopus retina. *Neurosci. Lett.*, **Suppl. 4**, 563.

Tu, Y. and Budelmann, B. U. (1994) The effect of L-glutamate on the afferent resting activity in the cephalopod statocyst. *Brain Res.*, **642**, 47-58.

Uemura, T., Yamashita, T., Haga, C., Miyazaki, N., Kondo, H. and Matsushita, M. (1987) Localization of serotonin-immunoreactivity in the central nervous system of *Octopus vulgaris* by immunohistochemistry. *Brain Res.*, **406**, 73-86.

Villegas, J. (1984) Axon-Schwann cell relationships. *Curr. Top. Membr. Trans.*, **22**, 547-571.

Villegas, G. M. and Villegas, G. (1974) Acetylcholinesterase localization in the giant nerve fibre of the squid. *J. Ultrastruc. Res.*, **46**, 149-163.

Walker, R. J. and Holden-Dye, L. (1989) Commentary on the evolution of transmitters, receptors and ion channels in invertebrates. *Comp. Biochem. Physiol.*, **93A**, 25-39.

Walker, R. J. and Holden-Dye, L. (1991) Evolutionary aspects of transmitter molecules, their receptors and channels. *Parasitology*, **102**, S7-S29.

Wells, M. J. (1966) Learning in the octopus. *Symp. Soc. Exp. Biol.*, **20**, 477-507.

Wells, M. J. (1978) *Octopus – Physiology and Behaviour of an Advanced Invertebrate*. London: Chapman & Hall.

Wells, M. J. (1983) Circulation in cephalopods. In *The Mollusca Vol. 5 (Part 2)*, ed. A. S. M. Saleuddin and K. M. Wilbur, pp. 239-290. New York: Academic Press.

Wells, M. J. and Mangold, K. (1980) The effects of extracts from neurosecretory cells in the anterior vena cava and pharyngo-opthalmic vein upon the hearts of intact, free-moving octopuses. *J. Exp. Biol.*, **84**, 319-334.

Williamson, R. (1989) Electrophysiological evidence for cholinergic and catecholaminergic efferent transmitters in the statocyst of octopus. *Comp. Biochem. Physiol.*, **93C**, 23-27.

Williamson, R. and Chrachri, A. (1994) The efferent system in cephalopod statocysts. *Biomed. Res. (Tokyo)*, **15, suppl. 1**, 51-56.

Young, J. Z. (1939) Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **229**, 465-503.

Young, J. Z. (1965) The organization of a memory system. *Proc. R. Soc. Lond. B Biol. Sci.*, **163**, 285-320.

Young, J. Z. (1971) *The Anatomy of the Nervous System of* Octopus vulgaris. Oxford: Clarendon Press.

Young, J. Z. (1973) The giant fibre synapse of *Loligo*. *Brain Res.*, **57**, 457-460.

Young, J. Z. (1974) The central nervous system of *Loligo*. I. The optic lobe. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **267**, 263-302.

Young, J. Z. (1976) The nervous system of *Loligo*. II. Suboesophageal centres. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **274**, 101-167.

Young, J. Z. (1977) The nervous system of *Loligo*. III. Higher motor centres: the basal supraoesophageal lobes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **276**, 351-398.

Young, J. Z. (1979) The nervous system of *Loligo*. V. The vertical lobe complex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **285**, 311-354.

Young, J. Z. (1983) The distributed tactile memory system of *Octopus*. *Proc. R. Soc. Lond. B Biol. Sci.*, **218**, 135-176.