L-Glutamate and its Ionotropic Receptors in the Nervous System of Cephalopods

A. Di Cosmo^{1,*}, C. Di Cristo² and J. B. Messenger³

¹Department of Structural and Functional Biology, University of Naples "Federico II", Italy, ²Department of Biological and Environmental Sciences, University of Sannio, Italy, ³Department of Zoology, University of Cambridge, UK

Abstract: In several species of cephalopod molluscs there is good evidence for the presence of L-glutamate in the central and peripheral nervous system and evidence for both classes of ionotropic receptor, AMPA/kainate and NMDA.

The best evidence for glutamate being a transmitter in cephalopods comes from pharmacological, immunohistochemical and molecular investigations on the giant fibre system in the squid stellate ganglion. These studies confirm there are AMPA/kainate-like receptors on the third-order giant axon. In the (glial) Schwann cells associated with the giant axons both classes of glutamate receptor occur.

Glutamate is an excitatory transmitter in the chromatophores and in certain somatic muscles and its action is mediated primarily via AMPA/kainate-like receptors, but at some chromatophores there are NMDA-like receptors.

In the statocysts the afferent crista fibres are also glutamatergic, acting at non-NMDA receptors.

In the brain (of *Sepia*) a neuronal NOS is activated by glutamate with subsequent production of nitric oxide and elevation of cGMP levels. This signal transduction pathway is blocked by D-AP-5, a specific antagonist of the NMDA receptor.

Recently immunohistochemical analysis has demonstrated (in *Sepia* and *Octopus*) the presence of NMDAR2A /B – like receptors in motor centres, in the visual and olfactory systems and in the learning system. Physiological experiments have shown that glutamatergic transmission is involved in long term potentiation (LTP) in the vertical lobe of *Octopus*, a brain area involved in learning. This effect seems to be mediated by non-NMDA receptors. Finally in the CNS of *Sepia* NMDA-mediated nitration of tyrosine residues of cytoskeletal protein such as α -tubulin, has been demonstrated.

Key Words: Cephalopods, octopus, squid, cuttlefish, glutamate, NMDA receptor, AMPA receptor.

INTRODUCTION

Glutamate (hereafter L-glu) is now known to be a major excitatory neurotransmitter in the CNS of vertebrates and occurs widely throughout the invertebrates [48]. It has long been accepted as the excitatory transmitter at the nerve-muscle junction of insects and crustaceans.

Over the last decade or so it has become clear that L-glu is an important transmitter in both gastropods and cephalopods. In such gastropods as *Aplysia* and *Helix* there is ample evidence, not only that L-glu is an excitatory transmitter but that it can act at AMPA/kainate or NMDA receptors, and, more importantly in the light of mammalian studies, that the latter receptors in *Aplysia* are implicated in long-term potentiation (LTP) of the gill-siphon withdrawal reflex [3, 4, 23, 34]. It is not clear, however, whether the NMDA and AMPA receptors are co-expressed at the same site or interact by shaping postsynaptic electrical activity to regulate Ca^{2+} influx differentially [3].

In cephalopods, too, there is good evidence that L-glu is an important excitatory transmitter, usually acting at AMPA/kainate-like receptors. Experiments with loliginid squids (*Loligo Alloteuthis*, *Lolliguncula*), the cuttlefish (*Sepia*) and

Octopus have revealed several glutamatergic systems. These include the squid giant synapse, the neuro-muscular junction of certain somatic muscles, the chromatophores, the statocysts, the ink-gland and the central nervous system. It will be convenient to consider these separately.

THE SQUID GIANT SYNAPSE

Probably the best evidence for L-glu being a transmitter in cephalopods derives from a series of studies on the stellate ganglion of the squids *Loligo* and *Alloteuthis*. Here a single second-order giant fibre from the brain branches to make synaptic contact with 10-12 third-order fibres originating in the ganglion (Fig. 1). The most medial third-order fibre is the largest, and the synapse onto this is the so-called "giant synapse", the largest synapse in the animal kingdom. De Santis & Messenger [19] bath-applied L-glu and a series of glutamate agonist and antagonists to the ganglion while recording intracellularly from the third-order fibre; they found that L-glu and its AMPA/kainate agonists reversibly blocked transmission, presumably by de-sensitising the synapse to the endogenous transmitter. CNQX and DNQX also blocked transmission reversibly, though no NMDA antagonists had an effect. Ionophoretic application of L-glu, quisqualate, kainate and AMPA causes weak depolarisation of the postsynaptic membrane [40] but L-glu released at the synapse from "caged" glutamate (by flash photolysis) depolarises the membrane sufficiently for action potentials to be generated in the third-order fibre (Fig. 2), demonstrating directly that

*Address correspondence to this author at Department of Structural and Functional Biology, University of Naples "Federico II", Via Cinthia - Complesso Monte Sant'Angelo, 80100 – Naples, Italy;
E-mail: dicosmo@unina.it

Fig. (1). Diagram of squid stellate ganglion of *Loligo vulgaris* to show the 2nd-order giant fibre (gf2, black) branching to synapse with (in this specimen) 10 postsynaptic 3rd-order giant fibres (gf3sL, the largest of the 3rd-order fibres; L-1, the next largest, L-2...L-9, the smallest). Scale bar 1 mm. Modified from Ref. [50].

L-glu mimics the effect of the endogenous transmitter strongly [16]. Subsequently Di Cosmo *et al.* [21] not only showed by HPLC that the second-order fibre contains significantly more L-glu than does the third-order fibre, but also demonstrated by immunocytochemical staining, a crucial difference between the presynaptic and postsynaptic fibres. Application of an antibody raised against L-glu resulted in positive immunofluorescence in the second-order fibre only, whereas an antibody raised against mammalian GluR1 with GluR2/3 led to fluorescence in the third-order fibre only (Fig. 3).

Recently Battaglia *et al.* [5] cloned a putative glutamate receptor subunit, SqGluR, from the stellate ganglion of the squid *Loligo opalescens*. They showed that its primary structure was homologous to mammalian AMPA-subunits GluR1-GluR4 (44-46% amino-acid identity) and also that intracellular injection of the C-terminal 15- amino-acid peptide of

SqGluR into the post-synaptic region of the third-order fibre inhibited synaptic transmission at the giant synapse (Fig. 4). They also showed by *in situ* hybridisation that SqGluR mRNA is localised in many cells in the stellate ganglion, especially in neurons in the giant fibre lobe that give rise to the third-order fibres. Finally, the effect of L-glu, D-glu, L-asp, and D-asp has been studied on human embryonic kidney cells expressing SqGluR [10]. Only L-glu is able to evoke an inward current, while aspartic acid (both L and D isomers) slows the time course of activation and inactivation of L-glu gated current. This let suggest a role for aspartic acid as neuromodulator during glutamatergic transmission [10].

SOMATIC MUSCLES

In a paper that was the first to implicate L-glu as a putative neurotransmitter in cephalopods Bone & Howarth [6] applied drugs to strips of muscle cut from the mantle and

Fig. (2). Postsynaptic action of photolytically released L-glutamate at pH 7.8 at the giant synapse of squid stellate ganglion. Depolarization of postsynaptic fibre by photolysis of 20mM caged glutamate by 1ms 300-350nm light pulse at the time indicated by the arrow. Modified from [13].

Fig. (3). Anti-L-glutamate and anti-glutamate receptor GluR1-GluR2&3 immunofluorescence in 25 μ m sagittal sections of *Loligo vulgaris* stellate ganglion. **(a)** Anti glutamate immunoreactivity is particularly intense (arrows) in the terminal region of the presynaptic (2nd-order fibre). Scale bar, 320 μ m. **(b)** Anti-glutamate receptor GluR1-GluR2&3 immunoreactivity is absent from the presynaptic fibre (triangle) and confined to the postsynaptic fibre (square). Scale bar, 350 μ m. Modified from [17].

fins of two squids (*Loligo* and *Alloteuthis*) and the cuttlefish, *Sepia*. They showed rapid twitches, often superimposed on slower contractures, after bath-application of 50 μ M L-glu. Such responses were reversibly blocked by 2APB. They also observed that topical application of L-glu to the skin caused sustained expansion of all colour classes of chromatophore (see below), while its application to such visceral muscles as the branchial hearts, the gut and ink-sac duct caused “immediate repetitive contractions”. These findings have never been followed up physiologically but it is significant that Palumbo *et al.* [43] have shown the presence of NMDAR-like immunoreactivity in the wall of ink sac duct (see below).

Collins [12, 13] has confirmed and extended Bone & Howarth’s findings on the effect of L-glu on squid somatic muscles. It should be noted that in cephalopods, which lack an internal or external hard skeleton, the muscles fibres in the fins, mantle, arms and tentacles are orthogonally arranged in all three planes, constituting a “muscular hydrostat” [29]. Collins found, in four species of loliginid squids, that all three types of fin muscle (transverse, longitudinal and dorso-ventral) responded to L-glu (25-50 μ M) though not

ACh. In the mantle, however, the longitudinal and radial muscles are cholinergic but the circulars glutamatergic, contracting strongly with 25-75 μ M L-glu; moreover the responses of the circular muscles are reduced or abolished by CNQX (Fig. 5). In the arms and tentacles Collins [12, 13] found that all muscle types were cholinergic. The use of different excitatory transmitters in the same muscle block is, of course, most curious by vertebrate or arthropod standards [and one can only speculate on its significance]; Bone *et al.* [7] suggest it may have arisen because the opposing fibres are so closely apposed in the squid mantle muscle that only the use of different transmitters could ensure isolated contraction during successive stages of contraction in the jetting cycle.

CHROMATOPHORE MUSCLES

Bone and Howarth’s discovery that the chromatophores expand in the presence of L-glu has been amply confirmed and extended, first by Florey *et al.* [24] and later by Messenger and his colleagues [14, 30-33, 39, 41]. Cephalopod chromatophores differ from those of vertebrates in that they

Fig. (4). Functional effects of the C-terminal peptide of SqGluR on synaptic transmission at the giant synapse. Effect of injected peptide Sqglu15. Postsynaptic responses to presynaptic stimulation: **(a)** control; **(b)** 10 min after injection; **(c)** response to antidromic stimulation in presence of the peptide. Modified from [5].

Fig. (5). Tension records showing the response of the central zone circular mantle muscular fibres of *Loligo vulgaris* to 50 μ M L-Glu in artificial sea water (ASW). The slice was then washed for 5 min in ASW. After five minutes continuous perfusion of ASW with 100 μ M CNQX, the response to 50 μ M L-Glu was reduced but not completely blocked. Modified from [10].

are complex organs, comprising a sac of pigment granules to which are attached 15-25 radial muscles, innervated directly from the brain. When the muscles contract, the chromatophore expands; when they relax, elastic energy stored in the sac causes the chromatophore to retract. A variety of evidence has accumulated that all points to L-glu being the endogenous excitatory transmitter at the chromatophore nerve-muscle junction: (1) staining of the chromatophore nerves with an antibody to L-glu; (2) topical application of a range of (mammalian) glutamate agonists and antagonists to intact and long-term denervated chromatophores; (3) following the behaviour of intracellular- Ca^{2+} levels during L-glu application to isolated radial muscle fibres (for summary, see [38]). AMPA, kainate quisqualate and domoate all expand the chromatophores, but this effect can be blocked by CNQX and DNQX (50 μ M). In *Sepia* Loi & Tublitz [35] have also shown that Joro spider toxin can block the effects of L-glu, so that there is overwhelming evidence that at the majority of chromatophores L-glu acts *via* AMPA/kainate-like receptors.

This was further supported by the work of Lima *et al.* [33] who showed that AMPA/kainate-like receptors are present in squid skin by immunoblotting with an antibody raised against a squid AMPA receptor (sGluR). However, more interestingly, these workers also identified a sub-population of chromatophores in the dorsal mantle skin of two species of squid where neurotransmission was partly blocked either by CNQX or DNQX or by the NMDA receptor antagonists AP-5 and MK-801, and completely blocked by the simultaneous application of both classes of antagonist. Using isolated muscle fibres from this same area they iontophoretically applied L-glu to reveal fast inward currents (sensitive to CNQX and DNQX) and slow outward glutamate-sensitive currents (Fig. 6). This slow component was voltage dependent and sensitive to external magnesium and glycine (Fig. 7); moreover L-glu also causes a fast, followed by a slow transient increase in Ca^{2+} , similarly sensitive to AP-5, glycine and magnesium. These findings strongly suggest that in the muscles of these chromatophores NMDA-like and AMPA/kainate-like receptors co-exist, and on the basis of these

results Lima *et al.* [33] propose that such an arrangement enables the conspicuous dorsal chromatophores of these squids to maintain expansion as they contribute to a variety of body patterns for concealment or for signalling [15].

THE STATOCYSTS

The cephalopod statocysts are complex organs comprising two separate equilibrium receptor systems, hair cells on the macula responding to linear-acceleration and those on the crista responding to angular-acceleration [11]. The sensory epithelia receive massive afferent innervation and in the crista Tu & Budelmann [47] have shown that this is glutamatergic; they also provided good evidence that the receptors were of the AMPA/kainate type.

THE INK GLAND

According to the observations of Bone & Howarth [6] topical application of glutamate to the muscles of the ink-gland duct caused "immediate repetitive contractions". Subsequent immunohistochemical staining, with antibodies to rat NMDAR2/3 subunits, in the regions of the anterior and posterior sphincters of this duct, labelled a few fibres from the visceral nerves that reach the outer circular muscle layer [43]. Somewhat more intense and widely distributed immunoreactivity was detected in fibres reaching the inner longitudinal muscle layer.

All along the wall of the ink sac and below the sphincters, NMDAR2/3-immunopositive fibres reach the trunks of the longitudinal muscle layer but not the circular muscle layer of the ink sac wall. The antibody against subunit 1 of the NMDA receptor did not show any positivity when tested on sections of the ink sac [43].

THE BRAIN

It is clear that the cephalopod brain contains glutamatergic systems, but the evidence to date is fragmentary and incomplete. The first experiments to implicate L-glu came from Andrews *et al.*, [1, 2]. Using an aortic perfusion technique, by which small "pulses" of drug can be introduced

Fig. (6). Ionic currents evoked by ionophoretic application of L-glutamate in isolated chromatophore muscle cells from mid-dorsal skin of squid. **(A)** Glutamate-evoked currents showing a voltage-independent single component. **(Aa)** Leak-subtracted traces recorded at holding potentials between -70 and +70 mV (20-mV step); **(Ab)** peak current values obtained in all the voltage steps (10 mV) applied in Aa; **(Ac)** current-voltage relationship of whole-cell currents, obtained from an isolated chromatophore muscle cell before and after reduction in external Na⁺ ([Na⁺]_e). Black dots, [Na⁺]_e = 157 mM; grey triangles, [Na⁺]_e = 78.3 mM. **(B)** Glutamate-evoked current showing a fast voltage-independent component and a slow voltage-dependent component. **(Ba)** Leak-subtracted traces recorded at holding potentials between -60 and +60 mV (20-mV steps); **(Bb)** Voltage relationship of currents obtained in Ba. Black dots are peak current amplitude and grey triangles represent current values measured 800 ms after the end of the ionophoretic pulse (a delay of $<6 \times$ the decay time constant of the fast glutamate evoked currents). **(Bc)** Curve fittings from the currents recorded in Aa under -60 and +60 mV holding potential. τ , time constant of exponential decay functions. Modified from [35].

directly into the blood stream of an octopus, these workers obtained dramatic (though transient) motor effects in the arms and mantle with L-glu, kainate and quisqualate. These same substances also expanded the black chromatophores, leading to conspicuous darkening. Control experiments, such as lesioning the pallial nerve to uncouple the mantle muscles on one side (including those of the chromatophores), emphasise that to produce these effects the drugs were acting directly on the CNS.

Subsequently D'Aniello *et al.* [17] and D'Aniello & Messenger (unpublished data) directly measured L-glu and L-aspartate levels in different regions of the *Octopus* brain and showed that L-glu was widely distributed in the lower, intermediate and higher motor centres (including those regulating the chromatophores), in the optic lobes and in the superior frontal - vertical lobe system (involved in learning) (see [37]). There are complementary findings for *Sepia* [43].

Immunohistochemical staining of brain sections with antibodies raised against L-glu has unfortunately produced inconsistent results although L-glu positive cell bodies seem to be widespread. This powerful technique for localising putative transmitter distribution has, however, been more successful with antibodies to glutamate receptors and Di Cosmo *et al.* [22] have excellent evidence that certain NMDA-like receptors are widely distributed in the brains of *Octopus* and *Sepia*. In summary we can note that positive immunoreactivity specific for NMDA receptor subunits 2A and 2B was found in the cell bodies and the neuropil of many (but not all) of the lobes that Boycott [8] defined as Lower and Intermediate Motor Centres: for example the pedal, magnocellular, palliovisceral and (in *Sepia*) the fin lobes. Similarly widespread specific staining was observed in the Higher Motor Centres, such as the anterior, lateral and median basal lobes and in the "silent areas" - those lobes that

Fig. (7). Ratio (F340/F380) measurements of Fura-2 AM-loaded squid chromatophore muscle fibres. **(a)** CNQX (100 μ M) blocked L-glutamate (20 mM)-evoked Ca^{2+} rise, which was obtained after cell was washed in ASW for 5 min. **(b)** Removal of external Mg^{2+} enhanced L-glutamate-evoked Ca^{2+} rises. Addition of L-glutamate (5 μ M) evoked a dual component increase in cytoplasmic Ca^{2+} . After washing for 4 min, the cell was superfused with Mg^{2+} -free ASW and exposed to 5 μ M L-glutamate. **(c)** Glycine increased the glutamate evoked response. Addition of L-glutamate (10 mM) evoked dual increase in cytoplasmic Ca^{2+} during constant superfusion of ASW. After recovery, glycine (20 μ M) was added to superfusion, after which L-glutamate was again added, evoking a larger and more sustained increase in Ca^{2+} . **(d)** NMDA- and L-glutamate-evoked increase in cytoplasmic Ca^{2+} . In ASW, addition of NMDA (200 μ M) evoked an increase in cytoplasmic Ca^{2+} , which increased further with subsequent addition of 20 μ M L-glutamate. **(e)** NMDA-induced Ca^{2+} increase depended on depolarization of muscle cell. Brief square-wave pulses (bottom trace) were applied through a glass microelectrode filled with bathing solution (ASW) and placed close to Fura-2-loaded fibres. Stimulation frequency, 0.2 Hz. Modified from [35].

give no response to electrical stimulation but which are important in the setting up of memories, visual or tactile: the

vertical, subvertical, superior frontal and (in *Octopus*) the subfrontal lobes [22]. Finally it is interesting to note that immunopositive cells and fibres are also found in several brain areas that influence the reproductive system *via* the optic gland, a gonadotropic endocrine gland.

These results are supported by SDS-PAGE analysis of brain extracts (from *Sepia* and *Octopus*), which revealed a single sharp band at 170 kDa immunoreactive to NMDAR2/3 receptor subunit antibodies. A similar immunoreactive band was detectable in extracts from optic lobes. An exceedingly weak response seems to rule out the presence of the R1 subunit in the cephalopod brain [22].

Complementary results have been obtained by Battaglia *et al.* [5] on the squid *Loligo*. Having cloned a glutamate receptor subunit, SqGluR (see above) they also showed by *in situ* hybridisation that SqGluR mRNA occurs in the second-order "giant" visual cells in the inner and outer granular layers, and in what appears to be the subvertical lobe.

Taking a biochemical approach, Palumbo *et al.* [43, 44] have shown that exposure of intact brains to either 1.5 mM glutamate or 1.5 mM NMDA caused a rapid increase in the levels of cyclic GMP after the activation of a neuronal nitric oxide synthase (NOS) and the production of nitric oxide (NO), all peaking within 1 min and then rapidly dropping to basal values within 3 min. Significant suppression of the activating effect of NMDA was observed in the presence of the NOS inhibitor L-NA, as well as the glutamate/NMDA antagonist D-AP5 [43].

In recent experiments it has been also demonstrated that glutamate, *via* the NMDA/NO signal transduction pathway, is involved in post-translational modification of cytoskeletal protein such as α -tubulin [20, 44]. It has been showed that glutamate/NO mediated tyrosine nitration at the C-terminus of α -tubulin is specifically inhibited by D-AP5. The incorporation of nitrotyrosine induces α -tubulin reversible degradation. This temporary process could be associated with physiological mechanism of cytoskeletal protein turnover. Such a mechanism could be involved in microtubule assembly, which is critical for axonal transport, growth, degeneration as well as in synaptic plasticity [20].

Physiological evidence for a glutamatergic system in the cephalopod brain comes also from a very interesting paper by Hochner *et al.* [25]. Their experiments centred on the superior-frontal/vertical lobe system in *Octopus*, a region of the brain that has been known for over 50 years to be closely involved in learning and memory [9]. By recording field potentials in brain slices of this area of the brain Hochner and his colleagues obtained good evidence of activity-dependent synaptic plasticity, very similar to the well known, NMDA-mediated, long-term potentiation (LTP) in the mammalian hippocampus [36]. However, LTP in the *Octopus* vertical lobe apparently does not involve NMDA receptors although the receptors are clearly glutamatergic: CNQX, kynurenic acid and DNQX block post-synaptic field potentials, and L-glut occludes them (Fig. 8). The same authors, however, do not exclude the presence of currents through NMDA receptors, though D-AP5 insensitive. In fact, for *Aplysia*, AP5-insensitive NMDA currents have been reported [18,

Fig. (8). LTP in the *Octopus vulgaris* involves non NMDA glutamate receptors. The response during HF [inset (1)] was completely blocked by a mixture of 20 mM kynurenic acid plus 200 μ M CNQX. After rinsing, the test fPSP was highly potentiated with respect to control, and additional HF [inset (2)] led only to small additional potentiation. Modified from [22].

42]. If so, this could reconcile the LTP results with the immunocytochemical data.

Thus a combination of different experimental strategies clearly implicates L-glu as a neurotransmitter in specific areas of the cephalopod brain: biochemical evidence, linking glutamate and its receptors to nitric oxide, immunocytochemical studies and a classical electrophysiological approach all demonstrate the role of L-glu and show that it exerts its effect *via* both NMDA and non-NMDA, AMPA/kainate-like receptors.

CONCLUSION

Cephalopods are well known to neuroscientists primarily because of the squid giant axon. Re-discovered in the 1930s by J.Z. Young [49], this was used in the elegant experiments of Hodgkin and Huxley [26-28] to establish the ionic basis of the action potential in nerves, work that gained them a Nobel Prize and that today still constitutes a cornerstone of modern neurophysiology.

However the sense organs, nervous system and behaviour of these animals have been studied in depth for more than a century. Such studies were given impetus by the realisation that octopuses had learning abilities comparable to those of lower vertebrates and were excellent laboratory animals [9]. As a result there is a substantial literature on the neurobiology of cephalopods including their putative transmitters, notably the "classic" vertebrate transmitters: ACh, dopamine and noradrenaline. This early pharmacology has been admirably summarised by Tansey [46], but shortly after her review was published it began to emerge that L-glu was also an important neuroactive agent in several systems of squids and octopuses (for review see [37]).

In the last ten years this has been confirmed beyond doubt. As this review has shown, cephalopods utilise L-glu as a transmitter at some (but not all) types of nerve-muscle junction; in some sense organs; in the peripheral nervous system; and in the CNS. What is remarkable in the brain is that L-glu occurs in so many systems: in lower, intermediate and higher motor centres; in learning centres; in the visual system; and in the olfactory-endocrine-reproductive regula-

tory system. In all these its effect appears to be excitatory, as it is in mammals [45].

Evidence is also emerging about the nature of the glutamate receptors in cephalopods. At first it seemed that they had only AMPA/kainate type receptors, but it is now clear that in several systems L-glu acts *via* receptors similar to the vertebrate NMDA type. The evidence for the presence of NMDAR2A-like and NMDAR2B-like subunits is particularly strong.

At this point it may be worth reminding mammalian neuropharmacologists that molluscs and vertebrates have been evolving independently for some 400 Myr so that it is highly unlikely that their receptors will be the same. More important are the facts that they are sufficiently similar (a) for new glutamate agonists and antagonists developed by mammalian pharmacologists to be physiologically active in cephalopods; (b) for antibodies raised against rabbit NMDAR to label cell bodies and fibres in the cephalopod brain; (c) for Western blot analysis of membrane proteins from *Octopus* and *Sepia* brains to reveal an immunoreactive band at 170 kDa, and (d) for there to be a link between glutamate and NMDAR-mediated NO production in *Sepia*.

REFERENCES

- [1] 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. B. Biol. Sci.*, **213**, 93-99.
- [2] 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. Ass. UK*, **63**, 355-370.
- [3] Antzoulatos, E.G. and Byrne, J.H. (2004) Learning insights transmitted by glutamate. *Trends Neurosci.*, **27**, 555-60.
- [4] Antzoulatos, E.G., Cleary, L.J., Eskin, A., Baxter, D.A. and Byrne, J.H. (2003) Desensitization of postsynaptic glutamate receptors contributes to high-frequency homosynaptic depression of aplysia sensorimotor connections. *Learn. Mem.*, **10**, 309-13.
- [5] Battaglia, A.A., Nardi, G., Steinhardt, A., Novakovic, A., Gentile, S., Iaccarino Idelson, P., Gilly, W.F. and de Santis, A. (2003) Cloning and characterization of an ionotropic glutamate receptor subunit expressed in the squid nervous system. *Eur. J. Neurosci.*, **17**, 2256-66.
- [6] Bone, Q. and Howarth, J.V. (1980) The role of L-glutamate in neuromuscular transmission in some molluscs. *J. Mar. Biol. Ass. UK*, **60**, 619-626.

- [7] Bone, Q., Packard, A. and Pulsford, A.L. (1982) Cholinergic innervation of muscle fibres in squid. *J. Mar. Biol. Ass. UK*, **62**, 193-199.
- [8] 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.
- [9] Boycott, B.B. and Young, J.Z. (1950) The comparative study of learning. *Symp. Soc. Exp. Biol.*, **4**, 432-453.
- [10] Brown, E.R., Piscopo, S., Chun, J.T., Francone, M., Mirabile, I. and D'Aniello, A. (2006) Modulation of an AMPA-like glutamate receptor (S_qGlur) gating by L- and D-aspartic acids. *Amino Acids*, in press.
- [11] Budelmann, B.U. (1990) *The statocysts of the squid*, In *Squid as experimental animals*, D.L. Gilbert, W.J. Adelman and J.M. Arnold, Editors., Plenum: New York. 421-439.
- [12] Collins, T.F.T. (2002) *Mechanisms of control in cephalopod muscle*, University of Sheffield: Sheffield.
- [13] Collins, T.F.T. and Tsutsui, I. (2003) Neurotransmitters of mantle and fin muscles in spear squid, *Loligo bleekeri*. *J. Mar. Biol. Ass. UK*, **83**, 857-860.
- [14] Cornwell, C. and Messenger, J.B. (1995) *Neurotransmitters of squid chromatophores*, in *Cephalopod Neurobiology*, N.J. Abbott, R. Williamson and L. Maddock, Editors., University Press: Oxford. 369-379.
- [15] Cornwell, C., Messenger, J.B. and Hanlon, R.T. (1997) Chromatophores and body patterning in the squid *Alloteuthis subulata*. *J. Mar. Biol. Ass. UK*, **77**, 1243-1246.
- [16] Corrie, J.E., DeSantis, 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 a 'caged' L-glutamate. *J. Physiol.*, **465**, 1-8.
- [17] D'Aniello, A., Nardi, G., De Santis, A., Vetere, A., Di Cosmo, A., Marchelli, R., Dossena, A. and Fisher, G. (1995) Free amino acids and aspartate content in the nervous system of Cephalopoda. A comparative study. *Comp. Biochem. Physiol. B*, **112**, 661-666.
- [18] Dale, N. and Kandel, E.R. (1993) L-glutamate may be the fast excitatory transmitter of Aplysia sensory neurons. *Proc. Natl. Acad. Sci. USA*, **90**, 7163-7.
- [19] 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**, 219-22.
- [20] Di Cosmo, A. (2003) A new model to study the role and the biochemical properties of nNOS: the nervous system of cephalopods. *Rec. Res. Dev. Biochem.*, **4**, 405-421.
- [21] Di Cosmo, A., Nardi, G., Di Cristo, C., De Santis, A. and Messenger, J.B. (1999) Localization of L-glutamate and glutamate-like receptors at the squid giant synapse. *Brain Res.*, **839**, 213-20.
- [22] Di Cosmo, A., Paolucci, M. and Di Cristo, C. (2004) N-methyl-D-aspartate receptor-like immunoreactivity in the brain of *Sepia* and *Octopus*. *J. Comp. Neurol.*, **477**, 202-19.
- [23] Ezzeddine, Y. and Glanzman, D.L. (2003) Prolonged habituation of the gill-withdrawal reflex in Aplysia depends on protein synthesis, protein phosphatase activity and postsynaptic glutamate receptors. *J. Neurosci.*, **23**, 9585-94.
- [24] Florey, E., Dubas, F. and Hanlon, R.T. (1985) Evidence for L-glutamate as a transmitter substance of motoneurons innervating squid chromatophore muscles. *Comp. Biochem. Physiol. C*, **82**, 259-68.
- [25] Hochner, B., Brown, E.R., Langella, M., Shomrat, T. and Fiorito, G. (2003) A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. *J. Neurophysiol.*, **90**, 3547-54.
- [26] Hodgkin, A.L. and Huxley, A.F. (1952) The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol.*, **116**, 473-96.
- [27] Hodgkin, A.L. and Huxley, A.F. (1952) Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.*, **116**, 449-72.
- [28] Hodgkin, A.L. and Huxley, A.F. (1952) The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol.*, **116**, 497-506.
- [29] Kier, W.M. and Smith, A.M. (1985) Tongues, tentacles and trunks: the biomechanics of movement in muscular-hydrostats. *Zool. J. Linn. Soc.*, **83**, 307-324.
- [30] Lima, P.A. (2000) *Neuronal and local control of squid chromatophore muscle*, University of Sheffield: Sheffield.
- [31] Lima, P.A., Messenger, J.B. and Brown, E.R. (1997) Monitoring cytoplasmic Ca²⁺ in cephalopod chromatophore muscles. *J. Physiol.*, **504** P, 2P.
- [32] Lima, P.A., Messenger, J.B. and Brown, E.R. (1998) 5-HT suppresses Ca²⁺ release from ryanodine-sensitive stores in squid chromatophore muscle. *J. Physiol.*, **513** P, 127P.
- [33] Lima, P.A., Nardi, G. and Brown, E.R. (2003) AMPA/kainate and NMDA-like glutamate receptors at the chromatophore neuromuscular junction of the squid: role in synaptic transmission and skin patterning. *Eur. J. Neurosci.*, **17**, 507-16.
- [34] Lin, X.Y. and Glanzman, D.L. (1994) Hebbian induction of long-term potentiation of Aplysia sensorimotor synapses: partial requirement for activation of an NMDA-related receptor. *Proc. Biol. Sci.*, **255**, 215-21.
- [35] Loi, P.K. and Tublitz, N.J. (2000) Roles of glutamate and FMRFamide-related peptides at the chromatophore neuromuscular junction in the cuttlefish, *Sepia officinalis*. *J. Comp. Neurol.*, **420**, 499-511.
- [36] Malenka, R.C. and Nicoll, R.A. (1999) Long-term potentiation--a decade of progress? *Science*, **285**, 1870-4.
- [37] Messenger, J.B. (1996) Neurotransmitters of cephalopods. *Invert. Neurosci.*, **2**, 95-114.
- [38] Messenger, J.B. (2001) Cephalopod chromatophores: neurobiology and natural history. *Biol. Rev. Camb. Philos. Soc.*, **76**, 473-528.
- [39] Messenger, J.B., Cornwell, C. and Reed, C. (1997) L-Glutamate and serotonin are endogenous in squid chromatophore nerves. *J. Exp. Biol.*, **200** (Pt 23), 3043-54.
- [40] Messenger, J.B., De Santis, A. and Ogden, D.C. (1995) *Chemical transmission at the squid giant synapse*, In *Cephalopod Neurobiology*, N.J. Abbott, R. Williamson and L. Maddock, Editors., Oxford University Press: Oxford. 283-297.
- [41] Messenger, J.B., Katayama, Y., Ogden, D.C., Corrie, J.E. and Trentham, D.R. (1991) Photolytic release of glutamate from "caged" glutamate expands squid chromatophores. *J. Physiol.*, **438**, 293P.
- [42] Murphy, G.G. and Glanzman, D.L. (1999) Cellular analog of differential classical conditioning in Aplysia: disruption by the NMDA receptor antagonist DL-2-amino-5-phosphonovaleate. *J. Neurosci.*, **19**, 10595-602.
- [43] Palumbo, A., Di Cosmo, A., Poli, A., Di Cristo, C. and d'Ischia, M. (1999) A calcium/calmodulin-dependent nitric oxide synthase, NMDAR2/3 receptor subunits and glutamate in the CNS of the cuttlefish *Sepia officinalis*: localization in specific neural pathways controlling the inking system. *J. Neurochem.*, **73**, 1254-63.
- [44] Palumbo, A., Fiore, G., Di Cristo, C., Di Cosmo, A. and d'Ischia, M. (2002) NMDA receptor stimulation induces temporary alpha-tubulin degradation signaled by nitric oxide-mediated tyrosine nitration in the nervous system of *Sepia officinalis*. *Biochem. Biophys. Res. Commun.*, **293**, 1536-43.
- [45] Sahai, S. (1990) Glutamate in the mammalian CNS. *Eur. Arch. Psychiatry Clin. Neurosci.*, **240**, 121-133.
- [46] Tansey, E.M. (1979) Neurotransmitters in the cephalopod brain. *Comp. Biochem. Physiol. C*, **64**, 173-182.
- [47] 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.
- [48] Walker, R.J., Brooks, H.L. and Holden-Dye, L. (1996) Evolution and overview of classical transmitter molecules and their receptors. *Parasitology*, **113** (Suppl.), S3-33.
- [49] Young, J.Z. (1939) Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. *Proc. R Soc. Lond. B. Biol. Sci.*, **229**, 465-503.