ELSEVIER

Comparative Biochemistry and Physiology Part C 134 (2003) 139–146

CBP

www.elsevier.com/locate/cbpc

Effect of nitric oxide synthase inhibition on the manipulative behaviour of *Sepia officinalis*

M.P. Halm[b], M.P. Chichery[a], R. Chichery[a,*]

[a]*Laboratoire de Psychophysiologie, Université de Caen, 14032 Caen Cedex, France*
[b]*CREC, Luc sur Mer 14530, France*

Received 25 March 2002; received in revised form 22 July 2002; accepted 15 October 2002

Abstract

Nitric oxide (NO), produced by nitric oxide synthase (NOS) in brain tissue, is essential for a variety of kinds of learning in vertebrates. In invertebrates, there are clear examples of an association between NO signalling and olfaction, feeding behaviour and learning. The role of NO as a neurotransmitter in the manipulative behaviour of *Sepia officinalis* was tested. Manipulative behaviour requires extensive chemotactile sensory processing, fine motor control and probably motor learning processes. NADPH-diaphorase activity (a reliable histochemical marker for nitric oxide synthase) was found in sensory epithelia and in the axial nerve cord of the arms. NOS inhibitor injections (L-NAME) produced an increase in the latency of prey paralysis. By placing mechanical constraints on the base of the fifth periopods of the crab, we prevented the cuttlefish from injecting cephalotoxin and, thus, forced it to change injection sites. We showed that L-NAME pretreatment did not affect the flexibility of the manipulative behaviour. The implications of the involvement of NO in the acquisition of chemo-tactile information and in the programming of the motor skills of the manipulative behaviour is discussed.

Keywords: Behavioural flexibility; Cephalopods; Chemo-tactile processing; Learning; L-NAME; Manipulative behaviour; Nitric oxide; *Sepia officinalis*

1. Introduction

Nitric oxide (NO) has been identified as a messenger molecule that mediates neuronal communication in the mammalian brain, with possible roles in the acquisition of sensory information and synaptic plasticity. Along with the numerous studies on the mammalian central nervous system (CNS), evidence has been accumulating in these last years showing that nitric oxide synthase (NOS) occurs in the nervous system of many invertebrates (Jacklet, 1997), and especially molluscs. NADPH-dependant diaphorase histochemistry and/or immunochemistry were used to localise NOS in a number of different mollusc preparations. NOS seems to be characteristic of molluscan chemosensory systems, and there are clear examples of an association of NO signalling with olfaction and feeding behaviour in gastropod molluscs (Gelperin, 1994; Moroz et al., 1993). Some gastropod molluscan studies also suggest that NO is involved in learning, especially in conditioning (Teyke, 1996).

In cephalopod molluscs, results concerning the presence of NOS often depend on the methods. In *Sepia officinalis,* NADPH-diaphorase positive staining (a reliable histochemical marker for NOS)

*Corresponding author. Tel.: +33-2-31-56-55-19; fax: +33-2-31-56-56-00.
E-mail address: chichery@scvie.unicaen.fr (R. Chichery).

1532-0456/03/$ - see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S1532-0456(02)00223-5

was demonstrated in the spines of the anterior basal and peduncle lobes (Chichery and Chichery, 1994). The anterior basal lobe, as well as the peduncle lobe, have been compared with the vertebrate cerebellum on account of similarities between their cytoarchitecture and connectivity networks (Hobbs and Young, 1973; Woodhams, 1977; Young, 1976). In *Octopus,* removal of the peduncle lobe led to motor dysfuntions: movements were imprecise and jerky (Messenger, 1967). In *Sepia*, extensive lesions of the spine of the anterior basal lobe induced defects in manipulative behaviour (Chichery and Chichery, 1987). By using immunochemistry, Di Cosmo et al. (2000) showed a nitric oxide synthase-like protein in these lobes but also in the buccal system (comprising the inferior frontal lobe) and in the suckers where numerous tactile and chemo-receptors are located (Graziadei, 1964). The occurrence of NOS and glutamate NMDAR2/3 receptors in the regions of the *Sepia* nervous system controlling the inking system has also been demonstrated (Palumbo et al., 1999). The experiments of Robertson et al. (1994, 1995, 1996) in *Octopus* suggested that NO may be involved in tactile and visual learning. The inhibition of NOS (and consequently the inhibition of NO release) by intramuscular L-NAME injections blocked touch learning without affecting retrieval of memory and without producing sensory or motor dysfunction.

Thus, three lines of evidence caused us to initiate the work presented here: (1) the multiple possible roles of NO in the molluscs (chemosensory processing, feeding behaviour and learning); (2) the presence of NADPH-diaphorase staining in a structure that is comparable to the vertebrate cerebellum; and (3) the complex manipulative behaviour of *Sepia* that includes chemo-tactile sensory processing, multiple motor actions, and probably motor learning processing. In this study, we aimed to:

- test for the presence of NADPH-diaphorase positive staining in the nerve cord of the arms and in organs (suckers and lips) that contain numerous chemoreceptors (Graziadei, 1964); and
- test by using pharmacological methods, for evidence that NO plays a role in the programming of manipulative behaviour and in its flexibility.

2. Materials and methods

2.1. Animals

Cuttlefish (*S. officinalis*) used in this research were collected by a trawler several miles off the Ouistreham coast between May and October. They were maintained in individual plastic tanks ($80\times60\times40$ cm) with open seawater circulation. Any cuttlefish that showed signs of injury or that did not feed within 1 week were discarded. During the experimental period, all cuttlefish were fed with crabs smaller than the limit of their refusal threshold (Duval et al., 1984).

2.2. NADPH-diaphorase staining

Cuttlefish ($n=5$) were anaesthetised with 2% ethanol in seawater and killed. The first right arm and the lip were rapidly removed.

We localised the NADPH-diaphorase according to the method described by Chichery and Chichery (1994). Tissues were fixed, incubated in 30% sucrose in 0.1 M Tris–HCl buffer, frozen and cut in serial sections (20 μm). Arms and lips were cut along different planes [cross ($n=3$) and horizontal ($n=2$) sections for the arms, cross ($n=5$) section for the lip]. The slides were treated with a staining solution of NADPH sodium salt, nitroblue tetrazolium and Tris–HCl buffer. Treatment of controls included the replacement of NADPH with NADP, NADH or NAD and the exclusion of NADPH or nitroblue tetrazolium from the solution.

A Leitz-Aristoplan universal microscope was used for examination under light and dark-field illumination and for photography. The terminology of the regions was adopted from previous anatomical data (Graziadei, 1964).

2.3. Manipulative behaviour

Prey capture in *Sepia* is typically followed by manipulative behaviour; crabs are paralysed by the injection of a cephalotoxin (Cariello and Zanetti, 1977; Ghiretti, 1959) and are finally ingested (Chichery and Chichery, 1987). Prey capture of large crabs is followed by an initial, rapid manipulative phase that moves the cephalothorax–abdomen junction of the crab to the mouth of the cuttlefish. In this position, the cuttlefish can bite. The salivary toxins are probably injected into this wound and cause a paralysis of the prey approxi-

mately 50 s after its capture. The examination of crabs within 20 s of capture showed that the wound was generally localised in the proximal joints of the hind pereiopods (in the articular basi-ischio-coxopodite membrane) (Chichery and Chichery, 1988). In a second manipulative phase, the crab is reoriented to facilitate its ingestion.

Despite these apparent signs of stereotypy (localisation of the wound, stability of the delay of appearance of the paralysis), we were recently able to demonstrate behavioural flexibility by placing mechanical constraints on the base of the fifth pereiopods. This flexibility suggests the intervention of motor learning processes (Halm et al., 2000) in the predatory behaviour of *Sepia*.

2.4. Time course of test and injection procedure

Before each injection of NOS inhibitor—the N^G-nitro−arginine methyl ester hydrochloride (L-NAME; Sigma Chemical)—the cuttlefish were deliberately underfed for 3 days to increase their appetite for crabs. After these 3 days, cuttlefish were tested by presenting a crab within the cuttlefish's anterior visual field. The crab was removed as soon as the cuttlefish began its predatory behaviour. Any cuttlefish that began a predatory sequence within 5 s after the introduction of the crab was injected.

Cuttlefish were anaesthetised with 2% ethanol in seawater. L-NAME was dissolved in filtered seawater. Injections were made with a 2-ml syringe in the vena cava of the cuttlefish. The injection volume was never greater than 1.5 ml.

Predatory behaviour was again tested 1–2 h after the injection. The delay prevented interference from anaesthesia and the stress of the injection.

2.5. Test of an implication of NO in the programming of manipulative behaviour

To find the threshold concentration of L-NAME necessary to obtain behavioural effects, and thus to evaluate the implication of NO in the programming of manipulative behaviour, different concentrations (from 50 to 500 mg/kg) were injected. Each cuttlefish was injected only once with a determined concentration of L-NAME.

Cuttlefish were fed with crabs and the outcome of the predatory event was recorded: successful capture followed by paralysis or, the failure of predation (rejection of the crab); the latency to fibrillation (=latency between prey capture and onset of quivering of pereiopods, indicating onset of paralysis); and the cephalotoxin injection site. For each cuttlefish, the cephalotoxin injection site was controlled by removing the captured crab after the beginning of the quivering of pereiopods. The control group ($n=12$) consisted of cuttlefish injected with 1 ml of seawater. The data were compared between the different treatment groups.

2.6. Test for an implication of NO in the flexibility of manipulative behaviour

This second experiment allowed testing for a role for NO in the flexibility of manipulative behaviour. Cuttlefish were injected either with the previously determined optimal concentration of L-NAME for 'L-NAME group', or with 1 ml of seawater for the control group. Both these groups were fed crabs; in this experiment, the entire proximal section of the 5th pair of pereiopods had been covered by plastic tubes glued to the legs, which made it impossible for the cuttlefish to bite into the articular basi−ischio−coxopodite membrane (Halm et al., 2000).

The success of capture followed by paralysis or, conversely, the failure (rejection of the crab) was compared between control ($n=17$) and experimental 'L-NAME' ($n=23$) groups, as was the latency to fibrillation.

All statistical analyses were carried out following Siegel and Castellan (1988) using 'statview' software.

3. Results

3.1. Localisation of NADPH-diaphorase activity in the arms and lips of S. officinalis

The substitution of NADH for NADPH was followed by a general positive reaction on all structures. The other controls gave a negative reaction (not shown).

The axial nerve cord of cuttlefish arms is composed of a peripheral cortex surrounding a central neuropile consisting of numerous bundles of fibres. We found a positive NADPH-diaphorase reaction in each of these different parts. Sparse cell bodies were slightly stained (not shown). Some fibres also showed a positive staining, particularly in the dorsal bundles (Fig. 1a).

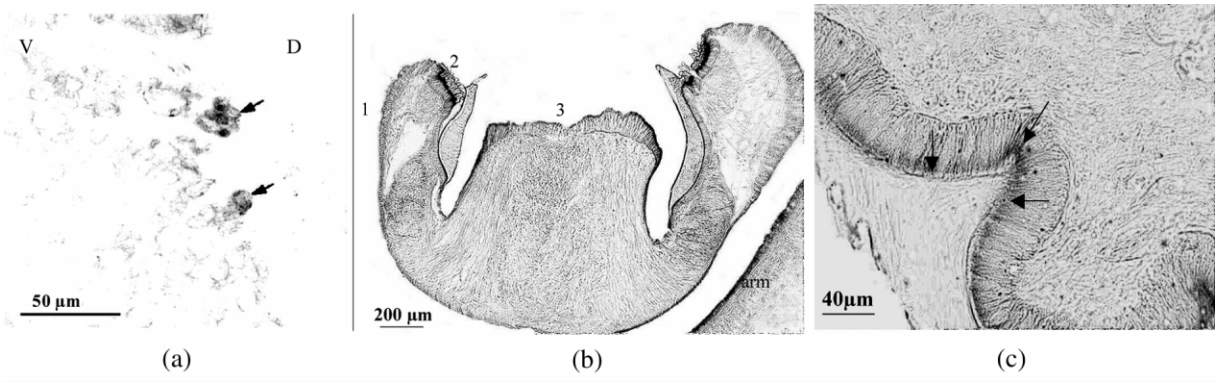

Fig. 1. Positive NADPH-diaphorase staining. (a): Transverse section in the axial nerve cord of the arm I of *Sepia officinalis*. Positive NADPH-diaphorase staining of a dorsal bundle of fibres (black arrows), D; dorsal part, V; ventral part. (b) Positive NADPH-diaphorase staining in one of the suckers (sagittal section) of arm I of *S. officinalis*. Zones 1, 2 and 3, where receptors are stained. Note the strong reaction of zone 2, the positive label of zone 3 and the slighter reaction of zone 1. (c) Positive NADPH-diaphorase staining (arrows) in the epithelium of the internal wall of the lip of *S. officinalis* (transverse section).

The sucker of the cuttlefish is a cuplike muscular organ. Its sensory apparatus consists of primary receptors that lie within the covering epithelium. These receptors can be grouped into three main zones: the external side of the sucker (zone 1); the flat rim of the cup (zone 2); and the bottom of the cup (zone 3) (Graziadei, 1964). Zone 2 showed a very strong positive reaction (Fig. 1b). The positively staining cells were spindle-shaped with a size of 40–60 μm in length and 1.5–3 μm in width. The NADPH-diaphorase activity was particularly concentrated in the apical pole. The lateral parts of zone 3 also showed positive activity. Zone 1 was distinguished by a lower level of activity. Finally, no positive reaction was found in the acetabular ganglion and in the muscles of the sucker.

The lip of the cuttlefish is a circular, musculocutaneous fold around the tip of the beak. The free surface has several finger-like papillae which contain a great number of receptors (Graziadei, 1964). In the internal (Fig. 1c) and external (not shown) walls of the lip, the epithelium showed a clear positive reaction in the apical part of the cells.

3.2. Effect of NOS inhibition on the programming of the manipulative behaviour

After injected doses above 200 mg/kg, some cuttlefish showed extensive body contractions and continuous ink discharge through the funnel, and some died. The rate of capturing crabs decreased and some cuttlefish did not eat. Moreover, the pH of the L-NAME solution decreased proportionally with the increase of concentration of the NOS inhibitor (pH < 6 for L-NAME concentrations above 200 mg/kg). These behavioural and chemical observations suggested a general toxic effect of L-NAME. Consequently, we decided to carry out analysis only for doses between 50 and 200 mg/kg where no sign of toxic effect on general behaviour was observed. In this case, the rate of capturing crabs was 100% for concentrations between 50 and 100 mg/kg and 92% for concentrations of 200 mg/kg of L-NAME. All the cuttlefish showed success in the capture of crab (one rejection was observed with 200 mg/kg) and concentrated their bites on the articular basi–ischio–coxopodite membrane of the crab's fifth pair of pereiopods. The crabs were always ingested in a reference position (Chichery and Chichery, 1988).

Injections of L-NAME produced an increase in the latency to fibrillation (Fig. 2). Statistical analysis revealed a highly significant difference between the different groups (Kruskal–Wallis test: $H_4 = 23.95$, $N = 59$, $P < 0.0001$). Except for the 50-mg/kg group, all L-NAME injected groups (and particularly the 75-mg/kg group) were significantly different from the control group [Mann–Whitney U-test: $U = 6.5$, $P < 0.0001$; $U = 35.5$, $P < 0.05$; $U = 27$, $P < 0.01$; between the control group ($N_1 = 12$) and the 75 mg/kg ($N_2 = 12$), 100 mg/kg ($N_2 = 12$) and 200 mg/kg ($N_2 = 11$) groups, respectively]. A 75-mg/kg concentration of L-NAME was necessary to produce this increase

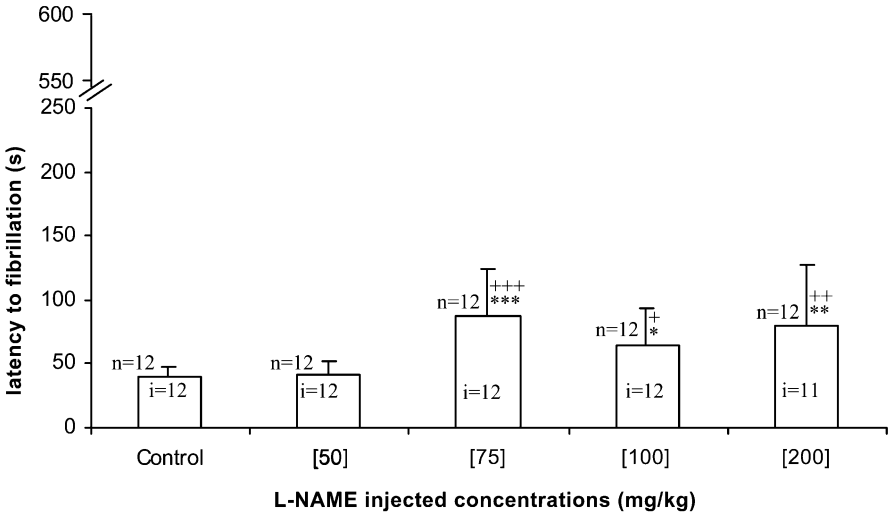

Fig. 2. Effects of L-NAME injections on the latency to fibrillation. N = number of injected cuttlefish, i = number of injected cuttlefish which successfully captured the crab. Vertical bars indicate standard deviation (S.D.). *Significant difference with the control group (*$P<0.05$,**$P<0.01$,***$P<0.001$) (Mann–Whitney U test). +Significant difference with the 50-mg/kg group (+$P<0.05$, ++$P<0.01$, +++$P<0.001$) (Mann–Whitney U test).

(Mann–Whitney U-test: $U=6$, $N_1=N_2=12$, $P<0.001$ for 50 mg/kg vs. 75 mg/kg; $U=32.5$, $N_1=N_2=12$, $P<0.05$ for 50 mg/kg vs. 100 mg/kg; $U=22$, $N_1=12$, $N_2=11$, $P<0.01$ for 50 mg/kg vs. 200 mg/kg). We observed no significant difference between concentrations of 75 and 100 mg/kg, 75 and 200 mg/kg and 100 and 200 mg/kg (Mann–Whitney U-test). The latency to fibrillation reached a maximum with the dose of 75 mg/kg. An increase in the variability of latency to fibrillation was also observed.

3.3. Experiment 2: effects of inhibition of NOS on the flexibility of manipulative behaviour

Based on the preceding results and the pH values of L-NAME solutions, we chose a dose of 75 mg/kg of L-NAME as 'optimal concentration' (threshold concentration required to obtain a clear behavioural effect, pH 6).

Injections of L-NAME did not affect success in capturing crabs whose proximal sections of the fifth pair of periopods was covered with plastic tube (Fig. 3a) although the latency to fibrillation increased (Fig. 3b). Statistical analyses did not reveal any significant difference between the injected group and the control group for either the percentage of successful captures or for the latency to fibrillation, however, a significant increase in the variability of the latency to fibrillation was observed for the injected group (Test F, $P<0.0001$).

4. Discussion

In *Sepia*, Chichery and Chichery (1988) showed the importance of tactile or chemical sensory stimuli in manipulative behaviour. Cuttlefish actively explored the crabs with their arms and lips (Halm et al., 2000) and received information about the toughness of the carapace or the progress of paralysis of the crab owing to its numerous sensory-receptors of suckers and lips (Graziadei, 1964; Emery, 1975).

Our histochemical results revealed NADPH-diaphorase activity in the sensitive parts of the organs (suckers and lips) involved in the manipulative behaviour. These results agree with those of Di Cosmo et al. (2000). In *Sepia* a NOS-like immunopositive staining was observed in areas that these authors defined as olfactory (olfactory organs and lobes) and chemotactile (epithelium of the sucker) and in the inferior frontal lobe. We stress that the frontal inferior lobe plays a part in both the regulation of feeding activities (Halm et al., 2002) and the treatment of sensory information from arms and mouth (Young, 1979). In *Lymnea stagnalis*, NOS has been molecularly characterised, and NO modulates synaptic transmission in the network controlling feeding behaviour. NO is col-

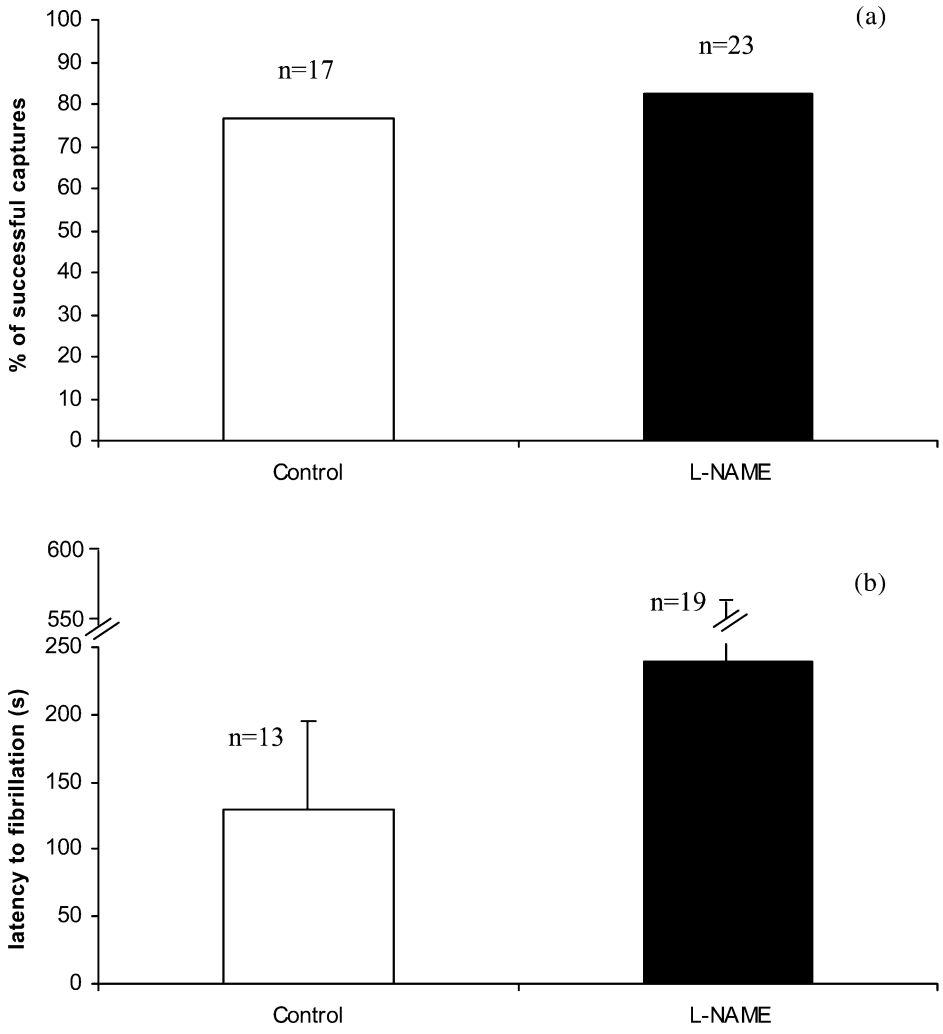

Fig. 3. Effects of inhibition of NOS on the flexibility of the manipulative behaviour. Cuttlefish were fed with crabs whose the fifth pereiopods were covered. (a) Effects of L-NAME injection on the percentage of successful captures; n = number of injected cuttlefish. (b) Effects of L-NAME injection on the latency to fibrillation; n = number of cuttlefish which successfully captured the crab.

ocalised with 5-hydroxytryptamine (5-HT) (Korneev et al., 1998), a fact of great interest because the spine of the anterior basal lobe also exhibits intense NADPH-diaphorase activity (Chichery and Chichery, 1994) and aminergic fluorescence (Tansey, 1980).

The manipulative behaviour involved fine motor control of highly coordinated arm movements that were appropriately timed by the neural circuits controlling them.

Injections of L-NAME did not produce defects in the preferential location of the wound on the fifth pereiopod or in the reorientation of crabs in the 'reference position' (Chichery and Chichery, 1988). The accuracy of manipulative movements seemed not to be affected by the NOS inhibitor. Therefore, a direct role for NO in the motor skills of manipulative behaviour is unlikely. Injections of NOS inhibitor could decrease the sensory information transmitted. This could produce some difficulties in correctly programming manipulative behaviour, resulting in a rejection of the crab for high concentrations, and an increase in the latency to fibrillation for low concentrations of L-NAME. NO could play an important part in the transmission of chemical and/or tactile information.

We could not exclude an indirect role of NO in the motor activities of the manipulative behaviour.

In *Aplysia*, cholinergic transmission is modulated by NO (Meulemans et al., 1995). We recently demonstrated the involvement of cholinergic networks of the inferior frontal lobe in the control of the manipulative behaviour (Halm et al., 2002). This lobe showed the most marked NOS staining of the entire feeding system (Di Cosmo et al., 2000). Moreover, the reduction of NO production could also lead to a decrease in the quantity or the efficacy of the cephalotoxin injected.

Cephalopods are well known for their tactile learning abilities. Blind *Octopus* are clearly able to learn to discriminate objects that differ by their surface texture (Wells, 1978). In *Sepia*, the manipulative behaviour seems to express a combination of predisposition and learning. The artificial protection on the base of the fifth pereiopods prevented cuttlefish from injecting cephalotoxin and, thus, forced it to change injection sites. In an earlier study, we demonstrated that cuttlefish are able to learn by tactile exploration to inject cephalotoxin in another area, thus, they maintain a minimum of flexibility for performing this behavioural task (Halm et al., 2000). Robertson et al. (1994, 1995) showed that tactile learning tasks could be abolished by intramuscular injection of NOS inhibitor L-NAME in *Octopus vulgaris,* although the authors admitted that they had no evidence for the site of action of L-NAME. In *Sepia,* we found no defect in the flexibility of the manipulative behaviour (with our criteria: percentage of successful captures and latency to fibrillation) by intra-venous L-NAME injections. However, the significance of NADPH-diaphorase activity in different motor centres (Chichery and Chichery, 1994) and of NOS-like immunoreactivity in learning centres remains to be discovered.

Acknowledgments

We are particularly grateful to S. von Boletzky and J. Boal for helping to correct the English translation.

References

Cariello, L., Zanetti, L., 1977. α- and β-cephalotoxin: two paralysing proteins from posterior salivary glands of *Octopus vulgaris*. Comp. Biochem. Physiol. 57C, 169–173.

Chichery, M.P., Chichery, R., 1987. The anterior basal lobe and control of prey–capture in the cuttlefish. Physiol. Behav. 40, 329–336.

Chichery, M.P., Chichery, R., 1988. Manipulative motor activity of the cuttlefish *Sepia officinalis* during prey-capture. Behav. Process 17, 45–46.

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

Di Cosmo, A., Di Cristo, C., Palumbo, A., d'Ischia, M., Messenger, J.B., 2000. Nitric oxide synthase (NOS) in the brain of Cephalopod *Sepia officinalis*. J. Comp. Neurol. 428, 411–427.

Duval, P., Chichery, M.P., Chichery, R., 1984. Prey-capure by the cuttlefish (*Sepia officinalis* L): an experimental study of two strategies. Behav. Process. 9, 13–21.

Emery, D.G., 1975. The histology and fine structure of the olfactory organ of the squid *Lolliguncula brevis* blainville. Tissue Cell 7, 357–367.

Gelperin, A., 1994. Nitric oxide mediates network oscillations of olfactory interneurons in a terrestrial mollusc. Nature 369, 61–63.

Ghiretti, F., 1959. Cephalotoxin: the crab-paralysing agent of the posterior salivary glands of cephalopods. Nature 183, 1192–1193.

Graziadei, P., 1964. Receptors in the sucker of the cuttlefish. Nature 203, 384–386.

Halm, M.P., Agin, V., Chichery, M.P., Chichery, R., 2000. Effect of aging on manipulative behavior in the cuttlefish, *Sepia*. Physiol. Behav. 68, 543–547.

Halm, M.P., Chichery, M.P., Chichery, R., 2002. The role of cholinergic networks of the anterior basal and inferior frontal lobes in the predatory behaviour of *Sepia officinalis*. Comp. Biochem. Physiol. 132A, 267–274.

Hobbs, M.J., Young, J.Z., 1973. A cephalopod cerebellum. Brain Res. 55, 424–430.

Jacklet, J.W., 1997. Nitric oxide signaling in invertebrates. Invertebrate Neurosci. 3, 1–14.

Korneev, S.A., Piper, M.R., Picot, J., Phillips, R., Korneeva, E.I., O'Shea, M., 1998. Molecular characterization of NOS in a mollusc: expression in a giant modulatory neuron. J. Neurobiol. 35, 65–76.

Messenger, J.B., 1967. The peduncle lobe: a visuo motor centre in *Octopus*. Proc. Roy. Soc. Lond. B167, 225–251.

Meulemans, A., Mothet, J., Schirar, A., Fossier, P., Tauc, L., Baux, G., 1995. A nitric oxide synthase activity is involved in the modulation of acetylcholine release in *Aplysia* ganglion neurons: a histological, voltammetric and electrophysiological study. Neuroscience 69, 985–995.

Moroz, L.L., Park, J.H., Winlow, W., 1993. Nitric oxide activates motor patterns in *Lymnaea stagnalis*. NeuroReport 4, 643–646.

Palumbo, A., Di Cosmo, A., Poli, A., Di Cristo, C., 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, 1254–1263.

Robertson, J.D., Bonaventura, J., Kohm, A., 1994. Nitric oxide is required for tactile learning in *Octopus vulgaris*. Proc. Roy. Soc. Lond. B256, 269–273.

Robertson, J.D., Bonaventura, J., Kohm, A., 1995. Nitric oxide synthase inhibition blocks octopus touch learning without producing sensory or motor dysfunction. Proc. Roy. Soc. Lond. B261, 167–172.

Robertson, J.D., Bonaventura, J., Kohm, A., Hiscat, M., 1996. Nitric oxide is necessary for visual learning in *Octopus vulgaris*. Proc. Roy. Soc. Lond. B263, 1739–1743.

Siegel, S., Castellan, N.J., 1988. In: McGraw, H. (Ed.), Non Parametric Statistics for the Behavioral Sciences. Mac Graw-Hill Book Company, St Louis.

Tansey, E.M., 1980. Aminergic fluorescence in the cephalopod brain. Phil. Trans. Roy. Soc. Lond. B291, 127–145.

Teyke, T., 1996. Nitric oxide, but not serotonin, is involved in acquisition of food attraction conditioning in the snail *Helix pomatia*. Neurosci. Lett. 206, 29–32.

Wells, M.J., 1978. *Octopus*: Physiology and Behavior of an Advanced Invertebrate. Chapmann and Hall, London.

Woodhams, P.L., 1977. The ultrastructure of a cerebellar analogue in *Octopus*. J. Comp. Neurol. 174, 329–346.

Young, J.Z., 1976. The 'cerebellum' and the control of eye movements in cephalopods. Nature 264, 572–574.

1979. The nervous system of Loligo V. The vertical complex. Phil. Trans. R. Soc. Lond. B 285, 311–354.