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Comparative Biochemistry and Physiology Part A 132 (2002) 267–274

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The role of cholinergic networks of the anterior basal and inferior frontal lobes in the predatory behaviour of *Sepia officinalis*

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Received 22 December 2000; received in revised form 6 December 2001; accepted 11 December 2001

Abstract

The predatory behaviour of the cuttlefish has been the subject of a few detailed studies and can be divided into several stages: prey detection, orientation, positioning, prey-seizing, prey-manipulation and ingestion. Nevertheless, the data about its control by the CNS remain fragmentary. By injecting a cholinergic agonist (nicotine) and antagonists (α-bungarotoxin, mecamylamine), the implication of cholinergic networks of the anterior basal and inferior frontal lobes in the control of predatory behaviour are demonstrated. Through these cholinergic networks, the anterior basal lobe takes an important part in the orientation and positioning. The inferior frontal lobe seems to play a role in the control of brachial manipulative and buccal mass activities. The implication of cholinergic networks of the anterior basal and inferior frontal lobes in the predatory behaviour and the pharmacology of nicotinic receptors are discussed.

Keywords: Cephalopods; CNS; Cholinergic drugs; Manipulative behaviour; Microinjections; *Sepia officinalis*

1. Introduction

The cuttlefish *Sepia officinalis* is an active predator, which is able to catch prey of a size that is large relative to its own. Typically prey-capture is composed of the following sequence of events (Chichery and Chichery, 1987): (1) prey detection starting with an ocular saccadic movement followed by (2) orientation of the head towards the prey and a rapid pivoting of the body and an ocular convergence; (3) a positioning phase characterised by the attainment of a specific attack distance; (4) the attack proper is achieved by one of two strategies: either by ejection of the two tentacles, or by a pounce upon the prey (Duval et al., 1984; Chichery and Chichery, 1991); (5) manipulation of the prey; and (6) ingestion.

Two phases of manipulation can be distinguished: an initial phase of manipulative arm movements which allows a rapid paralysis of the crab (approx. 55 s after capture) by injection of a toxic saliva. The second manipulative phase reorients the crab into a 'reference position' to facilitate its ingestion (Chichery and Chichery, 1988).

From a motor-control point of view, two groups of motor activities can be distinguished. The first group is constituted by postural–kinetic movements comprising the first four phases while the second group is characterised by manipulative movements leading to prey paralysis and ingestion (Chichery and Chichery, 1987).

However, little is known about the control of this predatory behaviour. Previous experiments involving acute and chronic electrical stimulations

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1095-6433/02/$ - see front matter
PII: S1095-6433(02)00002-8

or electrolytic lesions of the anterior part of the supra-oesophageal mass of the brain showed that the anterior basal lobe (AB) plays a prominent part in the control of this behaviour (Boycott, 1961; Chichery, 1983). In *Sepia*, Chichery (1983) reported that chronic electrical stimulation of the anterior part of the supra-oesophageal mass [AB and inferior frontal (IF) lobes] produces eyes movements, funnel movements and fin waves involving rotatory, backward or forward motions, the latter being linked with an ocular convergence and stretching of arms which point forward.

Some lesions done to the AB led to defects of all processes involved in the orientation of the cuttlefish towards the prey. Tentacle ejection still occurred but was often unsuccessful due to a directional miscalculation or to an underestimation of prey distance. Extensive lesions of the dorsal parts of this lobe also induced oculomotor and manipulation defects. In some cases, lesions of the anterior part of the AB lobe associated with destruction of the ventral cortex of the IF lobe produced rolling during the acceleration of the forward motions and manipulation defects (Chichery and Chichery, 1987). These results suggest an implication of the AB in the control of the motor activities linked to the execution of the predatory behaviour.

Cephalopod brains contains many putative neurotransmitters (Kime and Messenger, 1990; Messenger, 1996) but there is more limited evidence about their physiological implication. Acetylcholine and its associated enzymes: choline acetyl transferase (ChAT) and acetylcholinesterase (AChE) are present in high concentrations in the cephalopd CNS (Loe and Florey, 1966; Welsch and Dettbarn, 1972). In the supraoesophageal mass, maximal ChAT activity is observed in the IF (Bellanger et al., 1997). Moreover, this lobe and the anterior part of the AB show a highly positive reaction with histochemical staining of AChE (Chichery and Chichery, 1974), however, the presence of these enzymes is not proof that the structure is cholinergic.

Few biochemical studies carried out only on the optic lobes of squid have provided evidence for nicotinic-type receptors (Demushkin and Kotelevtsev, 1980). They revealed at least two types of binding sites, one for ACh that is more sensitive to agonists, the other for α-bungarotoxin that is more sensitive to antagonists (Chen et al., 1988).

Some motor and behavioural effects can be induced by injection of different nicotinic drugs into the cephalic aorta in *Octopus* (Andrews et al., 1983) and directly in the optic lobe of *Sepia* (Chichery and Chichery, 1985). Muscarine injections did not have a motor effect.

In the anterior part of the supraoesophageal mass, preliminary results show that micro-injections of acetylcholine (0.1 M) induce similar behaviours to those obtained by electrical stimulation. The chronical micro-injections of acetylcholine produce forward motions and grasping movements of the arms on any presented object (Chichery and Chichery, 1991).

Taken together, these results suggest that the predatory behaviour may be partly controlled by cholinergic nicotinic networks within the IF and/or AB lobes.

Therefore, the aim of the present qualitative study is to determine with a pharmacological approach any implication of putative cholinergic networks of each lobe (AB and IF) in the control of the predatory behaviour.

2. Material and methods

The animals used in this study were caught a few miles away from Luc sur Mer (France) between May and October. The animals ranged from 200 to 1500 g. The cuttlefish were placed in individual plastic tanks ($0.80 \times 0.60 \times 0.4$ m) connected to an open seawater circulation. Before any injection, the cuttlefish were deliberately underfed during 3 days to increase the appetency for crabs. After these 3 days, this appetency is tested with a crab presented to the cuttlefish in its anterior visual field. The crab is immediately taken away when the cuttlefish begins its predatory behaviour. Any cuttlefish that began a predatory sequence within 5 s after the introduction of the crab was injected.

The technique of micro-injections has been already described by Chichery and Chichery (1985). The animals are slightly anaesthetised by immersion in seawater containing 2% ethanol. The dorsal part of the cranium is exposed and a hole is drilled in the sagittal plane.

This hole allows the insertion of a microcannula (internal diameter: 0.075 mm, external diameter: 0.125 mm) in the CNS of *Sepia*. This microcannula is fixed through a small oval plate of chemically inert plastic (approx. 8×5 mm) and the part of microcannula under the plastic plate is adjusted

according to the weight of animals and to the correct depth to reach the appropriate lobe. The microcannula is connected into a catheter in its turn joined to a Hamilton syringe. After injection, the hole and the wound are sealed with a special glue (histoacryl), then the animal is placed in a tank containing fresh circulating seawater. The animals quickly recover (1–2 min) from the slight anaesthesia. Each animal was injected only once with the appropriate drug.

Pilot experiments made with micro-injections of nicotine (0.25; 0.5; 1 and 2 μl) allowed to find an optimal volume to be injected, namely 0.25 μl to avoid an excessive drug dispersion. Then, the effects of three nicotinic drugs currently used in vertebrates were tested: an agonist (nicotine) and two antagonists (α-bungarotoxin, mecamylamine). In vertebrates and insects, the snake venom α-bungarotoxin is a selective antagonist of both skeletal neuromuscular junction receptors and of neuronal nicotinic receptors with α7 subunits while mecamylamine is a central antagonist of nicotinic receptors which are not blocked by α-bungarotoxin. By using two specific antagonists of vertebrate nicotinic receptors, new information about the pharmacological profile of the nicotinic receptors of cephalopods were obtained. During pilot experiments several doses were tested for nicotine (0.0006 M, 0.003 M, 0.006 M, 0.06 M) and α-bungarotoxin (1.25×10^{-8} M, 1.25×10^{-6} M, 1.25×10^{-5} M, 1.25×10^{-4} M, 1.25×10^{-3} M). We chose 0.006 M concentration for nicotine (we obtained similar effects with the 0.003, 0.006 and 0.06 M concentrations) and a threshold of concentration of 1.25×10^{-3} M for α-bungarotoxin. Mecamylamine was injected at the same concentration as α-bungarotoxin in order to compare the affinity of these two drugs with putative nicotinic receptors. Drugs are always associated with 2% Evans Blue which allows a posteriori to detect the exact site of implantation of the microcannula. The control animals received a seawater injection with Evans Blue (2%).

The spontaneous motions and arm movements were analysed just after the micro-injection. The motor defects linked with the execution of the predatory behaviour were analysed from video recordings in an experimental glass aquarium ($1.5\times0.8\times0.35$ m) into which, a prey (shrimp and/or crab) was introduced 45 min after the micro-injection. In fact, the control animals showed a recovery of their predatory behaviour after this delay. The prey 'shrimp' allows one to observe the capture by tentacular ejection while the prey 'large crab' yields a capture by pouncing (Duval et al., 1984). Thus, the number of captures by pouncing is limited to only one due to satiation. The recorded criteria are: (1) the capacity of detection, orientation of the head, and positioning; (2) the capture success or failure; and (3) the capacity or incapacity to manipulate crabs. Here, only the first manipulative phase is observed. If a crab is paralysed by cephalotoxin injection within less than 60 s after the capture, the manipulative phase leading to the prey paralysis is considered as normal; for latencies of paralysis longer than 60 s, the first phase is considered as defective; if a cuttlefish fails to paralyse a crab, this phase is considered as impossible.

Following the experimentation, animals were killed (after anaesthesia with 2% of ethanol in seawater), the brain was removed and sectioned in a cryostat (20 μm). The localisation and the extent of the injection were studied in the light microscope; the site of injection appears blue under normal or red under fluorescent light.

3. Results

In this study, the results from 42 micro-injections affecting the AB lobe and/or the IF lobe are described. Micro-injections of seawater never induced a motor effect ($n=5$).

Micro-injections of different drugs accidentally injected into the vertical system (vertical, subvertical or superior frontal lobes) never induced any characteristic change on the predatory behaviour ($n=12$).

The main motor effects by micro-injections of nicotinic agonist and antagonists and the injection sites are summarised in Table 1.

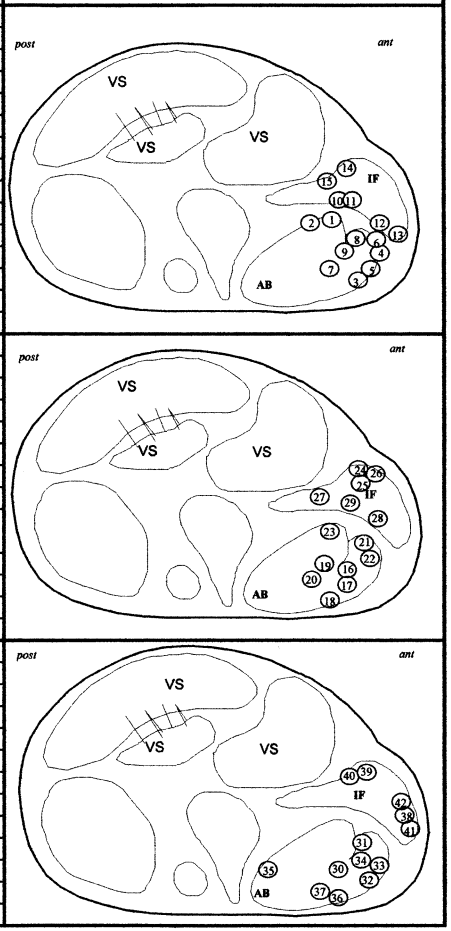
3.1. Nicotine micro-injections

Most of nicotine micro-injections ($n=15$) into the AB and IF lobes provoked some events of predatory behaviour sequences. Thus, many displacements and manipulative arm movements recalled chasing a prey or crab manipulation.

Micro-injections into the sagittal plane of the AB lobe induced forward motions of the cuttlefish with ocular convergence and antero-posterior fin waves (injections 1, 2, 3, 4). Micro-injections in the lateral parts of this lobe produced clockwise

Table 1
Main motors effects obtained on the predatory behavior of *Sepia officinalis* by micro-injections of cholinergic drugs

	Localisation of microinjections	postural-kinetic movements		manipulative movements leading to paralysis	sites of micro-injections in the supraoesophageal mass
		orientation	capture		
Nicotine	1 dorsal part of pAB	no effect	no effect	no effect	
	2 dorsal part of pAB	no effect	no effect	no effect	
	3 aAB	no effect	no effect	several manipulative phases	
	4 aAB				
	5 aAB	no effect	no effect	several manipulative phases	
	6 aAB				
	7 aAB				
	8 dorsal part of aAB	no effect	no effect	no effect	
	9 dorsal part of aAB	no effect	no effect	several manipulative phases	
	10 IF				
	11 IF				
	12 IF				
	13 IF	no effect	no effect	several manipulative phases	
	14 IF	no effect	no effect	ingestion without crab's paralysis	
	15 IF	no effect	no effect	ingestion without crab's paralysis	
α Bungarotoxin	16 aAB	impossible	no effect	no effect	
	17 aAB	impossible	no effect	no effect	
	18 aAB	impossible			
	19 pAB	impossible			
	20 pAB	impossible	no effect	no effect	
	21 dorsal part of BA	impossible	Failure of pouncing attack	no effect	
	22 dorsal part of BA	no effect	no effect	no effect	
	23 dorsal part of pAB	no effect			
	24 IF	impossible	Failure of attack (tentacular ejection + pounce)	initial phase impossible	
	25 IF	impossible	Failure of attack (tentacular ejection + pounce)	initial phase impossible	
	26 IF	impossible	Failure of attack (tentacular ejection + pounce)	no effect	
	27 IF	no effect	Failure of pouncing attack	initial phase impossible	
	28 IF	no effect	Failure of pouncing attack	initial phase impossible	
	29 IF	no effect	no effect	initial phase impossible	
Mécamylamine	30 dorsal part of AB	no effect	no effect	no effect	
	31 dorsal part of AB	no effect	no effect	no effect	
	32 dorsal part of AB	no effect	no effect	no effect	
	33 dorsal part of AB	no effect	no effect	no effect	
	34 dorsal part of AB	no effect	no effect	no effect	
	35 middle part of pAB	no effect	no effect	initial phase impossible	
	36 ventral part of aAB	no effect	no effect	slow manipulative movements	
	37 ventral part of aAB	impossible			
	38 IF	difficult			
	39 IF	no effect	no effect	no effect	
	40 IF	no effect	no effect	no effect	
	41 IF	no effect	no effect	slow manipulative movements	
	42 IF	no effect	no effect	slow manipulative movements	



Localisation of microinjections in the supraoesophageal mass: ant: anterior, AB: anterior basal lobe, aAB: anterior anterior basal lobe, pAB: posterior anterior basal lobe, IF: inferior frontal lobe, post: posterior, VS: vertical system
white compartment : no behavioral observation due to the defects in the previous behavioral phase (except for microinjection 23). For microinjections 4, 6, 7 and 10, 11, 12 : spontaneous motions and grasping, wriggling of the arms, cyclic activity of the buccal mass are respectively observed but cuttlefish refused to capture prey.

or anticlockwise rotary movements, the arms were crooked towards the side of micro-injection (injections 5, 6, 7, 8, 9).

For micro-injections 4, 6, 7, cuttlefish moreover showed a spontaneous grasping associated with co-ordinated activity of the buccal mass or grasping any object introduced between the arms of the cuttlefish.

When the micro-injections were made into the inferior frontal lobe (injections 10, 11, 12, 13, 14, 15), various motor effects were observed. The buccal mass shows a co-ordinated cyclic activity: the beaks started to open with protrusion of the gustative papilla, followed by the maximal opening of the beaks and protrusion of the radula and finally the beaks closed. Fifteen minutes after the injection, 10–15 cyclic movements of buccal mass/min were recorded. In contrast, the arms moved in a most disordered way, the cuttlefish showed spontaneous wriggling movements of the arms and a grasping of any presented object (piece of polystyrene), accompanied by salivation. These effects of nicotine appeared as soon as the cuttlefish woke up. They could last for approximately 1 h.

Cuttlefish which were injected into the AB and IF lobes were able to capture prey. The first phases of predatory behaviour were not affected by nicotinic micro-injections in spite of spontaneous rotary or forward movements. Nevertheless, the manipulative behaviour was disturbed. It was not co-ordinated, prey underwent several manipulative phases that increased the latency of the appearance of signs of paralysis (injections 3, 5, 9, 13). The prey was not held in a specific orientation. In two cases of micro-injections into the IF lobe (injections 14, 15), the first phase of manipulation proved impossible: crabs were eaten without being paralysed.

3.2. α-Bungarotoxin micro-injections

Micro-injections of α-bungarotoxin ($n=14$) into the AB and IF lobes produced numerous defects in the execution of the predatory behaviour. The duration of the drug's effect is approximately 1–2 days in *Sepia.*

Injected cuttlefish in the middle of the anterior part of the AB lobe (injections 18, 19, 20) showed continuous clockwise rotary movements associated with fin waves. Others did not show abnormal movements or remained quiescent on the bottom of the tank.

During the execution of predatory behaviour, postural–kinetic and/or manipulative movements were disturbed (Table 1).

3.2.1. Micro-injections in the middle of the anterior anterior basal lobe and posterior anterior basal lobe (injections 16, 17, 18, 19, 20; Table 1)

The detection phase still occurred but orientation, positioning and/or capture were difficult or impossible. These defects could be due to the continuous rotary movements previously observed (injections 18, 19, 20). In these cases the orientation of the head, the positioning and the capture proved impossible. Only two captures were observed (injection 20) when the prey remained in the frontal visual field of the cuttlefish. Similar captures were observed for micro-injections 16, 17. The spontaneous movements were normal but cuttlefishes did not reorientate their heads which seemed fixed in the body axis. The positioning remained possible but difficult. Once positioned, tentacle ejection and pouncing appeared without defect.

The manipulative behaviour was not disturbed by these micro-injections.

3.2.2. Micro-injections in the dorsal part of the anterior anterior basal lobe (injections 21, 22; Table 1)

Only the most dorsal micro-injection (21) produced defects of postural–kinetic movements, the injected cuttlefish moved 'crabwise' towards the prey. The pouncing attack thus was defective.

3.2.3. Micro-injections in the dorsal part of the posterior anterior basal lobe (injection 23, Table 1)

In this injected cuttlefish no behavioural defect was observed, tentacle ejection was possible and all the captures of shrimps were successful. Unfortunately, cuttlefish refused the prey when it was a crab so the manipulative behaviour could not be observed in this condition.

3.2.4. Micro-injections in the inferior frontal lobe (injections 24, 25, 26, 27, 28, 29, Table 1)

Both postural–kinetic and manipulative movements (except for micro-injection 29) were affected.

For micro-injections 24, 25, 26 the forward motions were blocked. The head reorientation was impossible when the prey moved to the lateral or posterior visual fields. Only the prey which were in the frontal visual field of the cuttlefish were potentially captured. The tentacle ejection was sometimes defective, tentacle clubs were shot above the prey (injections 24, 25, 26).

For the micro-injections 24, 25, 26, 27, 28 (Table 1), the cuttlefish were unable to pounce quickly on their prey. The 'pouncing distance' could be underestimated. As a result, cuttlefish touched the crabs only lightly.

For manipulative movements, the initial phase was totally impossible (injections 24, 25, 27, 28, 29, Table 1). The cuttlefish would maintain a crab without manipulative arm movements and the prey was able to escape from the arms of the cuttlefish. Captures and escapes of the crab were indeed observed. For micro-injection 26, the cuttlefish was successful in paralysing a crab but manipulative arm movements during the first phase were not observable. It was also unable to reorient the crab into 'a reference position'.

3.3. Mecamylamine micro-injections

The effects obtained by micro-injections ($n=13$) of this drug were different and less evident than those obtained with α-bungarotoxin (Table 1).

The spontaneous motions were not affected by the micro-injections.

For micro-injections into the AB lobe, the most dorsal (injections 30, 31, 32, 33, 34) never induced postural–kinetic defects in the execution of predatory behaviour. Only the micro-injection 37, in the ventral part of the AB lobe, prevented the capture of prey because the orientation phase proved impossible. Surprisingly, similar defects were observed for micro-injection 38 into the inferior frontal lobe. The manipulative behaviour appeared difficult (injection 36) or impossible (injection 35) when micro-injections were made in the ventral or middle part of the AB lobe.

When micro-injections reached the inferior frontal lobe, postural kinetic movements were not disturbed.

The manipulative behaviour was disturbed only by two micro-injections into the inferior frontal lobe (injections 41, 42). This behaviour was still possible and led to normal ingestion of prey. Only the execution speed of manipulative movements was reduced, the first signs of paralysis appeared within 60 s after capture.

All the effects of mecamylamine disappeared 4–6 h after the micro-injection.

4. Discussion

4.1. Implication of cholinergic networks of anterior basal and inferior frontal lobes in the control of predatory behaviour

In our experiments, micro-injections into the vertical system never induced motor effects. This result can be compared with those of Boycott (1961) and Chichery (1983) where electrical stimulation of the vertical system does not produce motor effects. Moreover, extensive lesions of this system (Sanders and Young, 1940) produced no changes in the execution of predatory behaviour. Our results confirm that these structures do not play a direct part in the control of motor behaviour and emphasises also the specificity of our local micro-injections. Micro-injections of nicotine or α-bungarotoxin into the dorso-posterior part of the anterior basal lobe produced no motor effects. These results are not surprising given the very slight reaction to the acetylcholinesterase staining (Chichery and Chichery, 1974) and provide a new argument for the non-cholinergic nature of this structure.

The effects of micro-injections of nicotine and α-bungarotoxin suggest a cholinergic implication for the different lobes of the anterior supraoesophageal mass in the control of postural–kinetic and manipulative movements linked with predatory behaviour.

However, we must stress that our results are qualitative ones, since in this animal model it is impossible to obtain several micro-injections rigorously localised in the same anatomical site. Moreover, IF and AB lobes are not only cholinergic, numerous putative neurotransmitters have been identified in theses structures (Kime and Messenger, 1990). The duration of the effect of the drug can depend on its concentration but also on the position of the micro-injection with regard to the cholinergic networks. These facts explain the difficulty to obtain a clear 'dose-effect'.

Previous experiments involving acute and chronic electrical stimulations (Boycott, 1961; Chichery, 1983) or electrolytic lesions (Chichery and Chich-

ery, 1987) of the AB lobe have shown that it plays an important part in programming the complex motor activities associated with predatory behaviour. The cholinergic networks in this structure are disturbed by micro-injections of nicotine or α-bungarotoxin.

Some micro-injections of α-bungarotoxin in the AB lobe block the orientating behaviour. This lobe receives tracts originating in the optic lobe. The projection of these optic fibres respects the retinotopic organisation of the optic lobe, thus these fibres might correspond to an eye–head co-ordination pathway in cephalopods (Young, 1977). The defects in orientation observed with these micro-injections of the nicotinic antagonist could be due to a partial or a total blocking of synaptic contacts of these tracts which might be cholinergic.

Some nicotinic micro-injections into the AB lobe induce an increase of forward motions, associated with co-ordinated fin waves. When micro-injections stimulated the lateral part of the posterior part of the AB, cuttlefish showed rapid rotations. The AB lobe sends efferent fibres to the posterior pedal and palleovisceral lobes implicated, respectively, in the control of the position of the funnel and in the control of fin waves (Boycott, 1961). Our results associated with previous anatomical and physiological data suggest that the cholinergic networks of the AB lobe might act upon these suboesophageal lobes in the directional processes. They might have a prominent part in the chasing behaviour. This behaviour is distinguished by a complex visuo-motor co-ordination, requiring a right position of eyes, head, arms and overall motions of cuttlefish.

In conclusion, the anterior basal lobe of *Sepia officinalis* is implicated, through cholinergic networks, in the postural–kinetic movements (Table 1) and particularly in the orientation phase and pursuit behaviour.

Micro-injections of nicotine into the IF lobe produce an increase of arm and buccal mass movements. The present results can be compared with those of Boycott (1961), where acute electrical stimulations in the anterior part of the supraoesophageal mass, which reached probably the IF lobe, provoked manipulative movements. The micro-injections of nicotine induce co-ordinated movements of the buccal mass and could suggest, for this behaviour, the existence of a central pattern generator (CPG) which might be cholinergic in the IF lobe. Another possibility is that the IF lobe contain the higher-order-interneurons (which might be cholinergic), so the CPG could be situated in the buccal lobes.

Injection of α-bungarotoxin induce opposite results to those obtained by electrical stimulation and prevent the manipulative behaviour (Table 1). These defects are due to an impossibility to move the arms and/or the buccal mass. In this last case, our micro-injections have probably blocked the CPG of the buccal mass movements.

Microinjections of α-bungarotoxin induced a total blockage of arms movements, the IF lobe might act upon the suboesophageal lobes (pedal and brachial lobes) which contain motor neurons connected with the nervous system of the arms (Budelmann and Young, 1987).

Defects in the postural–kinetic movements are also obtained by micro-injections of α-bungarotoxin into this lobe (Table 1). In this case, the drug may reach and diffuse into the AB lobe. Another explanation could be that the efferences towards the median basal lobe are attained by the drug. Previous experiments suggest that this lobe indeed controls the directional and propulsive process (Boycott, 1961; Young, 1977).

However, we cannot exclude the possibilities of cross-talk interactions with other neurotransmitter systems which could play a role in the control of predatory behaviour.

4.2. Pharmacology of nicotinic receptors

Micro-injections of the different antagonists do not produce the same behavioural defects and, contrary to α-bungarotoxin micro-injections, mecamylamine micro-injections induce only weak defects.

These results suggest two hypotheses that are compatible with one another. On the one hand, cholinergic receptors of cephalopods might have a different pharmacological profile from that of vertebrates. The investigations of Gebauer et al. (1999) into the cholinergic neuroregulation of the branchial heart of *Sepia* suggest the existence of cholinergic receptors which have mixed nicotinic/muscarinic properties. Motor and behavioural effects induced by cholinomimetic drug injections (neostigmine, succinyldicholine, carbachol) into the optic lobe of *Sepia* also show that the cholinergic receptors of this lobe have different pharmacological properties (Chichery and Chichery, 1985). On the other hand, the existence of two

subtypes of central nicotinic receptors have to be considered. In cephalopods, a pharmacological study on the optic lobe of *Loligo pealii* (Chen et al., 1988) revealed more than one type of neuronal nicotinic receptor according to their affinity for different ligands ([^{3}H]acetylcholine, [^{125}I]α-bungarotoxin). Finally, studies of properties of membrane bound acetylcholine receptors from optic ganglia of a squid (*Berryteuthis magister*) speak for this second hypothesis (Demushkin and Kotelevtsev, 1980). The receptors from the optic ganglia of *B. magister* were blocked neither by decamethonium nor by α-neurotoxin II (a close analog of α-bungarotoxin). Behavioural effects obtained by mecamylamine injections might be due to the nicotinic receptors with this pharmacological profile.

Binding experiments are in progress in our laboratory in order to obtain more informations about the localisation and pharmacological profile of these nicotinic receptors.

Acknowledgments

We are grateful to Dr Sigurd von Boletzky for helping to correct the English translation.

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