

Effect of aging on manipulative behavior in the cuttlefish, *sepia*

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Received 29 July 1998, received in revised form 28 September 1999; accepted 27 October 1999

Abstract

The cuttlefish is an active predator that is able to catch crabs of a size that is large relative to its own. The capture is followed by a complex manipulative behavior leading to paralysis of the prey by injection of a cephalotoxin. This manipulative behavior is relatively stereotyped, and earlier research has shown that the cuttlefish concentrates its bite on the articular basi-ischiocoxopodite membrane of the crab's fifth pair of pereopods. By placing mechanical constraints on the base of the fifth pereopods, we were able to demonstrate that this manipulative behavior presents a marked degree of stereotypy but is not rigidly fixed. Substantial behavioral differences, however, were observed between subadult and senescent cuttlefish. The existence of a reduction in behavioral flexibility in the older animals in reaction to the constraints is discussed. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: *Sepia officinalis*; Cephalopods; Predation; Manipulative behavior; Behavioral flexibility; Aging

1. Introduction

Many studies have provided an insight into the major characteristics of the predatory behavior of the cuttlefish. There are two capture strategies: tentacle strikes used generally for prey capable of a rapid escape (shrimps, fishes), and the “jump” mode used for less mobile prey (crabs) [1–3].

From a motor control point of view, two stages may be distinguished, contrasting posturokinetic motor activities that correspond, first, to the movement of the body and/or ejection of the tentacles for prey capture; second, manipulative motor activities allow the paralysis of the prey before its ingestion [4]. However, up until now, little is known about the control of this manipulative behavior. It appears to be especially complex in the “jumping mode,” following the capture of large-sized crabs with claws that may injure the predator. Such prey is often captured in the “jumping mode”: the cuttlefish jumps forward at its prey and covers it with its eight arms [2]. The crab is then moved to the buccal bulb and manipulated by the cuttlefish's arms in such a way that the pereopods of the crab cannot clinch to the substratum [5]. Two clear manipulative phases are observed. Prey capture is followed by an initial, very rapid manipulative phase that moves the cephalothorax–abdomen junction of the crab to the mouth of the cuttlefish. In this position, the cuttlefish can inflict a wound. The salivary toxins are proba-

bly injected into this wound and cause a paralysis of the prey about 55 s after capture. The examination within 20 s after the capture of the crabs shows that this wound is generally localized in the proximal joints of the hind pereopods [5]. The second manipulative phase reorients the crab to facilitate its ingestion [5]. The limited variability of the appearance of the first indications of paralysis (fibrillation time latency) and the preferential location of the wound on the fifth pereopod speak for a stereotyped organization of this behavior. This stereotypy is also found in other behaviors of cephalopods, such as body patterns and digging in *Sepia*; however, the latter is relative flexible [6]. In addition, in *Octopus*, different experimental studies have shown a nonrandom location of drill holes on different species of snails [7] and a large flexibility in the choice of opening the shells either by force or by drilling. These results suggest some complexity in the manipulative behavior of *Octopus*.

One might stress here the surprising learning capacities of the cephalopods [8–11]. In cuttlefish, Wells [12] and Messenger [13] showed that this animal can quickly learn to inhibit its predatory behavior towards shrimps enclosed in a glass tube. Visual or tactile learning have been described in *Octopus*. Blind octopuses are clearly able to learn to discriminate between objects that differ by their surface texture; in contrast, they failed to learn to discriminate by touch between objects of different sizes, shapes, or weights. Thus, *Octopus* does not seem to use haptic information, as this species does not integrate proprioceptive with surface contact information [11]. These data seem inconsistent with the

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behavioral observations on the flexibility of the opening of shells by force or by drilling [7, 14–17].

The aim of the present study is to investigate the flexibility of an apparently stereotyped behavior of *Sepia*, and to what extent this flexibility depends on the age of the animals.

2. Material and methods

2.1. Animals

The cuttlefish (*Sepia officinalis*) used in this study were collected off Luc-sur-Mer (France) between May and October. The cuttlefish were isolated in individual plastic tanks ($80 \times 60 \times 40$ cm) connected to an open seawater circulation. Any cuttlefish showing signs of injury or refusing to feed after 1 week was discarded. During the experimental period all the animals were fed with crabs of an appropriate size [2].

2.2. Experiment 1

Three groups of cuttlefish (dorsal mantle length = 12–26 cm) were used.

2.2.1. Protected fourth pereopods = P4 group (n = 15)

Cuttlefish were fed with crabs whose entire proximal section of the fourth pair of pereopods was covered by plastic tubes that were glued to the legs and thus made it impossible for the cuttlefish to bite into the articular basischirocoxopodite membrane. This group was used to verify that the presence of a leg cover is not, by itself, an aversive stimulus, and does not modify the predatory behavior of the cuttlefish.

2.2.2. Protected fifth pereopods = P5 group (n = 30)

Cuttlefish were fed with crabs whose entire proximal sections of the fifth pair of pereopods were covered by plastic tubes. Fifteen animals were used to analyze fibrillation time latency (latency between prey capture and onset of quivering of pereopods, indicating onset of paralysis), and 15 animals to check the cephalotoxin injection site. To do this, the prey were removed immediately on the first sign of paralysis.

2.2.3. Control group (n = 15)

The animals were fed with crabs without plastic leg covers.

2.3. Experiment 2

All the animals of this experiment (except the control group) were fed in each trial with crabs with the fifth pair of pereopods covered by plastic tubes. The animals were either senescent (aged around 22–23 months; captured during their reproductive phase and shortly before their natural death) or sexually immature subadults (aged around 10–11 months). The age of the animals was estimated on the basis of the hatching peak of juveniles at the end of July in the natural environment [18]. The control group consisted of animals aged about 20 months, and fed with crabs without plastic protection.

Nine trials were performed. For each trial the number of animals in each group varied because of survival problems (subadult animals died because they were often injured during trawling, and senescent animals died because of their age)—subadult group: 29 at trial 1, 7 at trial 9; senescent group: 33 at trial 1, 11 at trial 9; control group: 18 in all trials.

The success of capture followed by paralysis or, conversely, the failure (rejection of the crab) were noted, as was the fibrillation time latency. When the cuttlefish succeeded in paralyzing the crab, it was allowed to eat it to avoid any negative reinforcement. Thus, the number of trials was limited to one per day to avoid saturation of the animals.

All statistical analyses were carried out following Siegel and Castellan [19] and with “systat” and “Statview” softwares. The calculation of Spearman’s coefficient of correlation allowed the detection of a possible correlation between the rejection percentage and the mean fibrillation time latency.

3. Results

3.1. Experiment 1: Evidence of behavioral flexibility

The fibrillation time latencies for the control (48 ± 25 s) and the P4 (58 ± 30 s) groups were almost the same (Fig. 1); a statistical analysis showed no significant difference (Mann–Whitney *U*-test). In contrast, the fibrillation time latency in the P5 group increased (133 ± 84 s), as is evident from the significant differences between the control and P5 groups and between the P4 and P5 groups ($p < 0.001$ and $p < 0.005$, respectively; Mann–Whitney *U*-test). In addition to this increase, the cuttlefish of the P5 group made a detailed exploration of their prey with their arms and buccal bulb. In the 15 crabs paralyzed and removed from the cuttlefish of the P5 group, bite marks were seen on the plastic cover of the pereopods right up to either end. The shape of the bite marks indicate that they were done with the beak and not with the radula.

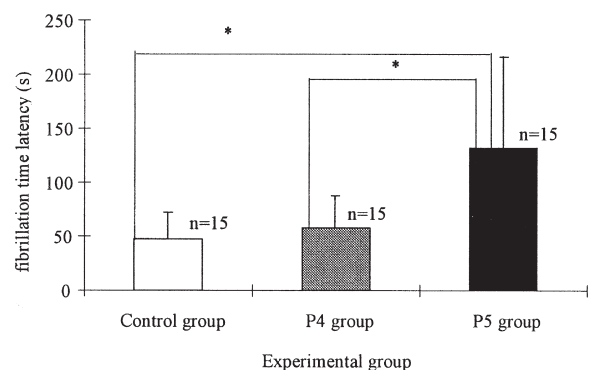


Fig. 1. Mean Fibrillation time latencies \pm SEM for the different groups of cuttlefish as a function of the type of crabs presented (control group fed with normal crabs; P4 group fed with crabs with a cover on the fourth pereopods; P5 group fed with crabs with a cover on the fifth pereopods). n: number of animals; *indicates a significant difference $p < 0.005$; Mann–Whitney *U*-test.

3.2. Experiment 2: Behavioral flexibility and aging

3.2.1. Analysis of successful captures

The capture of normal crabs by the control group is nearly always followed by paralysis of the prey. The experimental animals, fed with crabs with the fifth pair of pereopods covered, showed a decrease in successful captures, with the senescent animals having a lower success rate than the subadults.

Figure 2 shows the percentages of successful captures for the different trials. Figure 2 (left) includes all animals, whereas Fig. 2 (right) represents only those animals that survived up to the ninth trial; for both groups the performance profiles are almost identical. The χ^2 or Fisher's exact method tests (correlated to the number of animals in each trial) show a highly significant difference (Fig. 2, left) not only between the subadult and senescent cuttlefish ($p < 0.005$ for trials 1, 2, 3; $p < 0.05$ for trials 4, 5, 7), but also between the control and the senescent animals ($p < 0.0001$ for the trials 1, 2, 3; $p < 0.005$ for trials 4, 5, 6, 7, 8, 9). However, the difference between the senescent and the subadult animals disappears for the trials 6, 8, and 9, and does not exist between the control and subadult animals for any trial. An analysis of the performance curves of cuttlefish that have survived throughout the nine trials (Fig. 2, right) shows a significant difference between the senescent and the subadult animals for trials 2, 3 ($p < 0.005$, $p < 0.05$, respectively; Fisher's exact method test) and between the senescent and control animals for each trial. The Kolmogorov–Smirnov test ($n = 7$, $p < 0.05$) confirms that the subadult animals show a higher success rate than the senescent animals.

3.2.2. Evidence of an improvement of successful captures across the trials

The performances appear to improve from one trial to the next, reaching a maximum value (approximately 90% for the subadults and 60% for the senescent) at the fourth test (Fig. 2, left), although there is no significant difference across the different trials (Cochran Q -test).

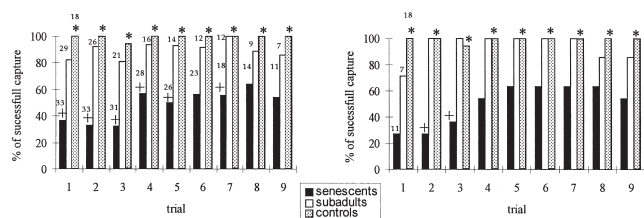


Fig. 2. Percentage of captures followed by paralysis plotted against the different trials. Left: all the cuttlefish analyzed. Right: only those cuttlefish analyzed that survived all nine trials. Senescent: animals fed with crabs with covers on the fifth pereopods; subadults: animals fed with the same type of crabs as senescent; controls: animals fed with crabs without a leg cover. Arabic numerals: number of animals in each trial. + indicates a significant difference between the subadult and the senescent animals; * indicates a significant difference between the control and the senescent animals (χ^2).

3.2.3. Analysis of fibrillation time latency

The fibrillation time latency obtained in animals that were fed with crabs with the fifth pereopod covered (for all the animals and mingled trials) confirms the results of Experiment 1: the fibrillation time latency is substantially higher than in the control animals (150 ± 27 s for the senescent cuttlefish, 128 ± 33 s for the subadults, and 50 ± 2 s for the control animals). The statistical analysis shows a significant difference (Fig. 3, left) within each cuttlefish group ($p < 0.001$ for trials 2, 5, 6, 7; $p < 0.005$ for trials 1, 3, 4, 8, 9; Kruskal–Wallis test). The Mann–Whitney U -test ($p < 0.05$) indicates that the control animals are responsible for these differences (except for trial 3 between subadult and control groups); they paralyse the crabs more quickly than subadult and senescent animals, which have similar fibrillation time latencies. Taking into account only those animals that underwent all nine trials (Fig. 3, right), the same trend is found and the Kolmogorov–Smirnov test confirms the previous results: a significant difference appears between the control group and the animals fed with crabs with the fifth pereopods covered (adult and senescent groups) ($n = 7$, $p < 0.005$); no significant difference was seen between the senescent and subadult animals (Fig. 3, right).

3.2.4. Evidence of an improvement of fibrillation time latency across the different tests

We first pooled the senescent and subadult animals that were fed with crabs with the fifth pereopod covered based on the absence of significant differences between the fibrillation time latency of these animals, and estimated the occurrence of learning processes across the different trials. When all animals were considered, the results showed an improvement of performance only between trials 2–3 ($p < 0.05$; Wilcoxon test). The animals that survived up to the ninth trial, failed to show a significant improvement between trials (Wilcoxon test). When the animals of different age were separately considered, a significant improvement was seen only for subadult animals between trials 2–3 ($p < 0.05$; Wilcoxon test).

The calculation of Spearman's coefficient of correlation shows that there is no correlation between the rejection percentage and the mean fibrillation time latency. The success of

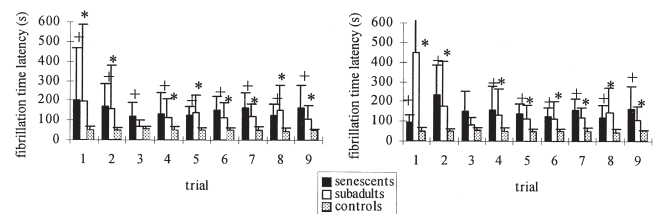


Fig. 3. Mean fibrillation time latencies \pm SEM plotted against the different trials. Left: all the cuttlefish are analysed. Right: only those cuttlefish analysed that survived all nine trials; * indicates a significant difference between control and subadult animals; + indicates a significant difference between control and senescent animals (Mann–Whitney U -test).

failure of manipulative behavior in any given trial has no effect on the fibrillation time latency during subsequent trials.

4. Discussion

The fibrillation time latencies for the control and P4 groups were almost the same. In contrast, the plastic cover on the fifth pereopod provoked a significant increase of the fibrillation time latency. Such results strengthen our earlier conclusions on the location of the cephalotoxin injection site on the crab's fifth pair of pereopods [5]. The systematic biting (or attempted biting) of the fifth pereopod suggests stereotypic motor programs that are responsible for the initial manipulative phase. Stereotypic motor programs are also found in other cephalopods, such as *Eledone*, which concentrates the boreholes for saliva injection close to the midline and on the posterior half of the crab's carapace [20], and *Octopus*, which drills holes into bivalves preferentially in the region of the heart [21].

The fact that the P4 group shows no significant difference from the control group, and the subadults show a rate of successful captures that is identical to that of the control animals indicates that the leg cover itself is not an aversive stimulus. The absence of any difference in fibrillation time latency between senescent and subadult cuttlefish rules out the possibility of reduced effectiveness or liberation of the cephalotoxin.

The protection of the articular basi-ischiocoxopodite membrane of the fifth pereopod prevents the cuttlefish from injecting cephalotoxin and, thus, forces it to change the injection site. The cuttlefish seem to try to perforate the plastic tube, but fail to do so; consequently, they explore alternative areas for perforation, which results in a longer fibrillation time latency. The present results can be compared with those of Wodinski [7]. He demonstrated that *Octopus* that were fed with *Strombus gigas* first try to pull out the snail's body by force; in case this fails, they drill a hole into the shell. When dental acrylic was used to cover the spire of the shell where the hole is usually made, the boring site was in the uncoated portion of the shell. When the shell was completely coated with acrylic, the animal pulled out the body of the snail by force without boring. Very small octopuses sometimes drilled the hole in nonsensical areas such that the toxin was released directly into the seawater. This phenomenon, however, did not occur more than once in a given animal, suggesting that *Octopus* may learn rapidly where to drill [7]. According to Mather's suggestion [22], the manipulative behavior of these animals may be an example of a combination of predisposition and learning. There may be a critical period during which juvenile cuttlefish learn through exploration to inject cephalotoxin into an area of least resistance (the basi-ischiocoxopodite junction on the fifth pereopod) that then causes rapid paralysis of the prey. The absence of any significant difference in percentages of captures followed by paralysis among subadults fed with crabs with the fifth pereopod covered and control

animals suggests that once this motor skill has been learned and the manipulative behavior has become automatic (which makes it look stereotyped), the subadult animals may maintain a minimum of flexibility to perform this behavioral task. The significantly higher number of rejections in senescent animals may result from a reduction in this flexibility of performance. The improvement between trials 2–3 of the fibrillation time latency indicates a processes of learning.

The manipulative behavior of the cuttlefish presents a certain degree of stereotypy but is not rigidly fixed. This flexibility of behavior seems more important in subadults than in senescent animals. The loss of flexibility in manipulative behavior in senescent animals could be due to a degeneration of the nervous structures involved in this behavior [1,4]. A marked terminal degeneration had been described in senescent cuttlefish in the neuropil of the anterior basal and inferior frontal lobes [23]. The manipulative behavior seems to be partly controlled by cholinergic networks within the inferior frontal lobe [1], and a substantial reduction in the choline acetyltransferase activity in senescent animals was recently demonstrated in that lobe [24]. Also, senescent cuttlefish have considerable behavioral deficits with aging, including an underestimation of tentacle ejection distance and a loss of long-term memory [23]. Thus, degeneration due to age and/or chemical modifications to the neuronal circuitry that controls this behavior may explain a reduced behavioral flexibility.

Acknowledgment

We are particularly grateful to E. T. Mackenzie for helping to correct the English translation.

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