

Short report

## Evidence for a specific short-term memory in the cuttlefish, *Sepia*

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### Abstract

Cuttlefish quickly learn to inhibit their predatory motor pattern when shown prawns in a glass tube. The available literature suggests that cuttlefish show an excellent retention between 2 and 8 min, a recovery of the predatory responses around 20 min and good retention after 1 h of the training phase. These results have been considered as the product of two separate short- and long-term memory stores. In this study, we have investigated the fact that the retention seen after a brief delay of the training phase corresponds to a true effect of learning. We compared animals under three experimental conditions. In two, there was a unique training trial of different duration (5 or 20 min), a third group served as controls. Our results demonstrate that the control situation failed to reduce the level of attack; in contrast the short-term retention, obtained after a single learning trial, is related to a specific short-term memory process. © 1998 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

The class Cephalopoda is the most highly evolved taxon within the Mollusca. Cephalopods exhibit complex behaviours and possess a sophis-

ticated central nervous system with an extreme fusion of ganglia, so that their ‘brain’ is subdivided into numerous lobes interconnected by tracts and commissures. Furthermore, some of their behavioral abilities, such as making detours (Wells, 1964; Moriyama and Gunji, 1997), reversal learning (Mackintosh and Mackintosh, 1964) and observational learning (Fiorito and Scotto, 1992), are equal to those of lower vertebrates.

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Among cephalopods, *Sepia officinalis* has been studied mainly for the neurobiology and behavioural aspects of its predatory behavior. Cuttlefish actively prey upon shrimps and capture them by shooting the two tentacles for a strike. This behaviour is visually driven (Messenger, 1968; Chichery and Chichery, 1987) and it has been extensively studied in laboratory conditions, where a shrimp is placed in a glass tube in the middle of the experimental tank and the cuttlefish attacks its prey without obtaining it (Sanders and Young, 1940; Wells, 1958, 1962; Messenger, 1971, 1973a; Chichery and Chichery, 1992a,b). In such a condition, *S. officinalis* quickly learns to inhibit the predatory motor pattern (Messenger, 1971, 1973a, 1977). Messenger (1973a) clearly demonstrated that the waning of the number of strikes was not the result of motor fatigue or a temporary incapacity to make a tentacle strike. The inhibition of the species-specific predatory behavior has been considered as a form of associative learning since the striking of the tentacles against the glass represents a negative reinforcement ('painful' stimulus) for the cuttlefish (Messenger, 1973a, 1977).

Using different durations of a unique training trial, Messenger (1973a) demonstrated that the level of learned performance is directly related to the amount of negative reinforcement that *S. officinalis* received during the training. He also reported differential performances of adult animals at different times of testing for memory recall: cuttlefish showed excellent retention between 2 and 8 min, a recovery of the predatory response at around 20 min and again a very good retention after 1 h of the training phase. The biphasic curve resulted from these experiments (Messenger, 1971, 1973a, 1977) has been considered as a product of two separate memory stores, a short-term memory (STM) and a long-term memory (LTM), such as have been found in other animals (e.g. honeybees: Menzel, 1984; slugs: Yamada et al., 1992).

However some questions can be raised about the possibilities that some non-specific effects—such as stress or 'contextual fear' in the experimental conditions, or disturbance caused by the experimenter's manipulation of the apparatus—

may induce inhibition of predatory behaviour. Cuttlefish appear to be extremely sensitive animals. Quite small changes in the experimental or maintenance tank, for instance, may cause enough disturbance to the animal to inhibit the spontaneous emergence of predatory behavior. The aim of this paper is to identify whether contextual and other non-specific factors could affect cuttlefish learning performances and particularly to ascertain whether the good retention performances observed following short-term intervals do indeed correspond to the expression of a STM store.

## 2. Materials and methods

### 2.1. Animals

*S. officinalis* juveniles reared in the laboratory and aged from 15 to 30 days (10–17 mm of mantle length) and subadults/adults, trawled in coastal waters off Ouistreham (France) (23–140 mm of mantle length), were maintained individually in opaque plastic tanks (9 × 24 × 6 cm for the youngest animals and 80 × 60 × 40 cm for the others) with running seawater kept sterilized by UV lamps. Animals were fed ad libitum with *Mysis* for the youngest and shrimps for the others. We discarded any animals showing external scars or not eating regularly. After acclimatization (3 days), animals were assigned to three experimental groups, each divided into two subgroups on the basis of their age. The different age groups were distributed in equal numbers in the three experimental batches. Each tested animal was naive and used only once.

### 2.2. Apparatus

The experimental apparatus is identical to that described by Messenger (1971, 1973a). A transparent glass tube filled with seawater and containing prey (*Mysis* for the 15/30-day old animals, shrimps for the others) was placed in the cuttlefish's tank. A slight seawater current in the tube ensured that the prey was kept constantly moving throughout the experiment (Chichery and Chichery, 1992a,b). The glass tube was introduced into

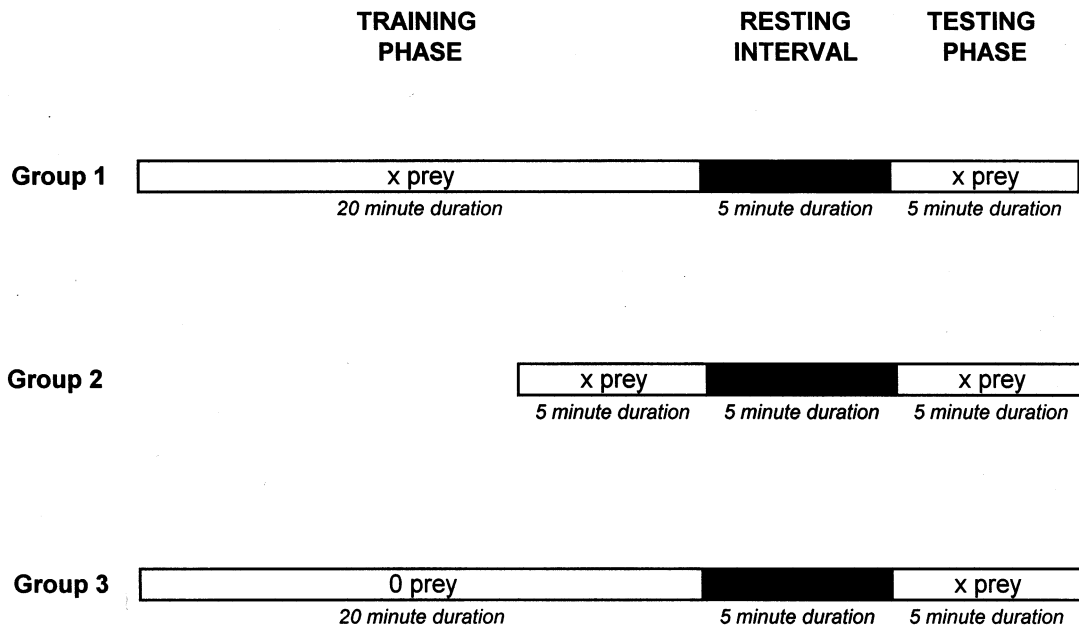


Fig. 1. Schematic representation of the experimental procedure. The number of strikes is counted during the first 5 min of the training phase and during the total duration of the testing phase.

the cuttlefish tank at least 12 h prior to the start of the experiment, during which time the prey was concealed behind an opaque plastic cylinder placed around the tube. The same opaque cylinder was placed around the glass tube in the interval between the learning and the retention experimental phases.

### 2.3. Procedure

A schematic view of the experimental procedure is given in Fig. 1. The first group of animals ( $N = 33$ ) were treated with a protocol identical to that utilized by previous works (Messenger, 1971, 1973a).

The glass tube containing the prey was opened to the view of cuttlefish for a single trial of 20 min (training phase). During this time, the number of strikes that cuttlefish exhibited during the first 5 min were counted. At the end of the training phase, the opaque cylinder was again gently placed around the glass tube (in 3–4 s) and left for 5 min. At the end of this resting interval, the opaque cylinder was removed again and we

counted the number of strikes during a single 5-min trial (testing phase), to determine the level of memory recall of *S. officinalis* for the inhibition of their striking predatory behaviour.

The second group of cuttlefish ( $N = 33$ ) were tested with the same protocol as the first experimental group, with the exception that the training phase was reduced to 5 min only.

*S. officinalis* of the third experimental group ( $N = 33$ ) had the same exposure to the experimental conditions as group 1, but no prey was presented to the animals during the training phase and it represents the control group. In brief, the glass tube was exposed with no prey inside during the 20 min of the training trial and after a 5 min interval, animals were tested for their predatory behavior during a single trial (5 min, testing phase), where prey were included in the glass tube of the apparatus. The number of strikes that cuttlefish made toward the glass tube were measured during both training and testing phases.

All statistical analyses were carried out following Siegel and Castellan (1988), Zar (1984) and using SYSTAT.

### 3. Results

Fig. 2 presents the mean number of strikes exhibited by all animals, grouped by age category and experimental groups, during the training and testing phases.

The data of the number of strikes exhibited by *S. officinalis* during the first 5 min of the training phase (groups 1 and 2) and those of the

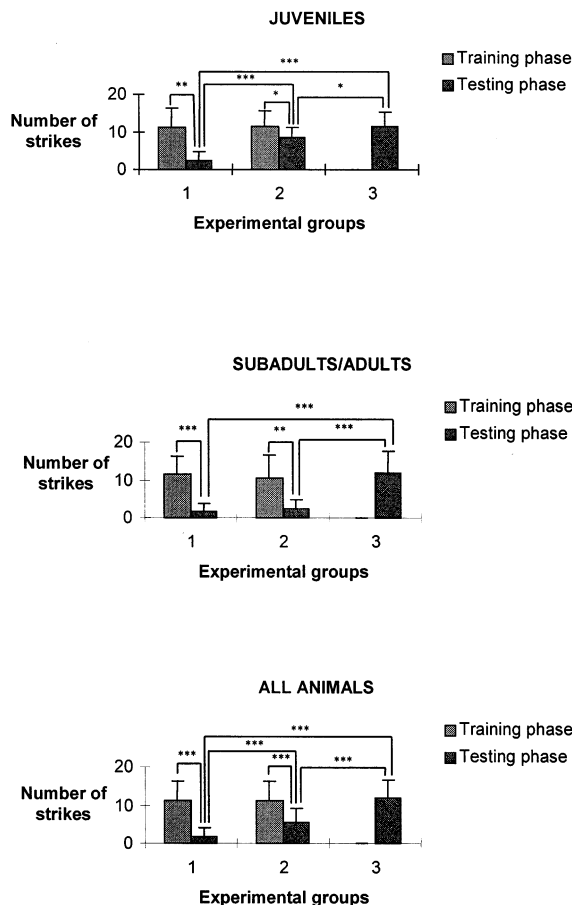


Fig. 2. Mean number of strikes ( $\pm$  S.E.M.) in the first 5 min of training and during the testing phase. \* indicates a significant difference (Wilcoxon test for matched paired data between training and testing phases within a group, Mann–Whitney U-test between groups; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). In juvenile animals,  $n = 14, 16, 17$  for groups 1, 2 and 3 respectively; in subadult/adult animals,  $n = 19, 17, 16$  for groups 1, 2 and 3 respectively; in all animals,  $n = 33$  in each group.

testing phase of group 3 were compared by non-parametric Mann–Whitney U-test. All animals show a high number of capture attempts and no statistical difference resulted between the three groups or the different age categories or pooled animals. The number of strikes was also compared using data from the animals at the testing phase. The analysis shows that no statistical difference appears between groups 1 and 2 for subadult/adult animals (Mann–Whitney U-test). In contrast we obtain statistical difference between the three groups in the juveniles (Mann–Whitney U-test,  $U = 12.5$ ,  $n_1, n_2 = 14, 16$ ,  $P < 0.001$ ;  $U = 5$ ,  $n_1, n_3 = 14, 16$ ,  $P < 0.001$ ;  $U = 69.5$ ,  $n_2 = n_3 = 16$ ,  $P < 0.05$ ), between groups 1 and 3 and 2 and 3 for the subadult/adult animals (Mann–Whitney U-test,  $U = 8$ ,  $n_1, n_3 = 19, 16$ ,  $P < 0.001$ ;  $U = 10$ ,  $n_2, n_3 = 17, 16$ ,  $P < 0.001$ ) and between all groups in pooled animals (Mann–Whitney U-test,  $U = 234$ ,  $n_1 = n_2 = 33$ ,  $P < 0.001$ ;  $U = 27$ ,  $n_1 = n_3 = 33$ ,  $P < 0.001$ ;  $U = 168.5$ ,  $n_2 = n_3 = 33$ ,  $P < 0.001$ ).

In order to verify whether learning affects the level of striking, we compared the predatory behavior (number of strikes) of the animals during training and testing phases (Fig. 2) for the two experimental groups (1 and 2) that were exposed to both conditions. Wilcoxon matched pair test demonstrates that retention is significant for juvenile animals of groups 1 and 2 (Wilcoxon test,  $Z = -3.301$ ,  $n_1 = 14$ ,  $P < 0.01$ ;  $Z = -2.358$ ,  $n_2 = 16$ ,  $P < 0.05$ ), for subadult/adult animals of groups 1 and 2 (Wilcoxon test,  $Z = -3.728$ ,  $n_1 = 19$ ,  $P < 0.001$ ;  $Z = -3.469$ ,  $n_2 = 17$ ,  $P < 0.01$ ) and for pooled animals of groups 1 and 2 (Wilcoxon test,  $Z = -4.942$ ,  $n_1 = 33$ ,  $P < 0.001$ ;  $Z = -4.210$ ,  $n_2 = 33$ ,  $P < 0.001$ ).

Fig. 3 shows the retention performances of animals in groups 1 and 2. These are expressed as a percentage of the number of strikes performed during the testing phase compared to the number of strikes registered during the first 5 min of the training phase. Non-paired student's  $t$ -tests using arcsin transformed values indicate that there is no statistical difference between the retention performances in subadult/adult animals of groups 1 and 2. However the retention performances are significantly better for group 1

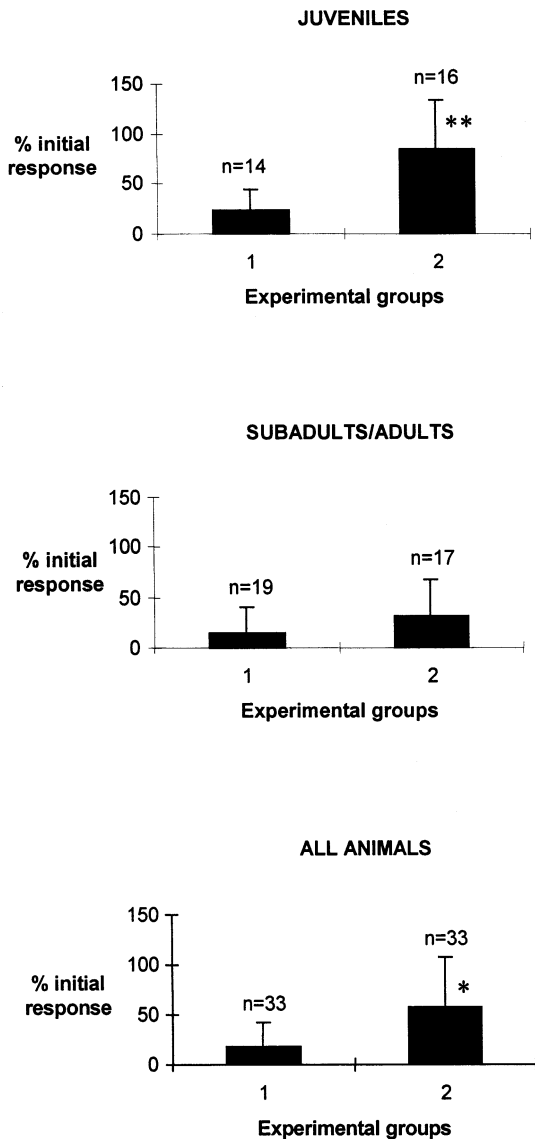


Fig. 3. Mean retention performances ( $\pm$  S.E.M.) at 5 min in experimental groups 1 and 2. *n* indicates the number of animals; \* indicates a significant difference with arcsinus transformed values (Non-paired *t*-test), \**P* < 0.01, \*\**P* < 0.001.

animals than group 2 animals in juvenile animals (Non-paired *t*-test,  $t_{12} = -4.608$ ,  $P < 0.001$ ) and also in pooled animals (Non-paired *t*-test,  $t_{12} = -3.446$ ,  $P < 0.01$ ).

#### 4. Discussion

Our experiments show that the number of strikes made by cuttlefish when first presented with prey in a glass tube is very similar (Fig. 2) and that the number of strikes to the tube is reduced to zero when the prey is not introduced in the glass tube (group 3). This result clearly shows that different possible factors—feeding motivation, type of prey used, different times at which experiments are performed—do not interfere with the emergence of predatory behavior in *S. officinalis* tested using the ‘prey-behind the glass tube’ protocol.

Our data on pooled animals confirm earlier observations with a 24 h retention delay (Messenger, 1973a) in showing that there is indeed an inverse relationship between the number of strikes and the duration of the initial training trial, i.e. an increase in the time of exposure to the glass tube containing the prey corresponding to a proportional decrease in the number of strikes exhibited by animals during the retention phase. However, in our conditions for the juvenile animals, a 5 min duration of the training phase is probably too short to obtain good retention performances. In contrast, this duration allows sufficient learning for subadult/adult animals, which could explain the absence of statistical difference between the retention performances of groups 1 and 2 (Figs. 2 and 3). Messenger (1973b) has already stressed the low learning capacities in juvenile animals. Overall, the response of cuttlefish during the retention tests should appear to be a direct consequence of a specific learning process.

The experiment presented in this paper highlights the fact that manipulations made to the experimental apparatus between the training and testing phases have no apparent effect on the animals’ responses. In fact, when *S. officinalis* are subjected to the same amount of manipulation of the experimental apparatus (animals of the group 3), there is no inhibition of their predatory capture attempts during the second experimental session (first exposure to a prey in the tube during the testing phase).

The results of this research clearly show that the good short-term retention performances of *S.*

*officinalis*, subjected to an initial 20 min presentation, definitely correspond to a specific learning effect. The validation of the experimental protocol used in earlier works (Wells, 1958, 1962; Messenger, 1971, 1973a) is a necessary starting point before undertaking a closer investigation of the memory recall processes of juvenile cuttlefish and before undertaking a study of the neurobiological correlates that underpin these short-term retention processes.

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