LEARNING PERFORMANCES AND AGING IN CUTTLEFISH (*SEPIA OFFICINALIS*)

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Abstract — The use of an associative learning protocol with negative reinforcement allowed us to compare the performances of three groups of animals: young, middle-aged, and senescent. Statistical analysis of the results does not enable us to assert that a significant difference exists between the learning speeds of young and senescent animals. On the other hand however, the latter show poor scores in a retention test carried out 24 h after the learning session. These results suggest that the long-term memory processes are affected during the aging of these animals.

Key Words: learning, aging, long-term memory, *Sepia officinalis*, cephalopod, mollusc

INTRODUCTION

LEARNING STUDIES performed in mammals have revealed many age-related changes. Despite numerous studies in this field the interpretation of these modifications remains an open question (Sarter, 1987). For many years now, numerous studies have been carried out on nonmammalian models researching aging (Lints, 1985). The ontogenesis of the behavior of cephalopods has been the subject of a few detailed studies. Among these, the evolution of predatory behavior (Wells, 1958, 1962) and the evolution of cognitive capacities are to be noted (Messenger, 1973b, 1977). For the latter, an improvement in learning experiments has been correlated with a large increase in the vertical system during the postembryonic period of the *Sepia officinalis* (Wirz, 1959; Messenger, 1973b, 1977). Contrary to this, no study has been carried out on the evolution of behavior during aging. However, the decrease in cognitive capacities with age has been shown in various animal species. With Mollusca, various experiments (especially dealing with the *Aplysia*) have shown a decrease in performance during nonassociative or associative learning in the period of aging (Bailey *et al.,* 1983). The cephalopods are by far the most highly developed of the molluscs. Their cognitive capacities are well-known and certain criteria like detour experiments (Wells, 1964) and improvement in reversal discriminative learning (MackIntosh and MackIntosh, 1964) allow us to position them alongside the vertebrates.

The life cycle of the cuttlefish in the English Channel (Richard, 1971) is characterized by a succession of relatively homogeneous population cohorts. The adults aged between 21–22 month (average length of the mantle: 24–25 cm) migrate towards coastal waters from the beginning of April to the beginning of May for reproduction. These animals then have a very rapid phase of senescence which leads to their death at the end of June or the beginning of July. The maximum peak of egg laying is in May and the maximum peak of hatching is at the end of July or the beginning of August. The young cuttlefishes then grow quickly and leave the cold coastal waters at the end of October. The following spring (at the end of May or the beginning of June) these cuttlefish (average length of the mantle: 10–11 cm) return to the coastal waters and undergo a new phase of extremely rapid growth. Then, the animals once again leave the coastal waters before the winter. At this stage they average 16 months in age. The males are all mature, and the females are undergoing sexual maturation (average length of the mantle: 16–17 cm). These animals return to the coastal waters the following spring for reproduction. Thus, according to the size of the animal and the date of its capture, an age can easily be assigned to it, and in the case of the older animals, an estimation can be given of the likely date of its natural death.

In this paper we will deal with the influence of age on memory in a situation of associative learning with negative reinforcement.

MATERIALS AND METHODS

Experimental animals

Cuttlefishes *(Sepia officinalis)* were caught off Luc-sur-Mer. They were first placed for a few days in big circular tanks (1.75 m diameter × 0.7 m height) filled with seawater. After a period of adaptation to this environment, we discarded any animals showing external scars or not eating regularly. The remaining animals were selected and kept separately in experimental opaque plastic tanks (0.80 × 0.60 × 0.40 m) containing running and filtered seawater to a depth of 0.30 m. The cuttlefish is a highly active predator. Prey is detected by visual input, the latter playing an important role in the various stages of predatory behavior. After orientating and positioning phases, the cuttlefish attacks its prey choosing one of two distinct strategies (Boycott, 1958; Messenger, 1968; Duval *et al.,* 1984; Chichery and Chichery, 1987, 1991). Fish and shrimp are usually captured by tentacle ejection (when not engaged in predatory behavior the two long tentacles are folded into subocular pockets). Large crabs are captured by the eight short arms of the predator after the latter has pounced on its prey.

Pilot experiments

Various authors (Sanders and Young, 1940; Wells, 1958, 1962; Messenger, 1971, 1973a) have shown that adult cuttlefish quickly learn to inhibit their predatory behavior toward shrimp enclosed in a glass tube. Messenger (1973a) has demonstrated that this learning is an associative process, the tentacle ejection on the glass tube delivering a pain input to the brain. During the spring of 1989, the first group of animals (29 animals aged 21–22 months) was chosen for preliminary experiments. Our protocol is simple and identical to that described by Messenger (1973a). A glass tube filled with seawater and containing three shrimps was placed in the tank with the cuttlefish. The training procedure

was the following: Naive cuttlefishes were shown shrimps in a tube for eight successive trials of 3 min each, separated by an interval of 30 min. A retention test was practiced 24 h later. During each trial, we counted the number of tentacle ejections. Careful examination of individual learning curves showed great variability. This variability may be a handicap when attempting to carry out reliable comparisons between animals of different cohorts. Two factors seem partly responsible for this variability. Many authors (e.g., Sarter, 1987) have emphasized the first of these two factors, which concerns food motivation (difficult to control). In our test with the preliminary group, we compared the performances, after four trials, of animals who made 12 or more capture attempts during the first trial ($M = 13.2$, highest level of food motivation) and animals having made 9 or less capture attempts during the first trial ($M = 7.3$, lowest level of food motivation). Statistical analysis (Mann–Whitney test) showed no significant difference between these two groups. Thus, the control of the length of fasting (according to the size of the animals and the temperature of the water), of preliminary food consumption, and of the level of capture attempts during the first trial should minimize the effects of this first factor. The second factor seemed more important, for, in this protocol, the movement of the prey is not constant throughout the learning experiment. The cuttlefish, as is the case for many visual predators, is sensitive to the movements of the prey. Thus, a temporary halt in the movement of the prey may lead to a decrease in capture attempts without there being any link to the learning process whatsoever (and vice versa). Consequently, during 1990 we redid the experiment, modifying the conditions to eliminate this artifact.

Primary experiments

Three shrimp were enclosed in a glass tube (7 cm in diameter) with a basal tibulation. This tibulation was connected to a small pump which provoked a water flow sufficient enough to cause the permanent movement of the shrimps throughout the experiment. The learning protocol was identical to that described above: eight trials (3 min each) separated by 30 min, and a retention test 24 h later. The experiments were carried out in plastic tanks used for breeding so that the animals were perturbed as little as possible. Between each trial, an opaque plastic cylinder was carefully placed around the glass tube. The whole device was placed in the tank the day before the experiment.

During different trials in his work, Messenger (1973a) counted the number of tentacle ejections. The all-or-none behavior was clearly distinguishable and consequently easily quantifiable. Messenger eliminated the capture attempts that employed the "jumping strategy," as the cuttlefish, which were studied in the Bay of Naples during his experiment, only rarely used this strategy. In our pilot experiments the cuttlefish showed greater behavioral flexibility, possibly as a result of their previous experience and of a different diet. It thus seemed essential to us to count all capture attempts regardless of the strategy (the tentacle ejection strategy remained nonetheless predominant). Three age groups were used: young adults (about 11 month old, $N = 14$, Group Y), middle-aged animals (about 16 month old, $N = 14$, Group M), and old animals (about 22 month old, $N = 15$, Group O), this last group having been tested 1 month before the average peak of mortality in this population cohort.

In order to compare the results as best as could, we estimated the different performances of each trial as a percentage of the level of the initial response.
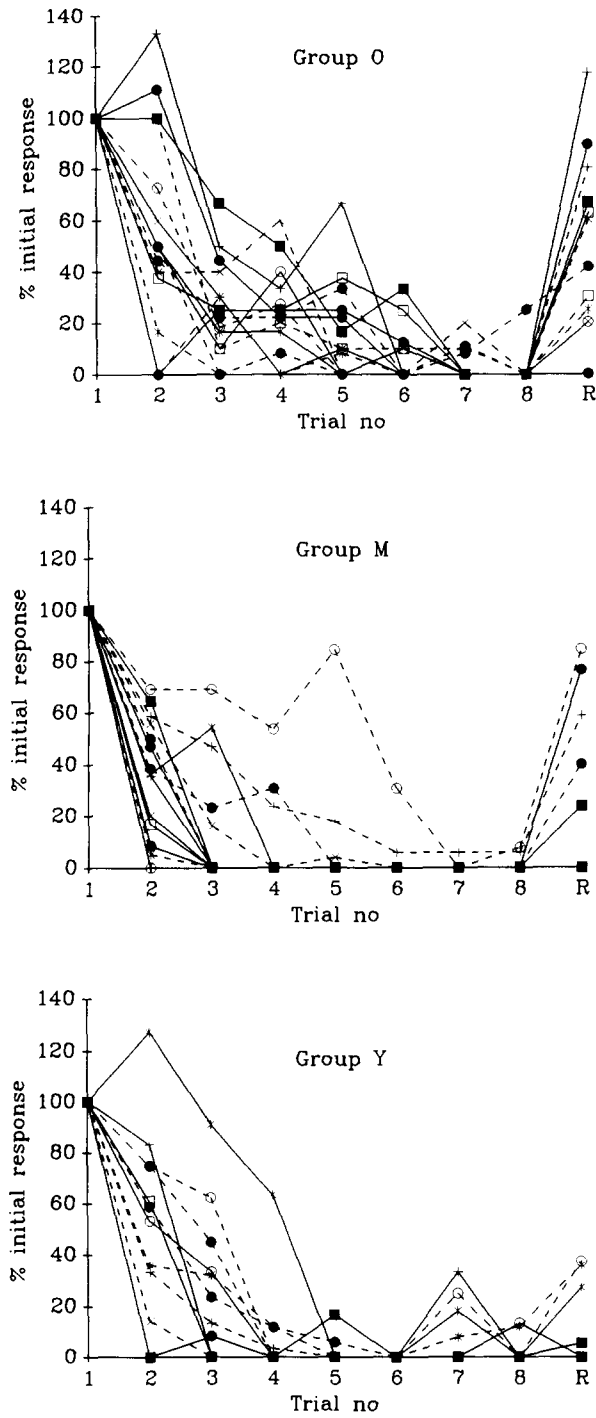
FIG. 1. Individual curves of the learning of old (aged about 22 month: Group O, $N = 15$), middle-aged (aged about 16 month: Group M, $N = 14$), and young animals (aged about 11 month: Group Y, $N = 14$).
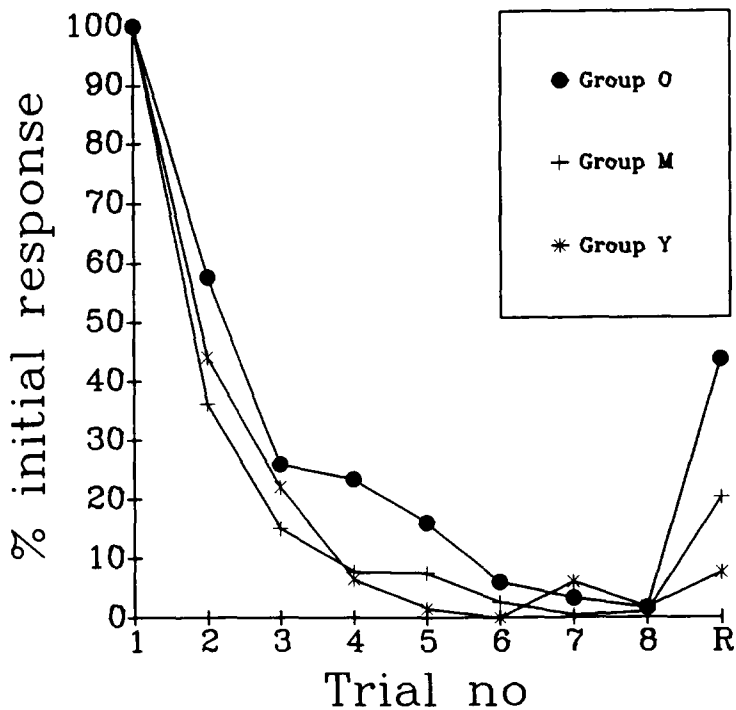
FIG. 2. Average performance curves of the three experimental groups.

RESULTS

Figures 1 and 2 summarize the main results. The three groups of animals behaved similarly at the eighth trial. There is no significant difference between the Groups Y and O in the number of captures made during the second and third trials. Indeed, a significant difference appears only during the fourth and fifth trials ($U = 41$, $U = 44.5$, $p < 0.01$, Mann–Whitney test). The animals' performances are once again noted as almost identical during the sixth, seventh, and eighth trials. In the retention test a significant difference ($U = 38$, $0.001 > p < 0.01$, Mann–Whitney test) appears between Groups Y and O, and a greater dispersion in the scores of the older animals is also to be noted (Group O, mean $= 43.7 \pm 36.5$; Group Y, mean $= 7.6 \pm 14.3$). The comparison of animals in Groups O and M gives almost identical results. The difference only becomes significant during the fourth ($U = 49.5$, $p < 0.01$ Mann–Whitney test) and the fifth ($U = 57.5$, $p < 0.025$ Mann–Whitney test) trials. This difference disappears during the sixth, seventh, and eighth trials. The retention test also indicates a significant difference ($U = 56.5$, $p < 0.025$ Mann–Whitney test). The comparison of Groups Y and M shows no significant difference whatsoever, regardless of the trials taken into account.

This statistical analysis, however, does not allow a good comparison of the pattern of learning across trials by each cohort. The comparison of the 5 first trials between groups O and Y and groups M and O shows the biggest differences, but these remain nonsignificant ($K_D = 5$, NS, Kolmogorov–Smirnov two-sample test, significant at $K_D = 7$, $N = 14$, $p \leq 0.05$).

DISCUSSION

The results of this study show that the common cuttlefish learns the experimental test easily. It is possible to consider the learning process as established from the sixth trial regardless of the group of animals tested. From the third trial onwards, a considerable inhibition in predatory behavior was noted. The average curves show a more definite negative slope than those obtained by Messenger (1973a). Our device, which controlled the movement of the prey, increased the number of background stimuli most probably perceived by the animal and thus facilitated its learning. The level of performance reached at the end of the experiment was almost identical for the three groups of animals. Although the slope of the average learning curve is more definite for Groups Y and M, the Kolmogorov–Smirnov test does not confirm a significant difference which would allow us to assert that the learning process is slower in the oldest animals. Additional research on a larger number of animals should allow us to check this point. The retention test of these older animals shows higher and more dispersed scores, revealing deleterious effects on the long-term memory process. Some 22-month-olds learn very quickly. Our method does not, however, enable the control of their earlier experiences. The breeding of this species is possible (Richard, 1975; Forsythe et al., 1991), and may eliminate this variability and allow a comparison of the results with those of captured animals. Many authors, however, have underlined the greater variability of cognitive capacities with increasing age in various animal models (Gage and Bjorklund, 1986). Some of the old animals performed as well as the young animals.

This significant difference is maintained between the older and middle-aged animals with the latter behaving in the same way as the youngest adults used in this study.

From this first study, it would seem that the senile process of the cuttlefish is not linear and that it tends to appear late in the biological development of this species. Present day research associating the use of different learning protocols and investigations of the correlations in the central nervous system should allow us to discover whether or not the common cuttlefish represents an interesting model for the study of aging mechanisms.

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