Ludovic Dickel*
Jean G. Boal
Bernd U. Budelmann
National Resource Center for Cephalopods
Marine Biomedical Institute
University of Texas Medical Branch
Galveston, TX 77555

The Effect of Early Experience on Learning and Memory in Cuttlefish

Received 14 April 1999; accepted 10 August 1999

ABSTRACT: *The effect of early experience on the growth and ontogeny of memory in cuttlefish* (Sepia officinalis) *was studied using an associative learning protocol. Five groups of cuttlefish were reared in different conditions (standard conditions, SC; impoverished conditions, IC; enriched conditions, EC; impoverished then enriched conditions, I/EC; enriched then impoverished conditions, E/IC) from birth to the 3rd month of postembryonic life. Acquisition and retention of the learning task were assessed at 1 and 3 months. Growth was slower and maturation of memory abilities occurred later in cuttlefish from Group IC than in cuttlefish from Group EC, with the maturation rate of memory in cuttlefish from Group SC intermediate between these two groups. Retention performances of cuttlefish from Groups I/EC and E/IC indicated that the environment of rearing during the 2nd and/or 3rd months of life was crucial for the development of memory.* © *2000 John Wiley & Sons, Inc.* Dev Psychobiol 36: 101–110, 2000

Keywords: *associative learning; ontogeny of memory; cephalopods*

INTRODUCTION

Among invertebrates, the coleoid cephalopods (octopuses, cuttlefishes, and squids; hereafter, referred to as "cephalopods") show particularly good learning and memory abilities. These abilities, and the neurobiology associated with them, have been studied most extensively in adult *Octopus vulgaris* (for review, see Hanlon & Messenger, 1997; Sanders, 1975). Learning and memory abilities have been less thoroughly explored in cuttlefish (for review, see Hanlon & Messenger, 1997; Messenger, 1977) even though the common cuttlefish, *Sepia officinalis*, is relatively easy to rear and keep under laboratory conditions (Forsythe, DeRusha, & Hanlon, 1994; Forsythe, Hanlon, & DeRusha, 1991). Unlike *Octopus vulgaris*, which has planktonic hatchlings that later settle to a benthic way of life, *Sepia officinalis* hatchlings show the same basic behavior as adults (Boletzky, 1987; Wells, 1958, 1962); this makes a single learning paradigm applicable in this species at each different stage of its postembryonic development.

Messenger (1971) devised a simple learning protocol to test learning retention in adult cuttlefish. During training, cuttlefish were offered prey items enclosed in a glass tube. Cuttlefish normally capture mobile prey by rapidly shooting out two long tentacles. In the experimental apparatus, the cuttlefish were unable to capture the prey but, instead, hit the glass tube. During a continuous 20-min training session, the

**Present address:* Laboratoire de Psychophysiologie, Université de Caen, 14032 Caen Cedex, France
Correspondence to: L. Dickel
Contract grant sponsor: FYSSEN Foundation
Contract grant sponsor: NIH (NCRR)
Contract grant number: RR 0124

cuttlefish showed a substantial waning of capture attempts. By using cuttlefish with their tentacles removed and a small electric shock, Messenger (1973a) demonstrated that the waning of the response during training was related to the amount of negative reinforcement received at each attempted capture. Messenger concluded that inhibition of tentacle strikes during learning was, at least partially, related to the "pain" following each hit of the tentacle clubs on the glass. After a 24-hr delay (during which the cuttlefish were starved), the cuttlefish were again presented with the prey in the glass tube. The inhibition of the predatory behavior of the cuttlefish toward the prey in the glass tube remained highly effective, demonstrating 24-hr retention of learning.

Deficiencies in acquisition processes have been reported in juvenile cuttlefish, as compared to adults (Agin, Dickel, Chichery, & Chichery, 1998; Wells, 1962). In juvenile cuttlefish, memory abilities increase throughout early and late postembryonic development (Dickel, Chichery, & Chichery, 1997, 1998; Messenger, 1973b). These developmental changes correlate with the late maturation of brain structures (vertical lobe complex) associated with visual learning and memory (Dickel et al., 1997; Messenger, 1973b; Wirz, 1954).

Rearing environment plays a crucial role in the maturation of learning and memory processes in mammalian and avian species (for reviews, see Renner & Rosenzweig, 1987; Rosenzweig & Bennett, 1996). Because this important factor has not yet been studied in invertebrates, the aim of this study is to determine the impact of different rearing conditions on subsequent retention of learning in juvenile cuttlefish.

METHODS

Animals

Cuttlefish (*Sepia officinalis*) hatchlings were obtained from the National Resource Center for Cephalopods at the Marine Biomedical Institute in Galveston, TX. Immediately after hatching, the cuttlefish were placed into one of two groups. In the first group ("impoverished conditions," Group IC), cuttlefish ($n = 40$) were individually isolated in bare, opaque plastic tanks (120 × 90 × 75 mm). They were fed ad lib during the first 2 weeks of life with mysid shrimp, and thereafter with *Palaemonetes* of suitable size. To accommodate the growth of the cuttlefish, the tanks were enlarged after 60 days (160 × 90 × 75 mm). In the second group ("enriched conditions," Group EC) cuttlefish ($n = 40$) were placed together in two opaque rectangular tanks (600 × 400 × 200 mm; $n = 20$ in each tank). The bottom of each tank was covered with fine sand (1–2 cm thick; enough to allow the cuttlefish to bury), and rocks, shells, and plastic seaweeds were liberally distributed throughout each tank. Cuttlefish from this second group were fed ad lib in the same manner as the impoverished group.

At 1 month of age, 13 cuttlefish from the impoverished group were transferred to enriched conditions (Group I/EC) and 12 cuttlefish from the enriched group were transferred to impoverished conditions (Group E/IC).

All cuttlefish were maintained in the same system of recirculating oxygenated seawater (a mix of natural seawater from the Gulf of Mexico and artificial seawater made from Fritz sea salts; temperature 22.5 ± 1°C; salinity 33–35 ppt). Illumination from natural light was supplemented with overhead artificial florescent lights from 8 a.m. to 6 p.m.

In addition, one further group of 20 cuttlefish was hatched and reared in standard laboratory conditions ("standard conditions," Group SC) at the National Resource Center for Cephalopods (Forsythe et al., 1994; Forsythe et al., 1991). This group was reared communally with several hundred other hatchlings in a single, barren tank (3.65 × 1.82 × 0.49 m, water temperature 20 ± 1°C; salinity 33–35 ppt). Cuttlefish were fed ad lib with shrimps of suitable size.

Experimental Procedures

The dorsal mantle lengths (DML) of the cuttlefish from Groups IC, EC, I/EC, and E/IC were measured every month with a dial caliper (0.1 mm accuracy). Growth was compared between groups using Student's t tests for nonpaired data initially, and Kruskal–Wallis one-way ANOVAs followed by multiple comparisons based on a Mann–Whitney U test (Siegel & Castellan, 1988; Sokal & Rohlf, 1969). Daily growth rates used for comparisons were determined using the instantaneous coefficient of growth (Forsythe & Van Heukelem, 1987) calculated from the equation:

$$ln(DML_2) - ln(DML_1)/T_2 - T_1$$

where DML_2 is the mean dorsal mantle length at the age T_2 and DML_1 is the mean dorsal mantle length at the age T_1. Individual growth rates could not be computed because individuals were not tagged.

The cuttlefish reared in standard laboratory conditions (Group SC) were measured only immediately after behavioral testing. This group was not included in comparative analyses of growth because growth rate is strongly influenced by temperature (Richard, 1971) and the water temperature for this group was consistently lower than the water temperature for the other four groups.

Cuttlefish were trained and then tested for retention of learning 24 hr later at 1 month (between 25 and 35 days of age; Groups IC30, EC30, and SC30) and at 3 months (between 83 and 97 days of age; Groups IC90, EC90, I/EC, E/IC, and SC90). All cuttlefish were tested only once.

The experimental apparatus consisted of a small glass container enclosing several shrimp. The shrimp were kept constantly moving by a current of water. Before and after training sessions, an opaque plastic cover was placed over the glass container to hide the prey.

At the start of training sessions, cuttlefish were placed individually into test tanks (opaque tanks—120 × 90 × 75 mm at 30 days and 160 × 90 × 75 mm at 90 days) and fed normally for at least 48 hr. The glass container was then introduced, with the opaque cover in place. For the next 24 hr, the cuttlefish were underfed (only two shrimps about 20 mm length at 1 month and about 30 mm length at 3 months) to insure that the cuttlefish were hungry at the start of the trial.

The training session was begun by removing the opaque cover, allowing the cuttlefish to observe the moving, unreachable prey in the glass container. The exact time that training was considered to have commenced was the time of the first tentacle strike on the glass. Cuttlefish that did not make any attempt to capture a shrimp within the first minute were set aside, fed with two shrimps, and trained the following day.

To insure that the same criterion of acquisition was used for all cuttlefish, the glass container was presented continuously to each cuttlefish until it made only one strike in 2 consecutive min after the 18th min (i.e., minimum duration of initial training was 20 min). At the end of the training session, the opaque cover was refitted onto the apparatus.

Retention tests were performed after 24 hr, during which time the cuttlefish were not fed. The cover was removed from the glass container so that the cuttlefish could again see the prey, and the number of tentacle strikes was recorded. The time limit for the retention test was 6 min after the first tentacle strike, or 15 min if no strike occurred.

To insure that the behavior of the cuttlefish was not inadvertently influenced by the experimenter, all acquisition and retention sessions were videotaped for later analysis (Sony camera mounted 50 cm above the test tanks and connected to a remotely located JVC monitor and Panasonic VCR).

Analysis of Behavioral Data

Only tentacle strikes were used to measure learning and retention performances because only with a tentacle strike did the cuttlefish have the opportunity to learn the negative association between strike and "pain" (Messenger, 1973a). The numbers of tentacle strikes were plotted in 3-min blocks (minimum of six 3-min blocks during training, T1 to T6, and two 3-min blocks during retention test, R1 and R2).

To evaluate the acquisition performances within groups, the number of tentacle strikes (in absolute terms) observed during T1 of the training session was compared with the number observed during T6. To evaluate 24-hr retention, the number of tentacle strikes (in absolute terms) observed during T1 was compared with the number observed during R1. For cuttlefish showing poor 24-hr retention, reacquisition was evaluated by comparing the numbers of strikes observed during T2 with the number observed during R2. In each case, the statistical significance of differences between the two time periods was evaluated using a Wilcoxon signed-ranks test for matched samples (Siegel & Castellan, 1988).

To compare acquisition and retention performances between groups, it was necessary to correct for any potential differences in the initial levels of predation of the cuttlefish reared in different conditions. Thus, numbers of tentacle strikes during T6 and R1 were expressed as a percentage of T1. In the case of multiple comparisons (for 90-day groups), a Kruskal–Wallis one-way ANOVA was used to determine any rearing condition group effect on learning and retention. The statistical significance of differences between any two groups being compared was evaluated using multiple comparisons based on Mann–Whitney *U* tests (Siegel & Castellan, 1988; Sokal & Rohlf, 1969).

RESULTS

Effect of Environment on Growth

The DML of all cuttlefish were measured within 3 days of hatching ($N = 80$), and at 30 days ($N = 77$), 60 days ($N = 54$), and 90 days ($N = 33$) posthatching. The cuttlefish from Group SC (standard conditions) measured approximately 8 mm DML at hatching, 29.4 ± 0.9 mm DML at 1 month, and 57.7 ± 1.2 mm DML at 3 months.

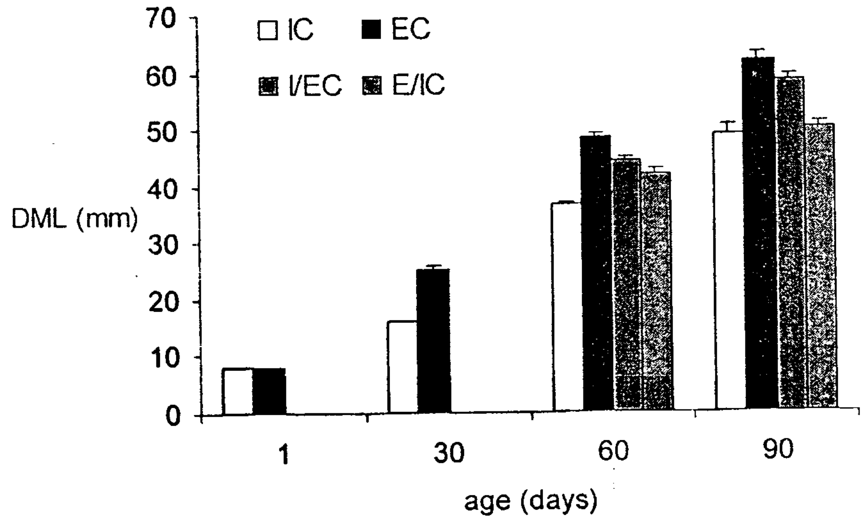

FIGURE 1 Dorsal mantle length (DML) plotted against age. Vertical bars indicate *SEM*.

The hatching size of cuttlefish from the IC and EC groups was approximately 8 mm DML. By Day 30, cuttlefish kept in enriched conditions (Group EC) were significantly larger than those reared in isolation, Figure 1, $t = -22.379$, $df = 75$, $p < .001$. This difference persisted throughout the study, 60 days: $U = 256$, $n1 = n2 = 16$, $p < .001$; 90 days: $U = 70$, $n1 = 7$, $n2 = 10$, $p < .01$; Figure 1.

The effect of rearing conditions on cuttlefish DML was again significant at 60 days, $H = 41.123$, $df = 3$, $p < .001$. At this time, the sizes of cuttlefish in the two switched groups (I/EC and E/IC) were similar to each other and intermediate between Groups IC and EC. Each was also significantly different in size from the group it originated from, Groups IC and I/EC: $U = 208$, $n1 = 16$, $n2 = 13$, $p < .001$; Groups EC and E/IC: $U = 137$, $n1 = 9$, $n2 = 16$, $p < .001$; Figure 1.

The effect of rearing conditions on cuttlefish DML remained significant at 90 days, $H = 23.644$, $df = 3$, $p < .001$. At this time, the two switched groups were no longer similar to each other, $U = 62$, $n1 = n2 = 8$, $p < .001$. The cuttlefish that had been transferred to impoverished conditions (Group E/IC) were similar in size to those reared continuously in impoverished conditions (Group IC). The cuttlefish transferred to enriched conditions had not yet reached the size of those that had been reared continuously in enriched conditions, $U = 62$, $n1 = 10$, $n2 = 8$, $p = .05$; Figure 1.

Daily growth rates also differed between groups.

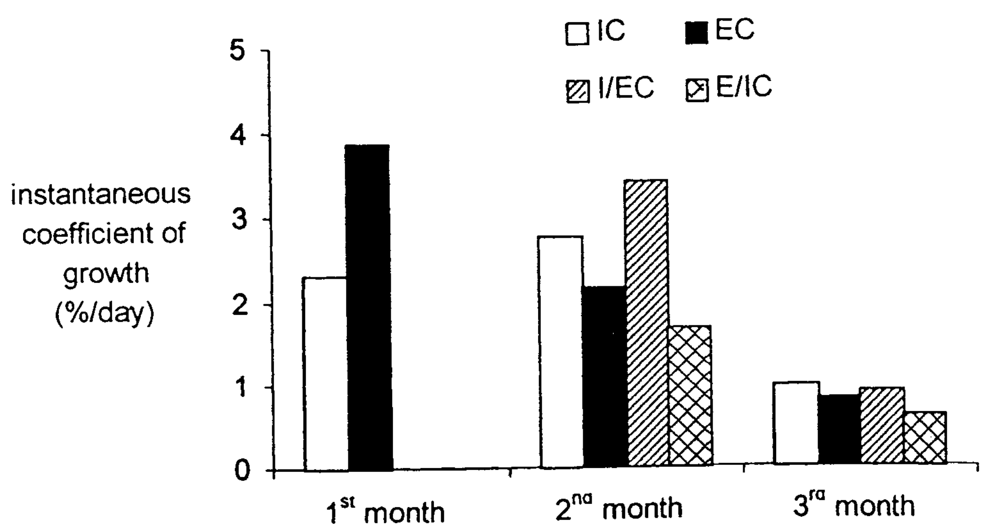

FIGURE 2 Instantaneous coefficient of growth of cuttlefish from Groups IC (impoverished conditions), EC (enriched conditions), I/EC (impoverished/enriched conditions), and E/IC (enriched/impoverished conditions) during the first 3 months of life.

During the 1st month, the daily growth rate was higher in Group EC (3.9%) than in Group IC (2.3%, Figure 2). During the 2nd month, the growth rate in Group E/IC was the lowest (1.7%) and in Group I/EC the highest (3.4%), with those of Groups IC and EC in-between (2.8% and 2.2%, respectively). During the 3rd month, the growth rates for Groups IC, EC, and E/IC were essentially the same (1%, 0.9%, and 0.9%, respectively) with the growth rate of Group E/IC somewhat lower (0.6%).

Correlation Between Initial Strike Rate and Total Acquisition Time

There was a small but significant positive correlation between the number of strikes observed during the first 3 min of the initial learning and the time needed to reach the criterion of acquisition, Pearson coefficient of correlation, $N = 71$, $df = 1$, $r = 0.277$, $p < .05$; Figure 3. Thus, a high initial level of strikes during training could increase the time required for acquisition.

Learning Performance

At 1 Month.

Within Groups. Cuttlefish from all three groups showed significant acquisition (comparison between T1 and T6) during the first 18 min, Group IC30: $Z = -2.556$, $n = 10$, $p < .05$; Group EC30: $Z = -2.807$, $n = 10$, $p < .01$; Group SC30: $Z = -2.814$, $n = 10$, $p < .01$; Figure 4. Retention at 24 hr (comparison between T1 and R1) was evident only for cuttlefish reared in enriched conditions, Group EC30: $Z = -2.255$, $n = 10$, $p < .05$. For Groups IC30 and SC30, reacquisition (comparison between T2 and R2) was not significantly faster than initial learning.

Between Groups. Rearing conditions had a significant effect on initial acquisition, $H = 6.954$, $df = 2$, $p < .05$, but no effect on retention at 24 hr. Acquisition (T6 expressed as a percentage of T1) was significantly better for Group SC30 than for the other two groups (Figure 5; IC30 and SC30: $U = 81.5$, $n1 = n2 = 10$, $p < .05$; EC30 and SC30: $U = 77$, $n1 = n2 = 10$, $p < .05$, whereas retention (R1 expressed as a percentage of T1) was not different between the groups.

At 3 Months.

Within Groups. Acquisition (comparison between T1 and T6) was significant for all five groups, Figure 6; Group IC90: $Z = -2.556$, $n = 7$, $p < .05$; Group EC90: $Z = -2.684$, $n = 9$, $p < .01$; Group I/EC: $Z = -2.366$, $n = 8$, $p < .05$; Group E/IC: $Z = -2.53$, $n = 8$, $p < .05$; Group SC90: $Z = -2.67$, $n = 9$, $p < .01$. Only the cuttlefish kept in enriched conditions for at least 2 months (Groups EC90 and I/EC) and those from standard conditions (Group SC90) displayed significant retention, comparison between T1 and R1; Group EC90: $Z = -2.536$, $n = 9$, $p < .05$; Group I/EC: $Z = -2.536$, $n = 8$, $p < .05$; Group SC90: $Z = -2.374$, $n = 9$, $p < .05$. Of the two groups that did not display any apparent retention at 24 hr (Groups IC90 and E/IC), only cuttlefish reared for 3 months in impoverished conditions (IC90) displayed

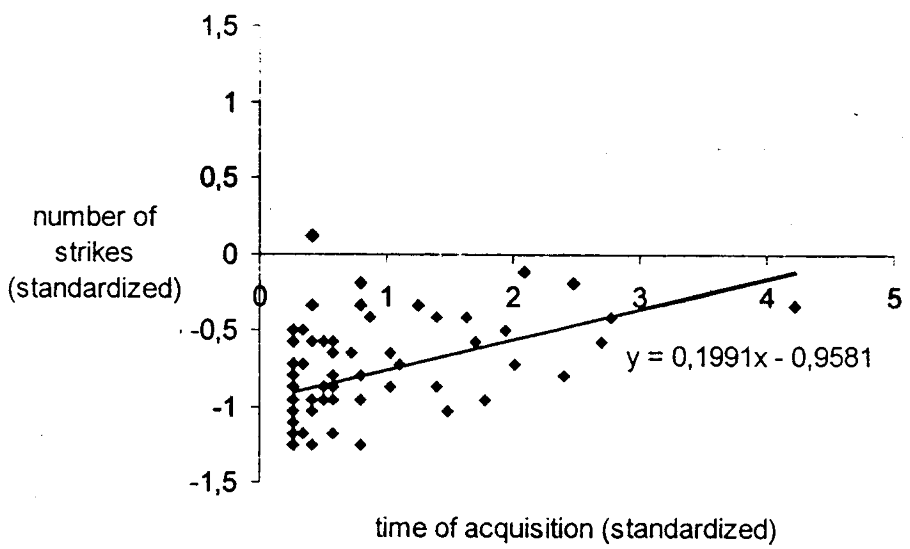

FIGURE 3 Number of tentacle strikes during T1 (initial 3-min block of training) plotted against the total time needed to reach the criterion of acquisition. All data were standardized. Linear trendline was added. All animals pooled ($N = 71$).

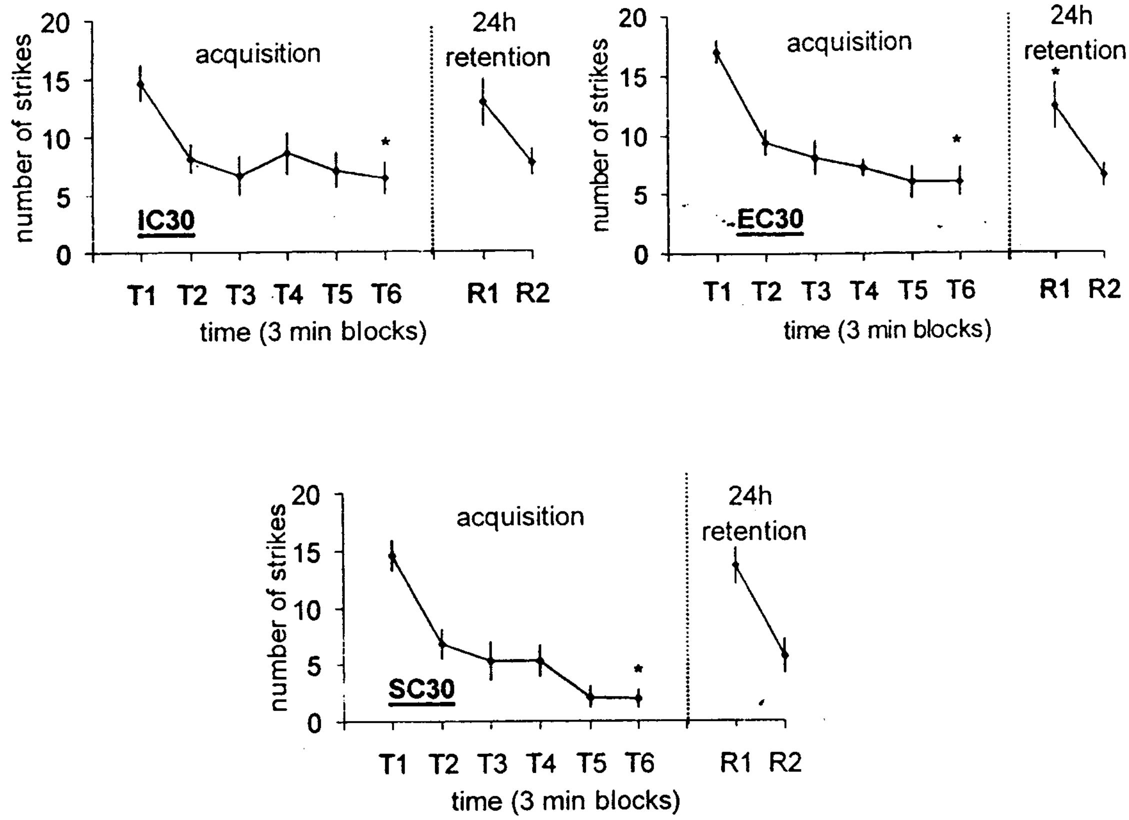

FIGURE 4 Training (T1–T6) and retention (R1–R2) curves of 1- month-old cuttlefish during the first 18 min of the training. Groups IC30 = impoverished conditions, EC30 = enriched conditions, SC30 = standard conditions. Asterisk indicates significant difference with the initial 3-min block of training (T1, Wilcoxon test for matched paired data, $p < .05$). Vertical bars indicate *SEM*.

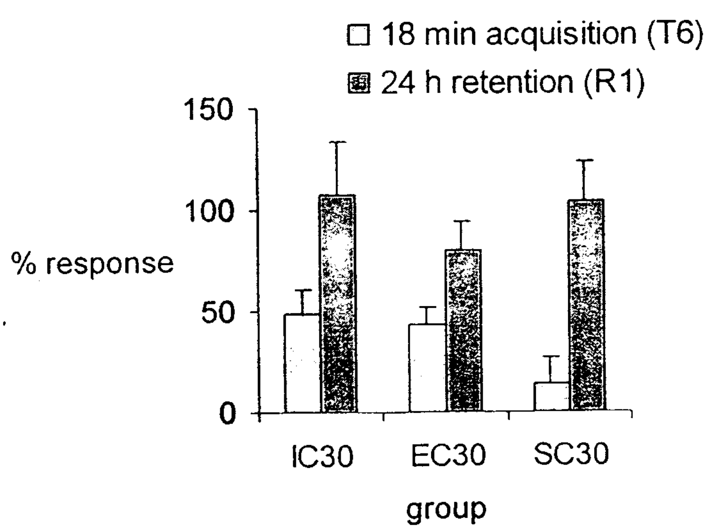

FIGURE 5 Tentacle strikes observed during the sixth 3-min block of training (T6) and the first 3-min block of retention test (R1) at 1 month (expressed as a percentage the number of strikes during the first 3-min block of training, T1). Vertical bars indicate *SEM*.

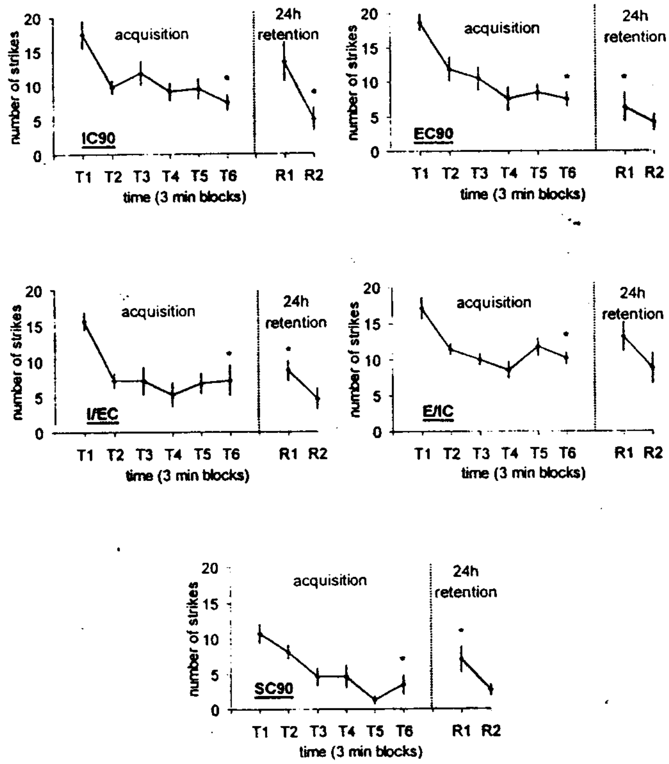

FIGURE 6 Training and retention curves of 3-month-old cuttlefish during the first 18 min of the training. Groups IC90 = impoverished conditions, EC90 = enriched conditions, I/EC = impoverished/enriched conditions, E/IC = enriched/impoverished conditions, SC90 = standard conditions. Asterisk indicates significant difference with the initial 3-min block of training (T1, Wilcoxon test for matched paired data, $p < .05$). ° indicates significant improvement in reacquisition (T2–R2), evaluated only when retention (T1–R1) is insignificant. Vertical bars indicate *SEM*.

significant improvement in acquisition, comparison between T2 and R2; $Z = -1.980$, $n = 7$, $p < .05$.

Between Groups. At 3 months, a significant effect of rearing condition on acquisition was found within the first 18 min of training, $H = 9.942$, $df = 4$, $p < .05$. Cuttlefish from Group E/IC showed significantly poorer acquisition (comparison between T1 and T6) than either Group EC90, $U = 10.5$, $n1 = 8$, $n2 = 9$, $p < .05$, or Group SC90, $U = 63$, $n1 = 8$, $n2 = 9$, $p < .001$, Figure 7.

Differences between groups in retention perform-

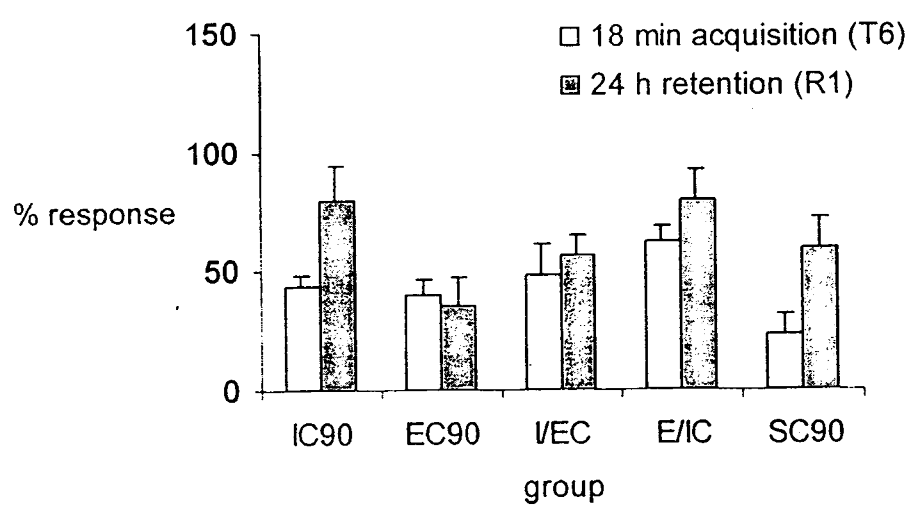

FIGURE 7 Tentacle strikes observed during the sixth 3-min block of training (T6) and the first 3-min block of retention test (R1) at 3 months (expressed as a percentage the number of strikes during the first 3-min block of training, T1). Vertical bars indicate *SEM*.

ances appeared pronounced but appeared only marginally significant, $H = 8.822$, $df = 4$, $p = .066$. Cuttlefish reared continuously in enriched conditions (Group EC90) showed significantly better retention (comparison between T1 and R1) than those reared in impoverished conditions, either continuously, Group IC90; $U = 12.5$, $n1 = 9$, $n2 = 7$, $p < .05$, or for the last 2 months, Group E/IC. $U = 13$, $n1 = 9$, $n2 = 8$, $p < .05$. Retention was also better for cuttlefish that had been shifted from impoverished to enriched conditions (Group I/EC) than those that had been in impoverished conditions continuously, Group IC90; $U = 45$, $n1 = 7$, $n2 = 8$, $p < .05$. Retention performances for cuttlefish in standard conditions (SC90) did not differ significantly from those reared continuously in either enriched (EC90) or impoverished (IC90) conditions.

Comparison Between 1 and 3 Months. No significant improvement in acquisition (comparison between T1 and T6) with age was found for any group (Figures 5 and 7).

Improvement in retention with age was significant for Group EC, enriched conditions; $U = 72$, $n1 = 10$, $n2 = 9$, $p < .05$, marginally significant for Group SC, standard conditions: $U = 68$, $n1 = 10$, $n2 = 9$, $p = .066$, and insignificant for Group IC (impoverished conditions). No significant improvement in retention was found between cuttlefish reared in enriched conditions (Group EC30) and those transferred to impoverished conditions (Group E/IC), or between those reared in impoverished conditions (Group IC30) and those transferred to enriched conditions (Group I/EC).

DISCUSSION

Effect of Environment on Growth

Growth was faster among cuttlefish reared in enriched, social conditions. This result is consistent with previous research demonstrating faster growth in group-reared cuttlefish than in isolated cuttlefish (Warnke, 1995). This difference in growth cannot be a result of differences in cuttlefish density (ratio between animal and tank surface areas, Richard, 1971). During the 1st month, the tank surface area per cuttlefish was initially 144 cm^2 for cuttlefish from Group IC and 120 cm^2 for cuttlefish from Group EC. During that same period, Group IC showed an increase in DML of approximately 99% (mean DML from 8 ± 0.1 mm to 16 ± 0.2 mm) whereas Group EC showed an increase of DML of nearly 219% (mean DML from 7.9 ± 0.1 mm to 25.4 ± 0.4 mm).

Consequently, the relative density of Group EC was higher throughout this period than it was for Group IC, yet Group EC grew more. Furthermore, cuttlefish from Group IC showed an increasing growth rate during the first 2 months of life even though the relative space in their tanks decreased (Figure 2).

The growth rate of our cuttlefish appears instead to be strongly related to the environment of rearing. The presence of conspecifics could induce, by competition and/or recruitment, an increase in alimentary motivation ("social facilitation"). During the 1st month of life, Warnke (1995) found a decrease in latency to attack a prey and a higher number of prey items caught among cuttlefish reared with conspecifics as compared with those raised in isolation. The relatively high growth rate of cuttlefish from Group I/EC after being moved to enriched conditions and the decreased growth rate of cuttlefish from Group E/IC after being moved to impoverished conditions supports this hypothesis (Figures 1 and 2).

Experimental Methods for Assessment of Learning

The number of tentacle strikes displayed in the present study was higher than that observed in previous studies, both at the beginning of training (T1: 8.4 ± 0.4 strikes during the 1st min, 15.7 ± 0.5 strikes during the first 3 min, all cuttlefish pooled, $N = 71$; as compared with Agin et al., 1998; Dickel et al., 1998; Messenger, 1973a, 1973b) during T6 (as compared with Messenger, 1973a using adults and the same protocol of learning) and during retention trials (as compared with Dickel et al., 1998 using juvenile cuttlefish). Water temperature during experiments can strongly affect the predatory behavior of the cuttlefish (Boucaud-Camou & Pequignat, 1973). In the present experiment the water temperature was relatively high for the IC, EC, I/EC, and E/IC groups (22.5 ± 1°C as compared with 15–20°C in Messenger, 1973a and 20 ± 1°C in Dickel et al., 1998) and may explain both high initial levels of predation and slower acquisition of the task, as compared with previously published data.

Figure 3 shows that there was a significant, positive relationship between initial levels of attempted captures and the time needed to reach the criterion of acquisition. To assure the same level of acquisition for all cuttlefish, in this study each cuttlefish was required to reach a criterion of initial training prior to the retention test (only one strike in 2 consecutive min after the 18th min). This criterion differed from that used in previous studies (a fixed period of 20 min for acquisition; Agin et al., 1998; Dickel et al., 1998; Messenger, 1971, 1973a, 1973b). Applying an individual criterion such as this is important when comparing retention performances of cuttlefish of different ages, or reared under different environments or water temperatures.

The improvement in reacquisition found for Group IC90 (Figure 6, T2 and R2) indicates that even when the level of predation at the beginning of the retention test (R1) does not differ from that displayed at the beginning of training (T1; classically no retention; Messenger, 1971, 1973a, 1973b, 1977; Chichery & Chichery, 1992; Dickel et al., 1998), the forgetting of the task could be only partial. Thus, a simple comparison of the level of predation at the beginning of the training (T1) with predation at the beginning of the retention (R1) test can be an insufficient measure of 24-hr retention.

Learning Performances: Environment and Development

Effect of the Environment of Rearing on Training and Retention. Early experience clearly influences both acquisition and retention performances. For example, at 1 month of age, cuttlefish reared in enriched conditions (Group EC30) displayed significant retention performances at 24 hr whereas cuttlefish reared in impoverished conditions (Group IC30) and standard conditions (Group SC30) did not (Figure 4). The differences between groups at 1 month increased further at 3 months, with cuttlefish from the enriched group (Group EC90) displaying significantly better retention performances than those reared in any of the other groups (Figures 6 and 7).

Differences in acquisition and retention cannot be linked to the size of the cuttlefish. For example, cuttlefish from Group EC30 were smaller than those in Group IC90 (Figure 1), yet Group EC30 displayed significant 24-hr-retention performances (Figures 4 and 6) whereas Group IC90 did not. Thus, memory capacities appear related more closely to early experience than to cuttlefish size.

Effect of the Rearing Environment on Improvement in Retention. There was a substantial improvement in retention between 1 and 3 months for Group EC. The improvement in retention for Group SC was less pronounced (Figures 4 and 6). The presence of numerous conspecifics in the large but barren tank (standard conditions) during the 1st month of life (Group SC30) was not sufficient to obtain the level of memory abilities found in Group EC. These results have some implications for learning research using laboratory-cultured juvenile cuttlefish. In that way, it seems that rearing environment of the subjects has to be carefully considered. In particular, the additional presence of sand and obstacles (rocks, seaweed; Group EC) appears to have provided a superior environment for the development and refinement of behavior associated with predation. Detouring around obstacles during the pursuit of prey quite likely involves some visual memory processes (Dickel et al., 1997; Messenger, 1977; Sanders & Young, 1940) whereas the possibility of burrowing could allow for the development of different strategies of hunting.

Cuttlefish raised continuously in isolation (IC group) did not show such improvement in retention of learning with age. In the IC group, forgetting was only partial at three months, however because reacquisition was faster at 3 months than at 1 month (Figures 4 and 6). Surprisingly, cuttlefish reared for 1 month in enriched conditions (displaying significant 24-hr retention, Group EC30, Figure 4) did not show significant retention after they were moved to impoverished conditions (Group E/IC, Figure 6). Thus, impoverishment of the environment during the 2nd and 3rd months of life appears to reduce previously acquired memory capacities. Conversely, cuttlefish reared for 1 month in impoverished conditions (displaying no significant retention, Group IC30, Figure 4) were able to, at least partially, overcome previous memory deficits (Group I/EC, Figure 6).

In conclusion, memory formation and growth in cuttlefish is greatly affected by the conditions under which the animals are reared, particularly during the 2nd and/or 3rd months posthatching. Thus, cuttlefish are interesting and useful subjects for the study of the ontogeny of memory during development and the plasticity of memory when development occurs in different environments.

NOTES

This research was supported by a grant from the FYSSEN foundation to L. Dickel. We also thank the staff of the National Resource Center for Cephalopods and the Marine Biomedical Institute for their technical assistance. The cuttlefish were produced through a grant provided by the National Institutes of Health (National Center for Research Resources).

REFERENCES

Agin, V., Dickel, L., Chichery, M. P., & Chichery, R. (1998). Evidence for a specific short-term memory in the cuttlefish, Sepia. Behavioural Processes, 43, 329–334.

Boletzky, S.von (1987). Juvenile behaviour. In P. R. Boyle (Ed.), Cephalopod life cycles: Vol. II. Growth (pp. 45–84). London: Academic Press.

Boucaud-Camou, E., & Pequignat, E. (1973). Etude expérimentale de l'absorption intestinale chez Sepia officinalis L. (Mollusque Céphalopode) [An experimental study of digestive absorption in Sepia officinalis L.] Forma et Functio, 6, 93–113.

Chichery, M. P., & Chichery, R. (1992). Learning performances and aging in cuttlefish (Sepia officinalis). Experimental Gerontology, 27, 234–239.

Dickel, L, Chichery, M. P., & Chichery, R. (1997). Postembryonic maturation of the vertical lobe complex and early development of predatory behavior in the cuttlefish (Sepia officinalis). Neurobiology of Learning and Memory, 67, 150–160.

Dickel, L, Chichery, M. P., & Chichery, R. (1998). Time differences in the emergence of short- and long-term memory during postembryonic development in the cuttlefish, Sepia. Behavioural Processes, 44, 81–86.

Forsythe, J. W., DeRusha, R. H., & Hanlon, R. T. (1994). Growth, reproduction, and life span of Sepia officinalis (Cephalopoda: Mollusca) cultured throught seven consecutive generations. Journal of Zoology (London), 233, 175–192.

Forsythe, J. W., Hanlon, R. T., & DeRusha, R. (1991). Pilot large-scale culture of Sepia in biomedical research. In E. Boucaud-Camou (Ed.), The cuttlefish (pp. 313–323). Centre de publication de l'Université de Caen, Caen, France.

Forsythe, J. W., & Van Heukelem, W. F. (1987). Growth. In P. R. Boyle (Ed.), Cephalopod life cycles (Vol. II, Comparative reviews, pp. 135–156). London: Academic Press.

Hanlon, R. T., & Messenger, J. B. (1997). Cephalopod behaviour. Cambridge, UK: Cambridge University Press.

Messenger, J. B. (1971). Two-stage recovery of a response in Sepia. Nature, 232, 202–203.

Messenger, J. B. (1973a). Learning in the cuttlefish, Sepia. Animal Behaviour, 21, 801–826.

Messenger, J. B. (1973b). Learning performance and brain structure: A study in development. Brain Research, 58, 519–523.

Messenger, J. B. (1977). Prey capture and learning in the cuttlefish, Sepia. Symposia of the Zoological Society of London, 38, 347–376.

Renner, M. J., & Rosenzweig, M. R. (1987). Enriched and impoverished environments: Effects on brain and behavior. New York: Springer-Verlag.

Richard, A. (1971). Contribution à l'etude expérimentale de la croissance et de la maturation sexuelle de Sepia officinalis. Thése de Doctorat d'Etat. Lille.

Rosenzweig, M. R., & Bennett, E. L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. Behavioral Brain Research, 78, 57–65.

Sanders, F. K. (1975). The cephalopods. In W. C. Corning, J. A. Dyal, & A. O. D. Willows (Eds.), Invertebrate learning (Vol. III, pp. 1–101). New York: Plenum Press.

Sanders, G. D., & Young, J. Z. (1940). Learning and other functions of the higher nervous centre of Sepia. Journal of Neurophysiology, 3, 501–526.

Siegel, S., & Castellan, N. J. (1988). Nonparametric statistics for the behavioral sciences, 2nd ed. New York: McGraw-Hill.

Sokal, R. R., & Rohlf, F. J. (1969). Biometry. San Francisco: W. H. Freeman.

Warnke, K. (1995). Some aspects of social interaction during feeding in Sepia officinalis hatched and reared in the laboratory. Vie et Milieu, 44, 125–131.

Wells, M. J. (1958). Factors affecting reactions to Mysis by newly hatched Sepia. Behaviour, 13, 96–111.

Wells, M. J. (1962). Early learning in Sepia. Symposia of the Zoological Society of London, 8, 149–169.

Wirz, K. (1954). Etude quantitative sur le système nerveux des Céphalopodes. [Quantitative study of the central nervous system in Cephalopods.] Comptes Rendus de l'Académie des Sciences (Paris), 238, 1353–1355.