

Postembryonic Maturation of the Vertical Lobe Complex and Early Development of Predatory Behavior in the Cuttlefish (*Sepia officinalis*)

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In two groups of newly hatched cuttlefish, from eggs incubated at different temperatures, the emergence of predatory pursuit was correlated with the development of some characteristics of the vertical lobe complex (namely, the development of the vertical and superior frontal lobes and the appearance of the vertical–subvertical lobe tracts) and with the state of resorption of the inner yolk sac. The temperature of egg incubation influences the appearance of postnatal pursuit behavior. Expression of this predatory behavioral characteristic is concomitant with the appearance of the vertical–subvertical lobe tracts. In contrast, the growth of the vertical and the superior frontal lobes relative to the growth of the supraesophageal mass and final yolk absorption are not correlated with the appearance of pursuit. To maintain a prey in the frontal visual field during predatory pursuit, short-term memory processes must be involved. Thus, the development of the vertical–subvertical lobe tracts, which is concomitant with the emergence of pursuit, appears essential in the maturation of these short-term memory processes. © 1997

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INTRODUCTION

The ontogenesis of behavior in cephalopods has been the subject of relatively few detailed studies. Among these, work devoted to the development of predatory behavior in the cuttlefish (Wells, 1958, 1962) and of its cognitive capacities should be em-

phasized (Messenger, 1973; Wells, 1962). For the latter, an improvement in learning has been correlated with a large increase in the relative volume of the vertical and the superior frontal lobes during the postembryonic period in *Sepia officinalis* (Messenger, 1973). Messenger (1977) notes the absence of pursuit of prey in newly hatched cuttlefish and puts forward the hypothesis of a short-term memory defect to explain this behavior.

The duration of embryonic development depends primarily on the water temperature (Boletzky, 1983); development becomes increasingly rapid as temperature rises: at 20°C, the mean duration is 44 days, against 90 days at 15°C, and the corresponding dorsal mantle length of the cuttlefish at hatching is 7 and 9 mm, respectively. Literature data give no indication of any possible connection between the length of embryonic development and the maturation of predatory behavior or the central nervous system. Here we examine the influence of the length of embryonic development on postembryonic maturation of predatory behavior in the cuttlefish. In parallel, we focus attention on the maturation of some nervous structures (vertical and superior frontal lobes) involved in learning and memory (Sanders & Young, 1940). Furthermore, this study considers the probable role of short-term memory processes during pursuit behavior (Messenger, 1977). Finally, the influence of the resorption of the internal yolk sac on the onset of predatory behavior is assessed (Wells, 1958).

MATERIAL AND METHODS

Animals

Hatchling *S. officinalis* used for this research came from the same egg mass layed by a single fe-

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male; eggs were subdivided into two batches: (1) "17°C batch": the eggs were kept at environmental temperature in June and July, at a mean temperature of 17°C (15.4 to 18.6°C); development took 56 days (mean mantle length of the newly hatched animals: 8.4 ± 0.2 mm). (2) "20°C batch": the eggs were kept in thermoregulated water at a constant temperature of 20°C. Embryonic development lasted 40 days (mean mantle length of newly hatched animals: 7.9 ± 0.3 mm).

For each of the two batches, animals were taken from hatchlings of the day with the highest hatching rate. Thus, prematurely hatched animals, which generally are smaller, are not included in the experimental batches. After hatching, 40 individuals from each batch were reared in individual opaque plastic tanks ($90 \times 240 \times 60$ mm water depth) in an open system of well-oxygenated seawater which was sterilized by UV. The bottom of each tank was checkered with twenty-four 30×30 mm squares.

Behavioral Analysis

In order to study the emergence of predatory behavior, one prey (*Mysis* or fry fish) was introduced into the tank each day over a 9-day period.

The following elements were recorded: (1) Water temperature at the moment of testing; (2) the total number of prey detections per session (orientating head movement, color changes induced by the presence of the prey) for each batch; (3) the total number of pursuits per session for each batch; and (4) the total number of captures per session for each batch.

If no reaction was observed after 3 min and three passages of the prey close to the cuttlefish (namely, in squares adjacent to that of the subject), the test was stopped. To be certain that the animal was not underfed, a second prey was offered and removed 3 min after detection if it failed to trigger an attack.

Histological Analysis

Four animals were taken from each batch on the first, third, sixth, and ninth days after hatching.

The whole animals were fixed (neutralized 10% (w/v) Formalin in seawater) without dissection to avoid damage to the brain and displacement of the esophagus. The specimens were subjected to Cajal's silver impregnation (Young's protocol, 1971, the staining being satisfactory with 3% silver nitrate in distilled water after 72 h). After double inclusion (celloidin-paraffin), serial brain cross sections, 10 μ m thick, were made using a horizontal microtome. Embedding of the whole animals facilitates orientation of the specimens for the achievement of cross

sections perpendicular to the longitudinal axis. Sectioning starts posteriorly, and the cartilaginous structures surrounding the posterior part of the subesophageal mass permit readjustment of the cutting plane for perfect symmetry of the subsequent brain sections. Localization of the anterior and posterior limits of the structures studied thus becomes reliable.

One section in five was then analyzed by means of an image analyzer (computer set with a Leitz Aristoplan microscope, Biocom 200 and Bio3D station). For each structure considered, the anteriormost sections lying beyond the last 50- μ m interval were taken into account for the final volume calculation.

The following data were obtained: (1) Outline of the vertical lobe and the supraesophageal mass (of the structures situated above a horizontal straight line drawn just above the esophagus, laterally excluding the olfactory-peduncle lobes and optic lobes). An example of these contours is given in Fig. 1. (2) Vertical lobe volume/supraesophageal mass volume. The limits of the supraesophageal mass are defined in the following way: anteriorly by the appearance of the neuropil of the inferior frontal lobe; posteriorly by the disappearance of the neuropil of the median basal lobe. For the vertical lobe, the chosen limits are: anteriorly, the appearance of the neuropil in the anterolateral parts of the vertical lobe; posteriorly, the disappearance of the neuropil. These criteria are unambiguous using the Cajal stain method. (3) Superior frontal lobe volume/supraesophageal mass volume without the vertical lobe. Identification of the anterior limit of this lobe is easily achieved based on the appearance of the neuropil. In contrast, the posterior limit of the superior frontal lobe was set arbitrarily by the sudden fragmentation of the neuropil in the rearmost part of the lobe, which is recognizable by its very characteristic pattern. (4) Number of vertical lobe-subvertical lobe tracts. These tracts represent the main efferents of the vertical lobe with some afferent fibers from the subvertical lobe. Only those tracts localized at the junction between the vertical lobe and the subvertical lobe and visible after image smoothing were counted in order to avoid recording isolated or poorly recognizable fibers as tracts. The number obtained was corrected relating to the vertical lobe volume to eliminate any bias produced by the vertical lobe growth (only one section in five being sampled for evaluation of fiber tracts and determination of volume). Accordingly, the values obtained are indices of the level of maturation of this connection and are expressed in arbitrary units. (5) Volume of anterior part of the inner yolk sac. The volume of the inner

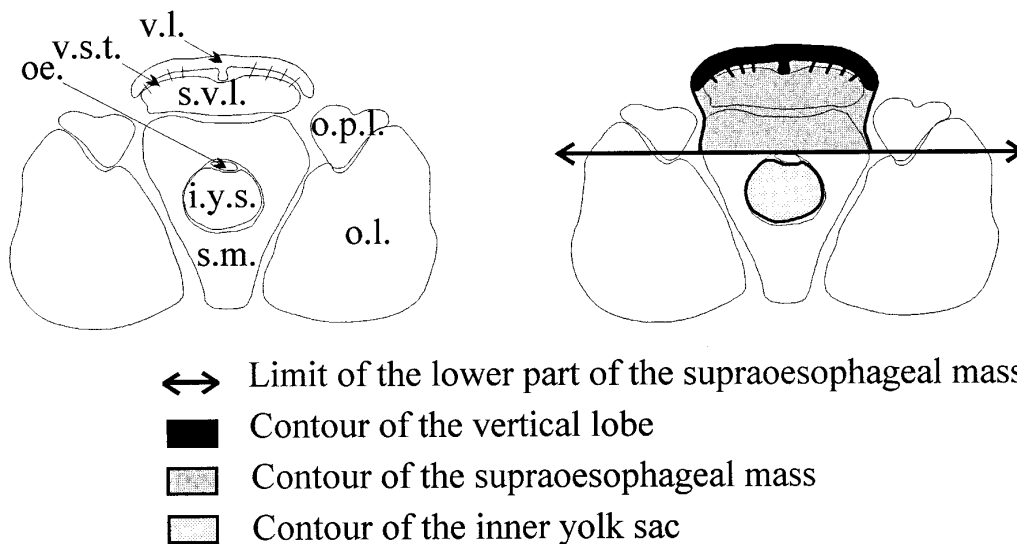


FIG. 1. Diagram of a cross section of the brain of a young cuttlefish: v.l., vertical lobe; s.v.l., subvertical lobe; v.s.t., vertical lobe-subvertical lobe tracts; oe., esophagus; o.p.l., olfactory-peduncle lobes; o.l., optic lobe; i.y.s., inner yolk sac; s.m., subesophageal mass.

yolk sac was determined from the same sections that had served to measure the volume of the supraesophageal mass.

In order to estimate the accuracy of the measurements produced by the image analyzer with the 50- μ m window, we determined the coefficient of variation of the volume of the vertical lobe relative to the volume of the supraesophageal mass in different animals (16 measurements for each animal): Batch 17°C, 1 day old, mean $7.03\% \pm 0.1$, coefficient of variation 1.55%; Batch 20°C, 1 day old, mean $7.29\% \pm 0.22$, coefficient of variation 3.1%; Batch 17°C, 3 days old, mean $7.84\% \pm 0.25$, coefficient of variation 3.21%; and Batch 20°C, 3 days old, mean $8.71\% \pm 0.05$, coefficient of variation 0.64%.

With the same animals the coefficients of variation were also determined for the maturation index of the vertical-subvertical tract: 1 day 17°C, 0.44%; 3 days 17°C, 3.01%; 1 day 20°C, 1.15%; 3 days 20°C, 1.34%, with eight measurements for each animal.

RESULTS

Behavioral Observations

Appearance of catching behavior. The number of animals having acquired a catching behavior is expressed as a percentage of the number of individuals tested (Fig. 2). The capture rate reaches a maximum (>90%) as early as 3 days after hatching for animals in the 20°C batch but only after 6 days for the 17°C batch. Initially, the 20°C batch individuals were 58% successful, and the 17°C batch individuals were 12% successful in catching behavior.

Appearance of pursuit behavior. The number of pursuits is expressed as a percentage of the number of detections (Fig. 3). The figure shows that appearance of pursuit behavior is earlier in animals of the 20°C batch. At Day 1, cuttlefish from the 20°C batch display 23% of pursuit-followed detections, against 5% for the 17°C batch. Levels higher than 70% are attained at age 3 days in the 20°C batch (72%), but only at age 7 days in the 17°C batch (88%).

Histological Observations

Development of the vertical lobe. The volume of the vertical lobe is expressed as a percentage of the volume of the supraesophageal mass (Fig. 4).

In both batches, the volume of the vertical lobe grows more rapidly than that of the supraesophageal

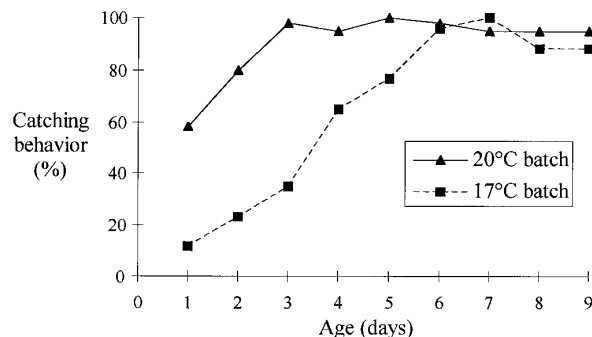


FIG. 2. Appearance of catching behavior. The total number of animals having captured a prey is expressed as a percentage of the total number of animals plotted against age ($n = 40$ for each batch).

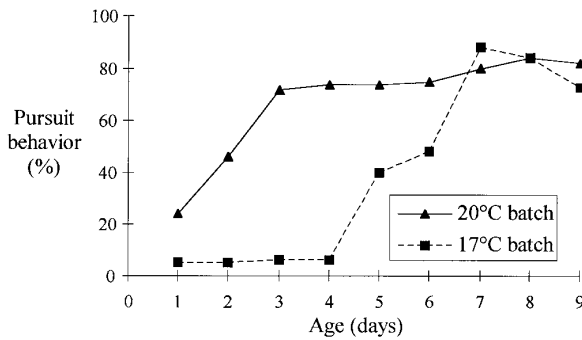


FIG. 3. Appearance of pursuit behavior. The total number of pursuits is expressed as a percentage of the total number of detections plotted against age ($n = 40$ for each batch).

mass (with a positive relation between age and vertical lobe volume in both batches, $n = 16$ for 17°C batch, $r = 0.83$; $n = 16$ for 20°C batch, $r = 0.74$, correlation coefficient of Pearson). A significant difference between the two batches was noted at 3 days (Fisher-Pitman test, $n = 8$, at 3 days $p < .05$). On the other hand, there was no significant difference either at hatching or at 6 and 9 days posthatching ($n = 8$ at 1, 6, and 9 days, Fisher-Pitman test).

Development of the superior frontal lobe. The volume of the superior frontal lobe is expressed as a percentage of the volume of the supraesophageal mass (minus the vertical lobe) to eliminate any bias due to the growth of the vertical lobe (Fig. 5). No significant difference between the two groups was noted whatever the age (Fisher-Pitman test for all ages). Furthermore, the superior frontal lobe grew at the same rate as the rest of the supraesophageal mass (with the exception of the vertical lobe) during the first 9 days.

Development of the connection between the vertical lobe and the subvertical lobe. At Day 1 (Fig. 6), the

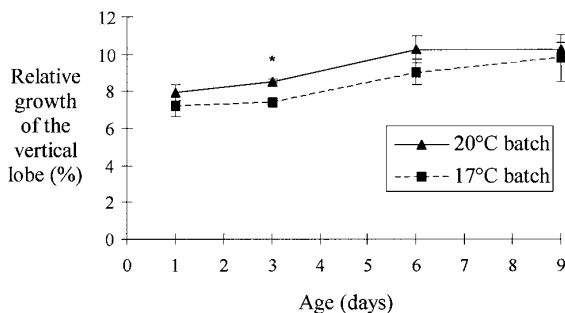


FIG. 4. Volume of the vertical lobe/volume of the supraesophageal mass plotted against age. Vertical bars indicate standard deviation. *Indicates significant difference between the two batches, $p < .05$, randomization test (Fisher-Pitman test).

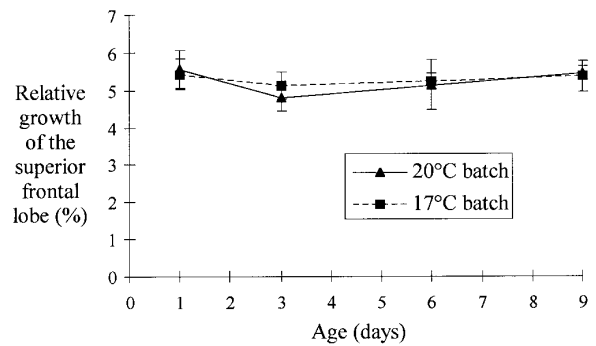


FIG. 5. Volume of the superior frontal lobe/volume of the supraesophageal mass except the vertical lobe plotted against age. Vertical bars indicate standard deviation.

index of the density of fiber tracts that connect the vertical lobe and the subvertical lobe is higher for cuttlefish from the 20°C batch than for those from the 17°C batch (Fisher-Pitman test, $n = 8$, significant difference $p < .05$). A maximum number of fiber tracts is attained after 3 days in the 20°C batch; in the 17°C batch the same number of tracts is attained after 9 days. The difference between the two batches is again significant at Days 3 and 6 (Fisher-Pitman test, $n = 8$ at 3 days; $n = 8$ at 6 days, $p < .05$) and not significant 9 days after hatching. The development of tracts is presented in Fig. 7.

Importance of the inner yolk sac. The inner yolk sac was sizable in all the animals (Fig. 8) and at all ages studied. Considering the yolk "neck" visible in the brain sections, on the first day of postembryonic life, individuals in the 20°C batch displayed a more voluminous inner yolk sac than those of the 17°C batch (Fisher-Pitman test, $n = 8$, $p < .05$). From the third day on, no significant difference was detected.

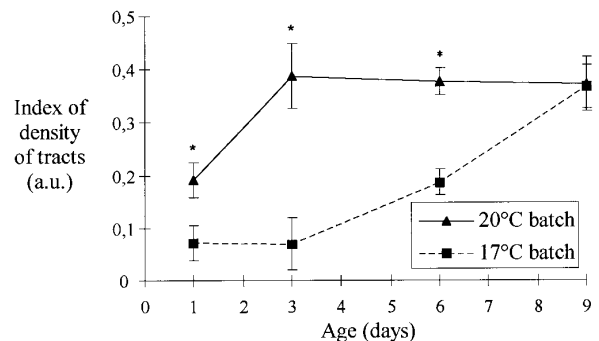


FIG. 6. Index of density of tracts between vertical and subvertical lobes (calculated as described in the text) plotted against age (a.u., arbitrary unit). *Indicates significant difference between the two batches, $p < .05$, randomization test (Fisher-Pitman test). Vertical bars indicate standard deviation.

The Correlation between Behavioral and Histological Data

The data are expressed as curves and compared using the Pearson waveform correlation (Borchers & Ewert, 1979). The analysis was restricted to the period of pursuit behavior development (1–3 days for the 20°C batch, 1–9 days for the 17°C batch, Fig. 3). Comparisons for each batch were made between the appearance of pursuit behavior and the growth of the vertical lobe, the development of vertical–subvertical lobe tracts, the volume of the superior frontal lobe, and the yolk volume recorded. The results are given in Table 1.

DISCUSSION

Early Predatory Behavior, Water Temperature, and State of Yolk Resorption

Figures 2 and 3 show that the environmental temperature during embryonic development has a major influence on the emergence of predatory behavior.

Different authors have noticed that there is a period of a few days before the first captures by young cuttlefish begin (Wells, 1958, 1962; Boletzky, 1983; Boucaud-Camou, Yim, & Tresgot, 1985), although there is some debate as to the duration of this period. These observations were based on animals that lived in either the Mediterranean sea or the English channel. The various delays recorded prior to the expression of predatory behavior can be probably explained by the difference in the temperature of incubation, below 20°C in the English channel and often higher than 20°C in the Mediterranean. Small females spawn regularly in the Mediterranean and it is known that the size of the eggs, i.e., the quantity of yolk available for the embryo, depends on female size (Richard, 1971; Boletzky, 1983; Ezzedine-Najai, 1985). We cannot exclude the possibility that the amount of yolk might influence the degree of maturation of the central nervous system of newly hatched cuttlefish.

Boucaud-Camou (1973) showed that the amount of food captured by cuttlefish increases with environmental water temperature; likewise, an increase in the speed of digestion was noted (Boucaud-Camou & Pequignat, 1973). However, in our study the water temperature measurements indicate that, at the time of testing individuals in the 20°C batch, the

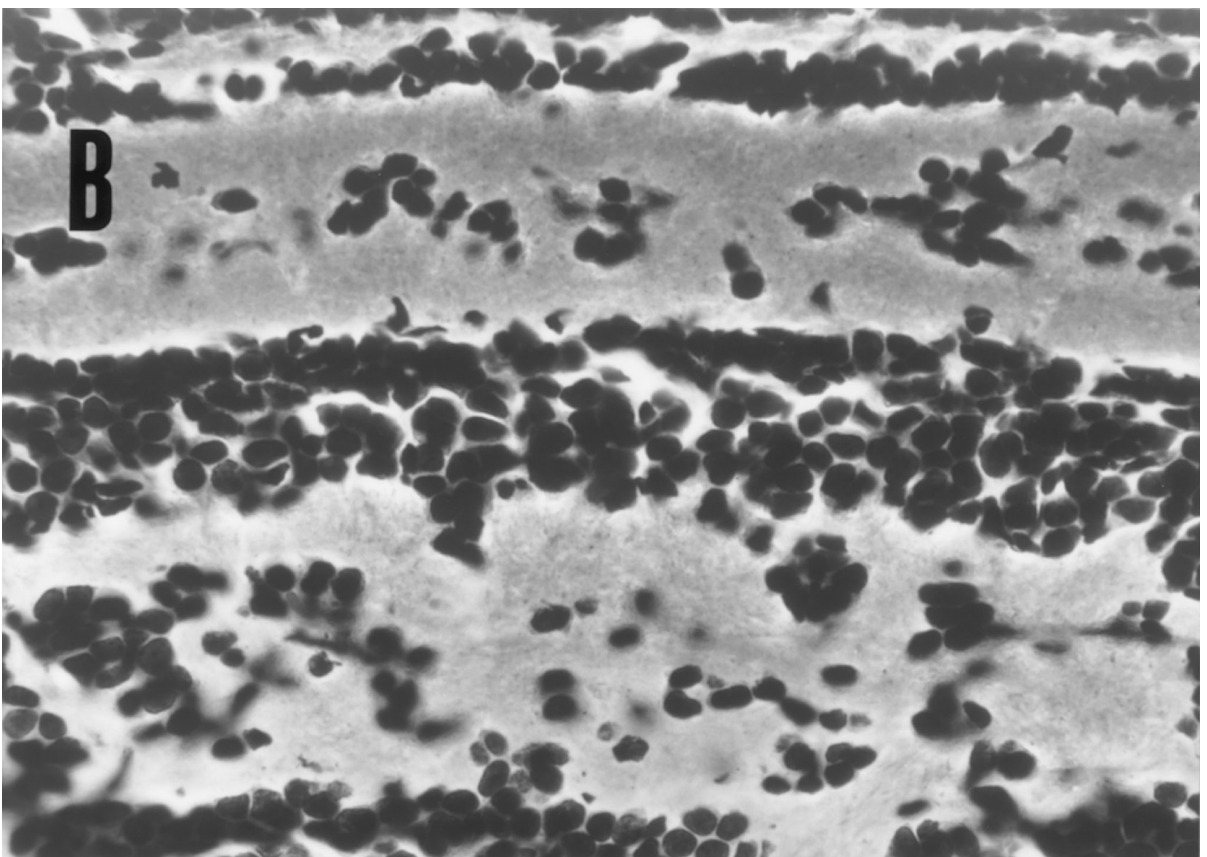
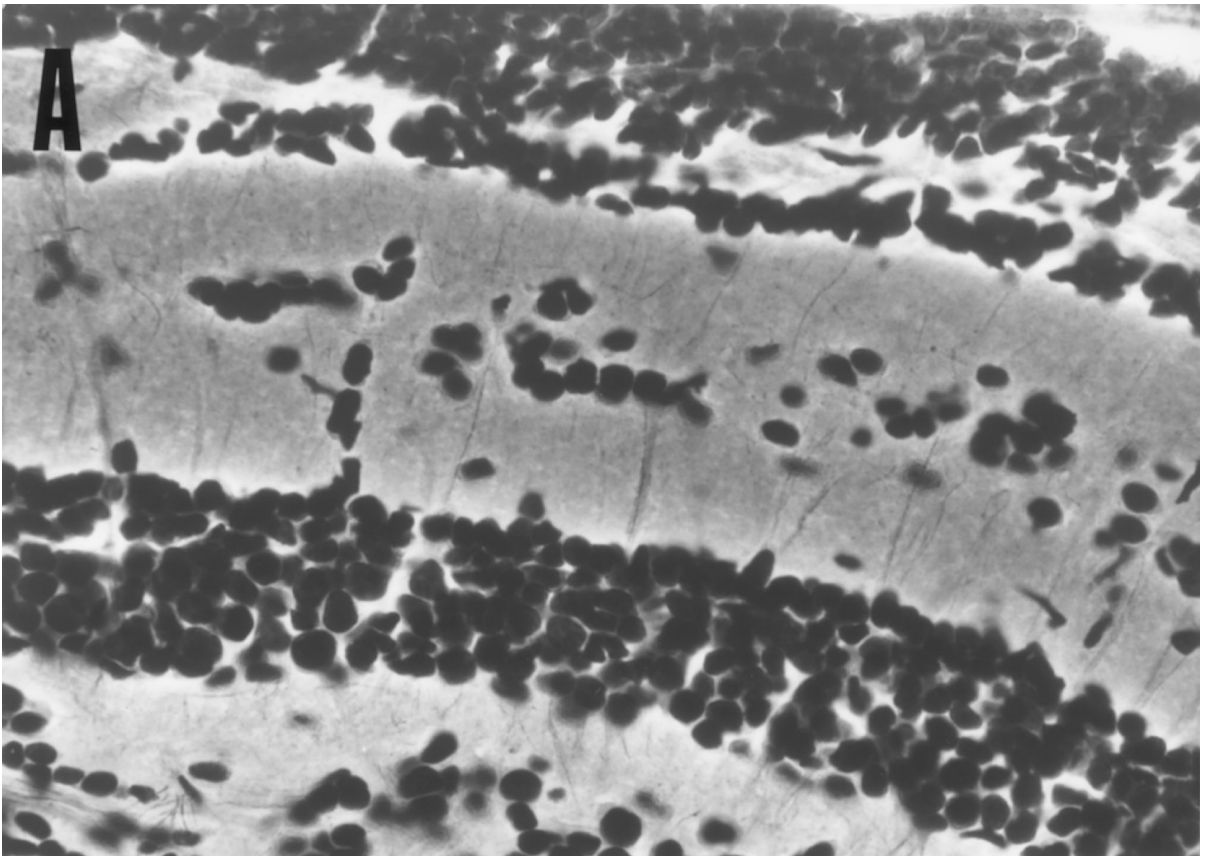
mean temperature ($17.7^{\circ}\text{C} \pm 0.1$) was lower than that for the 17°C batch ($18.6^{\circ}\text{C} \pm 0.2$). As the batch incubated at 20°C was more precocious, the differences between the two batches with regard to the triggering of predatory behavior and the initiation of pursuit cannot be attributed to water temperature during behavioral testing.²

In agreement with earlier data (Boletzky, 1975), we observed that young animals start feeding prior to the total resorption of the inner yolk sac. Our results show that the quantity of yolk remaining in the inner yolk sac is greater in animals in which embryonic development was rapid due to high temperature. These are the individuals that show the highest capture and pursuit rates on the first day of postembryonic life. Thus, contrary to the hypothesis of Wells (1958), the amount of yolk remaining in the inner sac would not appear to determine the triggering of predatory behavior; this conclusion is supported by the double correlation analysis on the pursuit maturation period, particularly for the 17°C batch (Table 1, $r_p[17^{\circ}\text{C}] = 0.92$, $r_y[17^{\circ}\text{C}] = -0.69$). Unlike the characteristics of the predatory behavior, yolk reserves do not differ significantly between the two batches at 3 days of age (Fig. 8).

Predatory Behavior and Maturation of the Vertical Lobe Complex

Vertical lobe and superior frontal lobe volumes. Our results again show that the volume of the vertical lobe grows more quickly than the volume of the supraesophageal mass (Fig. 4). However, a comparison of the volume of the vertical lobe at Days 1 and 6 between the two batches (no significant difference, Fisher-Pitmann test) shows that, contrary to Messenger's (1977) suggestion, the appearance of the

² Addendum: Two pilot experiments were performed in 1992 and 1993. Two batches of cuttlefish (from two different spawns) incubated at 15° and 20°C, respectively, were studied. These experiments yielded the same results as those described in this study, i.e., the earlier appearance of pursuit behavior in the animals from eggs incubated at 20°C (3 days for the 20°C batch versus 7/8 days for the 15°C batch). During the behavioral study the water temperatures were 18°C for the 20°C batch and 17°C for the 15°C batch. The different temperatures during testing observed in these three independent experiments support our interpretation that it is indeed the temperature of egg incubation (and not that after hatching) which influences the development of the pursuit behavior.



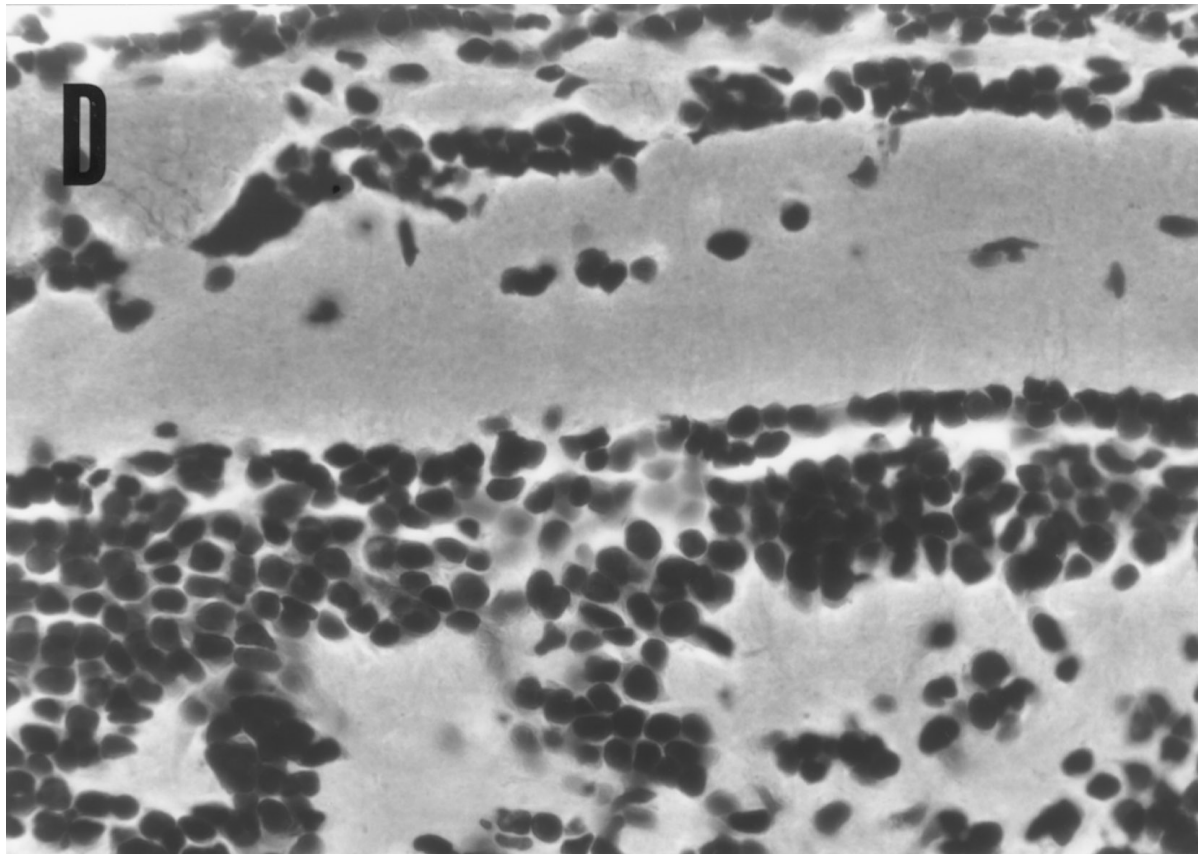
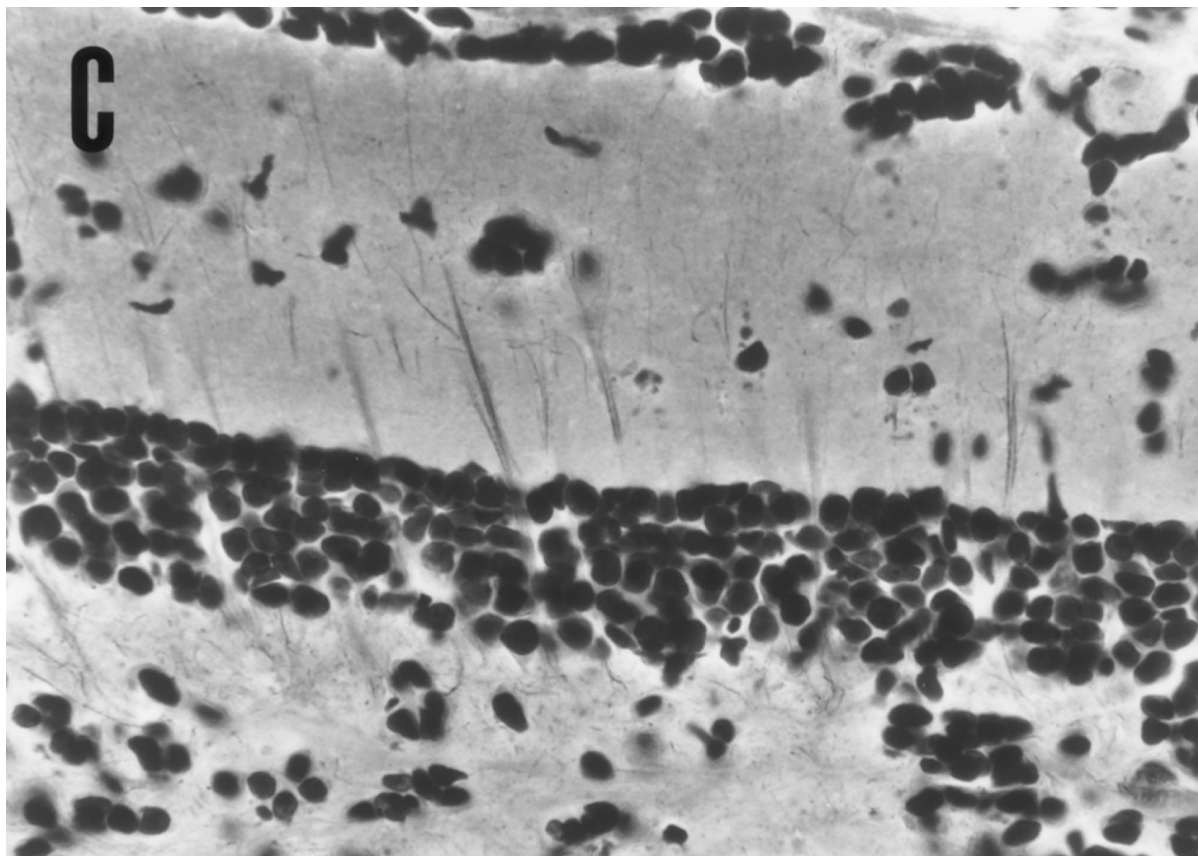


FIG. 7—Continued

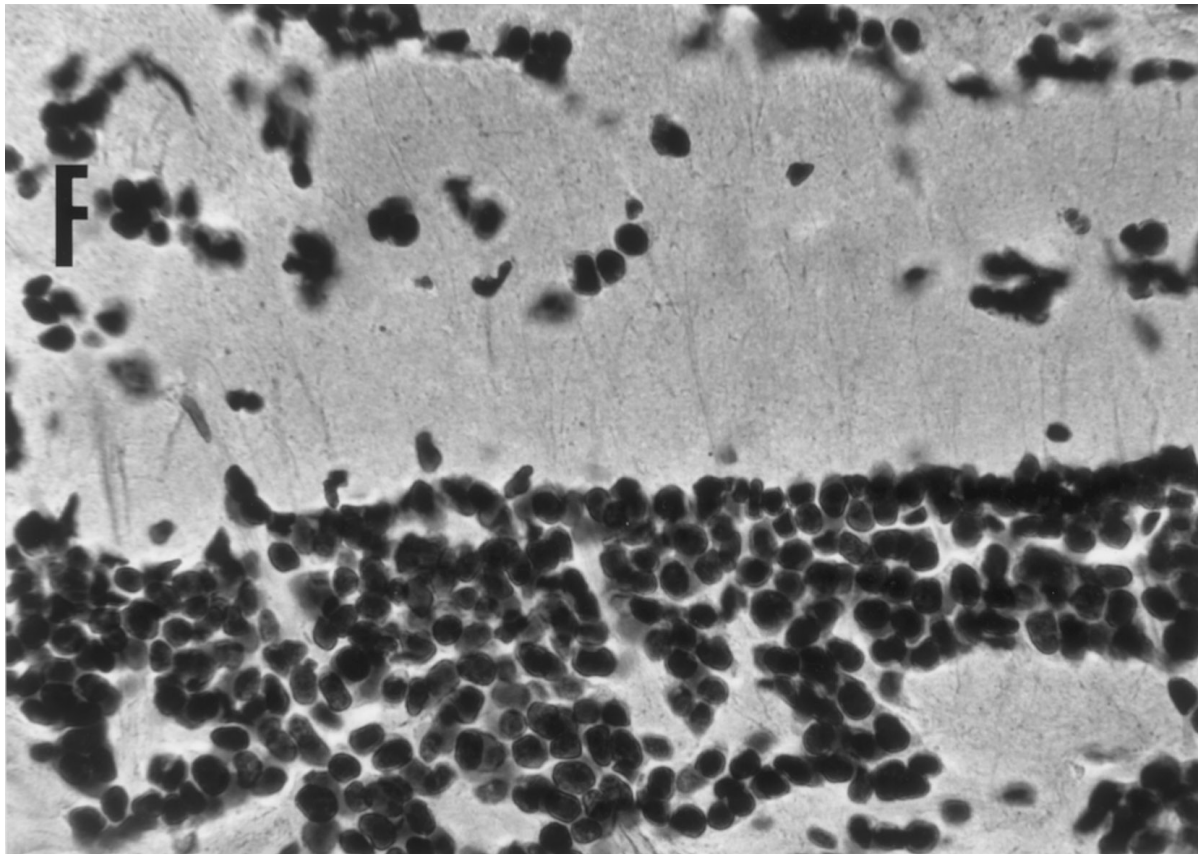
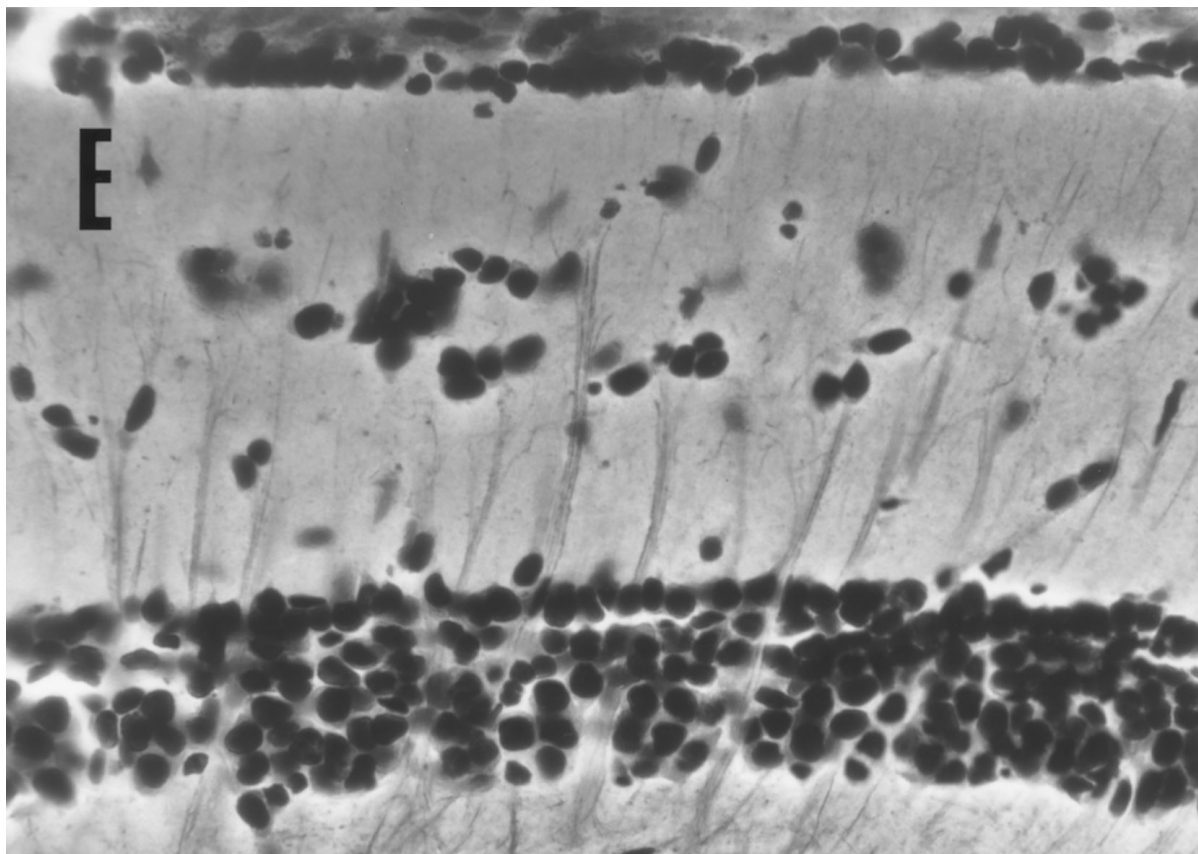


FIG. 7—*Continued*

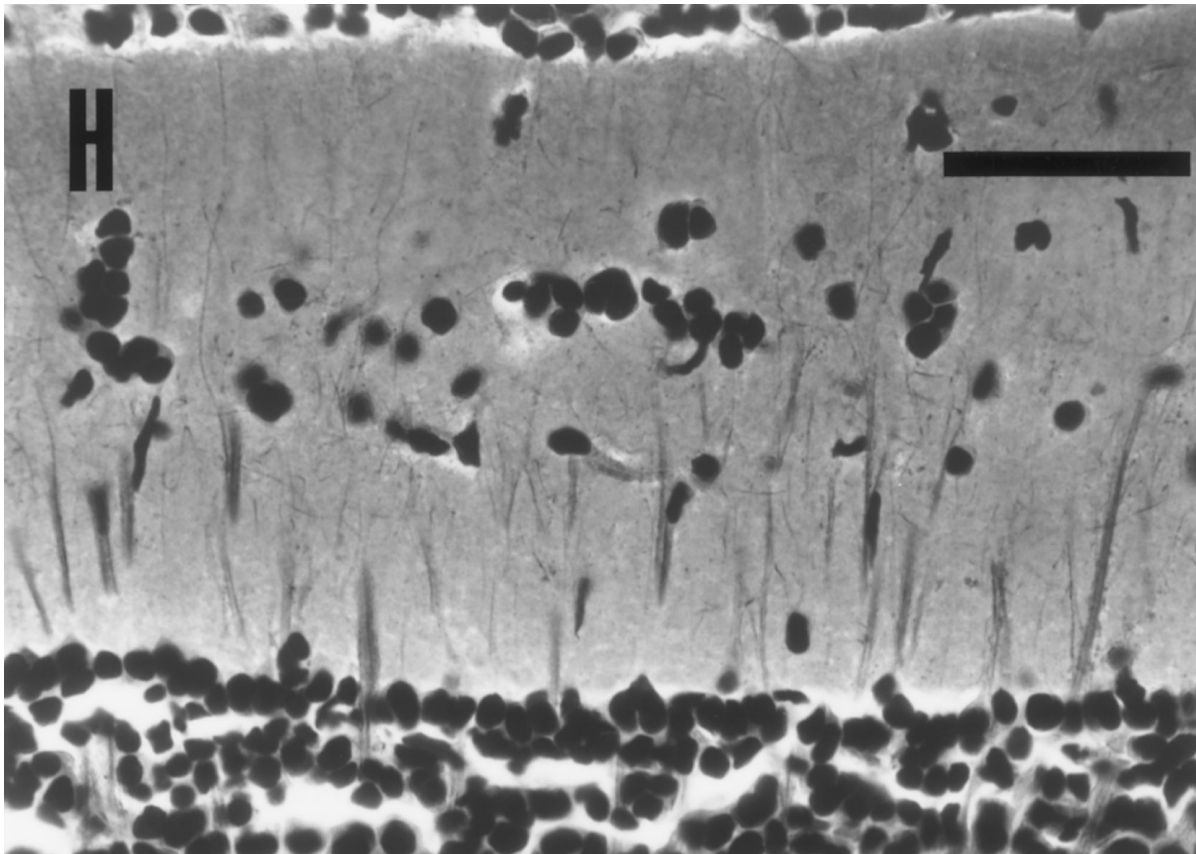
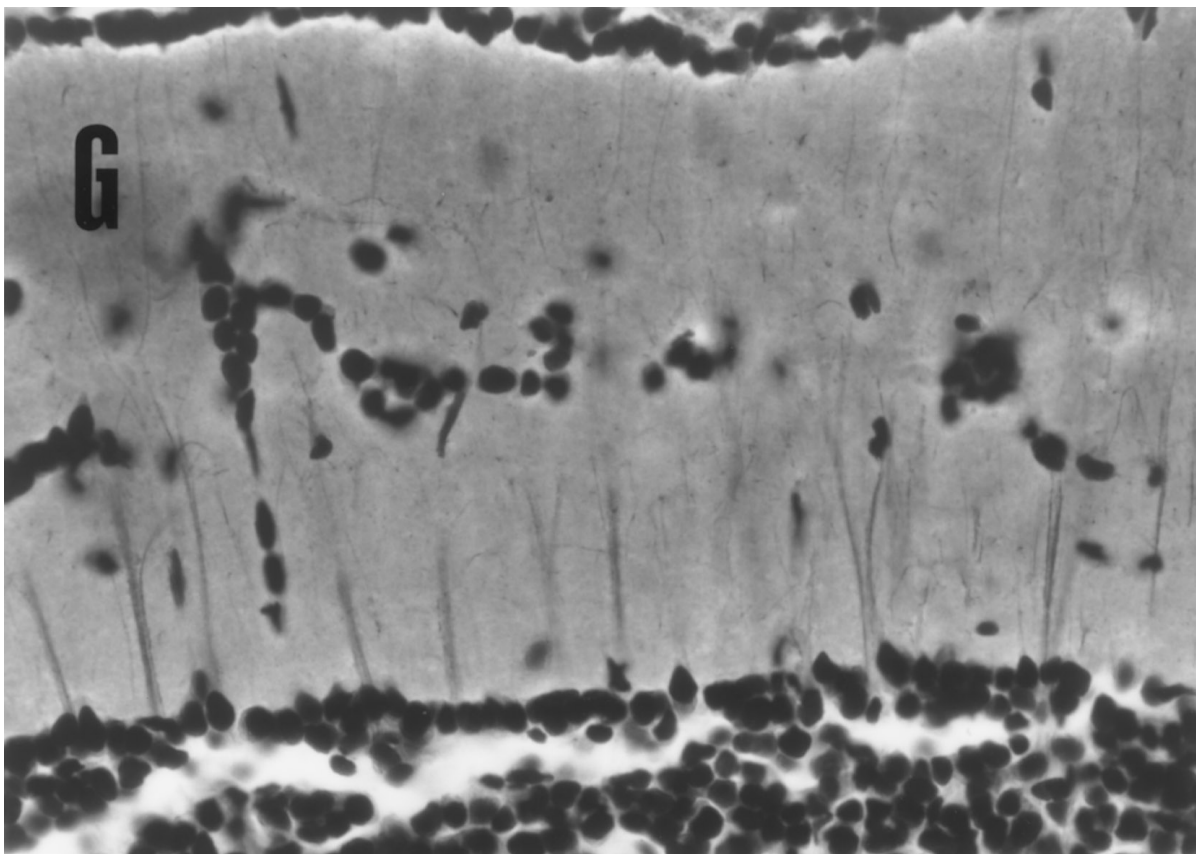


FIG. 7—*Continued*

pursuit behavior in cuttlefish would seem essentially independent of vertical lobe growth. The double correlation analysis supports this observation. Furthermore, Table 1 shows that r_p and r_{is} are very different between the two batches. These data would suggest that the development of the superior frontal lobe does not influence the appearance of active pursuit. The statistical analysis between the two batches at 1, 3, 6, and 9 days tends to support this hypothesis.

Development of the vertical-subvertical lobe tracts. A comparison of the correlation coefficient test (Table 1) for the density of fiber tracts between the vertical lobe and the subvertical lobe and the increase in pursuit rates would suggest the existence of a positive relation between the acquisition of the pursuit behavior and the maturation of this connection for both batches (Figs. 3 and 6). Furthermore, the significant differences detected at Days 1, 3, and 6 for this connection are related to important differences in the rates of pursuit observed between the two batches at the same developmental stage. This connection contains the principal efferents of the vertical lobe and some afferents (Young, 1979) from the subvertical lobe (the main input stems from the superior frontal lobe).

Pursuits, the Vertical Lobe Complex, and Short-Term Memory

Pursuit of a prey by a predator involves a certain ability of short-term memory when the prey moves out of the predator's frontal field of vision. This element of predatory behavior implies a rapid maturation of short-term memory processes. The presence of potentially self-reexciting chains of neurons has been reported in *Sepia* (Sanders & Young, 1940; Young, 1979). The maturation of the vertical-subvertical lobe tracts

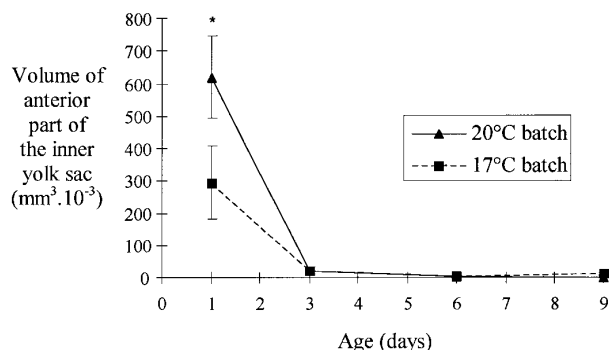


FIG. 8. Volume of inner yolk plotted against age. *Indicates significant difference between the two batches, $p < .05$, randomization test (Fisher-Pitman test). Vertical bars indicate standard deviation.

TABLE 1
Correlation Coefficient of Pearson (r) between Age and Behavioral and Histological Data

	17°C batch	20°C batch
Pursuit rate/age (r_p)	0.92	0.99
Vertical lobe percentage/age (r_v)	0.83	0.74
Frontal superior lobe percentage/age (r_{fs})	0.01	-0.30
Index of the density of tracts/age (r_t)	0.94	0.92
Volume of a part of the inner yolk sac/age (r_y)	-0.69	-0.96

during the first days of postembryonic life allows a more efficient functioning of the following circuit: optic lobe-superior frontal lobe-vertical lobe-subvertical lobe-optic lobe. The vertical lobe, acting via the pre-commissural lobe, might play a role in the control of the pursuit of prey; in fact, the precommissural lobe sends large fibers to the arm centers in the pedal lobe and also to the swimming centers in the magnocellular and palliovisceral lobes (Young, 1979). Via the large reverse pathway from the subvertical to the optic lobes (Young, 1979), the vertical lobe complex can have a reexciting effect on the "classifying cells" in the optic lobe (Young, 1971); this latter center sends efferents to the peduncle and anterior basal lobes which make appropriate adjustments of the eyes during pursuit movements (Young, 1976; Chichery, 1983).

Removal of the vertical lobe in the adult cuttlefish (Sanders & Young, 1940) results in a pursuit deficiency in the achievement of predatory behavior; this pursuit deficiency has also been reported in senescent cuttlefish (Chichery & Chichery, 1992). Neurohistological analysis indicates marked degeneration at the level of the vertical lobe-subvertical lobe tracts which seem crucial for the emergence of pursuit behavior. The much later maturation of long-term memory would appear to reach its optimum at the age of 4 months (Messenger, 1973). Research reported by Young (1979) has shown that the vertical lobe contains three types of cells: large cells around the edge of this lobe, smaller cells throughout the neuropil, and amacrine cells with axons restricted to the vertical lobe. The largest-diameter neurons (of the first type) are at the origin of the vertical to subvertical lobe tracts (Young, 1979). Early maturation of this pathway may underlie the rapid establishment of effective predatory behavior.

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