Post-Hatching Development of the Brain in Octopus ocellatus

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ABSTRACT—To investigate the post-hatching development of the brain in a benthic octopod, *Octopus ocellatus*, we performed volumetric analyses of the brain. The brain consisting of the supra- and subesophageal masses was divided into 5 regions according to the functions suggested for the brain of another benthic octopod *Octopus vulgaris*, and the volume of each region was estimated at three post-hatching ages. We found that the inferior frontal lobe system and the brachial lobe increased in relative volume as the animals grew, while the basal lobe system decreased in relative volume. This result suggests that increasing demand for processing tactile information after hatching is reflected in the higher developmental rate in the centers devoted for tactile sense and related learning. We also found that the inner neuropile layer mainly consisting of dendrites, synapses and axons showed great increases in volume compared with the outer neural-cell-body layer. Although the increase in volume of the inner layer was marked during 1 month after hatching in all brain regions examined, the extent of the increase varied among brain regions. Developmental changes in cell densities in the outer layer also varied among the regions. The present results suggest that the post-hatching development of the brain in *O. ocellatus* is not homogeneous but varies among brain regions depending on different roles in controlling the behavior.

Key words: brain, Cephalopoda, development, *Octopus*, volumetric analysis

INTRODUCTION

Cephalopods are known to have well-developed central nervous system (CNS). In the CNS, the "brain" consists of two large masses, a supraesophageal mass and a subesophageal mass, partitioned by the esophagus. Each of these masses is divided into a number of brain areas or "lobes" (Young, 1971). Some of the brain lobes are subdivided into the anterior, posterior, dorsal, ventral and central regions. In addition to the anatomical features, these lobes have been suggested to subserve particular functions (Young, 1971; Maddock and Young, 1987; Nixon and Mangold, 1996). Although not so many cephalopod species have been examined and the brain structures vary among species (Budelmann *et al.*, 1997), it seems that the functions assigned to particular brain lobes are similar to those of the counterparts in different species (Young, 1971; Budelmann *et al.*, 1997). For example, the supraesophageal mass is a combination of the lobes playing roles as higher centers. The subesophageal mass is a combination of intermediate or lower motor centers.

Although cephalopods don't have true larvae, post-hatching animals show various modes of growth depending on species. A well-studied octopod *Octopus vulgaris* spends planktonic life for a while after hatching (Boletzky, 1977). On the other hand, the hatchlings of typical sepioids and some octopods, such as *Octopus briareus* and *Octopus ocellatus*, begin nekto-benthic or benthic life as miniatures of the adults (Boletzky, 1977). For example, *O. ocellatus* swim vigorously after hatching and settle within several hr (Yamamoto, 1941).

Differences in the brain structures among cephalopods reflect not only phylogenetic relationships but also variety in life styles (Young, 1977, 1988; Maddock and Young, 1987). The effectiveness of higher functions of a brain depends on the number of nervous channels and the volumes of the parts of the brain devoted to different activities are significant functional parameters (Maddock and Young, 1987). Development of behaviors and changes in life style with growth may well be related to post-embryonic development of brain lobes (Nixon and Mangold, 1996; Dickel *et al.*, 1997; Shigeno *et al.*, 2001a, b). There are differences in ontogenetic changes in mode of life among species, and hence the modes of brain development are also distinct from species to spesies. Maddock and Young (1987) reported that a benthic species, *Octopus vulgaris* is distinct from pelagic species, largely on the basis of the greater brachial lobe and the inferior frontal lobe, which have been suggested to be concerned with tactile sense. *O. vulgaris* has planktonic

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phase of post-hatching development called paralarvae, and drastic morphological changes take place during this period. The changes in morphology with growth are also reflected in development of the brain lobes. In *O. vulgaris*, the neuropile mainly consisting of dendrites with synapses increases in volume in the tactile memory centers (Nixon and Mangold, 1996). Contrary, in the swimming centers of the same species, the neuropile layers decrease relatively around the time of the settlement of planktonic paralarvae (Nixon and Mangold, 1996).

A pelagic species, *Todarodes pacificus*, also spends a planktonic period as paralarvae before becoming juveniles. Relative volume of the lobes in the olfactory system decreases but that of the optic lobes increases with growth in the paralarvae (Shigeno *et al.*, 2001a). The change of dominance between olfactory and optic lobes during paralarvae in this species suggests the shift of the feeding mode from an olfaction-dependent to vision-dependent (Shigeno *et al.*, 2001a).

Embryonic development of brain lobes has been described in both benthic and pelagic cephalopod species, such as *O. vulgaris* (Marquis, 1989 cited by Shigeno *et al.*, 2001a), *Todarodes pacificus* (Shigeno *et al.*, 2001a), and *Sepioteuthis lessoniana* (Shigeno *et al.*, 2001b). However, there is little information about early post-embryonic development of the CNS in benthic cephalopods. Considerable difficulty in keeping hatched cephalopods is apparently a limiting factor of the study. In the present study, we chose *O. ocellatus* as experimental animals since the eggs and hatchlings are relatively easy to keep. In order to investigate development of the CNS in *O. ocellatus*, we examined the developmental changes in volumes of the brain and its parts in the course of the development. In addition, we compared our results in *O. ocellatus* with the post-hatching development of the brain in *O. vulgaris* (Nixon and Mangold, 1996), which is another benthic species but have a different mode of post-hatching development.

MATERIALS AND METHODS

Animals

Adult benthic octopods, *Octopus ocellatus*, were collected near the Ushimado Marine Laboratory located on the coast of Seto Inland Sea and transferred to the Fisheries Laboratory of Hiroshima University. A batch of eggs was obtained from a female octopod kept in the Fisheries Laboratory. The hatched larvae were divided into two groups and reared under different conditions. One group (group A) of 9 individuals was kept in a 450 l opaque plastic tank in which rocks and shelters were placed to enrich the rearing environment. Another group (group B) of 8 individuals was kept separately in 3.6 l transparent plastic tanks. Rocks and shelters were placed in each tank so that the environment except for social interactions was similar to that in group A. All rearing tanks were continuously supplied with filtrated sea water in 19–27°C. The animals were fed on hermit crabs, snails and clams. Care was taken so that the feeds were always available to all individuals.

Histology

On the next day of hatching, and 1 and 3 months after hatch-ing, 3 animals in each group were sampled for histological procedure. The animals were anesthetized in cold sea water (<5°C). After measuring the dorsal mantle length (ML), they were fixed in cold Gender's solution and kept in a fridge. The fixed animals were embedded in paraffin wax and cut into serial sagittal 10 μm thick sections. The sections were stained with Mayer's hematoxylin and eosin (HE) and then photographed using a digital camera through an optical microscope.

Identification of brain lobes

The brain of cephalopods consists of two large brain masses, the supraesophageal mass and the subesophageal mass, partitioned by the esophagus. Based on anatomical features, the masses are divided into "lobes" (Young, 1971). To identify the brain lobes in *O. ocellatus*, we referred to the results in another benthic octopod *Octopus vulgaris* (Young, 1971). We grouped the lobes (Table 1 and Fig. 1) into several units or systems according to the suggested functions of each lobe (Young, 1971; Hobbs and Young, 1973; Maddock and Young, 1987; Nixon and Mangold, 1996). The vertical lobe system (VERT) is a unit concerned with memory and learning. The VERT measured in the present study included the vertical lobe, subvertical lobe and superior frontal lobe. The inferior frontal lobe system (INFF) is a unit concerned with tactile learning, and, in the present study, it included the inferior frontal lobe and superior buccal lobe. The basal lobe system (BASAL) is a higher motor center receiving visual and static inputs. The BASAL measured in the present study included the median basal lobe and dorsal basal lobe. In the present experiments, lobes in the subesophageal mass, including the brachial lobe (Br) and the palliovisceral lobe (Pv), were measured separately. The former is a lower motor center for the arms, and the latter is concerned with diverse functions including the control of the mantle and viscera.

Table 1. Brain lobes measured in the present study and abbreviations of the lobes or lobe systems.

Supraesophageal mass
Vertical lobe system (VERT)
vertical lobe (VL)
subvertical lobe (SVL)
superior frontal lobe (SFL)
Inferior frontal lobe system (INFF)
inferior frontal lobe (IFL)
superior buccal lobe (SBL)
Basal lobe system (BASAL)
median basal lobe (MBL)
dorsal basal lobe (DBL)
Subesophageal mass
Brachial lobe (Br)
Palliovisceral lobe (Pv)

Measurement of the brain regions

Volumes of the brain regions were estimated from the areas measured on the pictures of the serial sagittal sections using an image analyzing software, NIH image, on an Apple Macintosh computer. In each lobe or lobe system, the outer layer mainly consisting of cell bodies of neurons and the inner layer consisting of neuropile were measured separately. To compare the developmental
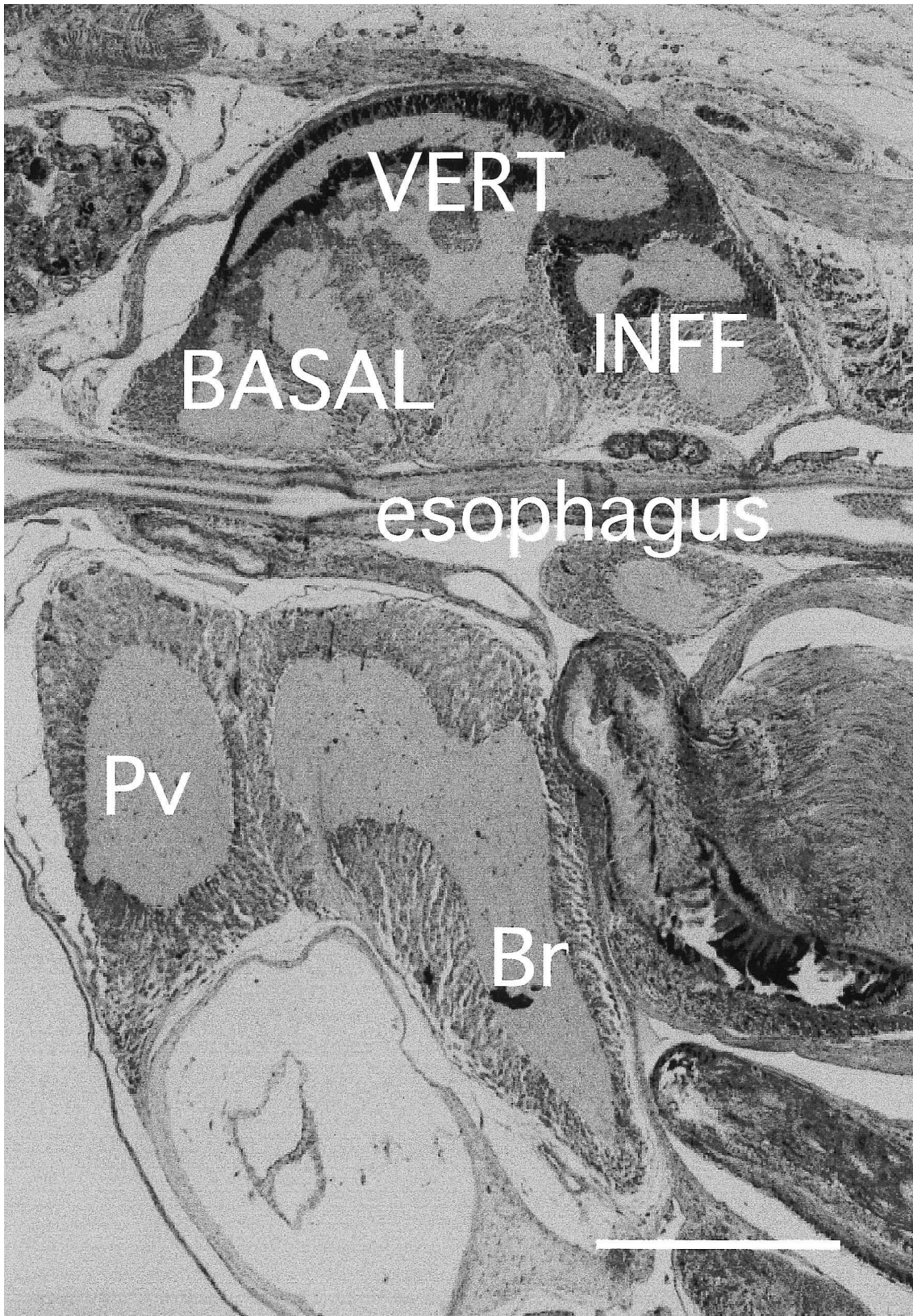
Fig. 1. Photomicrograph of a sagittal section of the brain in 1-month-old *Octopus ocellatus*, showing the brain regions measured in the present study. Dorsal is to the top, rostral to the right. For abbreviations see Table 1. Bar=500 μm.

Table 2. Mantle lengths and volumes of the brain regions in the individuals at three post-hatching ages. The values are expressed in mm for the mantle length (ML) and mm³ for the the others. JAH, just after hatching; 1M, 1-month-old; 3M, 3-months-old. Refer Table 1 for abbreviations.

		ML	VERT	INFF	BASAL	Br	Pv	Whole brain
JAH	1	2.73	0.31	0.15	0.27	0.36	0.50	2.37
	2	2.80	0.35	0.14	0.33	0.33	0.53	2.24
	3	3.20	0.39	0.21	0.39	0.39	0.56	2.68
1M	1	10.00	6.65	3.92	5.10	8.65	10.96	45.64
	2	10.40	5.72	5.66	8.10	11.06	13.50	50.86
	3	11.70	9.31	5.71	8.51	16.59	19.74	80.90
	4	12.20	10.20	4.77	2.95	6.83	10.53	51.00
	5	12.35	7.46	4.63	7.10	11.28	13.30	55.61
	6	12.40	8.38	4.83	7.91	8.92	12.20	59.15
3M	1	33.75	24.35	15.66	11.23	15.35	17.29	185.54
	2	39.85	22.73	15.72	13.68	34.85	35.03	163.03
	3	39.90	31.05	19.88	18.21	42.28	50.33	209.40
	4	41.70	36.24	32.25	27.31	75.64	71.33	322.71
	5	44.50	48.33	20.33	24.11	65.70	49.85	241.34

changes of the brain regions with each other, as well as the absolute volumes of the brain regions, relative volume of the each of the brain regions as a percentage of the whole brain was also calculated. In the vertical lobe system, the inferior frontal lobe system and the brachial lobe, we counted the number of cell bodies per unit area of the outer layer in the sagittal plane at the midline. Since it was difficult to discriminate neural cells from non-neural cells in HE-stained sections, we counted the number of all cell bodies per unit area.

Data analysis

In order to examine the differences in mantle length and brain volume between group A and group B, the Mann-Whitney U-test was used. The relationship of the volume in each brain region and mantle with growth was examined by regression analysis. Differences in the mantle length, brain volume and number of cell bodies among three developmental stages were analyzed using one-way ANOVA. In the cases where the differences were found to be significant, the Sheffe test was additionally used. In all tests used in the present study, the differences or the relationships were considered to be significant when $P<0.05$.

RESULTS

Post-hatching behavior of *Octopus ocellatus*

We confirmed the observation by Yamamoto (1941) on the behavior of juvenile *O. ocellatus*. Newly hatched *O. ocellatus* swam for a while and settled within one day after hatching, then began nekto-benthic or benthic life. The hatchlings with external yolks began to feed within a few days after hatching. They visually oriented themselves to prey animals as if they were miniatures of adult.

Development of body size and volume of brain lobes

There was no significant difference in mantle length and whole brain volume between the group A and group B. Thus, the data obtained from these two groups were pooled and subjected to the following analysis.

Table 2 shows the results of the measurement of the mantle lengths and the brain volumes. Fig. 2 shows the developmental changes of the mantle length and the whole-brain volume of *O. ocellatus* during the post-hatching period. Both the mantle length and the brain volume are markedly increased during the period from 1 to 3 months after hatching. Dorsal mantle lengths were 2.73–3.20 mm just after hatching (n=3), 10.00–12.40 mm at 1 month (n=6) and 33.75–44.50 mm at 3 month after hatching (n=5). The mantle length of 3-month-old young was comparable to that of the adults. Volumes of the whole brain including supra- and subesophageal masses were 2.24–2.68 mm³ just after hatching (n=3), 45.64-80.90 mm³ at 1 month (n=6) and 163.03–322.71 mm³ at 3 month after hatching (n=5).
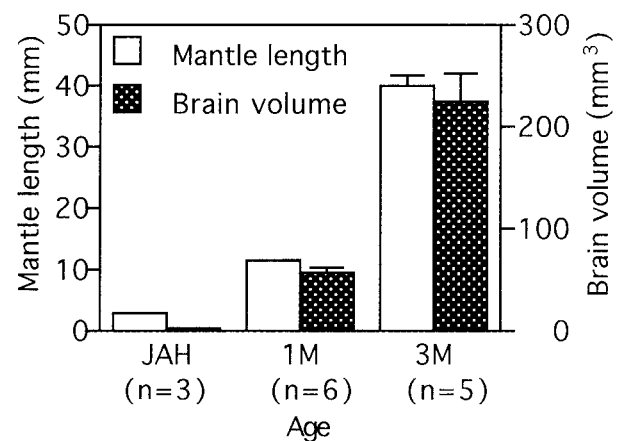

Fig. 2. The mantle length and the brain volume in *O. ocellatus* at three post-hatching ages. JAH, just after hatching; 1M, 1-month-old; 3M, 3-month-old. Bars indicate SE.

To compare the developmental changes of the brain regions grouped according to the suggested functions, we estimated relative volume of some lobes and lobe systems in relation to the whole brain.

Fig. 3 shows the relative volume of the brain lobes and the lobe systems in newly hatched and 1-month- and 3-month-old youngs. Although there were not dramatic changes in volume with growth in all lobe systems and lobes, the mode of the development in relative volume varied among brain regions. The inferior frontal lobe system and the brachial lobe tended to be relatively increased as the animals grew (Fig. 3). Regression analysis further confirmed that the relative volume of the inferior frontal lobe system and the brachial lobe significantly increased in the course of development (Fig. 4).

On the other hand, the basal lobe system was found to be decreased in relative volume from 1 month to 3 month after hatching (Fig. 3). Furthermore, the decrease in relative volume of the basal lobe system during the 3-month post-hatching period was found to be significant (Fig. 4). Other brain regions, the vertical lobe system and the palliovisceral lobe did not show obvious developmental changes in the relative volume.
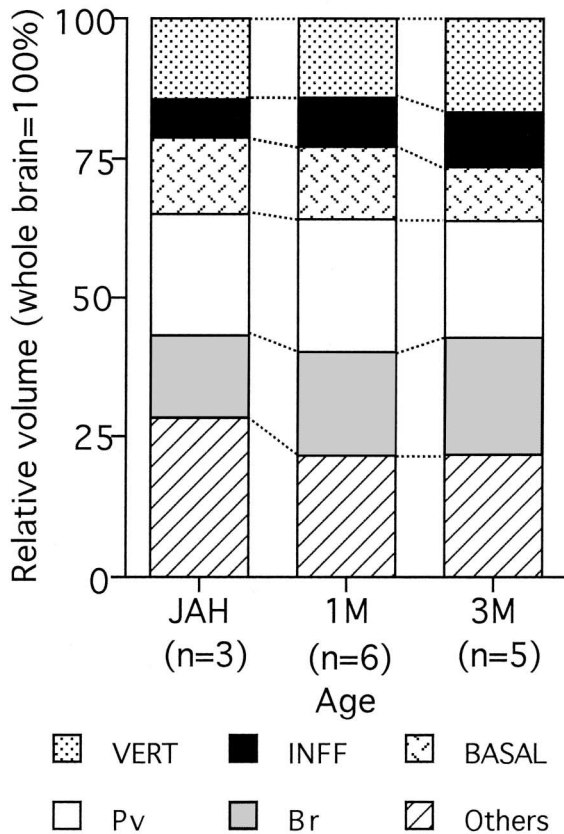

Fig. 4. Relationships between body size as expressed by mantle length^3 (mm^3) and relative volume of the inferior frontal lobe system (A), the brachial lobe (B) and the basal lobe system (C). Note that the mantle length^3 is shown on logarithmic scale. The solid lines indicate regression lines ($P<0.05$).

Development of layer structures in the brain lobes

In order to investigate further the course of development of the brain of *O. ocellatus*, we measured the volume of cell-body layer (outer layer) and neuropile layer (inner layer) in each brain region separately. As a result, relative volume of the inner layer was found to be increased in all brain regions as the animals grew (Fig. 5).

At hatching, there were little variations in the ratio of the outer layer volume to the inner layer volume among the brain regions (Fig. 5). In all regions examined, the outer layer was found to occupy more than 80% of the volume of each region at hatching (Fig. 5). By 1 month after hatching, however, proportion of the outer layer to the inner layer decreased in all regions examined. In the basal lobe system, the inner layer became larger than the outer layer in 1 month after hatching (Fig. 5). In the vertical lobe system, however, the inner layer was much smaller than the outer

Fig. 3. Relative volume of the brain regions in post-hatching *O. ocellatus* at three developmental ages. JAH, just after hatching; 1M, 1-month-old; 3M, 3-month-old. See Table 1 for abbreviations of the brain regions.
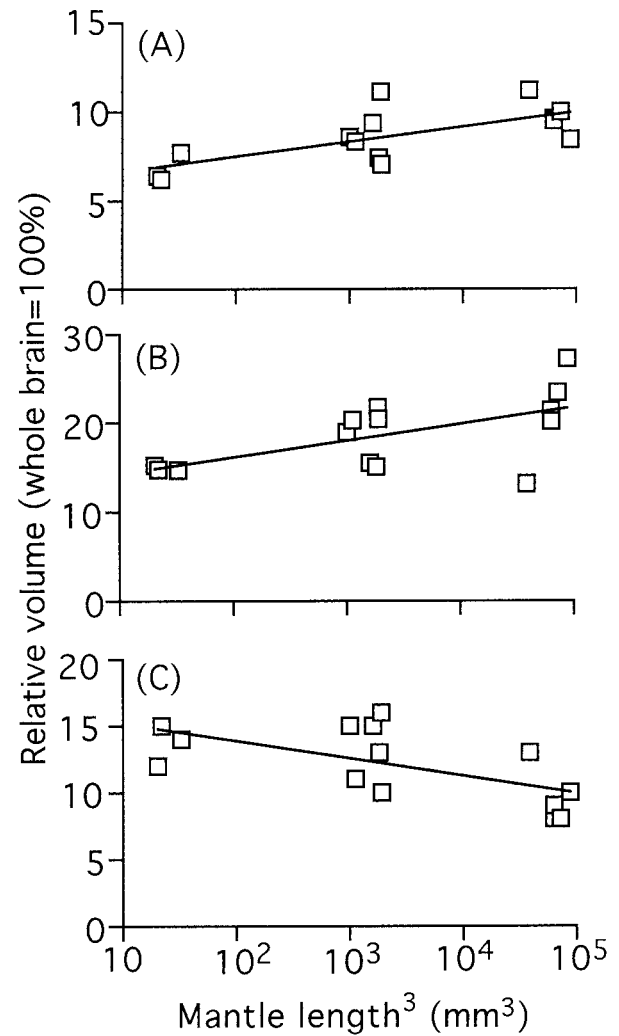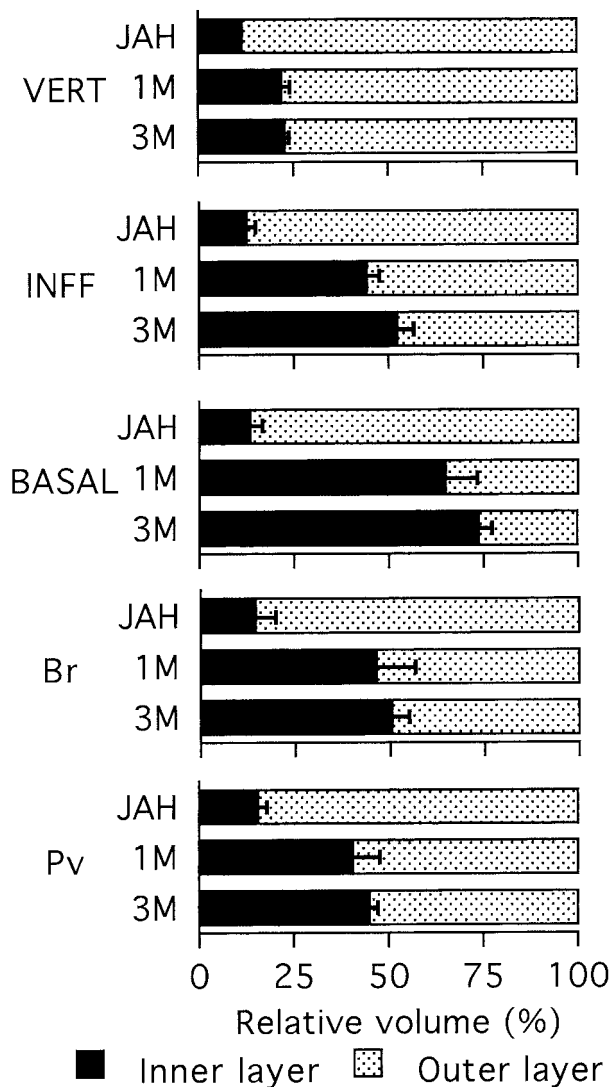
Fig. 5. Average relative volume of the inner layer and the outer layer in five different brain regions at three post-hatching ages. JAH, just after hatching (n=3); 1M, 1-month-old (n=6); 3M, 3-month-old (n=5). Bars indicate SE. For abbreviations see Table 1.

layer even in 3-month-old young (Fig. 5). During the period from 1 to 3 month after hatching, the inner layer did not show marked increase in the relative volume in all regions examined (Fig. 5).

Post-hatching changes of the size and density of neural cells

In order to investigate the development of cytoarchitectures in various brain regions, we counted the number of cell bodies per unit area (10^4 μm^2) in the outer layers of the vertical lobe, the inferior frontal lobe and the brachial lobe.

Fig. 6 shows the constructions of the outer layers in the vertical lobe, the inferior frontal lobe and the brachial lobe at three developmental stages. In the vertical lobe and the inferior frontal lobe, it was found that shapes of the cell bodies did not show marked changes as the animals grew (Fig. 6A,

B). On the other hand, in the brachial lobe, the size of the cell bodies was considerably larger in older animals with a larger variation than that in younger ones (Fig. 6C).

Fig. 7 shows the density of cell bodies in the three different brain lobes. The cell-body density in the vertical lobe was found to be increased during the period from 1 to 3 month after hatching, although the difference was not statistically significant. In the inferior frontal lobe, both shape and the density of the cell bodies did not show marked changes (Fig. 6B and 7). In the brachial lobe, on the other hand, the density was greatly decreased during the period from hatching to 1 month (P<0.05) (Fig. 7).

DISCUSSION

Cephalopods have various life styles depending on species (Boletzky, 1977). It has been suggested that differences in the brain structures among cephalopods reflect not only phylogenetic relationship but also variety in life styles (Young, 1977, 1988; Maddock and Young, 1987). Development of behaviors and changes in life styles with their growth are also related to post-embryonic development of brain lobes (Messenger, 1963; Frösch, 1991; Nixon and Mangold, 1996; Dickel *et al.*, 1997; Shigeno *et al.*, 2001a, b). In order to investigate the developmental changes of the brain in a benthic species, *Octopus ocellatus*, we performed a volumetric analysis of the brain regions in this species.

In *O. ocellatus*, post-hatching development in brain regions with their growth was found to be characteristic of each lobe or lobe system. Relative volume of the inferior frontal lobe system, which is concerned with tactile learning, and the brachial lobe, which is a lower motor center for arms, significantly increased. The increase in relative volume of the brachial lobe is partly due to the increase in the neuronal size in this region (see Fig. 6C), which probably reflects the increase in the muscular mass that the neurons in this lobe innervate. In contrast, relative volume of the basal lobe system, which is a higher center concerned with control of movements and viscera, decreased during the period from 1 to 3 month after hatching. Since *O. ocellatus* is a benthic species, which settles soon after hatching, increasing demands for processing tactile information may well be reflected in the development of the brain regions devoted to tactile sense and related learning. In *Sepia officinalis*, changes in relative volume in accessory lobes are related to emergence of predatory pursuits in the nekto-benthic young (Dickel *et al.*, 1997). Shigeno *et al.* (2001a) have suggested for the oegopsid squid, *Todarodes pacificus*, that there are relationships between differentiation of a certain lobe and onset of a certain behavior. The present results support the idea that the development of particular brain regions is related to the development of behavior.

In all brain regions, the ratio of neuropile layer to cell-body layer greatly changed during 1 month post-hatching. In mammals, dendrites and axons of young neurons grow extensively and form synapses. The biggest changes in
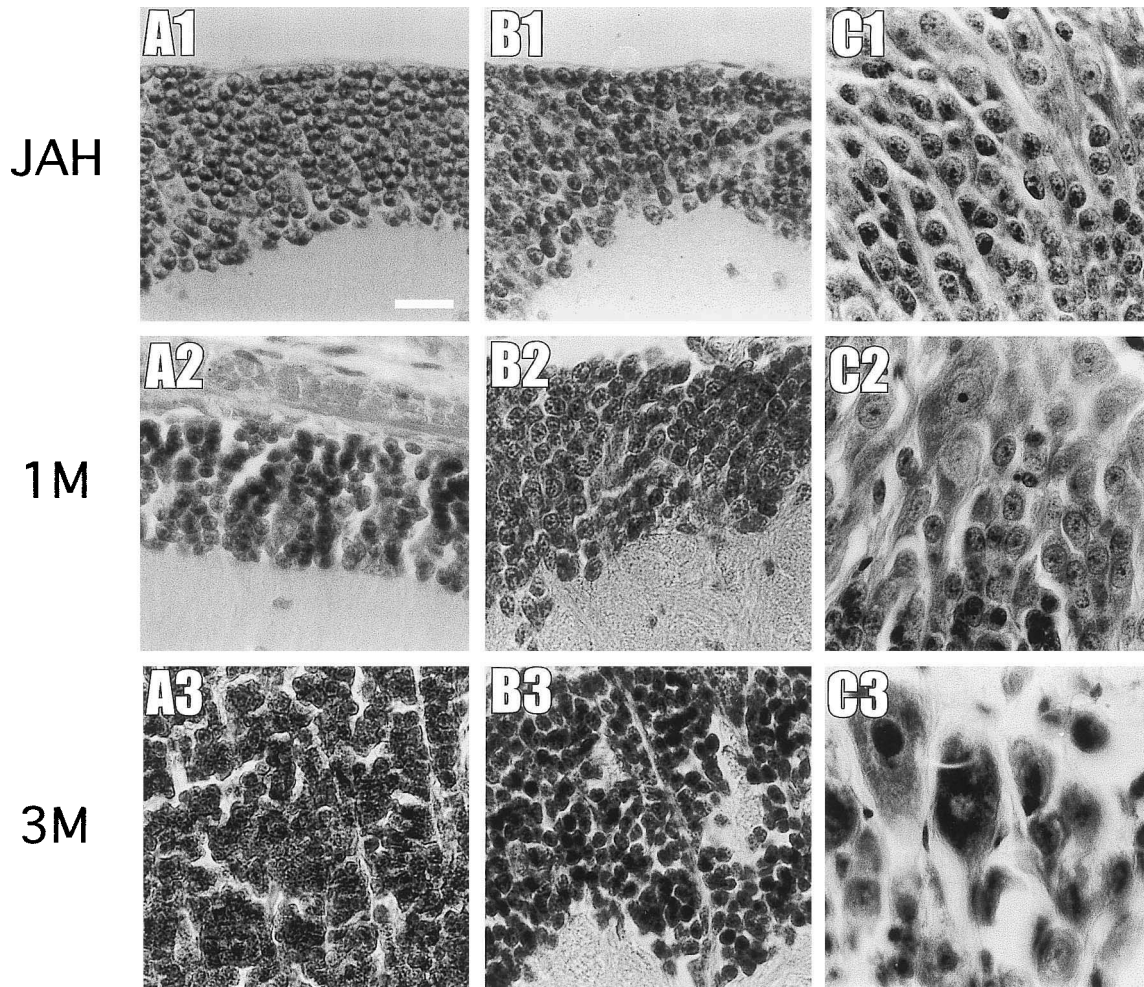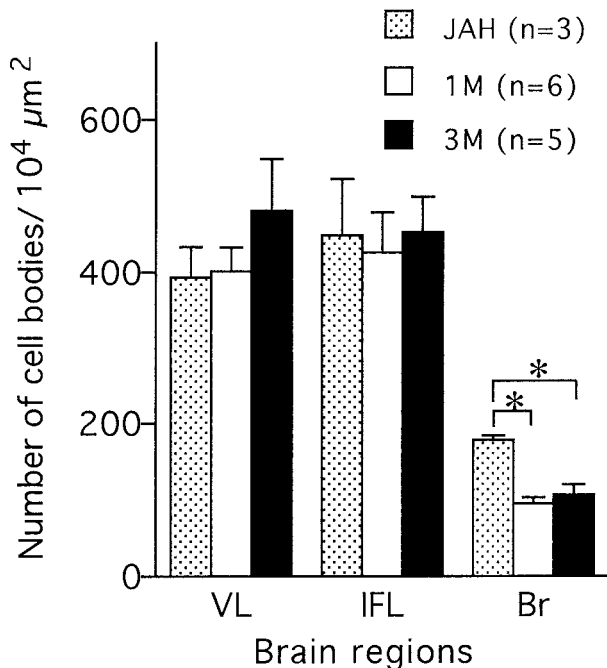
Fig. 6. Photomicrographs of the outer layers of the vertical lobe (A), the inferior frontal lobe (B) and the brachial lobe (C), showing the developmental changes of the morphology of neuronal somata. JAH, just after hatching; 1M, 1-month-old; 3M, 3-month-old. Scale bar=20 μm.

brain cells from birth to maturity take place in the branches and connections among neurons (Rosenzweig *et al.*, 1996). This must be the case in invertebrates as well. In *T. pacificus*, each lobe grows with an increase in volume of the neuropile from the late hatchlings to the juveniles (Shigeno *et al.*, 2001a). The great increase of the neuropile layer during 1 month after hatching suggests that this period is an important stage for *O. ocellatus* to establish an adaptive life style on the bottom of the sea.

Here we compare post-hatching development of the brain between *O. vulgaris* and *O. ocellatus*.

In *O. vulgaris*, which spend a planktonic phase after hatching for a while before settling (Boletzky, 1977; Young and Harman, 1988, cited by Shigeno *et al.*, 2001a), relationships between changes of the life style and the development of the CNS have also been studied (Nixon and Mangold,

Fig. 7. The density of neuronal somata in the outer layers of the vertical lobe (VL), the inferior frontal lobe (IFL) and the brachial lobe (Br) at three post-hatching ages. Bars=SE. Asterisks indicate significant differences between ages (P<0.05). JAH, just after hatching; 1M, 1-month-old; 3M, 3-month-old.
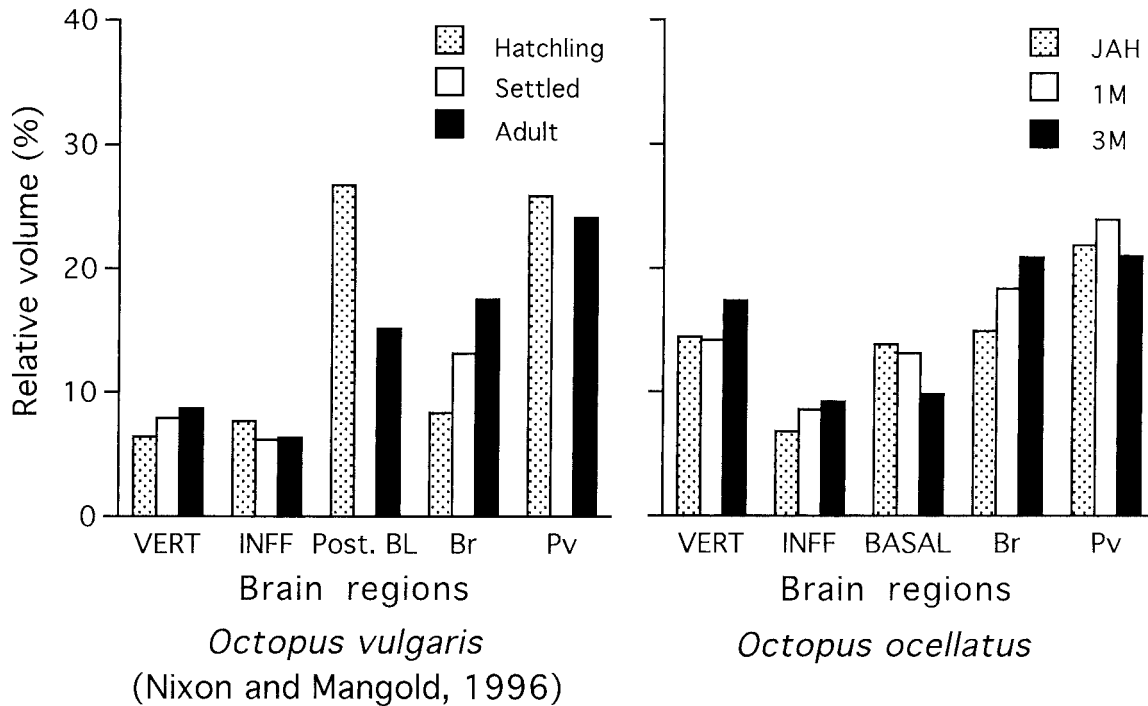
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Fig. 8. Comparison of developmental changes of the volumes of the various brain regions between *Octopus vulgaris* (adapted from table IV in Nixon and Mangold, 1996) and *Octopus ocellatus*. The posterior basal lobe (Post. BL) in *O. vulgaris* corresponds to the basal lobe system in *O. ocellatus*. Data of the posterior basal lobe and the pallioviseral lobe in the settled young of *O. vulgaris* are not available. JAH, just after hatching; 1M, 1-month-old; 3M, 3-month-old.

1996). Fig. 8 shows the comparison of the post-hatching development of the brain regions between two benthic octopod species, *O. vulgaris* and *O. ocellatus*. From an ecological point of view, a hatchling of *O. ocellatus* is equivalent to a newly settled young of *O. vulgaris*, because the settlement is the beginning of the life as benthic predators. At hatching, in *O. vulgaris*, the brachial lobe forms only 8.31% of the volume of the whole brain (Nixon and Mangold, 1996). However, after settlement, this lobe forms 13.12% of the volume of the whole brain and the increase corresponds to the rapid development of the arms at this time (Nixon and Mangold, 1996). In *O. ocellatus*, the brachial lobe occupies considerable part (about 15%) of the brain at hatching, reflecting early start of benthic life in this species. The basal lobes in *O. vulgaris* are relatively large and especially important while the paralarva is living and feeding in the water column, because they are concerned with the control of complicated movement (Nixon and Mangold, 1996). On the other hand, the basal lobe system is relatively small in the hatchling of *O. ocellatus*, which has no planktonic stage of the post-hatching development. The vertical lobe system, which is a part of the visual and tactile learning system (Young, 1971), is relatively large in *O. ocellatus* compared with that in *O. vulgaris*. Whether this difference reflects the difference in the species-specific life style is yet to be determined.

The present results on *O. ocellatus*, together with the comparison between two octopod species, *O. vulgaris* and *O. ocellatus*, demonstrate that the post-hatching changes of the life styles in cephalopods are reflected in the development of the brain regions each having different functions. In all brain regions the ratio of neuropile layer (inner layer) to the cell-body layer (outer layer) greatly changed during 1 month after hatching (see Fig. 5). The marked increase in the inner layer indicates the rapid formation of contacts between neurons during the early post-hatching period. Since the neural connections can be affected by postnatal experiences (Rosenzweig *et al.*, 1996), this period would be very important for the development of the brain and behavior in *O. ocellatus*.

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