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Research Report

Using ultrasound to estimate brain size in the cephalopod *Octopus vulgaris* Cuvier in vivo

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ABSTRACT

Ultrasound imaging was applied, for the first time, in the examination of the central nervous system of the cephalopod mollusc *Octopus vulgaris*, an invertebrate. Goals of this study were: *i.* to reveal and measure the cerebral masses in vivo, in their anatomical position; *ii.* to evaluate and compare the dimensions of the different parts of the octopus brain in vivo and postmortem, and *iii.* to test the reproducibility of the ultrasound method both in reaching a given sonographic plane in the same individual at two different times and in evaluating potential changes in brain size due to animal growth. Our results show that ultrasonography is a reliable method to measure the various parts of the octopus brain. Sonographic measurements of the brain masses in vivo were correlated with those determined postmortem. In addition, brain size estimation is reproducible via ultrasound: no significant difference resulted when measurements of the same brain were taken over consecutive days. Furthermore, when the time lapse between the two sonographic examinations was long enough (30 days), we were able to detect changes in brain dimensions in the same octopus.

1. Introduction

A great deal of research has been focused on the cephalopod mollusc *Octopus vulgaris*, an invertebrate, over the last 50 years. The high level of centralization of the nervous system coupled with the relative accessibility of the neural centers, pertaining to motor control and learning, has allowed *O. vulgaris* to be chosen as a model for studies on the neural basis of complex behavior (Boycott, 1954; for a review see for example: Young, 1961; Boyle, 1986; Hochner et al., 2006). The picture that has emerged from these works shows that octopus nervous system is organized in a series of neuroanatomical and functional matrices (each controlling chemio-tactile and visual sensorimotor processing) that work in parallel and that are considered analogous to the limbic system of vertebrates (Young, 1991).

Almost all the studies carried out to disclose the neural correlates of behavioral and learning capabilities of *O. vulgaris* were based on ablations (for a review see Sanders, 1975), which were favored by *Octopus* capability to recover promptly from massive brain surgery (Boycott, 1954; Young, 1971). A different approach, based on electrical recording or lesioning, was applied in a handful of studies by positioning electrodes or cannulas in specific areas of the cephalopod brain. However, this technique did not allow to pinpoint an internal target a priori so that the exact localization of the interference site of the neural circuit was possible only by post hoc histological examination (for example in *Sepia officinalis*:

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Boycott, 1961; Chichery and Chanelet, 1976; Chichery, 1983; Chichery and Chichery, 1985; Bullock and Budelmann, 1991; Halm et al., 2002; in *O. vulgaris*: Zullo, 2004; Kuba et al., 2006; Zullo et al., 2006).

The intrinsic limits of electrical recording, microstimulation, or lesioning, as described above, are related with the design of the cephalopod's brain. The central nervous system of the octopus consists of a neural mass, usually called "brain", positioned on the head between the eyes. In brief, the neural mass surrounds the anterior part of the esophagus, just behind the buccal apparatus (Pfefferkorn, 1915; Thore, 1939; Young, 1971). Three parts are clearly distinguishable: *i.* an upper helmet-shaped supraesophageal mass (dorsal to the esophagus); *ii.* a lower saddle-shaped subesophageal mass (ventral to the esophagus), and *iii.* two kidney-shaped optic lobes lateral to the supraesophageal mass (one for each side) resting on the floor of the eye sockets, just behind the eyes. The supra- and subesophageal masses are linked via the magnocellular lobes and other connectives and are enclosed in a tough cartilaginous box (called cranium) with its lateral ridges supporting the optic lobes and eyes. The optic lobes are sandwiched between the white bodies: multi-lobular hemopoietic glands positioned just behind the eyeballs (Young, 1971; Budelmann et al., 1997).

The absence of vertebrate skull-like structures does not allow to identify any superficial landmark and forces the opening of the cranium (mainly from the dorsal surface) to reach a target of the supraesophageal mass: a limit for the application of any preplanned approach (e.g. stereotaxis).

This limit can be overcome by ultrasonographic techniques. During the last decade, ultrasonography has been applied to cephalopods to examine structures internal to the mantle (*Eledone cirrhosa*, Davenport, 1993) or to study the role of the mantle contractions/expansions during locomotion and respiration (*O. vulgaris*, Tateno, 1993), or the contribution of the veins and mantle contractions in returning blood to the branchial hearts (*S. officinalis*, King et al., 2005). Here, we employed ultrasound imaging for the first time as support to behavioral neurobiology experiments in *O. vulgaris*. Our aims were: *i.* to identify and measure the cerebral masses in vivo; *ii.* to determine correlations between measurements of the brain derived via ultrasonography and those in situ taken postmortem; *iii.* to determine whether measurements taken at a given plane were repeatable (by ultrasound examination of the same animal over consecutive days); and *iv.* to check the feasibility in measuring changes in brain size due to changes in body size (by taking measurements in the same animal after a month). The final goal is the promotion of experiments where ultrasound may assist reaching internal targets without the need of massive brain surgery, thus allowing a planned interference of the circuit involved in neural processing.

2. Results

2.1. *Representative sonogram images of the whole brain*

In Fig. 1 typical sonographic images of the octopus brain are shown and compared with histological sections corresponding to the same anatomical plane. In the transversal plane we could see the brain entirely (Fig. 1A). The supraesophageal mass lying in median position, below the cranium (and its cavity), appeared clearly connected to the optic lobes, to the right and left, via the optic tract. The outline of the U-shaped subesophageal mass was also quite distinct despite the brightness of the esophagus (the bright circular spot in Fig. 1A) and reverberation due to the structures below (e.g. otoliths). The outline of the cranium surrounding the masses, together with other muscular and connective tissues, was also revealed.

In the longitudinal planes the shapes and dimensions of the supraesophageal mass and optic lobes were distinct (Figs. 1B, C). In contrast, the outline of the subesophageal mass was poorly distinguishable in this plane.

2.2. *Sonographic estimation of brain dimensions*

Sonographic measurements of the brain masses were correlated with those determined postmortem (Table 1). This was true both when the transducer was held by hand and when it was held by using the device, especially for the supraesophageal mass. However, correlation coefficients were worse when the device was used (Table 1).

Moreover, estimation of the brain's size was replicable in the same animal over two consecutive days (Table 2) independently from the method utilized to move the holder.

2.3. *Variation in brain dimensions*

Contrary to what resulted when measurements were taken over consecutive days, the linear coordinates of the sonographic planes changed in 54% of the scannings when Day 1 and Day 30 measurements were compared (Table 3). This corresponded to a significant increase in the length (AP SEM) and height (DV SEM) of the supraesophageal mass and the length of the optic lobe (AP OL). In contrast, we were not able to detect significant changes in the width of the supraesophageal mass (LL SEM) and height of the optic lobe (DV OL). Finally, on average the width of the optic lobe (LL OL) appeared decreased. At the same time the body size increased by about 9.5% on average (delta of body weight between Day 1 and Day 30: 4 to 27 g). This difference, although small, was significant (t-paired$_{24}$=3.039, P=0.006; data log-transformed) and corresponded to an average increase in mantle length of more than 10 mm.

3. Discussion

Sonography is well-recognized as a powerful tool, widely applied to several levels of research. Originally adopted only for classical clinical applications (Devey and Wells, 1978), ultrasound imaging is now considered an experimental support also for innovative clinical operations (Renner et al., 2005) and physiological studies, including behavioral biology, from invertebrates (Davenport, 1993; Tateno, 1993; King et al., 2005; King and Adamo, 2006) to vertebrates (e.g. Glimcher et al., 2001).

The aim of this work was to apply a non-invasive method to evaluate the dimensions of the brain in the cephalopod mollusc *O. vulgaris*. By providing images through the reflection