Hypoxia induced changes in the brain of hypoxic tolerant cuttlefish *Sepia officinalis*

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Introduction

Marine invertebrates are frequently used as model organisms in neurological research. Especially experiments on isolated neurons of snails or the giant axon of cuttlefish contributed to a basic and fundamental understanding in neuronal signal transduction and reception (e.g. Kupfermann & Kandel 1969). More recently, a limited number of NMR studies on isolated neurons of marine invertebrates were reported in the literature (Schoeniger et al. 1994, Grant et al. 2000, Grant et al 2001). Since MR imaging and spectroscopy become more and more important to study overall brain function and connectivity, the aim of this study was to develop a setup for MR studies of brain function in the cuttlefish *Sepia officinalis in vivo* under different physiological conditions. Cephalopods, although invertebrates, have specialized skills (e.g. independent coordination of different arms) and are believed to display neural abilities and complexity similar to those of small rodents. These characters are associated with an energy consuming lifestyle. Nonetheless, the cuttlefish *Sepia officinalis* is a hypoxia tolerant species and may thus be an ideal model species for the study of hypoxia tolerant brain functions.

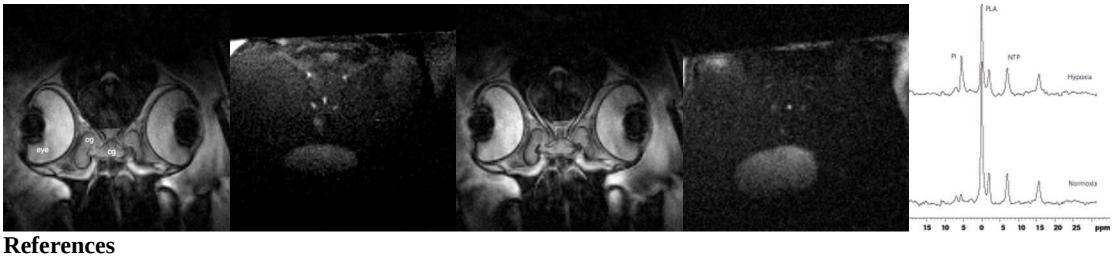
Materials and methods

Sepia officinalis from the North Atlantic were hatched in aquaria at the Alfred-Wegener-Institute and cultivated since 2002. Animals of approximately 20 cm size and ca. 360-450 g weight were used for MR experiments (n=4). Animals were placed in a flow through setup similar to Bock et al. 2002. Briefly, seawater was supported by hydrostatic pressure through a Perspex chamber designed with an adjustable slider. A triple tunable ^{1}H-^{13}C-^{31}P-NMR surface coil (diameter 5cm) was placed directly onto the chamber close to the head of the animal. The position of the head was centered via the slider under the surface coil for optimal localization and signal to noise ratios. Pilot scans were collected to check the position of the animal relative to the coil prior to experimentation and repeated regularly during the whole experimental procedure. Animals were kept for 24 hours minimum within the magnet to acclimate, excluding any effects of handling stress. After a control phase of around 5 hours, mixing nitrogen gas into the sea water supply induced hypoxia (98% N_2). Hypoxic conditions were confirmed by measuring the oxygen content in seawater with oxygen sensors before and after passing the animal chamber. The experimental protocol was as follows: blocks of in vivo ^{31}P-NMR spectroscopy flow weighted MRI and T_{2*} weighted MRI following ^{31}P-NMR spectroscopy was collected in repeated series. After one and a half hours of hypoxia, normoxic conditions were re-established and animals were monitored during a period of two hours of recovery.

Results and discussion

MR images from non-anaesthetized and unrestrained cuttlefish were obtained without any movement artefacts from the head of the animal. The high resolution allowed the differentiation of various brain structures in coronal MR images (Figure A). Flow weighted axial MR images revealed the network of blood vessels surrounding the cuttlefish brain in excellent agreement with former anatomical studies (Figure B). T_{2*}-weighted images were without any susceptibility artefacts giving the opportunity to investigate tissue oxygenation changes (Figure A+C). Interestingly, no obvious changes could be observed in T_{2*}-weighted images under hypoxic conditions (PO_2 <6,4 kPa), although flow decreased in brain vessels (Figure D) and concomitant ^{31}P-NMR spectra showed increased inorganic phosphate/phospho-l-arginine ratios indicating onset of anaerobiosis. Recovery to control conditions could be observed after 30 minutes of normoxia.

The BOLD contrast rely on oxygenation changes of the respiratory pigment, in particular haemoglobin, but cephalopods use haemocyanin as their oxygen carrier protein. Preliminary results from high resolution NMR spectra of haemocyanin samples at different oxygenation states indicate that the line width of the water signal will not be affected by the different oxygenation states, explaining the unchanged T_{2*} weighted image contrast.



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