A new set-up to investigate neurophysiological effects of CO2-induced ocean acidification on the brain of fish via MR imaging and spectroscopy

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Introduction

Excessive burning of fossil fuels will cause a sharp increase in the partial pressure of carbon dioxide (CO_2) within the worlds` atmosphere from a present level of 400 µatm to about 1000 µatm by the year 2100[1]. $CO_2$ leads not only to the warming of the worlds` climate, but dissolves also inside the oceans leading to their acidification, whereby polar, especially Arctic seas are expected to be most severely affected due to freshening and enhanced solubility of CO_2[2]. Recent studies found that such an increase of CO_2 leads to severe neurological disorders in marine fish such as impaired sensory systems and behaviour, significantly affecting predator prey interactions[3]. However, the exact mechanisms are yet to be investigated as well as the potential for acclimation or adaptation across generations. For deep insight into the neurological processes affected by ocean acidification, we developed an experimental setup for MR studies on the brain of fish. The setup includes a stereotactic holding device enabling dynamic *in vivo* MR imaging and spectroscopy of non-anaesthetized polar fish and providing the opportunity to analyse over several days the physiology of the brain and surrounding cranial tissues. We conducted a first study of the physiological responses of Antarctic black rock-cod *Notothenia coriiceps* to acute CO_2 exposure (hypercapnia).

Material and Methods

Notothenia coriiceps (length: 30.6±2.1 cm, weight: 329.3±100.6 g, n=4) were anaesthetized prior to experimentation using 170 mg MS222/l seawater. Subsequently, the animals got restrained using a stereotactic holding device consisting of three parts (Figure 1). The anaesthetized animals were placed on a 400x110 mm Perspex plate with milling grooves for adjustable plastic sticks (Polyoxymethylen, radius=5 mm, height=45 mm) every 3 cm. The sticks were mounted laterally and anteriorly of the animal leaving free space for the gills to ensure their unrestricted movement. This set-up was transferred into a chamber (length/width/height 400x103x179mm) with a volume of ~4l and two click valves for in- and outflow of seawater of defined chemistry. The chamber was closed with a Perspex lid and the plate gently lifted, using integrated plastic rods from the Perspex plate through the lid, to minimize the distance between lid and the animals` head. The chamber was connected to a recirculating water system with control conditions (0±0.5°C, ~390 µatm $CO_2$) and placed into the bore of a 4.7T Biospec DBX system. A 50 mm $^{1}H/^{31}P/^{13}C$ surface coil was placed directly over the animals` head for *in vivo* ^{1}H-MRI and ^{31}P-NMR spectroscopy. *In vivo* ^{31}P-NMR spectroscopy (^{31}P singlepulse, 4 min 16 sec/scan) was conducted for the next 12 hours to observe recovery of the acid-base and energetic status of the brain and surrounding tissues from anaesthesia and handling stress. RARE imaging was conducted for anatomical images (30 slices, slice/interslice thickness =1.5 mm, Field of View (FOV) = 4.99/8.64 cm). After two hours of defined control measurements the water supply was switched to a seawater reservoir preequilibrated with a pCO_2 of ~ 6,650 µatm. The physiological response of the brain and surrounding tissues to severe hypercapnia was monitored by continuously acquiring *in vivo* ^{31}P-NMR spectra for 24 hours after the onset of hypercapnic conditions.

Results and Discussion

Physiological effects following anaesthesia and handling stress were visible through a high P_i/PCr ratio immediately after the animals` were placed into the experimental chamber (data not shown). During recovery from anaesthesia the P_i peak fell simultaneously to a rise in PCr indicating an onset of the recovery process. P_i and PCr levels reached steady state after ~ 4 and 5 hours respectively. The rapid recovery of the P_i/PCr ratio and a pH_i similar to resting values reported in the literature[4,5] confirmed well-defined control conditions in this set-up. RARE imaging of the head enabled the identification of the most prominent brain regions (Figure 2), some of them being responsible for the neuronal processing of sensory cues such as *Bulbus olfactorius* (B.O.), *Subpallium* (SP), *Hypothalamus* (H) and *Tectum opticum* (TO). Further analysis of these regions during future studies (e.g. via fMRI) may shed light on the origins of sensory impairments in fish under ocean acidification scenarios. Onset of hypercapnic conditions immediately led to a drop of the pH_i in the brain and surrounding tissues that remained uncompensated for at least 24 hours at constant Pi/PCr ratios (Figure 3, Pi/PCr ratio not shown). This observation may indicate a low ability for acid-base regulation in the nervous and tissue of Antarctic fish.

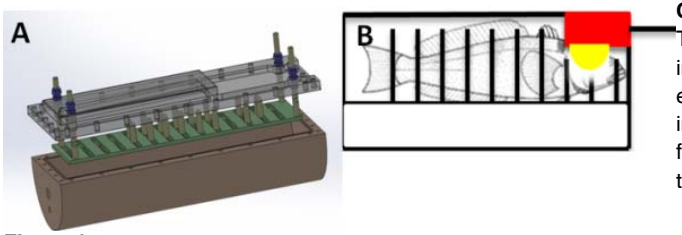

Figure 1

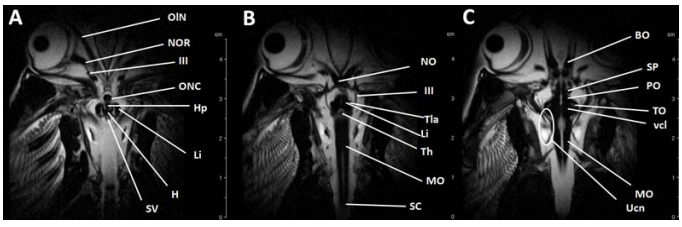

Figure 2

Conclusion

The presented experimental set-up allows long-time neurophysiological MR investigations under defined conditions and may be extremely useful for future experiments analysing the physiological background of ocean acidification induced neurological impairments in fishes. The avoidance of anaesthesia may facilitate the experimental procedure and might thus increase the power of other types of experiments such as fMRI.

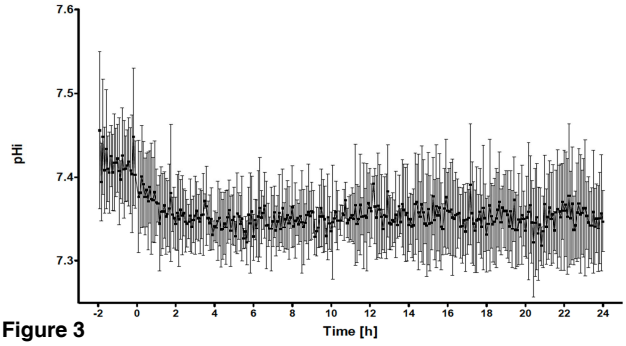

Figure 3

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