Stress response in the brain of a common carp submitted to a sublethal cold shock as measured by BOLD and CBV sensitive fMRI

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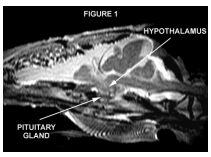
Introduction

Recently fMRI is not only used to reveal task activated responses of the brain in both animal and human subjects, but also as a means to determine brain structure responses following injection of pharmaceutical compounds (Pharmacological MRI) or nutrients [1]. It is also possible to study other phenomena with fMRI, such as stress induced brain activation. Stress is defined as a condition in which the dynamic equilibrium of an organism, called homeostasis is threatened or disturbed as a result of the actions of internal and external stimuli, commonly defined as stressors. The actions of stressors are twofold : they exert effects that threaten or disturb the homeostatic equilibrium, and they elicit a coordinated set of physiological and behavioral responses thought to be compensatory and/or adaptive, enabling the animal to overcome the threat. For the integrated stress response in fish, there is a distinction between primary, secondary, and tertiary responses. Primary responses represent the activation of particular brain centers, resulting in the massive release of catecholamines (CAs) and corticosteroids, whereas secondary responses usually are defined as the manyfold immediate actions and effects of these hormones at blood and tissue level. Tertiary responses extend to the level of the organism and population. Most research in stress physiology focuses on the secondary and tertiary stress response or the release of special hormones (CAs and cortisol) in the blood as the earliest indication of stress. We aim at studying the stress induced neuronal activity in particular brain regions of carp, expecting responses in the hypothalamic-pituitary-interrenal (HPI) axis, using both BOLD and CBV sensitive fMRI protocols.

Methods

Common carp (n=6, 40-69 g body weight) that were acclimated to 25ºC were submitted to the MRI protocol. A 7 Tesla MR system from SMIS (UK) was used in combination with a customised headphone RF transmission antenna and a circular surface receive antenna positioned on the head of the carp. The carp was maintained in a flow through system providing the fish with 500 ml water/min (to maintain continuous delivery of oxygen to the gills), containing 0.011% MS 222 (anaesthetic) [2]. For the CBV measurement 10 mg/kg NC100150 (Clariscan™, kindly given by Nycomed Imaging AS, Oslo, Norway), a USPIO class blood pool agent, was injected in the caudal vein/artery of the carp before they were inserted in the magnet.Sagittal and horizontal T_2-weighted SE high resolution images (TE/TR =40/2000, acquisition matrix 256*128, FOV=40, averages=2) were acquired in order to accurately identify different brain structures. For the BOLD experiment, 12 consecutive horizontal slices through the carp brain were taken (TE/TR = 10/450, acquisition matrix 128*64, FOV=40) with an acquisition time of 28.8 sec. These image acquisitions were continuously repeated every 30 sec during the entire experiment (100 acquisitions). After acquisition 20 (10 minutes of imaging) the temperature of the water supplied to the carp was switched from 25º to 16º C by using a 3-way valve. This low temperature was maintained during the rest of the experiment (80 acquisitions). For the CBV enhanced experiment the same MRI parameters were used.Data processing was performed on a PC using home developed programs in IDL (RSI, Boulder USA). To find the underlying functional activation it was necessary to substract the global signal intensity change as a result of the lower metabolic activity of the fish at lower temperatures from the signal intensity traces of well defined regions of interest (ROI) in the carps brain.



Results

A change in signal intensity was observed in the carp brain after induction of cold stress.

Figure 1 illustrates a mid-sagittal slice through the carp brain showing the different areas of interest.Figure 2 displays the time courses of signal intensity in 3 different ROIs (BOLD-fMRI) expressed as the percentage signal intensity change corrected for the global change observed in the entire brain, resulting from the lower metabolic activity and CBV at low temperature in ectotherms. The ROI in the pituitary gland and the hypothalamus show both a decrease in signal intensity as a result of the cold stress. The ROI in the optic lobes shows no significant signal change and is representative for other regions in the brain such as cerebrum, telencephalon, cerebellum and vagal lobes. In the contrast-enhanced experiments, the signal change displays the same decrease for both hypothalamus and pituitary gland (figure 3). The signal changes observed in both cases starts around 2 minutes after the cold shock and reaches it's maximum at 4-5 min after the shock for the hypothalamus and around 7-8 min for the pituitary gland.

Figure 2

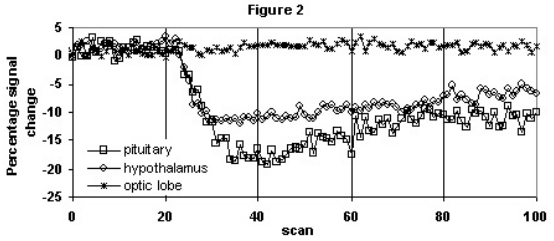
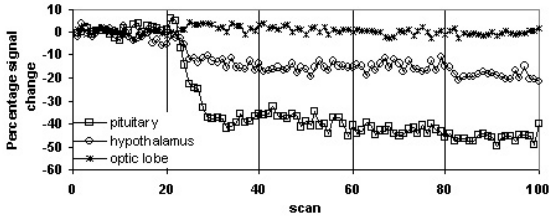


Figure 3



Discussion

Cold stress is a sophisticated stressor when studying fMRI in ectotherm animals. To analyze and interpret the brain activity it was necessary to adjust the entire data set for low temperature induced physiological changes such as the drop in CBV. The traces in the above figures show a drop in the BOLD signal (10-20%, figure 2) and a drop in the CBV weighted signal (20-40%, figure 3), clearly demonstrating a change in the activation states of both the pituitary gland and the hypothalamus. This agrees with the expected response to stress in the hypothalamic - pituitary -interrenal (HPI) axis which is a cascade of hormones, with cortisol as the physiologically important hormone responsible for the effects of stress. These data seem to match with data on cortisol levels in blood samples of carp submitted to the same experimental cold shock setup [3]. The results of this work illustrate the potential of fMRI to study the primary stress response in lower vertebrates

References

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