Brain Responses to Ambient Temperature Fluctuations in Fish: Reduction of Blood Volume and Initiation of a Whole-Body Stress Response

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INTRODUCTION

A constant body temperature facilitates the proper functioning of essentially all organisms. In ectothermic animals, spatial and temporal ambient temperature variations directly influence cellular biochemistry and thus the physiology of the organism. Many acclimation strategies have evolved throughout the animal kingdom to deal with temperature fluctuations (Crawshaw et al. 1985). In the vertebrate brain, the preoptic area is probably the most important thermoregulator (Boulant 2000; Crawshaw et al. 1985). It contains both warm- and cold-sensitive neurons, integrates temperature information from different parts of the body, and, in endotherms, determines a temperature set-point, a reference to which body temperature is adjusted (Boulant 2000). Many studies have been directed to the brain responses to temperature changes, which points to a compensatory mechanism within the neural system. Here we used high-resolution functional magnetic resonance imaging to study brain responses to a drop of 10°C of ambient water temperature in common carp. We observed a strong drainage of blood out of the brain as of 90 s after the onset of the temperature drop, which would be expected to reduce entry of cold blood arriving from the gills so that the change in brain temperature would be slower. Although oxygen content in the brain thus decreased, we still found specific activation in the preoptic area (involved in temperature detection and stress responses), the pituitary pars distalis (stress response), and inactivation of the anterior part of the midbrain tegmentum and the pituitary pars intermedia. We propose that the blood drainage from the brain slows down the cooling of the brain during an acute temperature drop. This could help to maintain proper brain functioning including sensorimotor activity, initiation of the stress response, and the subsequent behavioral responses.

METHODS

Animal handling

Carp (40–69 g body wt), acclimated to 25°C for ≥4 wk and kept in 40-l tanks under approved laboratory conditions (density, feeding, water quality, light regime), were lightly anesthetized with 0.011 wt% ethyl meta amino benzoate and sodium sulfonate acid salt (MS-222, Sigma) buffered to pH 7.4 with NaHCO3. Next the fish were either transferred to the MRI set-up (Antwerp experiments) or an MRI-like set-up (Nijmegen experiments) to assess blood cortisol responses. In both cases, a mouth piece was fitted that secured proper irrigation and aeration of the gills; in doing this, anesthesia also was controlled. The experimental procedures were approved by the Ethical Committees of the Universities of Antwerp and Nijmegen, following federal laws.

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fMRI

Anesthetized carp were inserted into a custom-built stereotactic apparatus combined with a customized headphone RF transmission antenna (53 mm) and a circular surface receive antenna (22 mm) positioned on the head of the carp but excluding the gills. The latter is required to avoid motion interference on the images due to water flow. The entire setup was mounted in the bore of the magnet while anesthesia and sufficient aeration of the gills was ensured by a flow-through system providing the fish, through a mouth piece, 500 ml water/min (containing 0.011% MS222). The temperature of the water supplied to the carp was switched rapidly from 25 to 15°C, which is within the natural preferred temperature range of this eury- thermic species (Elliot 1981), through a three-way valve connected to a second aquarium kept at 15°C; the 10°C-temperature drop took precisely 5 min (Fig. 5).

All MRI experiments \( n = 4 \) for both blood oxygenation level-dependent (BOLD) fMRI and cerebral blood volume (CBV) weighted fMRI were performed on a 7T horizontal SMIS MR microscope (SMIS, Guildford, UK) with a horizontal accessible bore of 80 mm diameter and shielded gradients with a strength of 1 Gauss/mm. Sagittal and horizontal T1- and T2-weighted SE high-resolution images [TE/TR = 40/2000 ms, acquisition matrix: 256 \( \times \) 128, field of view (FOV) = 40 mm, slice thickness = 1 mm, 2 averages] were acquired to accurately identify different brain structures. BOLD-fMRI experiments, which reveal changes in deoxyhemoglobin content and are used to estimate cellular activity (Ogawa et al. 1990), involved collection of 12 consecutive horizontal slices through the carp brain (TE/TR = 10/450 ms, acquisition matrix: 128 \( \times \) 64, FOV = 40 mm); the acquisition time was 28.8 s for each set. CBV-weighted MRI was accomplished in a similar way but after injection of 100 \( \mu l \) Clariscan (Amersham Biosciences Europe GmbH, Roosendaal, The Netherlands) into the caudal vessels.

Image acquisition was continuous and repeated every 30 s during the entire experiment (100 acquisitions). After acquisition 20 (10 min of imaging), the temperature was dropped and the low temperature maintained during the following 80 acquisitions. The threshold for plotting changes in BOLD- and CBV-weighted signal intensities was set at a 2 or 10% change relative to the signal intensity before the temperature drop.

In the pituitary gland, signal intensity changes were analyzed per pixel (pixel size is 234 \( \mu m \)) as a function of time, both in CBV- and BOLD-weighted MRI. From computing the slope (which is the change in time per unit of percent signal change relative to the signal intensity before the temperature drop) within the time-interval from 1 to 3 min after the onset of the temperature drop, an accurate functional map was obtained that allowed detection of regional slope changes within the small pituitary gland.

For angiography, gradient echo images were obtained in three different directions (sagittal, horizontal, and coronal) with 40 slices with the following parameters: TE 7 ms; TR 20 ms; FOV 30 mm; slice thickness 0.5 mm; and acquisition matrix 256 \( \times \) 256. Each set of 40 subsequent angiographic images was submitted to a maximum intensity projection algorithm (programmed in Interactive Data Language).

Brain structures in the images were identified by light microscopy on the basis of horizontal sections; nomenclature follows Meek and Nieuwenhuys (Meek and Nieuwenhuys 1998).

Cortisol measurements

To determine the effect of the temperature drop on the endocrine stress response (as measured by blood plasma cortisol levels), carp were divided into seven groups of six animals each. Samples were taken from carp from the first (control) group immediately after anesthesia to determine basal blood plasma cortisol levels. Fish from the second and third groups were anesthetized and placed individually for 30 or 75 min in the MRI-like setup (which included scan noise). The fish of the remaining four groups were treated similarly to the second group but were then subjected to a 10°C temperature drop by switching the water inlet irrigating the gills to 15°C. Blood of animals belonging to groups 3–6 were collected 1, 5, 15, and 45 min, respectively, after the onset of the temperature drop. From each animal, 300 \( \mu l \) of blood was collected by puncture of the caudal vessels using a 21-Gauge syringe with Na2EDTA to prevent clotting. After centrifugation (500 g, 10 min, 4°C), plasma was collected and stored at −20°C. Cortisol was measured by radioimmunoassay (Arends et al. 1998). The Mann-Whitney U test was used to assess the effects of handling prior to and during the temperature drop and to determine the effects of the temperature drop by testing the blood plasma cortisol values of fish from the shocked groups against these values of fish from the group that stayed in the MRI-setting for 30 min prior to the temperature drop.

RESULTS

Global brain responses

The temperature drop strongly reduced CBV (increased signal intensity due to decreased concentration of the applied paramagnetic blood pool agent) throughout the brain, from 1.5 min after the onset of the drop onwards (Fig. 1, A and B). Blood dissipated into two vessel systems, located rostroventrally to the brain, and caudally to the cerebellum and the vagal lobes (Fig. 1, A and C). Angiography showed that the blood vessels systems around the brain are sinuses, as judged by their broad appearance (Fig. 2). Further increase of blood volume was observed around the pituitary gland (Fig. 1A), where sinus-like vessels appeared absent (Fig. 2); blood redistribution in this region must therefore have occurred in a capillary network.

CBV redistribution was accompanied by an increased BOLD-signal in the brain (decreased deoxyhemoglobin content) and a decreased BOLD-signal in the blood vessels surrounding the brain (increased deoxyhemoglobin content; Fig. 1C). The close temporal relationship between blood volume and deoxyhemoglobin content strongly suggests that the BOLD signal in the brain is primarily determined by the amount of cerebral blood. The BOLD signal in the sinus-like vessels reaches a minimum after 3 min and then, although blood is still entering, increases to ~20%. This decrease of deoxyhemoglobin content is due to increased affinity of hemoglobin for oxygen at lower temperatures and is especially evident in sinus-like blood vessels, as the blood concentration per pixel is here extremely high when compared with brain tissue. Thus during a rapid temperature change, carp brain cells not only receive less oxygen because of the dramatic reduction of blood volume but also because of increased affinity of hemoglobin for oxygen. As less oxygen is available, it is possible that metabolic activity of brain cells is suppressed, but our BOLD-fMRI data do not discriminate among blood volume reduction, increased oxygen affinity of hemoglobin, and altered metabolic activity. However, by the use of newly developed data postprocessing protocols (Peeters and Van der Linden 2002), it is possible to correct for the CBV and BOLD changes observed throughout the brain, so that specific (metabolic) activities are revealed.

Specific brain responses

Specific responses in the brain were found in the preoptic area of the hypothalamus, the anterior part of the midbrain.
tegmentum, and the brain stem (Fig. 3). CBV decreased in the preoptic area but slightly slower than in the rest of the brain (1.5% decreased signal intensity in the corrected trace; Fig. 3B, left). Apparently, a mechanism is initiated to deliver more blood to this region to compensate for the temperature-induced drainage of blood out of the brain. A compensatory reaction is generally observed in mammalian brains to supply oxygenated blood to accommodate increased oxygen consumption by activated cells (Ogawa et al. 1992). Indeed, neurons in the preoptic area increased their metabolic activity in response to the temperature drop as evidenced by the immediate (within 30 s) and significant (up to 6% after 1.5 min) decrease of the BOLD-signal intensity (Fig. 3B, right). Activity of the preoptic area ceased over time during the experiment: blood volume decreased to values similar to those observed in the rest of the brain and the BOLD-signal intensity remained only 1.5% lower during the entire exposure period to 15°C. Similar negative BOLD-fMRI data were reported for hypothalamic areas in mammals (Becerra et al. 1999; Matsuda et al. 1999; Yokawa et al. 1995).

Metabolic activity in the anterior part of the midbrain tegmentum, a region relatively poor in cells, was transiently reduced, keeping in step with activation of the neighboring preoptic area (Fig. 3C). On the basis of timing of activity changes, it seems possible that reduced activation in the midbrain tegmentum serves to compensate for increased oxygen demand in the preoptic area in the early phase of the response to the temperature drop. This suggests that there must be connections between the midbrain tegmentum and the preoptic area, but those have not been investigated to date. Another or
alternative explanation for the reduced activity in the midbrain tegmentum is that this region is involved in an escape reaction as it is associated with locomotion (Meek and Nieuwenhuys 1998).

Part of the brain stem showed minor activation as judged by a small (2.5% 2 min after the onset of the temperature drop) decrease of BOLD-signal intensity (Fig. 3D). In many vertebrates, the brain stem is involved in thermoregulation (Crawshaw et al. 1985), but the small response observed here suggests that the preoptic area is by far the most important of the two thermo-sensitive systems in carp.

**Pituitary responses**

The small pituitary gland (diameter: 1.8 ± 0.15 mm; \( n = 8 \)) could easily be identified in anatomical and BOLD images, and its two lobes, the pars intermedia and the pars distalis (Wendelaar Bonga 1997), could be distinguished separately (Fig. 4A). This is of particular interest because the pituitary gland receives neuroendocrine input from the preoptic area and converts this input into an endocrine signal that directs many physiological processes throughout the body. Therefore we
studied responses in the pituitary pars distalis and pars intermedia, without correction for the over-all effects observed in the brain, as the pituitary gland is endocrine rather than neural tissue and situated outside the brain. It appeared that the pars intermedia and the pars distalis responded differently to the temperature drop, both on CBV- and BOLD-weighted MRI (Fig. 4A). The contrast-enhanced signal intensity revealed a decrease in blood volume (up to 29% signal intensity increase) in the pars intermedia (Fig. 4B). In contrast, the blood volume in the pars distalis increased almost immediately (within 90 – 120 s) and reached plateau values (36% lowered signal intensity) from 10 min onward (Fig. 4B). BOLD-fMRI also revealed, consistently, an opposite response in the pars intermedia as compared with the pars distalis (Fig. 4C). The BOLD-signal in the pars intermedia showed an initial transient increase of 4.9% within 1–4 min, followed by a second increase to a constant elevation of 14% during the exposure to the low temperature. The BOLD signal in the pars distalis decreased within 90–120 s after the onset of the temperature drop and stayed low (–26%) during the remainder of the experiment. Thus the pars distalis received more blood (Fig. 4B) and consumed more oxygen (Fig. 4C) after the rapid temperature drop and can therefore be considered as highly active from 2 min onward. Apparently, the increased blood delivery does not completely compensate for the increased oxygen consumption in this region. On the other hand, blood volume in the pars intermedia decreased simultaneously with an increase of the oxygenated hemoglobin versus deoxygenated hemoglobin ratio, characterizing an inactivation of this pituitary lobe.

One of the cell types located in the pars distalis are the corticotrope cells, which produce and release adrenocorticotropic hormone (ACTH). ACTH stimulates the release of cortisol by the interrenal cells in the head kidney (equivalent of the adrenal gland in mammals) (Wendelaar Bonga 1997). Corticotrope cells are under positive control of corticotropin-releasing hormone (CRH), which is released by neuroendocrine cells in the preoptic area during stress (Wendelaar Bonga 1997). The sequential activation of the preoptic area (within 30 s) and the pars distalis (within 90 – 120 s) may thus indicate the initiation of an endocrine stress response. To investigate whether a stress response occurred, we analyzed blood plasma samples for cortisol from carp experiencing fMRI analysis (including mild anesthesia, intubation, and scan noise). Plasma cortisol levels increased within 5 min after the onset of the temperature drop and remained elevated for the next 45 min (Fig. 5, stressed group), similar to cortisol responses seen in free-swimming carp (Tanck et al. 2000). The cortisol response to the temperature drop by far exceeded the mild elevation of cortisol levels after prolonged (75 min) residence in the MRI-like setup. We conclude that the sequential activation of the preoptic area and the pars distalis indeed reflects an endocrine stress response.

**DISCUSSION**

This paper describes, for the first time, an fMRI study in a lower vertebrate, and reveals new important insights into how an ambient temperature change affects the activity of the brain.
and pituitary gland of an ectothermic animal, the common carp. The possible effects of the light anesthesia on the brain and pituitary responses are likely small, as indicated by the strong cortisol response to the temperature drop, which was similar to that observed in free-swimming carp (Tanck et al. 2000). Also, the light anesthesia strongly reduced the cortisol response to handling and restraint that normally induce high blood plasma cortisol levels (Van den Burg et al. 2004). Thus the use of anesthesia makes it possible to study the effects of a temperature drop on cerebral blood volume and oxygenation in isolation from other stressful events.

The drainage of blood from the brain may serve to temporarily isolate the brain from the circulation and protect it against cooling down too rapidly. A similar thermoregulatory role of blood volume reduction during cold exposure is brought about by vasoconstriction in the mammalian skin, where most of the temperature between the body and the environment takes place (Charkoudian 2003; Johnson and Proppe 1996). In fish, body temperature is mainly determined by heat exchange between ambient water and blood in the gills, as the gills are the structures where blood gets into close contact with ambient water over the largest body surface area (in fact, only the buccal and opercular cavities are in close contact with water in our setup). Ambient water contacting the head hardly influences brain temperature, as the brain is insulated by a layer of fat (personal observation). Thus a reduced blood flow through the brain could provide a means to dampen a temperature drop, and keep brain temperature initially more constant. In contrast, cerebral blood flow in mammals (rodents) exposed to cold increases to accommodate increased metabolic activity of neuroglia in the brain (Szelenyi and Donhoffer 1978). It has been hypothesized that neuroglia, especially astrocytes, play a key role in thermogenesis within the brain (Szelenyi 1998).

It is well known that a constant brain temperature permits fish to expand their niche into colder environments: endothermy arose three times during evolution of large marine fish, and its development is always associated with a movement into colder water (Block et al. 1993). Some species, such as swordfish (Xiphias gladius) use cranial endothermy only; a constant brain temperature permits them to dive to colder waters, spanning a temperature difference of up to 19°C (Carey 1982). These observations suggest that the brain cannot withstand low temperatures without further adaptations. Thus isolation of the brain from incoming blood during a temperature drop, as we found in carp, could be a mechanism employed by aquatic ectotherms to dampen temperature fluctuations in the brain. It is likely, however, that it is not as efficient as (cranial) endothermy, and it will not keep brain temperature higher than ambient water temperature during the total period of acclimation. Also, this mechanism limits oxygenated blood entry into the brain and may thus compromise neuronal activity. It is therefore interesting that some brain regions were highly active during the temperature drop.

Activation of the preoptic area occurred within 30 s after the onset of the temperature drop. This brain region integrates peripheral and central temperature information (Boulant 2000) and contains thermo-sensitive neurons (Nelson and Prosser 1981a,b). These neurons could very well stimulate the neighboring neuroendocrine neurons that release CRH to elicit a stress response. The ambient temperature change during the first 30 s is considerable (2°C), and it is likely that the rapidity of the change activates cells in the preoptic area rather than the absolute temperature change. Our hypothesis is supported by the observation that the level of activity does not change during the final phase of the temperature drop and decreases again when the temperature drop is complete. Activation of the pituitary pars distalis likely results from a strong activation by the preoptic area during the initial phase of the response to the temperature drop and persists even though activity of the preoptic area ceases. It thus appears that brain initiates a stress response in the preoptic area, which persists while the preoptic area inactivates similar to all other brain regions.

The stress response is the first step in the process of acclimation, which leads to a number of biochemical adjustments of, among others, enzyme expression levels (Hazel 1993; Metz et al. 2003) and membrane lipid composition (Hazel 1993; Buda et al. 1994). However, temperature acclimation is energetically costly (Claireaux et al. 1995), and, if possible, fish (as well as other vertebrates) migrate to an environment of a temperature at or close to the initial temperature (Claireaux et al. 1995; Tanaka et al. 2000), before the temperature of the CNS is changed (Crawshaw 1980). Such a behavioral thermoregulatory response has been attributed to cellular activity changes in the anterior brain stem (reviewed by Crawshaw et al. 1985), but our fMRI data do not reveal any major activity changes in this region. This either means that the brain stem is not involved in temperature selection in carp, that the thermoregulatory nuclei are too small or diffuse to be separately detected, or that the behavioral response was suppressed by the mild anesthesia.

Neuronal activity during a rapid temperature drop not only depends on the supply of oxygen-rich blood but is also influenced by temperature itself. For instance, temperature directly influences conduction velocity (Harper et al. 1990), reliability of synaptic transmission (Hardingham and Larkman 1998), and probability of transmitter release (Volgushev et al. 2004). Recently it was shown in goldfish (Carassius auratus) that a 10°C temperature drop in 50 min changes properties of the Mauthner cell as well as of the feed-forward inhibition to this cell (Preuss and Faber 2003). As a result, startle-escape behavior to an acoustic stimulus changes such that the probability of an escape reaction is increased, but more often toward rather than away from the stimulus. From the preceding text it may be clear that ambient water temperature has a major impact on cellular and network responses in the brain to certain stimuli, and therefore it is important to limit temperature fluctuations as much as possible. The reduced CBV during the temperature drop we observed in carp may thus be one of the compensatory mechanisms present in the CNS of aquatic ectothermic vertebrates to maintain behavioral performance and sensorimotor function.

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