Activation of a sensorimotor pathway in response to a water temperature drop in a teleost fish

E. H. van den Burg[1,]*, M. Verhoye[2], R. R. Peeters[2], J. Meek[1], G. Flik[1] and A. Van der Linden[2]

[1]Department of Organismal Animal Physiology, Faculty of Science, Radboud University Nijmegen, Toernooiveld 1, 6525 ED, Nijmegen, The Netherlands and [2]Bio-Imaging Lab, Campus Groenenborger, University of Antwerp, Antwerp, Belgium

*Author for correspondence (e-mail: E.vandenBurg@science.ru.nl)

Summary

When common carp, *Cyprinus carpio* L., experience a rapid temperature drop, the cerebral blood volume is strongly reduced to dampen the temperature drop in the brain. Simultaneously, the preoptic area and pituitary gland are activated to launch whole-body adaptive responses. However, the preferred reaction of fish to a temperature change is an escape reaction, which implies activation of a sensorimotor pathway. Here, we used blood oxygenation level-dependent (BOLD)- and cerebral blood volume (CBV)-weighted functional magnetic resonance imaging (fMRI) to identify a sensorimotor pathway, during a 10°C temperature drop in common carp. Transient activation was observed in the region where the sensory root of the trigeminal nerve enters the brain, and in the valvula cerebelli. In both regions, metabolic activity increased (increased deoxyhemoglobin content demonstrated by a decreased BOLD signal) within 30 s after the onset of the temperature drop, peaked after 2–3 min, and then decreased, even though the temperature continued to drop for another 2 min. These brain structures appear to respond to temperature change, rather than to the absolute temperature. Thus, during a temperature drop, the sensorimotor pathway consisting of the trigeminal nerve, the primary sensory trigeminal nucleus, the valvula cerebelli and some motornuclei, is active, in line with perception of temperature change in the buccal cavity, leading to motor activity for escape. This pathway operates in parallel to an acclimation pathway, which involves the preoptic area to pituitary gland pathway.

Key words: fMRI, temperature, ectothermy, teleosts, brain, common carp, *Cyprinus carpio*.

Introduction

Ectothermy offers animals many advantages over endothermy, which include low energy costs for living, and therefore decreased foraging times and exposure to predators. The consequent minimization of locomotion, which consumes most of the energy budget, makes it possible for more energy to be directed to investment processes such as reproduction, growth, and development (Hochachka and Somero, 2002). The biggest disadvantage of ectothermy is that body temperature is not stable when ambient temperature varies. During evolution, ectotherms have developed several mechanisms to maintain constant body temperature, notably behavioral responses such as stimulation of locomotor activity to select a thermostable microclimate, basking in the sun to stay warm or moving to the shade to cool down (Hochachka and Somero, 2002).

The brain may be the most vulnerable organ to temperature changes. A constant brain temperature is essential for synaptic activity and conduction of action potentials. Studies on isolated synaptic vesicles from rainbow trout showed that transmitter uptake depends on temperature (Roseth and Fonnum, 1995). In carp it was found that conduction velocity of the vagal nerve correlates positively with temperature (Harper et al., 1990). Indeed, some species such as swordfish, (*Xiphias gladius*) have developed cranial endothermy to guarantee a more constant brain temperature, and this enables them to dive 600 m deep to hunt for prey. During these diving excursions, swordfish may experience a temperature drop as big as 19°C (Carey, 1982). Strictly ectothermic fish may protect the brain from a rapid temperature drop by reducing the cerebral blood volume. In this way, brain exposure to cold blood from the gills is limited, and the temperature drop in the brain will be more gradual than that in the water (Van den Burg et al., 2005). Simultaneously, the preoptic area, where thermosensitive neurons are located (Nelson and Prosser, 1981), is activated (Van den Burg et al., 2005). The preoptic area harbors corticotrophin-releasing hormone-positive cells (CRH cells) that govern the endocrine stress response (Wendelaar Bonga, 1997). CRH cells project to the pituitary gland, which is

activated during temperature stress (Van den Burg et al., 2005). The pituitary gland releases adrenocorticotropic hormone (ACTH) into the blood, which leads to a rise of blood plasma cortisol levels (Tanck et al., 2000; Van den Burg et al., 2005). In general, the consecutive activation of the preoptic area, the pituitary gland, and the rise of cortisol levels results in recruitment of energy from internal sources to cope with a stressor (Wendelaar Bonga, 1997).

The preferred response of ectothermic fish to an ambient water temperature change is escape, to prevent the multitude of biochemical and physiological consequences of exposure to a new temperature (Claireaux et al., 1995; Tanaka et al., 2000). We, therefore, hypothesized that a sensorimotor pathway should be active in the carp brain to stimulate locomotor activity when these fish experience a temperature drop. An escape reaction must rely on fast temperature detection and activation of a sensorimotor pathway, which suggests the presence of thermoreceptors in the skin, or the buccal cavity. To test this hypothesis, and if confirmed, to identify the sensorimotor pathway, we have conducted a functional magnetic resonance imaging (fMRI) study on the brain of common carp, *Cyprinus carpio* L., exposed to a rapid 10°C temperature drop.

Materials and methods
Animal handling

Carp *Cyprinus carpio* L. (40–69 g body weight, $N=6$), acclimated to 25°C for at least 4 weeks and kept in 40-l tanks under approved laboratory conditions (density, feeding, water quality, light regime), were lightly anesthetized with 0.011 mass% MS-222 (ethyl 3-aminobenzoate methanesulfonate salt; Sigma, St Louis, MO, USA) buffered to pH 7.4 with NaHCO₃. Next, the fish were transferred to the fMRI apparatus. A mouthpiece was fitted that secured proper irrigation of the gills; by doing so also anesthesia was controlled. Four other fish were anesthetized and perfused for histology purposes. The experimental procedures were approved by the Ethical Committees of the Universities of Antwerp and Nijmegen, following federal laws.

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 receiver 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 (Van der Linden et al., 1998; Van den Burg et al., 2005). The entire apparatus was mounted in the bore of the magnet and anesthesia and sufficient aeration of the gills was ensured by a flow-through system, *via* a mouth piece, providing 500 ml water min⁻¹ (containing 0.011% MS-222). The temperature of the water supplied to the carp was switched rapidly from 25°C to 15°C, which is within the natural preferred temperature range of this eurythermic species (Elliot, 1981), through a three-way valve connected to a second aquarium kept at 15°C. It took precisely 5 min to realize the 10°C temperature drop.

All fMRI experiments [$N=3$ for both blood oxygenation level-dependent (BOLD)-weighted 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 1×10^{-4} T mm⁻¹. Sagittal and horizontal T₂-weighted scanning electron high-resolution images [echo time/scan repeat time (TE/TR)=40/2000 ms, acquisition matrix: 256×128, field of view (FOV)=40 mm, slice thickness=1 mm, 2 averages] were acquired to accurately identify different brain structures. BOLD–fMRI experiments reveal changes in deoxyhemoglobin content and are used to estimate cellular activity (Ogawa et al., 1990). Twelve consecutive horizontal slices through the carp brain were collected (TE/TR=10/450 ms, acquisition matrix: 128×64, FOV=40 mm); the acquisition time was 28.8 s for each set. CBV-weighted fMRI was accomplished in a similar way but after injection of 100 μl 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 resolution of the pixels was 256 μm.

Image processing

All data processing was performed on a PC workstation using home-developed algorithms and programs in IDL (Version 6.0, RSI, Boulder USA). To correct for the global signal increase in both CBV- and BOLD-weighted fMRI observed throughout the entire carp brain during a temperature drop (Van den Burg et al., 2005), appropriate data post-processing protocols were used that cut out these physiological consequences of the severe temperature drop – i.e. massive vasoconstriction and increased affinity of hemoglobin – and exclusively reveal specific (metabolic) activities (Peeters and Van der Linden, 2002). To that end, the signal intensities (SI) of each pixel or the mean SI over a region of interest (SI_{local}) were linearly fitted to the mean SI of a global brain region (SI_{global}) of the same time point and this within a period that demonstrates mainly the physiological changes, i.e. 10–35 min after the onset of the cold drop: $SI_{local}(t)=a\ SI_{global}(t)+b$. From the regression parameters a and b, the corrected SI were calculated as: $SI_{cor}(t)=SI_{local}(t)-aSI_{global}(t)-b+SI_{local}$ (first data point), where t is time. The percentual signal change of the corrected (voxel or ROI) BOLD- or CBV-weighted data was calculated as the change of intensity relative to the mean SI measured before the temperature drop: $\%SI(t)=100[SI_{cor}(t)/SI_{cor-mean}(\text{period prior drop})-1]$. Subsequently, signal intensity (SI) and percentual SI change will always refer to the corrected SI.

Serial mean percentual difference maps showing only those voxels that demonstrated a 2–20% BOLD signal change

following the temperature drop were calculated for each consecutive 5-min period before and after the temperature drop. These maps are overlaid on the high-resolution images for a better localization. Apart from the visual analysis of the percentual difference maps, we analyzed the time course of the percentual signal change for two specific regions of interest:

the region where the trigeminal nerve enters the brain and the valvula cerebelli.

Histology

Following deep anesthesia, carp were perfused through the heart with saline, followed by Bouin's fixative. The brain was
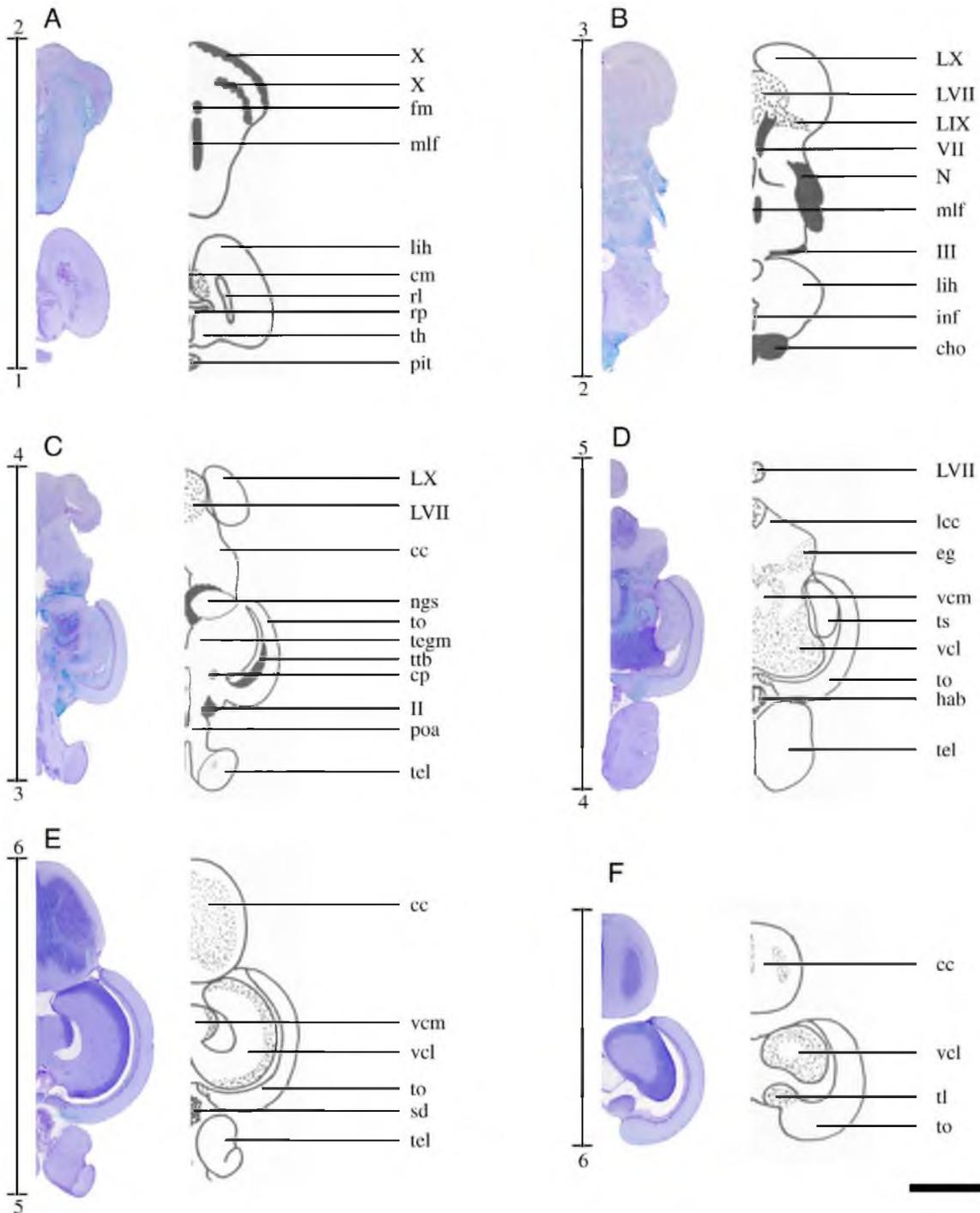

Fig. 1. Partial brain atlas of common carp. Horizontal sections of 10 μm were stained according to Kluver–Barrera. Horizontal distance between consecutive sections is 1 mm. Section A is the most ventral, and the bar on the left of the sections indicates corresponding sectioning of the magnetic resonance images shown in Fig. 2. The scale bar is 2 mm. See List for abbreviations.

then removed, embedded in paraffin wax, and 10 μm horizontal or transverse sections were cut and processed according to standard protocols. One of every 15 sections was stained according to Kluver–Barrera (Kluver and Barrera, 1953), and the horizontal sections were used to prepare an atlas and the transverse sections to make a lateral reconstruction of the carp brain. On the basis of this lateral view, the precise plane of sectioning to obtain the high-resolution images in the fMRI set-up was reconstructed. Nomenclature follows Meek and Nieuwenhuys and Wulliman et al. (Meek and Nieuwenhuys, 1998; Wulliman et al., 1996).

Results

Anatomy

With the atlas constructed, the brain structures observed in the magnetic resonance images were identified (Figs 1 and 2).

As the purpose of this paper is to detail neurophysiological responses to a temperature drop, and not to present an overview of carp brain anatomy, we do not give a thorough anatomical analysis. The identified brain regions and abbreviations are given in the List of abbreviations. The structures that showed specific responses to the 10°C temperature drop, other than those involved in the endocrine stress response (Van den Burg et al., 2005), are described in more detail below.

Entrance of the trigeminal, facial, acoustic and lateral line nerves

One brain region that became active during the temperature drop is the area where the trigeminal, facial, acoustic and anterior lateral line nerves extend from the brain (N, Fig. 3). In the magnetic resonance images, the entrances of these nerves cannot be discriminated individually (N, Fig. 2C3). Light microscopy revealed that the trigeminal nerve enters
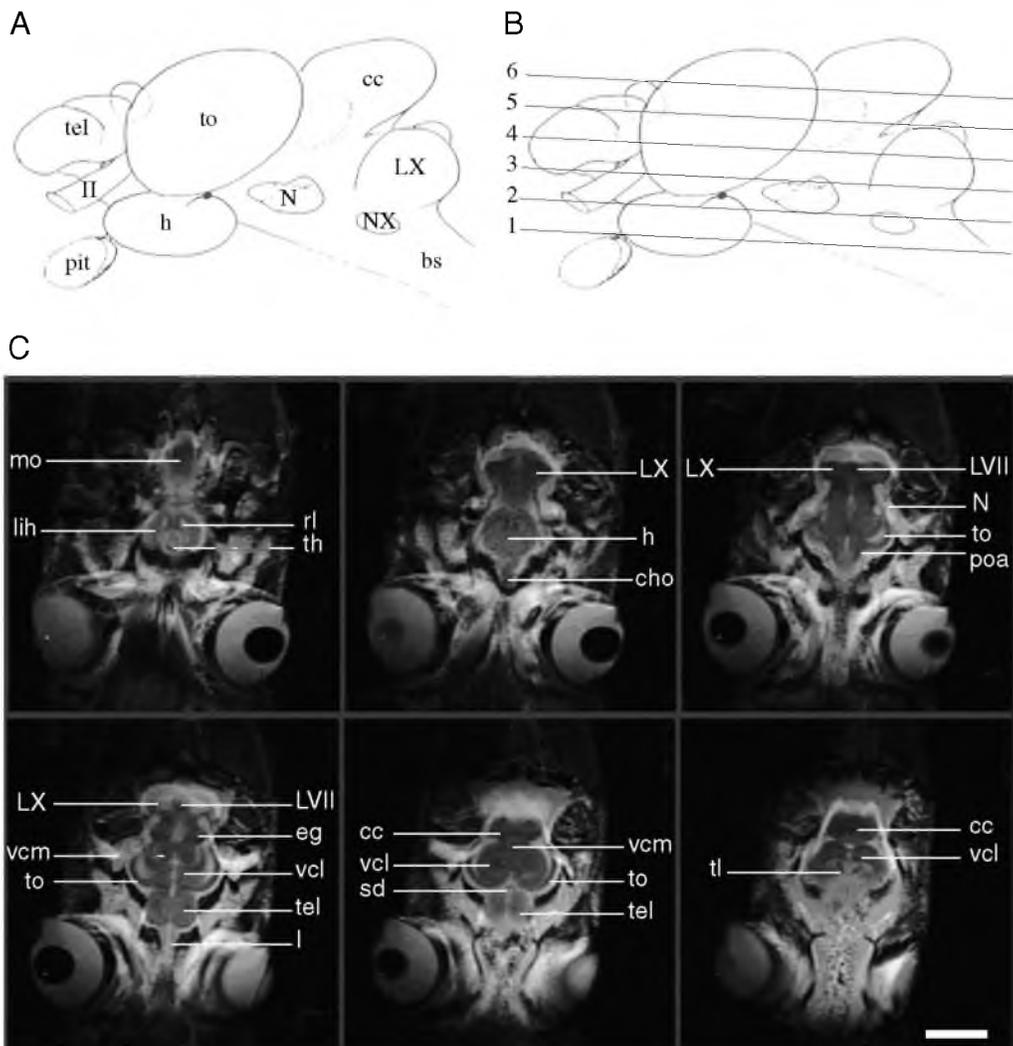

Fig. 2. Identification of brain structures in magnetic resonance images (MRI). (A) Lateral reconstruction of a carp brain, on the basis of transversal sections. (B) Lateral reconstruction with lines that indicate the middle of the acquired adjacent 1 mm fMRI slices through the carp brain. The number of each line corresponds to the image number C. (C) T$_2$-weighted high resolution spin echo images of horizontal 1 mm slices through the carp brain. Scale bar, 5 mm. See List for abbreviations.

most rostrally, whereas the other cranial nerves enter closely together more caudally (Fig. 1B) (Luiten, 1975).

BOLD–fMRI revealed that the brain area where the cranial nerves enter displayed a prominent drop in *SI* within 30 s following the onset of the temperature drop, which was maximal after 3.5 min (Fig. 3B,C). Then, *SI* increased again, and slowly returned to basal values after 14 min. The temporal pattern of the response in all of the three fish tested was similar, whereas the magnitude of the maximum response differed between individuals. The cerebral blood volume did not change significantly (Fig. 3D), so that the decreased BOLD signal, which reflects an increase of the deoxygenated hemoglobin content, is the result of increased cellular activity, rather than of reduced blood volume.

The resolution of the images does not allow identification of individual cell groups in the brain region where the cranial nerves enter. A horizontal and a transverse light microscopic section show that a significant part of this region is occupied by the sensory root of the trigeminal nerve and its associated nuclei (Fig. 4). The sensory root (Vs) contacts the dorsal and ventral part of the trigeminal motor nucleus (NVmd and NVmv) as well as the primary sensory trigeminal nucleus (NVs). The targets of the motor nucleus are the muscles of the jaws; the sensory nucleus projects to the valvula cerebelli (Wullimann et al., 1996). The sensory root of the trigeminal nerve bends rostrally around the secondary gustatory nucleus. The most caudal part of the activated region is occupied by the anterior octaval nucleus and the most rostral part of the medial octavolateral nucleus, which are associated with the acoustic nerve and lateral line nerve, respectively. Other identified brain structures that are outside the activated area are given in the List of abbreviations.

Valvula cerebelli

The valvula cerebelli (vcl, vcm; Fig. 1D–F, Fig. 2C4–6) is a rostral protrusion of the cerebellum in the midbrain ventricle, and has no homologue in mammals (Meek and Nieuwenhuys, 1998). Like the corpus cerebelli, the valvula cerebelli integrates and processes information from several sensory modalities (Wullimann and Northcutt, 1989; Meek and Nieuwenhuys, 1998).

The BOLD signal measured in the valvula cerebelli (vc; Fig. 5C) decreased within 30 s after the onset of the temperature drop. The negative response peaked 3.5 min after the onset of the temperature drop, and then declined. After 11 min, the BOLD signal reached the same basal value as the signal prior to application of the temperature drop. Simultaneously with the decrease of the BOLD signal, the CBV signal dropped (Fig. 5D),
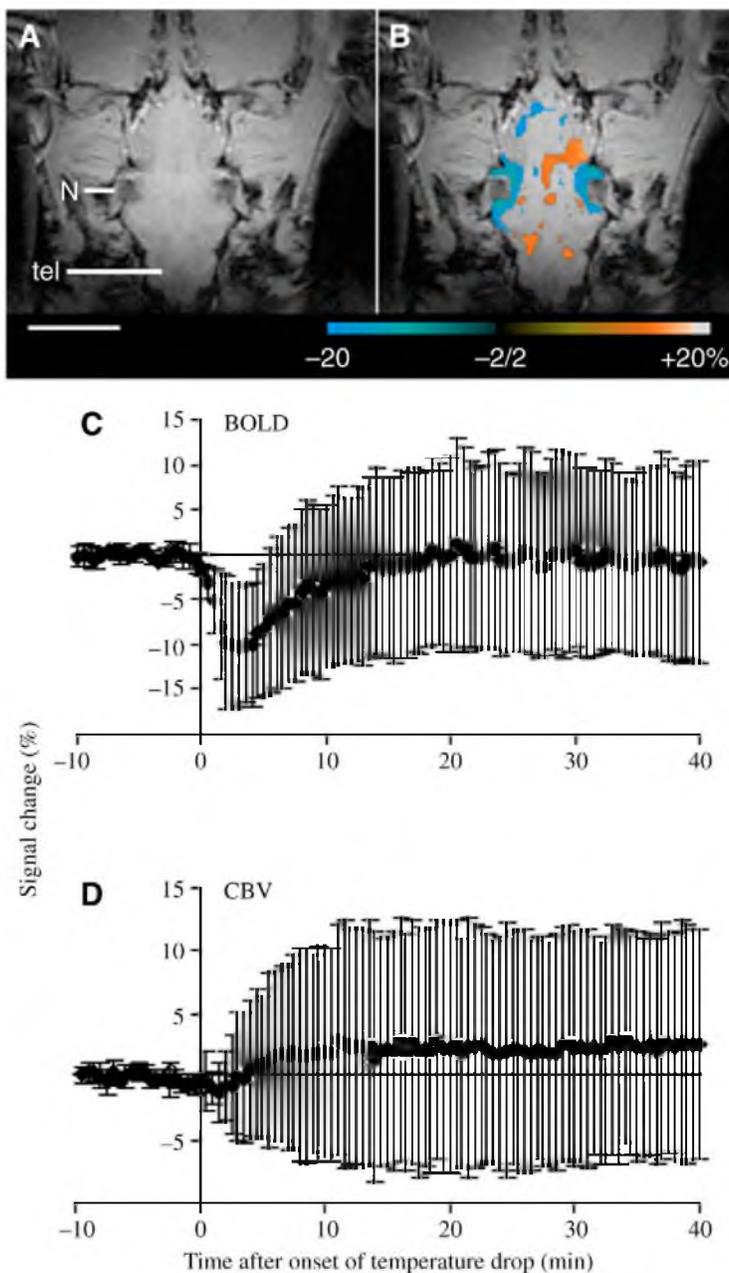

Fig. 3. Activation at the nerve entrance (N) following a 10°C temperature drop, as determined by blood oxygenation level-dependent (BOLD) functional magnetic resonance images (fMRI). (A) T_2-weighted high-resolution spin echo image showing the complex (N) of trigeminal, facial, acoustic and anterior lateral line nerve extending from the brain. The image resolution does not allow for separate identification of the cranial nerves. tel, telencephalon. (B) Average of the color-coded percentual BOLD difference maps within the first 5 min after the onset of the temperature drop overlaid on the high-resolution image. The brain region surrounding the entrance of the cranial nerves is transiently activated (blue, reduced BOLD signal intensities). Some increase in BOLD signal intensities (red) can sometimes be observed in other brain regions. Scale bar, 2.5 mm. (C) The transient decrease of BOLD signal intensity measured in the region surrounding the entrance of the cranial nerves is not accompanied by any substantial change in cerebral blood volume (CBV; D) from which it is concluded that the decreased BOLD signal is a result of increased cellular activity. (D) CBV signal intensity changes in the region surrounding the entrance of the cranial nerves. Values are means ± s.d. (*N*=3).

Fig. 4. Anatomy of the region where the cranial nerves extend from the brain. (A) Horizontal section, stained according to Kluver–Barrera. The orange line surrounds the region of activation. (B) Drawing of the section shown in A delineating the sensory root of the trigeminal nerve (Vs) and other areas and nerves. The V is located rostrally to the facial nerve (VIIs) and the anterior lateral line nerve (alln). It projects to the trigeminal motor nucleus (NVmd) that innervates the muscles of the jaws. (C) Transverse section in the region of the sensory and motor nuclei associated with the trigeminal nerve. (D) Drawing of the section shown in C, delineating the location of the primary sensory trigeminal nucleus (NVs) dorsally to the trigeminal motor nuclei (NVmd and NVmv), and ventrally to the commissure of the secondary gustatory nuclei (cgus). The NVs receives input from the trigeminal nerve and projects to the valvula cerebelli. For other abbreviations, see List of abbreviations. Scale bar, 0.3 mm (A,B) and 0.2 mm (C,D).
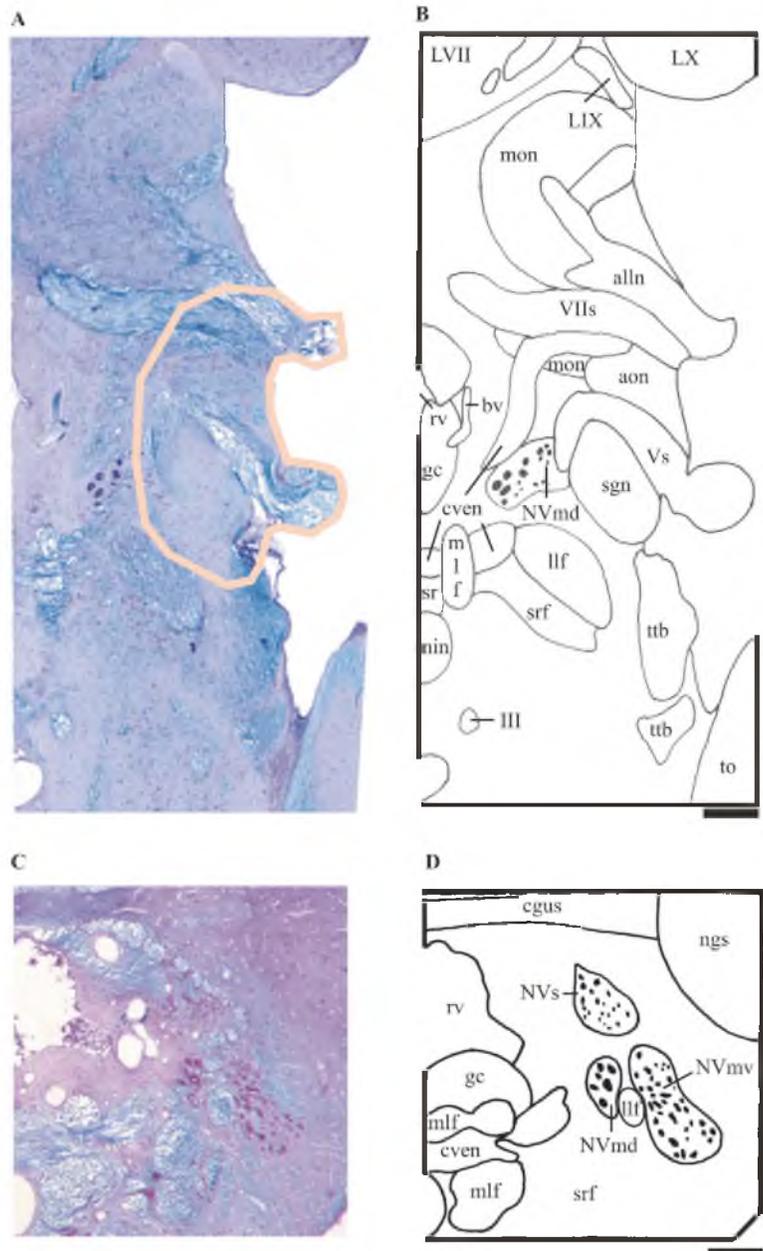
which indicates an increased blood volume. Thus, in the valvula cerebelli, the concentration of oxygenated hemoglobin decreased, yet it received more blood. The valvula cerebelli can therefore be considered activated following the rapid 10°C temperature drop.

The size of the area within the valvula cerebelli where the decreased BOLD signal was observed differed between individual fish, and in some fish BOLD and CBV signal drops occurred in slightly different regions. This partly reflects different durations of activation in individual fish on the one hand, and different timing of the onset of deactivation on the other hand. It thus appears that the valvula cerebelli does not respond as a homogeneous structure, but rather that differential responses occur in smaller subregions. The BOLD percentual difference map of the fish shown in Fig. 5B demonstrates some early signal increase (red colored areas) in the corpus cerebellum (cc; Fig. 1E,F, Fig. 2C, Fig. 5). The time course (data not shown) of both the BOLD and CBV percentual signal intensity differences measured in this region were, however, not reproducible for the fish measured. Apparently, the balance between activation and inactivation in the corpus cerebellum following a temperature drop is very delicate, and depends on cold-induced inhibition and disinhibition.

Discussion

This study tested the hypothesis that in the ectothermic common carp a fast neural pathway exists to allow a fast escape reaction to a rapid temperature drop. We show specific activity changes in discrete brain regions, in line with a sensorimotor pathway, next to an acclimation (or stress) pathway that becomes active when a carp cannot avoid the temperature drop.

The sensorimotor pathway

An escape reaction to a temperature drop obviously depends on rapid detection of a temperature change, and this detection should be reliably encoded when relayed to the brain. The rapid onset of activation in the area that harbors the sensory root of the trigeminal nerve indicates that thermotransduction is mediated by cold-receptors that are part of the trigeminal system. The head of teleost fish is densely innervated by trigeminal nerve endings, and there are at least five different trigeminal somatosensory receptor types that respond to mechanical, chemical and thermal stimulation (Sneddon, 2003). However, studies that include a search for cold receptors have not yet been conducted. We propose that cold receptors are at least present in the mouth, as in our set-up, the mouth and buccal cavity are the only parts of the carp body
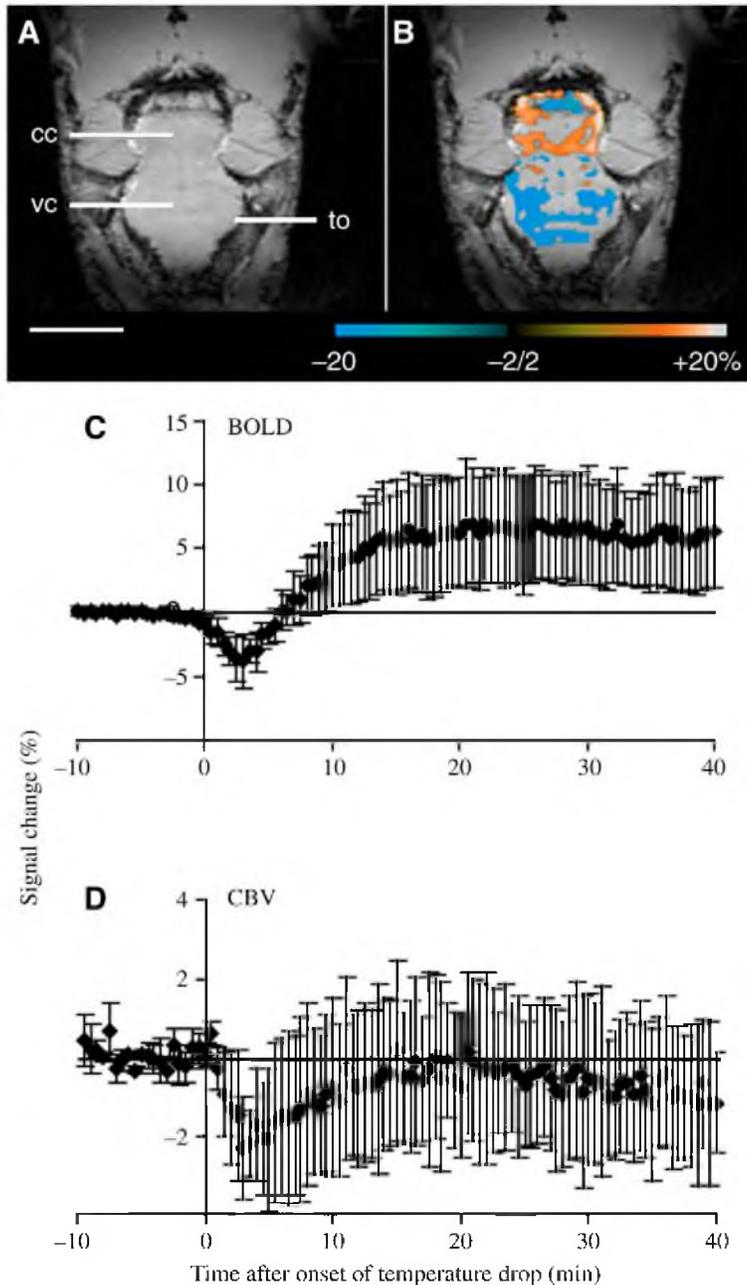
Fig. 5. Activation of the valvula cerebelli following a 10°C temperature drop as determined by blood oxygenation level-dependent (BOLD)-weighted and cerebral blood volume (CBV)-weighted functional magnetic resonance images (fMRI). (A) T$_2$-weighted high-resolution spin echo image showing the location of the corpus cerebelli (cc), the valvula cerebelli (vc), and the tectum opticum (to). (B) Average of the color-coded percentual BOLD difference maps within the first 5 min after the onset of the temperature drop overlaid on the high-resolution image. The valvula cerebelli, but not the tectum opticum shows a reduced BOLD signal intensity (blue). This fish (but not the other two examined) also demonstrated pronounced early BOLD signal increase (red) in the corpus cerebellum. (C) Biphasic change of BOLD signal intensity measured in the valvula cerebelli indicates activation followed by inactivation of this brain region following the temperature drop. (D) Transient decrease of CBV signal reveals increased blood supply to the valvula cerebelli, demonstrating that the decrease of the BOLD signal is caused by increased cellular activity. Scale bar, 2.5 mm (A,B). Values are means ± s.d. (*N*=3).

during the first phase of the temperature drop, when the temperature change was biggest.

It should be noted, however, that the activated area contains, in addition to structures associated with the trigeminal nerve, nuclei that are contacted by other cranial nerves or are part of a distinct sensory modality such as the secondary gustatory nucleus and the octaval nuclei. We believe that the activity observed in this brain region is caused by activity of the trigeminal system. The trigeminal nerve projects both rostrally and caudally to two motor nuclei and four sensory nuclei. The location of all of the sensory nuclei spans a region from the isthmic primary sensory trigeminal nucleus (Fig. 4) to the very end of the medulla oblongata, where the medial funicular nucleus emerges (Wullimann et al., 1996). The resolution of the functional magnetic resonance images is probably not sufficient to discriminate between small activated and non-responding structures.

In goldfish (*Carassius auratus*), another member of the family Cyprinidae, the sensory trigeminal nucleus receives primary trigeminal projections (Puzdrowski, 1988), and extensive connections from this nucleus to the valvula cerebelli have been described (Wullimann and Northcutt, 1989). In carp, the sensory trigeminal nucleus of Puzdrowski most probably corresponds to the posteromedial part of the lateral valvular nucleus described by Ito and Yoshimoto (Ito and Yoshimoto, 1990). Whether this nucleus should be regarded as a part of the trigeminal sensory nuclear complex or a part of the lateral valvular nucleus is, as yet, not clear. Thus, there is anatomical evidence for connections from the trigeminal sensory system to the valvula cerebelli in carp, comparable to those found in goldfish. The activation of the valvula cerebelli that we observed suggests that this pathway is functionally active when carp face a temperature drop. It has

that are in contact with water. In mammals, it has been shown that the trigeminal system conveys information from, amongst others, cold-receptors from the skin, tongue and eye (Hensel and Zotterman, 1951; Gallar et al., 1993; Hutchison et al., 1997). Studies in cold-sensitive trigeminal ganglion neurons in mouse show that activation during cooling is probably brought about by a block of background K$^+$ conductance, which leads to depolarization and action potential firing (Viana et al., 2002). Cold receptors differ from heat receptors in that they respond to temperature changes, rather than to the absolute temperature (McCleskey, 1997), which is in line with our observations in cooling of common carp. We found that the BOLD signal in any responding brain region changed most

been suggested previously that information relayed *via* the sensory root of the trigeminal nerve to the valvula cerebelli originates from the buccal cavity, and this pathway is believed to be involved in feeding behavior (Wullimann and Northcutt, 1989; Ikenaga et al., 2002).

In all vertebrate taxa, the cerebellum is believed to achieve sensorimotor coordination by processing and predicting sensory input to elicit appropriate behavioral responses to changing stimuli (Nixon, 2003). Thus, after processing of temperature information in the valvula cerebelli, one or more descending motor pathways should be activated to initiate swimming to avoid the 10°C temperature drop. Amongst the efferent targets of the valvula cerebelli are the nucleus of the medial longitudinal fascicle, the nucleus ruber and the reticular formation (Ikenaga et al., 2002). In salmon it has been shown that neurons of these nuclei project to the spinal cord (Oka et al., 1986), probably to initiate swimming (Uematsu and Todo, 1997). It thus appears that the expected descending motor pathway is present in fish, but we could not demonstrate its functionality during the temperature drop. The motor nuclei could be too small to be individually detected by fMRI, or they were not active because of the mild anesthesia.

The acclimation pathway

When fish cannot escape from temperature changes, they have to acclimate to a new temperature. In a recent paper, we proposed that the preoptic area initiates acclimatory responses
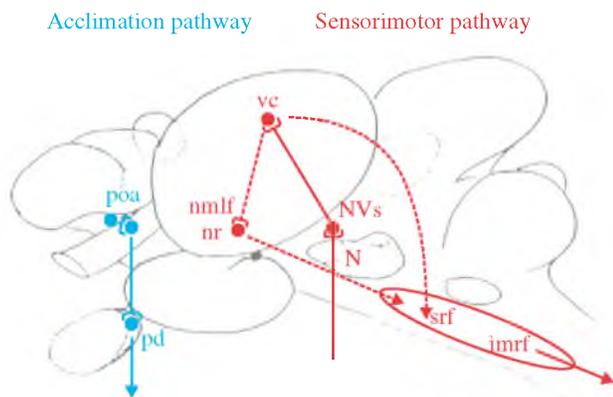

Fig. 6. Schematic representation of the adaptive and sensorimotor pathway as derived from our fMRI studies (this study) (Van den Burg et al., 2005), and from several neuroanatomical studies. The acclimation pathway is probably triggered by a reduction of the temperature of the cerebral blood, and starts with thermodetection in the preoptic area (poa), where also a subsequent stress response is initiated. This leads to activation of the pituitary pars distalis (pd; to stimulate the release of ACTH). The sensorimotor pathway is probably triggered by trigeminal cold-receptors in the buccal cavity, and involves sequential activation of the sensory root of the trigeminal nerve (N), the associated sensory nucleus (NVs) and the valvula cerebelli (vc). The vc probably stimulates one or more motor nuclei (the nucleus of the medial longitudinal fascicle (nmlf), the nucleus ruber (nr) and the reticular formation (srf and imrf) to initiate (broken lines) swimming behavior.

to a temperature drop (Van den Burg et al., 2005). The preoptic area contains thermosensitive neurons that change their firing rate as temperature changes. Some of these neurons have no apparent synaptic input, and are therefore considered to be 'true' thermodetectors, although endocrine and other humoral input cannot be excluded (Nelson and Prosser, 1981). As synaptic input to these cells is lacking, they can only change their firing rate in response to a brain temperature change. Brain temperature changes are probably brought about by temperature changes of entering blood that arrives directly from the gills, the principal site of thermoexchange in fish. The brain is insulated by a layer of fat, so that the temperature of the water that contacts the skin will have only limited influence on brain temperature. The onset of the acclimation response will thus be a little slower than the onset of the escape response, which we propose relies directly on neuronal information from the buccal cavity. Unfortunately, our experimental protocol did not allow for measurements within the first 30 s following the onset of the temperature drop. The preoptic area also constitutes the initiation site of the endocrine stress response, which is an adaptive response to environmental disturbances (Wendelaar Bonga, 1997). Axons of CRH cells end in the pituitary gland to stimulate the release of ACTH (this study) (Olivereau et al., 1984).

Perspectives

In this and a previous paper, we have presented evidence for an activation of two distinct neural pathways during a rapid temperature drop in common carp (Fig. 6). The first pathway is a sensorimotor pathway (this paper) that is probably activated upon detection of ambient temperature changes by cold receptors in the buccal cavity. This pathway could direct an escape reaction, which is the naturally preferred reaction to temperature fluctuations so as to avoid energetically costly investments to acclimate to a new temperature (Claireaux et al., 1995). The second pathway, the acclimation pathway (Fig. 6) is activated in response to cooling of the brain as a result of incoming cold blood from the gills, when the fish cannot avoid the temperature drop (Van den Burg et al., 2005). The acclimation pathway leads to elevated blood plasma cortisol levels, and may operate in concert with slower biochemical responses, such as conformational changes of enzymes, and cellular physiological responses, including altered gene expression and changes in the fatty acid composition of the cell membrane (Sonna et al., 2002; Airaksinen et al., 2003). Together with the reduction of cerebral blood volume (Van den Burg et al., 2005), these responses to fluctuating temperatures warrant survival of fish, as long as the new temperature is within (species-specific) physiological limits.

List of abbreviations

alln	anterior lateral line nerves
aon	anterior octaval nucleus
bv	blood vessel
cc	corpus cerebelli

cgus	commissure of the secondary gustatory nuclei
cho	chiasma opticum
cm	corpus mammillare
cp	central posterior thalamic nucleus
cven	commissura ventralis rhombencephali
eg	eminentia granularis
fm	fibers of Mauthner cells
gc	griseum centrale
h	hypothalamus
hab	habenula
inf	infundibulum
lcc	lobus caudalis cerebelli
lih	lobus inferior hypothalami
llf	lateral longitudinal fascicle
LVII	facial lobe
LIX	glossopharyngeal lobe
LX	vagal lobe
mlf	medial longitudinal fascicle
mo	medulla oblongata
mon	medial octavolateralis nucleus
N	complex of trigeminal, facial, acoustic and lateral line nerves
NVmd	trigeminal motor nucleus, dorsal part
NVmv	trigeminal motor nucleus, ventral part
NVs	primary sensory trigeminal nucleus
ngs	secondary gustatory nucleus
nin	interpeduncular nucleus
pit	pituitary gland
poa	preoptic area
rl	recessus lateralis
rp	recessus posterior
rv	rhombencephalic ventricle
sd	saccus dorsalis (or plexus chorioideus)
sgn	secondary gustatory nucleus
sr	superior raphe
srf	superior reticular formation
tegm	tegmentum
tel	telencephalon
th	tubercular hypothalamus
tl	torus longitudinalis
to	tectum opticum
ts	torus semicircularis
ttb	tractus tectobulbaris
vcl	tateral division of valvula cerebelli
vcm	medial division of valvula cerebelli
I	olfactory nerve
II	optic nerve
III	oculomotor nerve
V	trigeminal nerve
VIIs	sensory root of facial nerve
X	vagal nerve

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