

BODY COLOUR CHANGES INDUCED BY SPINAL THERMAL STIMULATION OF THE CRUCIAN CARP (*CARASSIUS CARASSIUS*)

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SUMMARY

1. Body colour changes of the crucian carp during spinal thermal stimulation were recorded photoelectrically.
2. Warming the spinal cord induced darkening, whereas cooling induced lightening of body colour.
3. After transection of the medulla oblongata posterior to the vagal lobe, the same colour responses as in intact fish were induced, apart from one of the seven responses to spinal cooling.
4. After spinal pithing, thermal stimulation of the spinal cord failed to induce the responses.
5. The present responses are considered to indicate influence of spinal thermal stimulation on the cutaneous sympathetic systems of this fish.

INTRODUCTION

The triggering of thermoregulatory behaviour by localized temperature displacements in the central nervous system of poikilothermic vertebrates (Hammel, Caldwell & Abrams, 1967; Duclaux, Fantino & Cabanac, 1973; Strømme, Myhre & Hammel, 1971; Crawshaw & Hammel, 1971, 1973, 1974) clearly indicates that these animals have a central thermodetecting mechanism. Recently, relationships between local temperature in the CNS and autonomic functions, besides the thermoregulatory behaviour mentioned above, have also been observed (Rodbard, Samson & Ferguson, 1950; Crawshaw, Hammel & Garey, 1973). Our previous observations have also demonstrated a relationship between temperature of the spinal cord and heart rate in cyprinid fishes (Iriki *et al.* 1976; Nagai & Iriki, 1977). These relationships found in poikilothermic vertebrates seem to have important evolutionary implications, because physiological thermoregulatory responses which appear in the higher vertebrates rely primarily on some autonomic mechanism.

In the present study we assess the effect of spinal thermal stimulation on body colour of the crucian carp. This is an excellent system for studying one of the effects of spinal temperature. First, it is primarily under nervous control, and secondly, the pathway of the effector neurones in the spinal cord and sympathetic chain is almost the same as in the minnow *Phoxinus laevis* (von Frisch, 1911a).

MATERIALS AND METHODS

The experiments were performed from August to September 1976 at Okayama University.

Crucian carp (*Carassius carassius*) of 16–21 cm body length were used. Animals were immobilized with 0.05–0.1 ml injection of 0.1 % gallamine triethiodide solution into dorso-lateral muscle and were held loosely in an experimental bath with a half-tone-coloured background. The gills were continuously perfused with tap water of room temperature at the rate of 1.5–1.7 ml/s. Throughout the experiments, the illumination was kept constant.

Thermal stimulation of the spinal cord and temperature measurement

A copper tube of 40 mm length and 3 mm diameter, tightly fixed along the right arc of the vertebral column, was used as a thermode. This thermode extended from the 5th or 6th vertebra in the caudal direction to not more than the 15th vertebra. Hot or cold water was perfused through the thermode during spinal thermal stimulation, and water of room temperature was perfused before and after the stimulation. The effects of these temperature changes in the thermode on the temperature inside the vertebral canal have been described previously (see Iriki *et al.* 1976).

Temperature measurements were performed using thermistor probes; thermode temperature was measured at its surface, deep body temperature was measured within the visceral cavity about 3–4 cm distance through the rectum; in some fish, the temperature of muscle adjacent to the portion of the body from which colouration was measured, was taken instead of the visceral temperature.

Recording and calculation

Body colour of the tail region of the left side of the trunk was measured photo-electrically by the method of Iwata & Fukuda (1973), after a little modification (Fig. 1). The system consists essentially of a microlamp, CdS photoconductor and light guides. Changes in the intensity of light reflected from the restricted body surface were measured. Thus, when the skin surface is darker, a decrease in reflected light results in a decrease in CdS conductivity. The CdS cell composed one arm of the bridge circuit, and the potential difference derived from fluctuations of the balance within the circuit was fed into a DC amplifier and recorded by a pen writer.

For further statistical analysis, body colour was evaluated semiquantitatively as mm of recorder deflexion. The reference body colour level was taken as the mean level during the last 4 min of the pre-stimulating period. Changes in body colour were shown as the difference in mean level during 4 min astride the point of termination of the stimulation. Significance of the data was examined by Student's *t* test.

Medullary transection and spinal pithing

In seven fish the medulla oblongata was transected 2–3 mm posterior to the vagal lobe. In four fish the spinal cord was pithed by inserting a cannula from top to tail into the entire length of the vertebral canal.

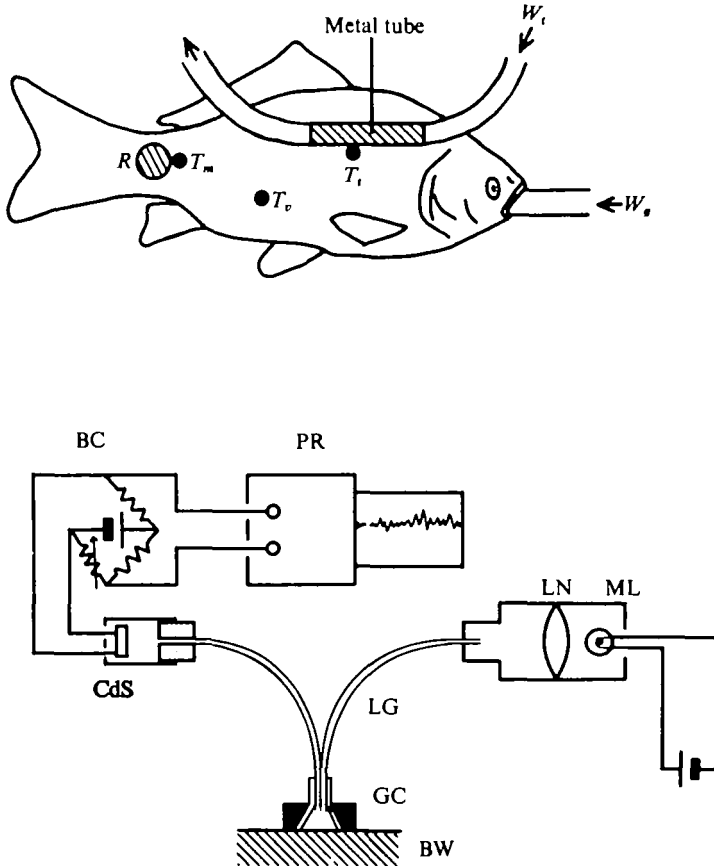


Fig. 1. Experimental arrangement (upper) and recording system of body colour (lower). A metal tube was fixed along the right arc of the vertebral column anterior to the 15th vertebra. Body colour was measured within a 17 mm diameter circle (R) on the tail region of the left side of the trunk. W_i : water perfusing a metal tube. W_v : water perfusing the gills. T_i , T_v , T_m , Measuring portions of the temperature of the metal tube, the visceral cavity and the muscle just adjacent to the colour recording position. BW, Body wall of the fish; GC, gummy covering; ML, microlamp; LN, lens; LG, light guide; CdS, cadmium sulphide photoconductor; BC, bridge circuit; PR, pen recorder.

RESULTS

(1) Results in intact fish

Thermal stimulation of the spinal cord was performed on five intact crucian carp; warming caused darkening of body colour and cooling induced lightening.

Fig. 2 shows original recordings of body colour and the sequence of temperature changes. 'Miniature pulsations', spontaneous oscillations of hue (Iwata & Fukuda, 1973), were observed; however, spinal thermal stimulation elicited definite responses. Generally, darkening of body colour was caused by exchanging the background colour tone from the experimental one to black and by spinal pithing. The amplitude of the darkening response during warming was about 50–60% of that during the former two conditions. The amplitude of the lightening during cooling was about 20–30% of the lightening induced by exchanging the background from the experimental half-tone to white.

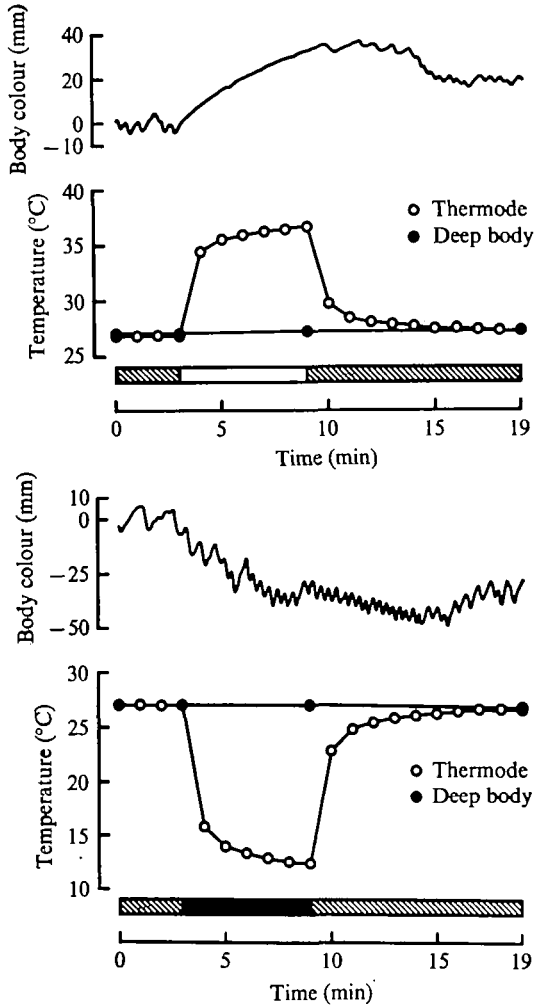


Fig. 2. Effects of spinal warming (upper) and cooling (lower) on body colour of intact fish. Ordinate shows temperature and body colour, and abscissa shows time course. Positive values for body colour indicate darkening, and negative values indicate lightening of body colour. White bar on horizontal block equals period of warming, black bar period of cooling. Results from one fish.

Table 1 summarizes the observations in intact fish. During warm stimulation mean temperature changes in the thermode and the visceral cavity were $+8.3 \pm 0.73$ °C and $+0.2 \pm 0.02$ °C respectively. During cold stimulation they were -12.1 ± 0.72 °C and -0.1 ± 0.08 °C respectively.

(2) Results in medullary transected fish

The medulla was transected in seven fish. Thermal stimulation of the spinal cord was performed 50–60 min after transection. Colour responses associated with spinal thermal stimulation and also miniature pulsations were observed in fish after medullary transection as shown in Fig. 3. Table 2 summarizes all results. During warm stimulation mean temperature changes in the thermode, the visceral cavity and the muscle

Table 1. *Effects of spinal warming and cooling on body colour in intact fish*

(+, Darkening; -, lightening. s, Data significantly different from the values before the stimulation ($P < 0.05$, Student's t test.)

Number	Body colour	Thermode temp. (°C)		Deep body temp. (°C)		50% recovery (min)
		Before	During	Before	During	
Spinal warming						
I-1	+26.3 ^s	26.8	36.6	27.0	27.2	10
I-2	+9.3 ^s	26.8	35.5	27.0	27.1	5
I-3	+8.4 ^s	26.2	31.8	26.4	26.6	—
I-4	+23.0 ^s	26.5	35.8	26.7	26.8	—
I-5	+12.0 ^s	26.6	34.9	26.8	27.0	4
\bar{x}	+15.8	26.6	34.9	26.8	26.9	
$S\bar{x}$	±3.7	±0.1	±0.8	±0.1	±0.1	
Spinal cooling						
I-1	-35.9 ^s	27.0	12.4	27.0	27.0	12
I-2	-14.6 ^s	26.8	15.4	27.0	27.0	—
I-3	-14.8 ^s	26.2	15.1	26.4	26.4	2.5
I-4	-30.4 ^s	26.6	15.9	26.8	26.8	12
I-5	-4.8 ^s	26.6	13.0	27.0	26.6	—
\bar{x}	-20.1	26.6	14.4	26.8	26.8	
$S\bar{x}$	±5.7	±0.1	±0.7	±0.1	±0.1	

were $+11.1 \pm 0.77$ °C, $+0.1$ °C and $+0.1 \pm 0.05$ °C respectively. During cold stimulation they were -13.0 ± 0.83 °C, -0.1 °C and -0.03 ± 0.03 °C respectively.

Warm stimulation induced darkening of body colour in all fish. Cold stimulation caused lightening in 6 fish and darkening in 1 fish.

(3) *Results in pithed fish*

The spinal cord was destroyed in four fish. This resulted in general darkening of the body, and the miniature pulsations became undetectable, because the pre-ganglionic pathway of melanophore effector neurones was eliminated. The spinal cord was heated or cooled at least 50 min after pithing, when darkening was complete.

During warming, mean temperature changes in the thermode and the muscle were $+11.9 \pm 0.56$ °C and $+0.1 \pm 0.04$ °C respectively. During cooling they were -12.8 ± 0.95 °C and -0.03 ± 0.03 °C respectively.

Neither warming nor cooling induced any significant response in body colouration (Fig. 4).

DISCUSSION

It is well known that many fish change their body colour adaptively in response to background colouration. In addition to such a visually induced response, thermal stimulation of the skin or an odour substance applied to the olfactory epithelium can elicit colour responses by affecting the melanophore effector system via an integrative centre for body colour (von Frisch, 1911*b*; Pye, 1964; Iwata & Fukuda, 1973).

The movements of pigments within the melanophore are likely to be achieved via the single innervation by sympathetic neurones, whose excitation induces the aggrega-

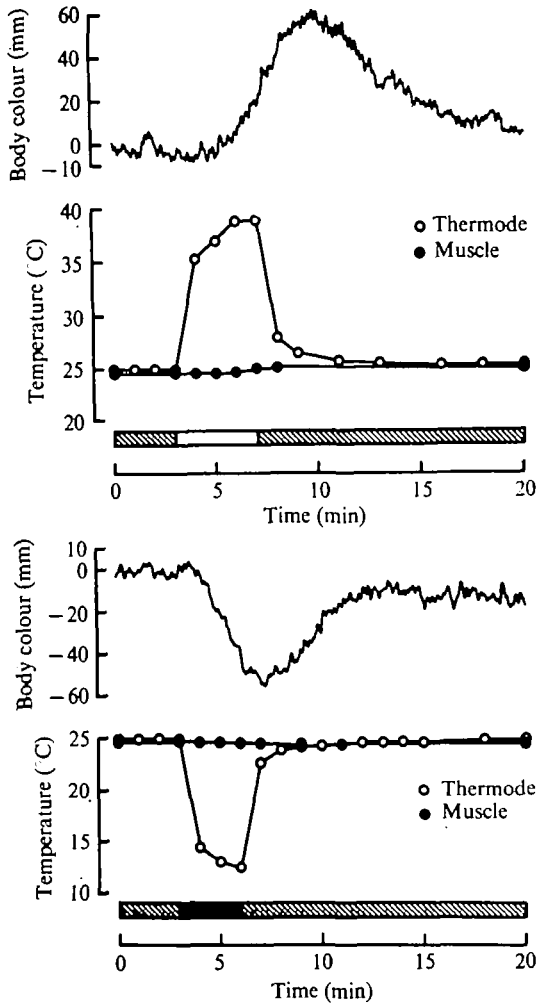


Fig. 3. Effects of spinal warming (upper) and cooling (lower) on body colour of medullary transected fish. Symbols as in Fig. 2.

tion of pigments, i.e. lightening of body colour; suppression causes the dispersion of pigments, i.e. darkening of body colour (reviewed by Iwata & Fukuda, 1973).

The pathway of these sympathetic aggregating neurones has essentially been established: the neurones originate from the medullary centre for body colour, run through the spinal cord to the 12th–15th vertebra, then extend within the sympathetic chain in both rostral and caudal directions, and innervate the melanophores through the spinal and trigeminal nervous pathways (von Frisch, 1911a; Healey, 1951, 1954). Thus, the body colour responses provide an excellent indicator for investigating the effects of spinal thermal stimulation on the cutaneous sympathetic system.

In the present experiments, colour responses were induced by thermal stimulation of the spinal cord anterior to the 15th vertebra, even after medullary transection, but not after the spinal cord was destroyed.

Any direct effect of temperature on the melanophore itself during thermal stimula-

Table 2. *Effects of spinal warming and cooling on body colour in medullary transected fish*

(+, Darkening; —, lightening. *s*, Data significantly different from the values before the stimulation ($P < 0.05$, Student's *t* test). *v*, Visceral temperature; *m*, muscle temperature.)

Number	Body colour	Thermode temp. (°C)		Deep body temp. (°C)		50% recovery (min)
		Before	During	Before	During	
Spinal warming						
M-1	+16.5 ^s	24.5	33.6	^v 24.5	24.6	2
M-2	+33.1 ^s	24.9	37.8	^m 24.5	24.8	6
M-3	+25.6 ^s	24.6	36.0	^m 24.4	24.4	4.5
M-4	+20.1 ^s	24.5	34.2	^m 24.0	24.0	27
M-5	+14.5 ^s	24.8	33.8	^m 24.8	24.8	13.5
M-6	+5.9 ^s	24.7	37.2	^m 24.8	24.9	2
M-7	+11.7 ^s	24.6	38.4	^m 24.5	24.5	16.5
\bar{x}	+18.2	24.7	35.9	24.5	24.6	
$S\bar{x}$	±3.4	±0.1	±0.8	±0.1	±0.1	
Spinal cooling						
M-1	-13.9 ^s	24.4	14.6	^v 24.4	24.3	5
M-2	-37.2 ^s	24.9	12.4	^m 24.6	24.4	4
M-3	-30.4 ^s	24.7	14.0	^m 24.4	24.4	7
M-4	-30.1 ^s	24.6	11.8	^m 24.1	24.1	—
M-5	-3.8 ^s	24.4	10.6	^m 23.8	23.8	—
M-6	+8.9 ^s	24.6	8.6	^m 24.5	24.5	5.5
M-7	-6.8 ^s	24.6	9.8	^m 24.5	24.5	—
\bar{x}	-16.2	24.6	11.7	24.3	24.3	
$S\bar{x}$	±6.4	±0.1	±0.8	±0.1	±0.1	

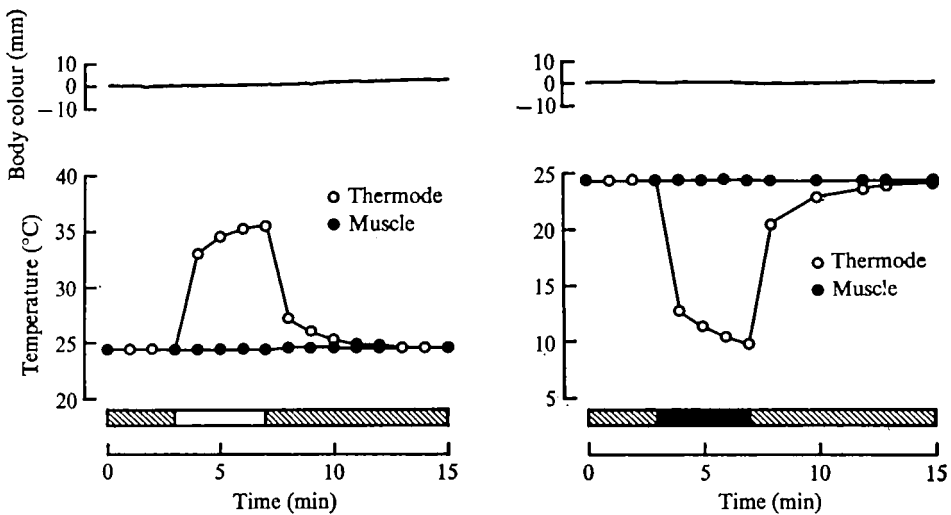


Fig. 4. Effects of spinal warming (left) and cooling (right) on body colour of pithed fish. Symbols as in Fig. 2.

tion has been precluded: temperature changes in the visceral cavity and the muscle near the recording portion were small, as shown in the tables, and further, the responses were not evoked after pithing (Fig. 4). The direct temperature effect on the melanophore effector neurones within the sympathetic chain must also be negligible, because the sympathetic chain remained intact even after pithing, and yet no responses were elicited. Therefore, although the integrative function of the brain is not necessary for the temperature responses (Table 2, Fig. 3), a neuronal conduction of thermal information to the melanophore effector system via thermosensitive structures in the spinal cord is essential.

The fact that warming the spinal cord caused darkening of body colour and cooling induced lightening indicates, according to the functional property of effector neurones, that spinal warming caused a decrease in the activity of cutaneous sympathetic neurones and cooling elicited an increase. In homeotherms, spinal warming results in a decrease in the activity of sympathetic efferents of cutaneous regions and spinal cooling produces an increase in the activity of these efferent sympathetic fibres, accompanying the thermoregulatory changes in vasomotor tone and blood flow of cutaneous regions (Walther, Iriki & Simon, 1970); this also occurs in decerebrated and spinalized animals (Walther *et al.* 1971*a*; Walther, Simon & Jessen, 1971*b*; Iriki & Kozawa, 1976). The present results in the crucian carp suggest that activity changes in cutaneous sympathetic efferents during spinal thermal stimulation would be mediated by the same neural mechanism within the spinal cord as in homeotherms.

One of the seven medullary transected fish revealed a darkening response during spinal cooling (Table 2), perhaps caused by the condition of the melanophores before stimulation or by a non-specific influence of the stimulus on spinal structures.

The present investigation of body colour changes in fish has provided additional evidence of central thermodetection in poikilothermic vertebrates; further, it is possible that the same spinal mechanism as that used in homeotherms contributes to changes in activity of cutaneous sympathetic neurones during spinal thermal stimulation.

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