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SHORT COMMUNICATION

THE HEART RATE VARIABILITY SIGNAL IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

BY LUIS DE VERA* AND IMANTS G. PRIEDE

University of Aberdeen, Department of Zoology, Tillydrone Avenue, Aberdeen, AB9 2TN, UK

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Records of heart rate often show short-term variation in beat-to-beat interval which can be plotted as a heart rate variability signal (HRVS). It has been suggested that, in mammals, analysis of the HRVS can be used as a quantitative means of investigating control of cardiac function (McDonald, 1980). An HRVS can be recognised in the electrocardiograms (ECGs) of teleost fishes (Armstrong *et al.* 1989*a*) and these signals have been recorded by telemetry from free-living fish in their natural environment (Armstrong *et al.* 1989*b*). Regulation of the heart is usually studied by blocking components of the system by surgical or pharmacological intervention or using *in vitro* isolated preparations (Farrel, 1984). Analysis of the HRVS may provide a unique insight into the function of the intact system and should be applicable to free-swimming fish in their natural environment.

The rainbow trout (*Oncorhynchus mykiss*=*Salmo gairdneri*) was chosen since it has been relatively well studied and has a dual cardiac innervation with both adrenergic and cholinergic elements (Gannon and Burnstock, 1969). Priede (1974) showed that heart rate variation in this species is abolished by section of the vagus nerves so we might expect the HRVS to be an indicator of neural mechanisms controlling heart rate in this species. *In vivo* and *in vitro* studies (Priede, 1974; Wood *et al.* 1979; Graham and Farrell, 1989) have shown that control of heart rate is affected by temperature. Therefore, we analysed the HRVS in fish acclimated to three different temperatures: 5, 10 and 15°C (±0.5°C).

Fifteen rainbow trout of either sex and weighing between 203 and 351 g were used. They were kept in well-aerated outdoor tanks and fed *ad libitum* until 24 h prior to experimentation. Experiments were carried out from February to June during a period of natural temperature increase from 5 to 15°C. Five fish were used at each temperature and times were chosen so that the fish were acclimated for at least 4 weeks to the appropriate temperature regime. Fish were anaesthetised in benzocaine ($0.1\,g\,l^{-1}$) and two stainless-steel wire electrodes were implanted

* Permanent address: Department of Physiology, Medical Faculty, University of La Laguna, Tenerife, Canary Islands, Spain.

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subcutaneously (Priede, 1974). The fish with electrodes attached were transferred to a temperature-controlled tank with flowing fresh water and allowed 24 h to recover before recording of ECGs began.

The ECG signal was amplified, passed through an R-wave detector and then *via* a S200 interface (Cambridge Research Systems) to an IBM-compatible PC which logged successive R–R intervals to an accuracy of 1 ms. For each fish, records of 30 min duration were made for one day, centred at 06:00 h, 12:00 h, 18:00 h and 24:00 h, so that the effects of any circadian rhythm could be accounted for. The series of R–R interval durations was first plotted as a function of the R-wave occurrence time and then converted to an equally spaced time series using linear interpolation (Rompelman *et al.* 1977).

Fourier analysis was then used to examine the oscillatory patterns in the HRVS series. Sampling rates were 2 Hz at 5 and 10 °C and 2.5 Hz at 15 °C to avoid aliasing effects taking into account the mean heart rate and Nyquist criterion (Rompelman *et al.* 1977; Gonzalez and De Vera, 1988). The effects of the value of the mean heart rate at the particular time of day or other slow changes were removed using the following techniques (Bendat and Piersol, 1971). Data were normalised by subtracting the mean R–R interval from each data point, any linear trend was removed and a 10 % cosine taper was applied. The power spectral density function (PSD) was calculated using a fast Fourier transform algorithm (Monro, 1976). Each PSD was frequency-smoothed by averaging the results for five contiguous spectral components. The estimates obtained from all the fish at each temperature were averaged, resulting in one ensemble-averaged PSD per temperature (Livnat *et al.* 1984). For data at each temperature, the mean centre frequency of the main spectral peaks and the mean power associated with those peaks were calculated in accordance with methods described by Bendat and Piersol (1971).

Mean heart rates increased with temperature (Table 1) and values were intermediate between basal and maximal values for this species (Priede, 1974). This reflects the expected effect of temperature on the cardiac pacemaker frequency. The heart rate was higher in the middle of the day (12:00 h) than at night (24:00 h). On visual inspection of 1-min HRVS samples it is clear that in all three groups there is heart rate variability and that the oscillation frequency increases with temperature (Fig. 1). The Kolmogorov–Smirnov test showed the variation to be non-random (95 % confidence limit) in the frequency range from 0.025 to 0.425 Hz or 2.3–40 s period. The power spectra of the HRVS at all three temperatures show two major peaks corresponding to two main periodic components in the signal. The two spectral peaks are variable, one or other disappearing in particular records and sometimes changing their relative power content and centre frequency. Overall frequencies and powers of the two spectral peaks did not vary significantly ($P>0.1$). Data were therefore pooled for analysis of ensemble spectra at the three temperatures (Fig. 2, Table 1). Both the high- and low-frequency peaks showed no significant change in centre frequency between 5 and 10 °C ($P>0.001$). Between 10 and 15 °C, however, there is a significant increase in centre frequency of both peaks ($P<0.001$). At 5 and 10 °C, the power of the low-

Table 1. *Characteristics of the main peaks that appear in the ensemble-averaged power spectra of the heart rate variability signal at the different temperatures*

		Low-frequency peak				High-frequency peak			
Temperature (°C)	Heart rate (beats min^{-1})	Frequency (Hz)	S.D.	Period (s)	Spectral power ($ms^2 \times 10^{-3}$)	Frequency (Hz)	S.D.	Period (s)	Spectral power ($ms^2 \times 10^{-3}$)
5	39.6	0.069	0.021	14.5	0.534 (0.437–0.674)	0.151	0.035	6.6	0.333 (0.254–0.400)
10	52.4	0.084	0.030	11.9	2.454 (1.933–3.036)	0.171	0.040	5.8	1.099 (0.866–1.360)
15	71.1	0.162	0.050	6.2	0.993 (0.738–1.201)	0.350	0.062	2.8	1.645 (1.313–2.128)

All data from all fish at each temperature have been pooled.
Figures in parentheses below the spectral power values are 95 % confidence intervals.

frequency peak was significantly greater ($P<0.05$) than that of the high-frequency one; but at 15 °C this was reversed and the high-frequency peak exhibited greater power.

The main feature of the HRVS spectrum of rainbow trout is the presence of two

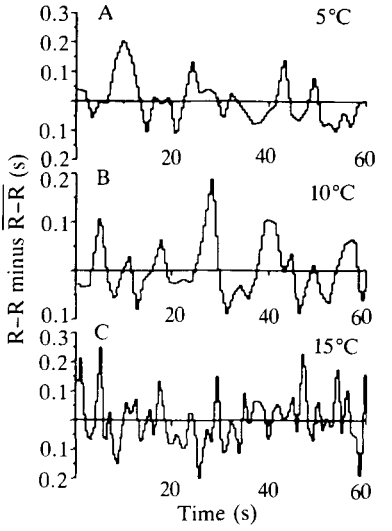

Fig. 1. Examples of heart rate variability signals at the three experimental temperatures. The ordinate is the normalised R–R interval. (A) 5 °C, (B) 10 °C and (C) 15 °C.

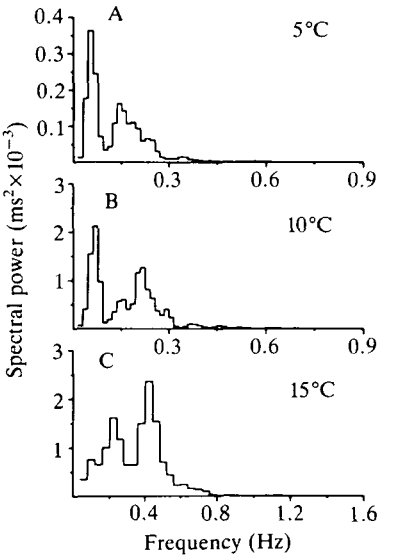

Fig. 2. Ensemble average relative power spectral density of the heart rate variability signal computed from 25 individual spectra for each fish. (A) 5 °C, (B) 10 °C and (C) 15 °C. Note the change in relative size of the spectral peaks and the shift in peak frequency.

spectral peaks. This contrasts with the report by Armstrong *et al.* (1989*a*) of only one HRVS spectral peak in pike (*Esox lucius*) and brown trout (*Salmo trutta*). In view of the extensive data set in this study we conclude that two spectral peaks are a normal feature of the HRVS in rainbow trout.

The observation of two spectral peaks, the relative importance of which changes with temperature, is very similar to previous observations for the lizard (Gonzalez and De Vera, 1988). In the lizard, the low-frequency peak is dominant at low temperatures and the high-frequency peak is dominant at higher temperatures, exactly as for rainbow trout in this study. By analogy with observations on mammals, the low-frequency peak was identified as a 'temperature' component related to the thermal vasomotor control system and the high-frequency peak as a 'pressure' component related to operation of the blood pressure control system (Gonzalez and De Vera, 1988). Mammals tend to have three HRVS spectral peaks and it has been shown that the high- and mid-frequency peaks are caused by parasympathetic activity whereas the low-frequency peak includes the sympathetic component together with a parasympathetic component (Akselrod *et al.* 1981).

Wood and Shelton (1980*b*) studied the cholinergic control of heart rate in rainbow trout and found that, during periods of low heart rate, tachycardia could be evoked in response to a fall in blood pressure. This was mediated by a decrease in parasympathetic vagal tone. As well as this baroreceptor reflex, the cholinergic pathway is also responsible for chemoreceptor responses. Experimentally induced anaemia (Wood *et al.* 1979) leads to tachycardia mediated by the parasympathetic vagus. A widespread phenomenon in fishes mediated by the parasympathetic pathway is the 'approach reflex' (Labat, 1966): a bradycardia triggered by external sensory stimuli. These parasympathetic effects are analogous to the pressure high-frequency component identified in the HRVS spectra of higher vertebrates. The period of the high-frequency peak, 6.6 s at 5 °C and 2.8 s at 15 °C, is commensurate with heart rate regulation over a time base of one or two beats, giving rise to the characteristic beat-to-beat variation observed in fish.

The adrenergic receptor in the teleost heart is of a beta2-type (Ask, 1983). Adrenergic stimulation is important in maintaining a regular heart beat in perfused isolated heart preparations (Graham and Farrell, 1989). In intact fish, adrenaline increases systemic resistance and decreases gill resistance (Wood and Shelton, 1980*a*). The heart may show reflex responses to these changes or direct responses of the myocardium to the catecholamine (Laurent *et al.* 1983). It is probable that the low-frequency peak in the HRVS corresponds to these adrenergic effects. The period of oscillation in this instance varies between 14.5 s at 5 °C and 6.2 s at 15 °C.

It is possible therefore to visualise the heart being regulated in the following way. The free-running pacemaker frequency increases with temperature and is intermediate between basal and maximal heart rate. The heart is controlled by a dual innervation with cholinergic inhibitory pathways showing feedback approximately twice as fast as adrenergic stimulatory pathways. The two pathways vary in importance according to temperature, with the cholinergic pathway (high fre-

quency) dominating at high temperature. This is at variance with the suggestion by Priede (1974) and Wood *et al.* (1979) that the cholinergically mediated parasympathetic inhibitory tonus dominates at low temperatures.

The precise origin of the spectral peaks in rainbow trout HRVS may be resolved by administration of pharmacological blocking agents. This study, however, has shown that the HRVS in rainbow trout is non-random, exhibits characteristics consistent with the presence of a functional dual innervation, and changes with temperature, reflecting patterns of adaptation of the cardiovascular system. HRVS analysis particularly in view of the possibilities of telemetry from free-living fish, promises to be a powerful new technique in the study of cardiovascular regulation in fishes.

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