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Unilateral vagal control of pulmonary blood flow in the rattlesnake, *Crotalus durissus terrificus*

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Complete vagotomy in the rattlesnake caused heart rate to rise and become unvarying, blood flow to increase in both the systemic and more markedly in the pulmonary circuit (a left to right shunt) and the breathing rhythm to slow, with greatly increased lung volumes (Wang *et al.* 2001). These data can be interpreted as loss of vagal tone on the heart and on the pulmonary sphincter, plus denervation of lung stretch receptors. As the rattlesnake only possesses a single functional lung, developed from the left side of the body, vagal efferent innervation of the sphincter on the pulmonary artery may be similarly unilateral. This interesting possibility was tested by peripheral stimulation of the left and right cervical vagi.

Rattlesnakes, 8 of either sex, mass 260 to 850 g, were fasted for more than one week prior to experiments. Snakes were terminally anaesthetised with 20 mg Nembutal kg^{-1} injected into a caudal blood vessel. A ventrolateral incision above the heart enabled non-occlusive cannulation of the right aortic arch, for measurements of blood pressure. Blood flow probes (Transonic Systems Inc.) were placed around the pulmonary artery and the left aortic arch. The cervical vagi was exposed and lifted onto platinum hooks for electrical stimulation (2 msec pulses at 2–10volts). A range of frequency/response determinations was then made for each nerve. Each stimulation was followed by a period of recovery that constituted the control conditions for the subsequent stimulation.

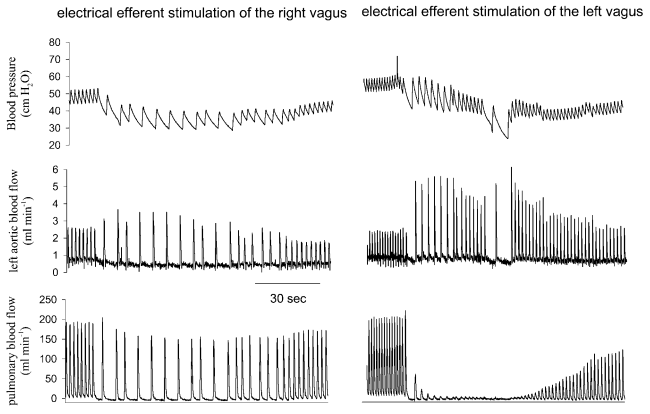

Figure 1. A recording of systemic arterial blood pressure, blood flow in the left aortic arch and blood flow in the pulmonary artery during peripheral electrical stimulation of the right or left cervical vagus in the anaesthetised rattlesnake *Crotalus durissus terrificus*.

The haemodynamic changes elicited by peripheral electrical stimulation differed substantially between the right and left vagi. Thus, while stimulation of either side led to pronounced reductions in heart rate and a drop in blood pressure, stimulation of the left vagus also led to large reductions in pulmonary blood flow. A recording from one experiment, shown in Fig. 1, highlights these differences.

This demonstration of vagal control of the heart and pulmonary artery in *Crotalus* is consistent with previous studies on turtles and other reptiles, but the unilateral influence of the left vagus on the pulmonary artery is novel.

Wang, T. *et al.* (2001). *Exp Physiol* **86**, 777–780.

All procedures accord with current UK legislation

C82

Does cardiovascular variability differ in normothermic and cold-acclimated anaesthetised rats during acute hypothermia?

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Heart rate variability (HRV) is described as the variation in R-R interval that occurs in consecutive waveforms of the electrocardiogram (ECG), which may act as a predictor of morbidity (Matthew *et al.* 2002). The power spectrum can be divided into low frequency (LF) and high frequency (HF) bands. In rats LF reflects sympathetic and parasympathetic control of blood pressure (BP), while HF is mostly due to parasympathetic stimulation and correlates with respiration frequency (Cerutti *et al.* 1991). The aim of the study was to compare HRV in rats without (normothermic, $n = 10$) and with prior exposure to lower ambient temperatures (cold-acclimated, $n = 7$) at normal core temperature (Tb) of 37°C, on cooling to Tb = 25°C (acute hypothermia) and on rewarming to Tb = 37°C.

Male Wistar rats, 295–310g, were anaesthetised with fluothane (2.5 % in O_2) and α-chloralose/urethane (32/450 mg kg^{-1} I.V.). BP, ECG and ventilation were recorded and stored onto a computer for offline auto-spectral and cross-spectral analysis in the frequency domain. Deep oesophageal Tb was regulated by means of a thermostatted plate connected to a temperature control unit. Rats were killed humanely with an overdose of sodium pentobarbitone. Data (mean ± S.E.M.) were analysed using ANOVA and significance taken at $P < 0.05$.

At Tb = 25°C, in normothermic and cold-acclimated rats there was a similar prolongation of R-R interval by ~40 % ($P < 0.001$ *vs.* 37°C) and a shift of LF and HF range by 50 % and 35 % ($P < 0.05$ *vs.* 37°C). Hypothermia reduced total power by 26 % ($P < 0.05$) and 66 % ($P < 0.01$) reflecting central depression of neural outflow and decreased sympathetic control of peripheral blood flow suggested by reduced LF power by 21 % and 57 % ($P < 0.05$) in normothermic and cold-acclimated rats, respectively. In contrast, there was a moderate increase in HF power in normothermic rats (1.12 ± 0.33 ms^2 *vs.* 1.07 ± 0.18 ms^2) and a reduction in cold-acclimated rats (0.35 ± 0.09 ms^2 *vs.* 0.82 ± 0.24 ms^2) implying a higher vagal tone in former. In both groups, cross-spectral analysis between ECG, BP and ventilation revealed a high coherence between signals at HF at all Tb. Hypothermia increased the phase lag between ECG and BP signals by 60 % and 50 %, and between ECG and respiration by 35 % and 18 % (all $P < 0.05$) in normothermic and cold-acclimated rats, respectively. On rewarming all power spectra variables were completely restored to precooling levels.

These results suggest maintenance of cardio-respiratory coupling at lower Tb, and an intact autonomic system that rapidly restores physiological functions to steady-state levels on rewarming that was more efficient following cold-acclimation.

Cerutti C *et al.* (1991). *Am J Physiol* **261**, H1292–1299.

Matthew CB *et al.* (2002). *Can J Physiol Pharmacol* **80**, 925–933.

All procedures accord with current UK legislation

C83

Investigating the control of heart rate variability in fishes using power spectral analysis

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The intrinsic heart rate (*f*H) in fish, like all higher vertebrates, is modified on a beat-to-beat basis by the level of vagal activity. Power Spectral Analysis (PSA) is used clinically to explore this regulation of cardiac performance noninvasively (Sands *et al.* 1989), and we have adapted this application for use with fishes using a similar approach to that described previously (Altimiras *et al.* 1995).

ECG electrodes were implanted under MS222 anaesthesia (0.5g/l). Anaesthesia was maintained during surgery, then animals allowed to recover. Animals were humanely killed after the experiments. PSA applied to recordings of instantaneous heart rate from resting fish (*Myoxocephalus scorpius*) displayed dual spectral peaks (0.02 Hz and 0.05 Hz). The effects of minor surgery or vagotomy decreased the mean R-R interval from 2946 ± 232 ms to 1912 ± 87 ms (mean ± S.E.M., $n = 5$). The variability in beat to beat (R-R) interval, and both frequency peaks were also abolished temporarily by disturbance and permanently by bilateral cardiac vagotomy, suggesting that much of the regulation of heart rate was under parasympathetic, cholinergic control. Interestingly, the mean and standard deviation of R-R interval (SDRR) determined by cholinergic tonus showed a close correlation with oxygen consumption (MO_2), indicating a high degree of cardio-respiratory coupling. This was further explored in 7 species of Antarctic fish, as the closely related Notothenioid group allows the physiological consequences of ecological differences between species to be evaluated without the difficulties posed by comparing fish with different phylogenetic backgrounds. Comparison between species showed that whilst mean *f*H and MO_2 rates were significantly different (ANCOVA, $P < 0.05$), linear correlation between relative *f*H and MO_2 were not. Furthermore, PSA analysis showed two underlying oscillations in the instantaneous heart rate, a higher frequency peak at 0.03–0.08 Hz that was *f*H dependent being similar in period between species, and a lower frequency peak at 0.02 Hz that was independent of *f*H. Thus the degree of cholinergic control on fH and heart rate variability was similar across the Antarctic species, but different from the closely related sub-Antarctic *Paranotothenia angustata* or the temperate *Myoxocephalus scorpius* which has a similar benthic habitat.

We conclude that cholinergic tonus on the fish heart shows retention of ancestral characteristics, with a degree of phenotypic plasticity that appears to be associated with species ecology.

Altimiras J *et al.* (1995). *Brazil J Med Biol Res* **28**, 1197–1206.

Sands T *et al.* (1989). *Circulation* **79**, 76–82.

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All procedures accord with current UK legislation

C84

Sarcomere length-tension relationships in single cardiac ventricular myocytes of the rainbow trout (*Oncorhynchus mykiss*)

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Increased cardiac output in fish is achieved largely by an increase in stroke volume. This is achieved by an increased end-diastolic ventricular volume and a stretch-induced increase in myocardial contractility known as the Frank-Starling mechanism (Farrell & Jones, 1992). Compared to mammalian myocardium, relatively few details of this mechanism are known for fish.

Trout (159 ± 9 g, mean ± S.E.M.) were killed humanely. Single ventricular myocytes were isolated enzymatically then placed in the chamber of an inverted microscope and superfused at 21–22 °C with a physiological saline solution containing 2 mM Ca^{2+} (pH = 7.8). Carbon fibre transducers were attached towards either end of the myocyte about 100 μm apart, in order to record tension and to apply axial stretch to the longitudinal axis of the myocyte. Stretch was measured as increased sarcomere length (SL) between the fibres (Hongo *et al.* 1996). Cells were stimulated by external electrodes at 0.5 Hz and contracted auxotonically.

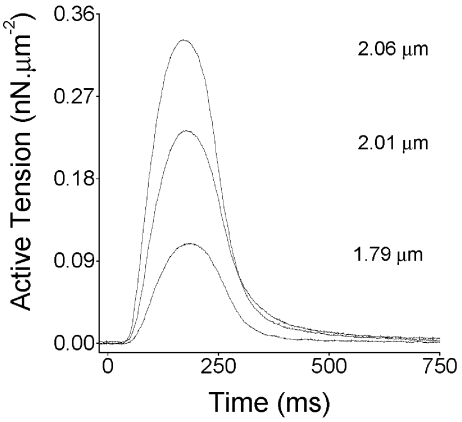

Figure 1. Active tension (normalized to cross-sectional area, cell width 17.5 μm) in a trout ventricular myocyte contracting auxotonically at 0.5 Hz and stretched from a slack SL of 1.79 μm to SLs indicated in figure.

The mean end diastolic SL was (1.84 ± 0.02 μm, $n = 19$ myocytes from 6 hearts) at slack length. When cells were progressively stretched there was a progressive increase in both the passive tension (during diastole) and the active tension (additional tension developed during systole, Fig. 1). Analysis of stretches in 19 cells predicted each 1% increase in SL above slack SL caused approximately a 20% increase in active tension (linear regression, $R = 0.75$, $P < 0.001$).

These are the first data measuring SL in contracting fish myocytes and demonstrating the Frank-Starling mechanism in single fish cardiac myocytes. Resting SL was similar to that typically reported in mammalian myocytes. The slope of our SL-tension relationship is consistent with data from trout cardiac muscle strips (Harwood *et al.* 1998) where active stress increased by approximately 350 % when length was increased from 70 to 90 % Lmax (the length at which maximum active stress is recorded).

Farrel AP & Jones DR (1992). *Fish Physiology* **XIIA**, 1–88.

Harwood CL *et al.* (1998). *J Exp Biol* **201**, 2723–2733.
Hongo K *et al.* (1996). *J Physiol* **491**, 599–606.

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All procedures accord with current UK legislation

C85

The cardiovascular changes associated with warming and cooling in the turtle, *Trachemys scripta*

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The cardiovascular changes associated with warming and cooling were investigated in seven freshwater turtles, *Trachemys scripta*. Animals were anaesthetised by isoflurane inhalation, a piece of the ventral carapace over the heart was removed with a bone saw, and blood flow probes (Transonic Systems Inc.) were placed around the left aortic arch and left pulmonary artery. The carotid artery was occlusively cannulated, using a PE50 catheter, for blood pressure measurements. Animals were then artificially ventilated until spontaneous ventilation resumed, and allowed to recover for a period of 48 hours. The turtles were warmed to 34 °C, using an infrared 150W heating lamp, and then allowed to cool to 24 °C, both before and 45 min after intrarterial injection of atropine (3 mg kg^{-1}). All experimental animals were humanely killed following the protocol using an overdose of Pentobarbitol (intrarterial injection of 200 mg kg^{-1}).

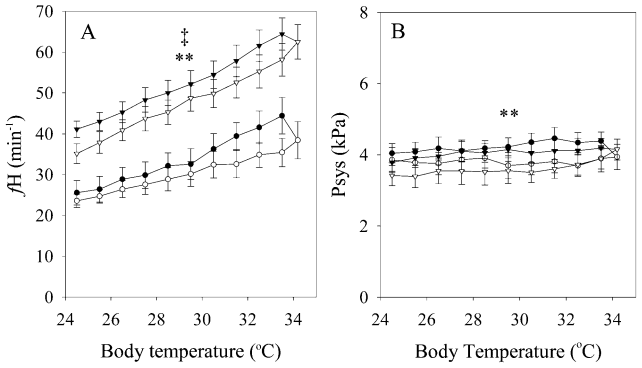

Figure 1. Heart rate (fH) (*A*), and systemic arterial blood pressure (Psys) (*B*), during warming and cooling in untreated (circles) and atropinised (triangles) animals. Values are mean with S.E.M. ($n = 7$). ** Indicates there is a significant difference between warming and cooling; ‡ indicates there is a significant difference between atropinised and untreated animals ($P < 0.05$).

All turtles warmed at a faster rate than they cooled. Animals exhibited a hysteresis of heart rate and blood flow to both the pulmonary and systemic circulations, which was not cholinergically mediated (Fig. 1A). Blood pressure remained constant during both warming and cooling (Fig. 1B), while systemic resistance decreased during heating and increased during cooling, indicating a barostatic response. Hicks (1998) described a large R-L shunt in *Trachemys scripta* which increased with heating and decreased with cooling in untreated animals, interpreting the shunt pattern as a passive consequence of altered resistances within the systemic circulation. However, in the present study, there was a large R-L shunt during warming and cooling in untreated animals which remained relatively constant. This may indicate an active regulation of cardiac shunts in order to sustain oxygen delivery. Atropinisation resulted in a large L-R shunt which decreased during warming and increased during cooling. However, the cooling periods were extended in atropinised animals, indicating that heat lost across the lung surface is not a determining factor in rates of cooling.

Hicks, J.W. (1998). In *Biology of Reptilia*, *Morphology* G. *Visceral Organs*, ed. Gans C & Gaunt A.S., pp. 425–483. SSAR Press, Ithaca, NY, USA.

All procedures accord with current UK legislation

C87

The development of the interstitial cells of Cajal in the equine fetus: an immunohistochemical study

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The interstitial cells of Cajal (ICC) are integral to the coordination of gastrointestinal motility by generating pacemaker activity and mediating neurotransmission in the gastrointestinal tract (Sanders 1996). There is currently no information available on the development of the ICC in the equine fetus. This study was carried out in order to determine whether ICC are detectable in the equine fetus, and if so, whether there are age-related changes in the patterns of ICC distribution, with respect to both the transmural and rostro-caudal distribution of ICC.

Tissues from 12 naturally aborted equine fetuses were used in this study. The ages of the fetuses ranged from 6–11 months (normal gestation length in the horse is 11 months). Sections of ileum, caecal base, pelvic flexure and distal small colon were obtained from each animal. All material was collected at post mortem and was surplus to that required for the routine investigation into the cause of abortion. Collected tissue was fixed in formalin and standard immunohistochemical labelling techniques were applied using an antibody against the c-Kit protein of the ICC (Oncogene Research Products, Cambridge, MA, USA).

ICC were present throughout the equine gastrointestinal tract at 6 months of gestation. The distribution of ICC in the small intestine was similar to that of the neonatal animal while in the large intestine changes were observed during development. A rostro-caudal gradient of immunoreactivity was evident, with the more distal part of the large intestine being less densely colonised by ICC in the younger fetuses compared to the near-term animals. A transmural gradient of ICC distribution was also evident within the large intestine, with the most luminal part of the muscularis externa appearing to be the last area of the intestine to be colonised by these cells. Indeed, even in the full term fetus this region did not appear fully developed compared to the neonatal animal. This transmural gradient observed in the horse does not appear to be present in other mammalian species that have been studied. Considering the proposed location of the pacemaker ICC at the submucosal border of the circular muscle layer (Rae *et al.* 1998), this may be of importance when investigating commonly observed problems in the equine neonate such as meconium impactions and motility disorders observed in dysmature and premature foals.

Rae MG *et al.* (1998). *J Physiol* **510**, 309–320.
Sanders KM (1996). *Gastroenterology* **111**, 492–515.

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PC72

NMDA receptor subunits immunoreactivity in the CNS of the cephalopods

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Excitatory amino acids, such as glutamate and aspartate, are present in many synapses of the vertebrate and invertebrates central nervous system (CNS). Glutamate has a depolarizing effect on neurons and this effect is rapid and can be mimicked by other amino acids. The speed of this effect suggested that glutamate and its agonist evoke depolarization by acting directly on receptor channel.It can be distinguish two main groups of receptor channel activated by glutamate: this distinction is based on the affinity of the receptor for glutamate selective structural analogs, notably for *N*-methyl-D-aspartate (NMDA). Thus, ionotropic glutamate receptors have been subdivided into *N*-methyl-D-aspartate (NMDA), and AMPA/kainate classes.

NMDA receptor subunits 2A&B (NMDAR2A&B) immunoreactivity is shown to be present in specific regions of the central nervous system (CNS) of the cephalopod molluscs *Sepia officinalis* and *Octopus vulgaris* (*n* of experiments = 10; all the animals were humanely killed). An antibody which recognizes both NMDAR2A and NMDAR2B subunits equally (Chemicon) was used. SDS-PAGE/Western blot analysis revealed for both animal an immunoreactive band at 170 kDa.

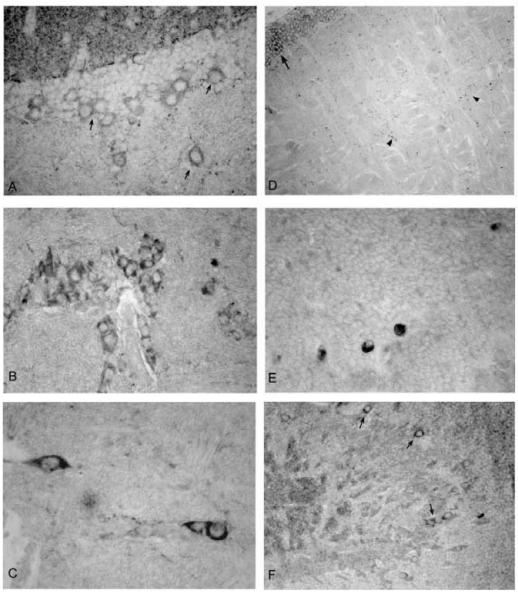

Figure 1. NMDAR 2A&B immunopositive staining in the vertical-superior frontal system of *Sepia officinalis* and *Octopus vulgaris*. A Immunopositive staining of cell bodies (arrows) and neuropil in the vertical lobe of *Sepia officinalis*. Note the intense immunostaining in the fibers. B Subvertical lobe of *Sepia officinalis*. Cluster of immunopositive neurons in the central region of the suvbertical lobe under the vertical lobe. C. Strong immunopositive neurons in the precommissural lobe of *Sepia officinalis*. D Median superior frontal lobe of *Octopus vulgaris*. Staining in small cell bodies (arrows) and scattered immunopositive staining in the neuropil (arrowheads). E Immunopositive neurons in the lateral superior frontal lobe of *Octopus vulgaris*. The immunopositive axons reach the neuropil of the lobe. F Vertical lobe of *Octopus vulgaris*. Positive staining of cell bodies (arrows) and in fibers running to the neuropil of the lobule. Note few immunopositive amacrine cells. Magnification: A, B, C, E x900; D, F x500.

This same antibody was then used for immunohistochemical staining of serial sections of the CNSs to reveal localized specific staining of cell bodies and fibers in several lobes of the brain. Staining was found in lower motor centers, mainly posterior pedal and palliovisceral lobes; in some higher motor centers (anterior basal and peduncle lobes); in learning centers (vertical lobe system (Fig.1) and, in *Octopus* inferior frontal system); and in the optic lobes. Immunopositivity was also found in the areas of brain which control the activity of the optic gland, a gonadotropic endocrine gland. These findings suggest that glutamate via NMDA receptors may be involved as a signalling molecule in motor, learning, visual and olfactory systems in the cephalopod brain.