P166

Interdependence between telencephalic nervous activity and respiratory activity in reptiles: a non-linear approach

Luis De Vera*, Ernesto Pereda† and Julián Jesús González*

**Laboratory of Biophysics, Department of Physiology, Faculty of Medicine, University of La Laguna, Canary Islands and †Department of Basic Physics, Faculty of Physics, University of La Laguna, Canary Islands, Spain*

Many physiological studies are interested in the complexity and non-linearity of the EEG and the respiratory activity (RA). Relationships between these signals in mammals, have been estimated using a non-linear approach (Burioka *et al.* 2001). Similar studies have been performed on the EEG of reptiles, in order to characterise its non-linear behaviour and to compare it with the human EEG during sleep (González *et al.* 1999). Nevertheless, no non-linear studies have been carried out in reptiles to describe the relationships between central nervous and respiratory activities. Therefore, cortical EEG and respiratory signals were simultaneously registered in six lizards (*Gallotia galloti*) at 25 °C, with the aim of studying the degree of interdependence between both physiological systems, by using a non-linear systems approach.

Table 1. Significance (σ_u) and nature (σ_b) of the interdependence between power signals

	Mean	−95 % CL	+95 % CL
σ_u: EEG-LF *vs.*RAS	4.114	3.821	4.407
σ_b: EEG-LF *vs.* RAS	0.903	0.771	1.034
σ_u: RAS *vs.* EEG-LF	4.412	4.096	4.729
σ_b: RAS *vs.* EEG-LF	−0.199	−0.290	−0.109

Number of recordings = 96; CL, confidence limit.

Table 2. *S* index between power signals

	Mean	S.D.
EEG-LF *vs.* RAS	0.187	0.026
RAS *vs.*EEG-LF	0.215***	0.050

Asterisks indicate asymmetry between signals (***$P < 0.001$). Number of recordings = 96.

Monopolar cortical EEG recordings were carried out by means of electrodes implanted on the right medial cortex. Simultaneously, the RA was recorded from the EMG of intercostal muscles. All surgical procedures were performed under ether anaesthesia. Every second, the EEG was digitised at 128 Hz and the EMG at 1 kHz, and the corresponding power spectra (PSD) were obtained via FFT, in order to calculate the power of the EEG low-frequency band (0.1–4 Hz), as well as the power of the main respiratory peak. This procedure was repeated every second during a 10 min period. In this way, two 600-data-point power signals (EEG-LF and RAS) were obtained. Continuous recordings from 10.00–14.00 h provided 24 consecutive power signals of these characteristics for each lizard.The state space of each power signal was reconstructed using time delay embedding ($\mu = 8$; $\tau = 3$). The interdependence was assessed by means of a non-linear index *S* (Arnhold *et al.* 1999). The eight nearest neighbours of each reference vector in the state space, and a Theiler window equal to 5 have been used to calculate *S*. The significance ($\sigma_u > 2$ indicates significance at 95 % confidence interval) and the nature ($\sigma_b < 2$ indicates linear nature) of the interdependence between the power signals, were assessed by means of a multivariate variant of the surrogate data test for non-linearity (Pereda *et al.* 2002). A paired *t* test was used to check the existence of asymmetry in the interdependence between the above mentioned power signals. Differences were considered significant if $P < 0.05$. The surrogate data test showed that the index S measured significant interdependence between EEG-LF and RAS, and that the interdependence was of linear nature (Table 1). There was asymmetry in the interdependence between EEG-LF and RAS (Table 2).

In conclusion, we have found that in reptiles the RA depends on the EEG-LF activity in a linear way. In humans during slow-wave sleep (SWS), the reduced EEG complexity is attributed to an increased regularity in breathing (Burioka *et al.* 2001). Our findings are in line with the hypothesis that reptilian waking could evolve into mammalian SWS.

Arnhold J *et al.* (1999). *Physica D* **134**, 419–432.

Burioka N *et al.* (2001). *Clin Neurophysiol* **112**, 1147–1153.

González J *et al.* (1999). *Am J Physiol* **277**, R86–93.

Pereda E *et al.* (2002). *IEEE Trans Biomed Eng* **49**, 548–555.

This work was supported by the grants BFI2002-01159 of the Spanish Ministerio de Ciencia y Tecnología, and PI020194 of F.I.S.

All procedures accord with current National and local guidelines.

P167

A telemetry system for ECG acquisition from diving mammals

M. Rodríguez *, A. Ayala *, M. Díaz † and J. García*

**Department of Fundamental and Experimental Physics, Electronic and Systems and †Department of Animal Biology, University of La Laguna, Tenerife, Spain*

One of the central physiological issues related to mammalian life in the aquatic environment is the development of cardiovascular adaptations during diving. Most of our knowledge on mammalian diving comes from studies performed in the laboratory. At present only a few reports have attempted to measure electrocardiograms (ECG) on freely diving sea mammals. The present work is focused on the design and implementation of a telemetry system prototype to allow the measurement and transmission of ECG from diving mammals, including humans. The system, also records relevant physical parameters from the diving environment, such as water temperature and hydrostatic pressure. These parameters are extremely useful for correlating ECG changes with diving and exercise activities. Measuring ECG signals on the body surface within a conductive media like seawater presents some intrinsic difficulties, such as attenuation of the signal amplitude, insulation of the associated electronics, etc. Therefore, as an initial step we needed to determine not only the levels of signal attenuation and the adequacy of different electrodes, but also the main features of real ECG from the different species. In order to obtain accurate determinations, we concentrated our studies on trained dolphins and human volunteers.

A portable acquisition system was designed consisting of a biopotential amplifier with adjustable gain and offset, A/D converter and a parallel port interface which let us to store and analyse, on a laptop computer, the ECG under real-time conditions by means of the implemented software. The system was fixed on the body surface and ECG measurement allowed us to obtain ECG waveforms, voltage levels and frequency bandwidth. These preliminary studies revealed differences between human and dolphins regarding wave amplitudes, heartbeat frequency and shape of the different ECG waves (Fig. 1) (Rodríguez *et al.* 2000).

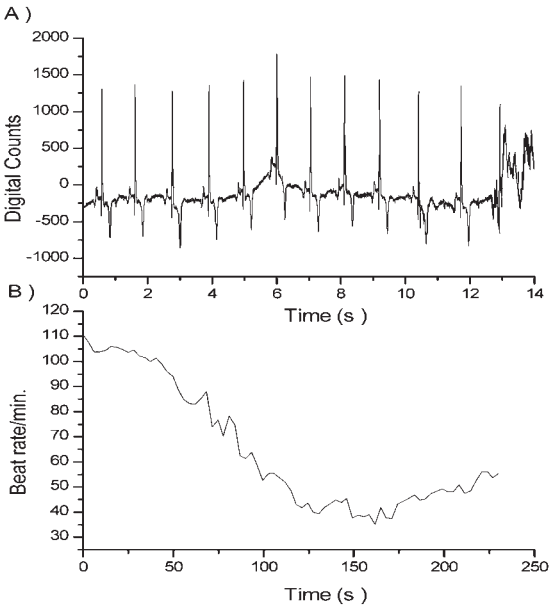

Figure 1. A, representative traces for a dolphin ECG. B, instantaneous heart frequency *vs.* immersion time for a human diver.

These preliminary data were used to develop the autonomous telemetry system. This system is based on a microcontroller that manages the measurement instrumentation, data compression/storage, and the radio frequency (RF) output stage. In addition, a compression algorithm, based on a Max-Lloyd non-uniform quantizer (Rodríguez *et al.* 2002), was implemented to optimize the limited resources of the system. ECG and physical data are stored in the system memory and transmitted to a receiving station via a UHF transmitter once the system detects the specimen has contacted the water surface.

Rodríguez *et al.* (2000). *Comp Meth Prog Biomed* **62**, 145–152.
Rodríguez *et al.* (2002). *Comp Meth Prog Biomed* (in the Press).

All procedures accord with current National and local guidelines.

P168

Kinetics of procalcitonin in neutropaenic rats after bacterial and viral fevers

M.L. Ojeda, R. Maldonado, E. Tavares, D. Chbihi, A. Fernández-Alonso and F.J. Miñano

Unidad de Investigación, División de Farmacología Experimental y Clínica, Hospital de Valme, Facultad de Medicina, Universidad de Sevilla, Ctra de Cádiz s/n, Sevilla, 41014, Spain

Procalcitonin (PCT) concentration increases in bacterial infections but remains low in viral infections. Studies to date have demonstrated that PCT is synthesized and release in response to appropiate stimuli in the face of immunosuppression and leukopaenia.

The purpose of this study was to investigate the dynamics of PCT and fever in a neutropaenic rat model of gram-negative (lipopolysaccharide, LPS) and virus (polyinosinic-polycytidylic acid, poly I:C) sepsis that mimics the series of pathophysiological events that may accompany the administration of cytoreductive chemotherapy in cancer patients.

Male Wistar rats were used (weighing 250–275 g; Charles River Laboratory, Barcelona, Spain). All of the experimental procedures were performed following guidelines from the European Union (86/609/EU) and Spanish regulations (BOE/67:8509/1988) for the use of laboratory animals in chronic experiments. Body temperature (T_b) (± 0.1 °C) was measured by biotelemetry using transmitters (Mini Mitter, Sunriver, OR, USA) implanted intraperitoneally (I.P.) at least 7 days before the onset of experimentation, the rats were anaesthetised with a mixture of ketamine and xylazine I.P. All drugs were injected I.P. (Miñano *et al.* 1996).

Neutropaenia (< 50 mm^3) was induced by injecting a first dose of cyclophosphamide (150 mg kg^{-1}) and a second one (50 mg kg^{-1}) at day 3. LPS (50 μg kg^{-1}), poly I:C (100 μg kg^{-1}) or an equivalent volume of pyrogen-free saline were administered 1 day after the second dose of cyclophosphamide (Bhattacharjee *et al.* 1994). Serum PCT levels were measured by immunoluminometric assay with a sensitivity of 0.1 ng ml^{-1}.

Intraperitoneal injection of either LPS or poly I:C produced fever of greater magnitude in neutropenic animals. The febrile response induced by LPS was significantly higher than that induced by poly I:C. Time–kinetics studies demonstrated that the production of PCT was markedly increased at 8 h after LPS challenge in non-immunosuppessed animals and at 8 h after poly I:C challenge in neutropenic rats.

These results confirm that PCT is a well-known tool for detection of bacterial gram-negative fevers in immunocompetent animals. However, in contrast to normal rats, PCT was significantly increased during fever induced by poly I:C in neutropaenic rats. Finally, present data suggest that different cellular and humoral mechanisms, which may have important therapeutic implications, are involved in the development of a febrile response triggered by a bacterial and viral infection in normal and neutropenic animals.

Bhattacharjee AK *et al.* (1994). *J Infect Dis* **170**, 622–629.
Miñano FJ *et al.* (1996). *Eur J Neurosci* **8**, 424–428.

All procedures accord with current National and local guidelines.

P169

Role of macrophage inflammatory protein-2 in a rat model of febrile neutropaenia

R. Maldonado, M.L. Ojeda, E. Tavares, D. Chbihi, A. Fernández-Alonso and F.J. Miñano

Unidad de Investigación, División de Farmacología Experimental y Clínica, Hospital de Valme, Facultad de Medicina, Universidad de sevilla, Ctra de Cádiz s/n, Sevilla, 41014, Spain

Gram-negative bacteriaemia is one of the most important causes of persistent fever and dose-limiting in the management of neutropaenic patients. The purpose of this study was to investigate and compare the role of macrophage inflammatory protein-2 (MIP-2), a powerful murine chemotactic factor for neutrophils, in the development of fever induced by bacterial endotoxin in both normal and neutropaenic animals. We show in a rat model of chemotherapy-induced neutropaenia that intraperitoneal injection of bacterial endotoxin (lipo-polysaccharide, LPS) produced a fever whose magnitude was equal to or greater than that observed in normal animals.

Neutropaenia was induced in male Wistar rats (250–275 g, CRIFFA, Barcelona) by two intraperitoneal injections of cyclophosphamide (CP) of 150 mg kg^{-1} on day −4 and

50 mg kg^{-1} on day −1 (before LPS treatment). In this model of neutropaenia, the absolute neutrophil counts are very low (< 50 mm^3) for 5–7 days. Also, the number of lymphocytes and monocytes are reduced, but not as strongly as the neutrophils (Bhattacharjee *et al.* 1994). In all experiments, the control groups received sterile saline as a placebo by the same route. Core body temperature (T_b) (± 0.1 °C) of each rat was measured by biotelemetry using precalibrated transmitters implanted intraperitoneally (Mini Mitter, Sunriver, OR), under aseptic conditions, at least 7 days before the onset of experimentation; the rats were anaesthetized with a mixture of ketamine and xylazine (Miñano *et al.* 1996). The kinetics of MIP-2 production were measured by specific ELISA (BioSource International, Camarillo, CA) in normal and CP-treated rats challenged with LPS.

For *in vivo* blocking experiments, a dose of 350 μg kg^{-1} neutralizing anti-MIP-2 (goat plyclonal antibody raised against rat MIP-2; Santa Cruz Biotechnology), was administered intraperitoneally, 15 min before LPS. Positive reference control animals received the same dose of an affinity-purified normal goat IgG (Santa Cruz Biotechnology). The present data show an increased MIP-2 production upon LPS challenge in normal and neutropenic rats. Although this enhanced production of MIP-2 is accompanied by an increased susceptibility of neutropenic rats to a febrile response with LPS, MIP-2 increase is responsible for the late phase of the febrile response induced by LPS in normal, but not in neutropenic, animals. By contrast, the specific neutralization of MIP-2 results in a partial reduction of the initial phase of LPS-induced fever in neutropenic rats but not in immunocompetent animals. Thus the present study indicates that blocking of MIP-2 bioactivity *in vivo* results in decreased endotoxin fever in normal but not neutropenic animals. These results are also the first demonstration that the chemokine MIP-2 is involved in the pathogenesis of a fever triggered by a gram-negative bacterial infection.

Bhattacharjee AK *et al.* (1994). *J Infect Dis* **170**, 622–629.
Miñano FJ *et al.* (1996). *Eur J Neurosci* **8**, 424–428.

All procedures accord with current National and local guidelines.

P170
The effects of acute stress on leukocyte activation

I. Montes*†, G.W. McLaren†, D. Macdonald† and R. Mian*

**School of Science and The Environment, Coventry University, Cox Street, Coventry CV1 5FB, UK and †Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

Acute stressors can activate circulating leukocytes and change their composition and number (Ellard *et al.* 2001; Mian *et al.* 2002). Here we report that transport stress causes leukocyte activation in wild badgers (*Meles meles*) that were trapped and transported as part of an ongoing long-term ecological study (Macdonald & Newman 2002). We also show that rest after transport results in a decrease in the number of activated circulating leukocytes.

The animals were caught during the night in cage traps baited with peanuts. Traps were checked at 06.30 h and trapped animals then experienced one of three handling regimes: anaesthetised (0.2 ml kg^{-1} of 100 mg ml^{-1} ketamine hydrochloride I.M.) at site of capture (T), $n = 5$; transported for less than 10 min to a field laboratory and anaesthetised (TR), $n = 9$; and as TR, but with a period of rest after transport of at least thirty min (TRR), $n = 8$. A sample of blood was taken from each animal (< 5ml), and all animals were then marked and examined as part of the ecological study, and released back into the wild after recovering from anaesthesia. Levels of leukocyte activation were determined using a nitroblue tetrazolium (NBT) staining test.

Nitroblue tetrazolium (Sigma 840-10) was diluted in phosphate buffered saline (PBS 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride pH 7.4) at a concentration of 1 mg of NBT in 1 ml of PBS buffer. This solution was then stored in the dark at 4°C when not directly in use. Immediately after the blood sample was collected from the animal a sample of 50 ml was transferred into an Eppendorf tube from the EDTA vial. This tube was incubated at 37°C for 45 min. After the incubation period, 50 ml of NBT was added to the tube, which was then incubated for a further 10 min at 37°C. After incubation, 15 ml was taken from the tube and smeared onto a microscope slide and left to dry.

The dry slides were flooded with 1 ml of Accustain Wright stain modified (0.3 % w/v buffered at pH 6.9 in methanol; batch no. 096 H4372) for 45 s. The slides where then washed with 1 ml of distilled water, allowed to stand for 1 min, washed with excess distilled water, and allowed to dry. Using the × 100 oil immersion objective, four areas of each slide were examined. The total number of activated and non-activated leukocytes were counted. Leukocytes were classified as active if they appeared irregular in shape and diffusely granular with intracytoplasmic formazan deposits. At least 100 neutrophils were counted in each slide; the count was repeated several times and the mean average taken. The number of NBT-positive cells from these counts is considered to be the percentage neutrophil activation.

Transport regime had a significant effect on leukocyte activation ($F_{2,19} = 12.4$, $P < 0.001$). Activation was highest immediately after transport when a mean (S.E.M.) of 62.6 % (2.6 %) of leukocytes were active. Non transported animals had significantly lower activation levels than transported (Tukey test, $P < 0.001$) and transported and rested (Tukey test, $P < 0.05$) animals. However, activation did not differ significantly between rested and non-transported animals (Tukey test, $P > 0.05$) where recorded mean (S.E.M.) activation levels were 51.8 % (2.4 %) and 42.4 % (3.6 %), respectively.

Thus leukocytes respond rapidly to transport stress by increasing their levels of activation, but this is a short-term response that is reversed by resting for 30 min. These results can help improve the welfare of wild animals handled for ecological and other research purposes.

Ellard DR *et al.* (2001). *Int J Psychophysiol* **41**, 93–100.
Macdonald DW & Newman C (2002). *J Zool* **256**, 121–138.
Mian R *et al.* (2002). *Stress* (in the Press).

All procedures accord with current UK legislation.

P171

Exhaustion stress and the circadian rhythms of melatonin, corticosterone and phagocytic activity

S.D. Paredes, S. Sánchez, A.B. Rodríguez, R.V. Rial* and C. Barriga

*Department of Physiology, Faculty of Science, University of Extremadura, 06071 Badajoz, Spain and *Laboratory of Physiology, Fundamental Biology and Health Science Department, University of Illes Balears, 07071 Palma de Mallorca, Spain*

Corticosterone and melatonin are true internal chemical pacemakers of different physiological processes, with both hormones having immunoregulatory effects and a circadian rhythm in most vertebrates (Rodríguez *et al.* 1999). Also, melatonin, due to its physiological antioxidant properties, acts as a buffer in stressful situations by quenching the adverse immunological effects of stress by way of its circadian release (Maestroni, 1993), whilst corticosterone is thought to be the main glucocorticoid secreted in response to stressful exercise (Simon, 1991). The present work was aimed at evaluating if swimming exercise-induced stress changes the circadian rhythms of melatonin, corticosterone, and phagocytosis in 3-month-old male Wistar rats.

Animals ($n = 6$) were maintained under a 12 h light and 12 h dark photoperiod and subjected every 2 h over one circadian period to a physical activity, which consisted of free swimming for 2 h and then rotating rod exercise until exhaustion, based on a modified version of the automated Porsolt test suggested by Nomura *et al.* (1982). Afterwards, they were killed by decapitation, and peritoneal cavity macrophage and plasma samples were taken for comparison with samples from control animals (the same conditions as the stressed individuals but in the absence of stressor). Radioimmunoassay was used to determine plasma levels of melatonin (IBL) and corticosterone (DRG Diagnostics). Prior to the melatonin assay, haemoglobin level of samples was measured to avoid any interference it might cause in the melatonin values. All experiments were carried out according to the guidelines of the European Community Council Directive 89/6091 EEC. Data are expressed as mean values ± S.D. and were compared by Student's unpaired t test. Values of $P \leqslant 0.05$ were considered significant.

The results showed that the circadian rhythm of the control animals for melatonin (peak at 02:00 h; 137 ± 14 pg ml^{-1}) and corticosterone (peak at 17:00 h; 285 ± 19 ng ml^{-1}) was lost by the stressed group, with the melatonin levels being lower (although the values were still higher at night than during the day) and the corticosterone levels much higher than those of the control group. Phagocytosis was determined as the latex-bead phagocytosis index (PI), i.e. the number of latex beads ingested by 100 macrophages, the phagocytosis percentage (PP), i.e. the percentage of cells that had phagocytosed at least one latex bead, and the phagocytosis efficiency (PE), i.e. the ratio PI:PP which indicates how effectively the phagocytes ingested the particles. The control animals' peritoneal macrophage PI also showed a circadian rhythm with maximum values at 03:00 h (684 ± 40 PI) because of a greater phagocytic efficiency of those cells. The stressed group displayed higher values than the controls at most hours of the night. These data confirm that melatonin and corticosterone act as modulators of the innate immune response.

Maestroni GJM (1993). *J Pineal Res* **14**, 1–10.

Nomura *et al.* (1982). *Eur J Pharmacol* **83**, 171–175.

Rodríguez AB *et al.* (1999). *J Pineal Res* **26**, 35–42.

Simon H, B (1991). In *Psychoneuroimmunology*, ed. Ader R *et al.*, pp. 869–895. Academic Press, New York.

The authors thank Elena Circujano Vadillo for technical assistance. The research was supported by the Consejería de Bienestar Social and the Consejería de Educación, Ciencia y Tecnología - Fondo Social Europeo (Junta de Extremadura, 2002).

All procedures accord with current National guidelines.

P172

Effect of tryptophan on circulating levels of melatonin and the innate immune response

S. Sánchez, S.D. Paredes, A.B. Rodríguez, M.I. Martín and C. Barriga

Department of Physiology, Faculty of Sciences, University of Extremadura, 06071 Badajoz, Spain

One of the important physiological functions of melatonin, the principal hormone secreted by the pineal gland, is its immunoregulatory action (Skwarlo-Sonta, 1996; Rodríguez *et al.* 1999). The hormone is synthesized during the period of darkness from tryptophan, an essential amino acid in the diet. We therefore studied whether the oral administration of tryptophan influences either circulating melatonin levels or the innate immune response by evaluating the phagocytic activity of macrophages.

To this end, we used 4-month old male Wistar rats subjected to a 12 h light–12 h dark cycle. The experimental group consisted of animals ($n = 6$) administered tryptophan (125 mg kg^{-1}) via an orogastric cannula half an hour before the start of the dark period (19.30 h). The control group consisted of animals under identical conditions but administered saline solution. Plasma was isolated from blood samples taken from the tail at 09.00, 21.00, and 02.00 h at the beginning of the treatment, half-way through (day 11), and at the end (day 21). On the last day, when the blood sample was taken, the animals were killed by decapitation, and macrophages were collected from the peritoneal cavity. All experiments were carried out according to the guidelines of the European Community Council Directive 86/6091 EEC. Data are expressed as mean values ± S.D. and were compared by Student's unpaired t test. Values of $P \leqslant 0.05$ were considered significant. Plasma melatonin levels were assayed by RIA (IBL) and the macrophage phagocytic activity was evaluated as the phagocytosis index (PI), i.e. the number of latex beads ingested per 100 macrophages, the phagocytosis percentage (PP), i.e. the percentage of cells that had phagocytosed at least one latex bead, and the phagocytosis efficiency (PE), i.e. the ratio PI:PP which indicates how effectively the phagocytes ingested the particles.

The results showed the phagocytic activity to be higher in the tryptophan group than in the controls, with maximum activity at 02.00 h (night) (PI tryptophan group 620 ± 51; PI control group 244 ± 26). The 02.00 h melatonin levels were higher in the tryptophan group both half-way through the treatment (100 pg ml^{-1}) and at the conclusion of the experiment (100 pg ml^{-1}) than in the control group (75 pg ml^{-1}). We may therefore conclude that tryptophan in the diet can raise nocturnal melatonin levels and enhance phagocytic activity during the period of darkness.

Rodríguez AB *et al.* (1999). *J Pineal Res* **26**, 35–42.

Skwarlo-Sonta K (1996). *Acta Neurobiol Exp* **56**, 341–357.

The authors thank Elena Circujano Vadillo for technical assistance. This research was supported by the Fundación Valhondo Calaff and the Consejería de Bienestar Social – Fondo Social Europeo (Junta de Extremadura, 2002).

All procedures accord with current National guidelines.

P173

Tryptophan and the innate immune response

J. Cubero, A.B. Rodríguez, J.M. Marchena, E. Ortega, J. Durán* and C. Barriga

*Department of Physiology, Faculty of Sciences, University of Extremadura, 06071 Badajoz, Spain and *Hospital Infanta Cristina-Materno Infantil, Badajoz, Spain*

The immune system function has a circadian rhythm. Exogenous administration of the amino acid tryptophan increases the release of the neurohormone melatonin (Hajak *et al.* 1991). This pineal hormone increases phagocytosis and decreases oxidative stress (Rodríguez *et al.* 2002). The purpose of the present work was to evaluate the ingestion capacity and oxidative metabolism in heterophils from ring dove (*Streptopelia roseogrisea*) after 7 days administration of 125 mg L-tryptophan (kg b.w.)$^{-1}$.

The birds ($n = 15$) were maintained under a 12 h light (3000 lux)–12 h dark cycle and handled according to the guidelines of European Community Council Directives 86/6091 EEC. Tryptophan was administrated at 19.00 h (1 h before darkness). Phagocytosis and superoxide anion levels were measured in heterophils (isolated by a density gradient with Ficoll) at 21.00 h (2 h after administration of the amino acid) and at 02.00 h, using MIF plates and the NBT reduction test respectively. All experiments were carried out according to the guidelines of the European Community Council Directive 89/6091 EEC. Data are expressed as mean values ± S.E.M. and were compared by Student's unpaired *t* test. Values of $P \leqslant 0.05$ were considered significant. Results are expressed as a phatocytosis index (PI, number of latex beads ingested by one hundred heterophils), phagocytosis percentage (PP, percentage of heterophils which have phagocytosed at least one latex bead), and phagocytic efficiency (PE, mean number of latex beads phagocytosed by each heterophil that has phagocytosed at least one latex bead). The NBT reduction test results are expressed as the percentage of the absorbency at 525 nm of the stimulated samples relative to the non-stimulated samples.

The results showed the PI after tryptophan administration to be higher ($P < 0.05$) at 02.00 h (79.38 ± 11.02 *vs.* 41.86 ± 10.65 control values), due to a greater phagocytic efficiency of the heterophils at that h (PE 2.52 ± 0.6 *vs.* 1.76 ± 0.18 control values). Superoxide anion levels were significantly lower in the tryptophan group ($P < 0.05$) at 02.00 h (59.24 ± 4.11) with respect to both the controls (100) and the values obtained at 09.00 h in the tryptophan group (107.77 ± 8.88).

In conclusion we suggest that administration of L-tryptophan increases the phagocytic capacity of heterophils and decreases superoxide anion levels at 02.00 h, at which hour there are elevated levels of melatonin.

Hajak G *et al.* (1991). *Pharmacopsychiat* **24**, 17–20.

Rodríguez AB *et al.* (2002). *Exp Gerontol* **37**, 421–426.

The authors thank Elena Circujano Vadillo for technical assistance and the Laboratories Ordesa S.L. for their collaboration. This research was supported by the Ministerio de Ciencia y Teconología AGL 2000-0182-P4-03.

All procedures accord with current National guidelines.

P174

Activity–inactivity cycles in ring dove (*Streptopelia roseogrisea*): effect of tryptophan

J. Cubero, V. Valero, C. Barriga, R. Rial*, M. Rivero† and A.B. Rodríguez

*Department of Physiology, Faculty of Science, University of Extremadura, 06071 Badajoz, Spain, *Animal Physiology Lab., Department FICS Biolgy, University of Illes Balears, 07071 Palma de Mallorca, Spain and †Laboratories Ordesa s.l. Barcelona, Spain*

Melatonin is the most important hormone regulating the rhythm of sleep–wake cycles; it has the essential amino acid tryptophan as precursor (Dajk & Lockley 2002). The aim of this study was to determine the possible correlation of tryptophan adminstration with circadian activity–inactivity rhythms as well as with body temperature and weight.

The birds, ring dove (*Streptopelia roseogrisea*; $n = 10$ per experimental group) with diurnal habit, were maintained under a 12 h (08.00–20.00 h) light (3000 lux) and 12 h dark cycle and handled according to the quidelines of European Community Council Directives 86/6091 EEC. Tryptophan was administered orally at doses of 125 mg or 300 mg L-tryptophan (kg b.w.)$^{-1}$ in single adminstration at 09.00 or 19.00 h. The anal temperature and the weignt of animals were measured at 10.00 h. The activity was recorded in pulses with one actometer per animal (two perpendicular infrared transmitters with a frequency of 4866 Hz). These activity pulses were logged every 5 min by a computer program (Das 16) throughout the experiment. The result are expressed as a comparison of the mean ± standard deviation (S.D.) activity between 10 animals admimistered tryptophan *versus* 10 control animals. Data were compared with Student's unpaired *t* test. Values of $P \leqslant 0.05$ were considered significant.

The result showed that the animals administered tryptophan at 19.00 h presented a decline in their activity during the period of light at both the 125 mg dose (20.01 ± 5.10 *vs.* 23.71 ± 6.18 control values) and the 300 mg dose (15.14 ± 1.62 *vs.* 20.37 ± 3.02). When the adminstration was at 09.00 h, the 300 mg dose group of animals presented a decline in their mesor (mean activity of the circadian rhythm; 8.37 ± 4.18 *vs.* 11.30 ± 6.64 control values). Tryptophan administration caused no significant changes in either the weight or the temperature relative to the controls. We can therefore conclude that the circadian activity–inactivity rhythms can be modified by tryptophan in the diet, and that this amino acid (the precursor of melatonin) could affect sleep by diminishing activity, if the administration is made at the precise moment.

Dijk DJ & Lockley SW (2002). *J Appl Physilol* **92**, 852–862.

The authors thank Elena Circujano for technical assistance. This research was supported by the Ministerio de Ciencia y Tecnología AGL 2000-0182-P4-03.

All procedures accord with current National guidelines.

P178

Modulation of intestinal Na^+–K^+-ATPase by polyunsaturated fatty acids in the fish *Sparus aurata*: effects on temperature dependence

E. Almansa, J.J. Sanchez, S. Cozzi, C. Rodríguez, R. Dópido and M. Díaz

Laboratory of Animal Physiology, Department of Animal Biology, University of La Laguna, 38206, S/C de Tenerife, Spain

The aim of the present study was to determine the thermodynamic properties of the Na^+–K^+-ATPase along the intestine of gilthead seabream (*Sparus aurata*) and to analyse the possible relationship between the fatty acid composition of the lipid microenvironment and the regional differences observed for the Na^+–K^+-ATPase activities. With this objective, the temperature dependence for the Na^+–K^+-ATPase has been examined in the proximal–distal axis of the intestine of gilthead seabream, i.e. pyloric caeca (PC), anterior intestine (AI) and posterior intestine (PI). The experiments were performed in accordance with the requirements of the European Convention for the Care and Use of Laboratory Animals.

Data derived from the Arrhenius plots showed differences in terms of temperature discontinuity points (T_d) (13.29, 16.39 and 17.48 °C for PC, AI and PI, respectively) and activation energy ratios (E_{a2}/E_{a1}) obtained at both sides of T_d (2.38, 1.98 and 1.78 for PC, AI and PI, respectively). The analyses of polar lipids showed differences in the levels of certain fatty acids among intestinal regions. The content of each fatty acid and different fatty acid ratios were correlated with the corresponding T_d and E_{a2}/E_{a1} values. Regression analyses revealed the existence of strong negative correlations between docosahexaenoic acid (22:6n-3, DHA) or the DHA/monoenes ratio and T_d. No obvious relationships were observed for other polyunsaturated fatty acids (PUFA) nor saturated fatty acids.

The results obtained in the present study indicate that the heterogeneous values of T_d displayed by the Na^+–K^+-ATPase along the intestinal tract could be related to a modulatory role of certain fatty acid within the lipid microenvironment of the enzyme.

All procedures accord with current National guidelines.

P179

Isolation and characterisation of enterocytes from the intestine of gilthead seabream (*Sparus aurata*): a comparative study of enzymatic activities

R. Dópido, C. Rodríguez, T. Gómez and M. Díaz

Department of Animal Biology, University of La Laguna, Tenerife, Spain

A protocol for the isolation and characterisation of highly pure viable enterocytes from *Sparus aurata* has been established. These enterocytes, are suitable for further physiological studies to elucidate the mechanisms of absorption and transport of nutrients, water and ions as well as the homeostasis and osmoregulation which take place in the intestinal epithelium. Considering that the physiology of the digestive tract varies along its proximal–distal axis, we have isolated cells from the three different intestinal regions, i.e. anterior intestine (AI), posterior intestine (PI) and the pyloric caeca (PC). Finally, it was intended to make a comparison between the activity of some digestive enzymes in both the isolated cells and the complete intestinal preparations, in order to establish the active role that isolated enterocytes play in these processes.

For cell isolation, the fish were killed in accordance with the requirements of the European Convention for the Care and Use of Laboratory Animals. The intestinal sections were incubated in a hyperosmolar, low sodium, high potassium containing (intracellular-like) solutions, to which EDTA and DTT (dithiothreitol) were added as disjunctive agents (Dópido *et al.* 2001). Results obtained from trypan blue exclusion, oxygen consumption, cellular ATP content and lactate dehydrogenase liberation were all traduced in a high viability rate of the isolated cells.

Morphological identification of isolated cells by light microscopy and image analysis, after differential staining showed that the high-yield epithelial cell preparations were all composed of more than 98 % enterocytes, which were slightly smaller in size in PI. The rest of cells were large mucous cells present in a lower proportions in the PI.

The digestive enzymatic activities measured in isolated enterocytes and complete intestinal preparations from the three intestinal regions were sucrase (S), maltase (M), phosphatase alkaline (PA), 5′-nucleotidase (5′-N), leucine aminopeptidase (LA) and γ-glutamyl transferase (γ-GT). The results showed higher enzymatic activities in enterocytes for S, M, 5′-N and LA than in whole intestinal homogenates. In addition, we observed a heterogeneous distribution of enzymatic activities along the digestive tract. The highest S, M and LA activities were observed in enterocytes isolated from AP, and the highest PA and 5′-N activities in enterocytes from AI.

This is the first report demonstrating the successful isolation of high-purity viable fish enterocytes from different intestinal regions. These cells provide an excellent experimental system for further metabolic and physiological intestinal processes at a cellular level.

Dópido *et al.* (2001). *Proc VII Cong Nac Acuac* **I**, V/22–V/25.

This work was supported by grant PI2001/059 from Gobierno Autónomo de Canarias.

All procedures accord with current National and local guidelines.

P180

A comparative study of lipid compositions from isolated enterocytes and subjacent tissues from the intestinal tract of gilthead seabream (*Sparus aurata*)

C. Rodríguez, R. Dópido, T. Gómez, E. Almansa and M. Díaz

Department of Animal Biology, University of La Laguna, Tenerife, Spain

In the present study, the lipid classes and the fatty acid compositions of the polar lipids from both isolated enterocytes and the underlying intestinal tissue (mainly smooth muscle) were analysed in gilthead seabream (*Sparus aurata*) following a procedure previously described (Dópido *et al.* 2001). The fish were killed in accordance with the requirements of the European Convention for the Care and Use of Laboratory Animals. Considering that the physiology of the digestive tract also varies along its proximal–distal axis, and in order to establish a possible relationship between the intestinal cellular functions and their lipid compositions, these analyses were performed on cells and tissues isolated from the three intestinal regions, i.e. pyloric caeca (PC), anterior intestine (AI) and posterior intestine (PI).

The smooth muscle from the three intestinal regions contained higher amounts of cholesterol and lower amounts of polar lipids (particularly phosphatidylcholine and phosphatidylinositol), than the corresponding enterocytes. Additionally, polar lipids from enterocytes were always richer in 22:6*n*−3 (DHA) and poorer in18:1*n*−9 (oleic acid) than the subjacent tissue, suggesting the relevant role for polar lipids and DHA in the enterocyte physiology.

When comparing the enterocyte lipid composition between the three intestinal sections, important differences were found adding more evidence to the well known proximal–distal differential physiology of the digestive tract. For instance, there was an increasing presence of free fatty acids in the enterocytes from the caeca to posterior intestine. However, phosphatidic acid as well as monoacylglycerides, which are both products of lipid digestion, followed the opposite trend. This seems to indicate that enterocytes from both AI and PC are important locations for lipid digestion and that enterocytes from AI and PI are involved in the absorption of these fatty acids.

In summary, a detailed analysis of lipids from isolated enterocytes and the subjacent intestinal tissues from the different intestinal segments reveals a high heterogeneity in both transverse and proximal–distal axis in terms of composition but also are indicative of a distribution of functions that clearly differ from the mammalian paradigm.

Dópido *et al.* (2001). *Proc VII Cong Nac Acuac* **I**, V/22–V/25.

This work was supported by grant PI2001/059 from Gobierno Autónomo de Canarias.

All procedures accord with current National and local guidelines.

P181

Increased mRNA expression of a potassium–chloride co-transporter in chronically epileptic rats

Begonia Morales-Aza, Gavin L. Woodhall, Roland S.G. Jones and Lucy F. Donaldson

Department of Physiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK

One factor which may determine the strength of GABAergic inhibition in cortical neurones is the expression of the electro-neutral neuronal-specific potassium–chloride co-transporter, KCC2 (Payne *et al.* 1996). KCC2 helps to maintain the transmembrane Cl^- concentration providing a driving force for $GABA_A$ receptor-mediated Cl^- currents, which are inward in adult cortical neurones. In states of altered neuronal excitability such as experimental epilepsy, numerous aspects of GABAergic function may be altered. In the present study we examined the hypothesis that altered KCC2 expression/function could contribute to epileptogenesis in specific neuronal areas.

An acute state of status epilepticus was induced in male Wistar rats by repeated (30 min intervals) low doses (5 mg kg^{-1}) of pilocarpine 24 h following administration of 320 meq kg^{-1} $LiSO_4$ (see Glien *et al.* 2001). Four to six weeks following this acute epileptic episode rats developed recurrent spontaneous seizures. Following a period of 4–6 weeks of chronic seizure activity the expression of KCC2 mRNA was determined using *in situ* hybridisation. Animals (2 epileptic, 2 age-matched controls) were anaesthetized with ketamine (120 mg kg^{-1} I.M.), decapitated and the brains removed. Brains were rapidly frozen, sectioned horizontally at 10 μm and subject to *in situ* hybridisation as previously described (Payne *et al.* 1996). mRNA levels were determined by silver grain counting of 12–15 fields from each brain area, in each of three sections from each animal. Regions studied were the superficial and deep layers of the lateral and medial entorhinal cortices (EC), hippocampal areas CA1, CA2, CA3 and the dentate gyrus.

KCC2 mRNA expression was high in both hippocampus and entorhinal cortex. There was no apparent change in expression in epileptic animals for lateral EC (control 27915 ± 4680 pixels/field (P/F), epileptic 33206 ± 6574 P/F; mean ± S.E.M.) or CA1–3 regions of the hippocampus (104–120 % control values). In contrast, KCC2 mRNA expression was slightly increased (control 28715 ± 4189 P/F *vs.* epileptic 36519 ± 5793 P/F; 127% control) in the deep layers of the medial EC and substantially increased in the superficial layers (control 29027 ± 4776 P/F *vs.* epileptic 49283 ± 8743 P/F; 170% control). In addition, KCC2 mRNA expression was also significantly increased in the dentate gyrus (control 86640 ± 11286 P/F *vs.* epileptic 122514 ± 11606 P/F; 141% control) of epileptic rats.

Assuming that KCC2 is localised to postsynaptic sites, these data could suggest that $GABA_A$-receptor mediated responses may be strengthened by an increased inward Cl^- gradient in the EC and dentate gyrus. The implications of this for epileptogenesis remain to be determined.

Glien M *et al.* (2001). *Epilepsy Res* **46**, 111–119.

Payne J *et al.* (1996). *J Biol Chem* **271**, 16245–16252.

All procedures accord with current UK legislation.