



## XIV European Biological Rhythms Society (EBRS) and IV World Congress of Chronobiology (WCC)

2 - 6 August 2015









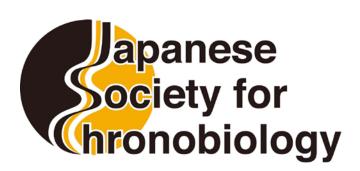
# **Programme**

Organised by the European Biological Rhythms Society in association with the Japanese Society for Chronobiology



**European Biological Rhythms Society** 

European Biological Rhythms Society



EBRS-WCC Congress 2015
Manchester, August 2<sup>nd</sup> to 6<sup>th</sup>, 2015





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# **Programme**

### Sunday August 2<sup>nd</sup> 2015

14:00–16:30 Registration + Posters Up

Room C16

**16:45–17:00** Opening Remarks
Hugh Piggins
Martin Humphries University of Manchester Vice-President
Till Roenneberg

17:00-18:00 PLENARY LECTURE:

 $\label{eq:michael} \mbox{MICHAEL H. HASTINGS (UK) "Cellular and Circuit-Level Circadian Pacemaking in the Suprachias matic Nucleus."}$ 

Introduced by: Charlotte Foerster (Germany)

Room C15

**18:00 – 20:00** Opening Reception





### Monday August 3<sup>rd</sup>, 2015

Room C16

08:30 - 09:20 PLENARY: THE GWINNER LECTURE

DAVID HAZLERIGG (Norway) "What do we Really Know About Circannual Clocks."

Introduced by: Paul Pevet (France)

Sponsored by: The Society for Endocrinology / Journal of Endocrinology

Room C16

#### 09:30-13:00 SYMPOSIUM 1: BEYOND MELANOPSIN

Chair: Tim Brown (UK)

**09:30–10:00** SAMER HATTAR (USA) "Retinal Photoreceptors' Contribution to Non-Image Forming Visual Function."

**10:00–10:30** STUART PEIRSON (UK) "The Effects of Light on Learning and Memory Depend Upon Both Melanopsin and Classical Photoreceptors."

**10:30–11:00** ETHAN BUHR (USA) "Photic entrainment of local circadian clocks in the mouse retina by non-canonical photoreceptors."

11:00-11:30 Coffee Break

Room C16

11:30-12:00 ROB LUCAS (UK) "Visual information reaching the SCN."

**Short Communications** 

**12:00–12:15** Birgitte Georg, Birgitte Falktoft, Jens Hannibal, Sarah B. Kristiansen, Thomas K. Klausen, Lauge Kellermann, and Jan Fahrenkrug (Denmark) "Phosphorylation of Rat Melanopsin at Ser-381 and Ser-398 by Light/Dark and its Importance for ipRGCs cellular Ca<sup>2+</sup> Signalling."

**12:15–12:30** Brock, O., Balint, K., Jager, P., Roska, B., and Delogu, A. (UK) "The Intergeniculate Leaflet: Development and Connectivity."

**12:30–12:45** Lydia Hanna, Michael Howarth, and Timothy M. Brown (UK) "A Novel in vitro Slice Preparation for Studying Geniculohypothalamic Signalling."

**12:45–13:00** Palus K., Chrobok L., and Lewandowski M.H. (Poland) "Orexins/Hypocretins modulate the activity of NPY-positive and -negative neurons in the rat intergeniculate leaflet via OX, and OX, receptors."

Room D7

#### 09:30-11:00 SYMPOSIUM 2: CLOCKS AND METABOLISM

Chair: David Bechtold (UK)

Sponsored by: The British Society for Neuroendocrinology

09:30-10:00 AK REDDY (UK) "Metabolic Oscillations in the Circadian Clockwork."

10:00–10:30 URS ALBRECHT (SWITZERLAND) "Liver Period 2 is Necessary to Regulate Food Anticipation."





10:30-11:00 JON JOHNSTON (UK) TBA

11:00-11:30 Coffee Break

**Short Communications** 

**11:30–11:45** Sangeeta Chawla and Emma Lord (UK) "Absence of effects of Sir2 knockdown on *Drosophila melanogaster* circadian rhythms."

**11:45–12:00** Priya Crosby and John O'Neill (UK) "Insulin resets the circadian clock in "non-metabolic" cells via induction of clock gene *PER2*."

**12:00–12:15** Gerben van Ooijen (UK) "Conserved circadian rhythms in intracellular [Mg²+] determine clock features and global energy metabolism."

**12:15–12:30** Yan Su, Ewout Foppen, Zhi Zhang, Eric Fliers and Andries Kalsbeek (Netherlands) "Effects of regular feeding and adrenalectomy on daily gene expression rhythms in rat white adipose tissue."

**12:30–12:45** Dirk J. Stenvers, Aldo Jongejan, Sadaf Atiqi, Jeroen P. Vreijling, Edward J. Bradley, Eelkje J. Limonard, Frank Baas, Perry D. Moerland, Eric Fliers, Andries Kalsbeek, Peter H. Bisschop (Netherlands) "RNA sequencing shows disturbed adipose tissue clock rhythms in patients with type 2 diabetes."

**12:45–13:00** Emma J. Wams, Igor Hoveijn, Heleen M. Rinsema, Laura van Rosmalen, Moniek Geerdink, and Roelof A. Hut (Netherlands) "Metabolism and its influence on human phase of entrainment and sleep coupling."

13:00-14:00 Lunch—Barnes Wallis Building

Room C16

#### 14:00-15:30 SYMPOSIUM 3: TEMPORAL NICHE

Chair: Etienne Challet (France)

**14:00–14:30** ETIENNE CHALLET (France) "Nocturnality versus diurnality: mechanisms within or downstream of the master clock?"

14:30–15:00 NICK FOULKES (Germany) "When to go fishing...?!"

**15:00–15:30** ROELOF HUT (Netherlands) "The Flexible Chronotype: Energy Balance and Predation Risk Determine the Circadian Phase of Entrainment."

15:30-16:00 Coffee Break

**16:00–16:30** LAURA SMALE (US) "Temporal niche transitions: From questions to data to speculation and back."

**Short Communications** 

**16:30–16:45** Eran Tauber and Mirko Pegoraro (UK) "New insights into the genetics of diurnal/nocturnal preference."

**16:45–17:00** Wataru Ota, Makiko Kashio, Makoto Tominaga, and Takashi Yoshimura (Japan) "Search for the thermosensors involved in temperature dependent negative masking behavior in mice."





**17:00–17:15** Pawan Kumar Jha, Hanan Bouaouda, Sylviane Gourmelen, Andries Kalsbeek, and Etienne Challet (France/Netherlands) "Sleep deprivation and caffeine treatment potentiate photic resetting of circadian clock in diurnal rodent, Sudanian grass rat (*Arvicanthis ansorgei*)."

**17:15–17:30** Patricia Tachinardi, Øivind Tøien, Veronica S. Valentinuzzi, C. Loren Buck, and Gisele A. Oda (Brazil) "Relationship between locomotor activity and body temperature timing and energetic challenges under energetic challenges: a lab and field approach in a subterranean rodent."

Room D7

14:00–15:30 SYMPOSIUM 4: MODELLING: FROM OSCILLATORS TO TOOLS FOR THE REAL WORLD Chairs: Casey Diekman (USA) and Daniel Forger (USA)

14:00-14:30 CASEY DIEKMAN (USA) "Modeling Circadian Rhythmicity of Cardiac Arrhythmias."

14:30-15:00 OLIVIA WALCH (USA) "A global assessment of sleep schedules using smartphone data."

**15:00–15:30** TILL ROENNEBERG (Germany) "Automatic Chronotyping."

15:30-16:00 Coffee Break

**Short Communications** 

**16:00–16:15** Hirokazu Fukuda and Kazuya Ukai (Japan) "Estimation of Cellular Phase Response Curve Through a Spatiotemporal Pattern in Plant Roots."

**16:15–16:30** Karolina Lech, Fan Liu, Katrin Ackermann, Victoria L. Revell, Debra J Skene, and Manfred Kayser (Netherlands) "Investigating rhythmicity of gene expression in human blood and application for estimating body and sampling time."

**16:30–16:45** Aurore Woller, Hélène Duez, Bart Staels, and Marc Lefranc (France) "Entrainment of the mammalian circadian clock by metabolism in peripheral organs: a quantitative mathematical model."

**16:45–17:00** Daniel Forger (USA) "Phase organization of clock neurons and its implications for mood disorders"

**17:00–17:15** Helga Schmidt (UK) "UCHRONIA: Practice-led research project at the intersection of design, chronobiology and chronosociology."

Room F14

#### 14:00-15:30 SYMPOSIUM 5: TIMEKEEPERS IN THE EYE

Chair: Mario Guido (Argentina)

14:00-14:30 MARIO GUIDO (Argentina) "Sensing Light and Time by Inner Retinal Cells of Birds."

14:30–15:00 GIANLUCA TOSINI (USA) "Circadian Organization of the Mammalian Eye."

15:00-15:30 ANNETTE ALLEN (UK) "Melanopsin Acts as the Retina's Light Meter."

15:30-16:00 Coffee Break





#### **Short Communications**

**16:00–16:15** Gema Esquiva Sobrino, Aaron Avivi, and Jens Hannibal (Spain/Israel/Denmark) "Non-image forming light detection by melanopsin, rhodopsin and L/M cone opsin in the eyes of the blind mole rat, the *Spalax Ehrenbergi*."

**16:15–16:30** Patrick Vancura, Tanja Wolloscheck, Kenkichi Baba, S. Anna Sargsyan, Gianluca Tosini, P. Michael Iuvone and Rainer Spessert (GERMANY/USA) "Circadian and dopaminergic control of  $Cpt-1\alpha$  expression in retina and photoreceptors."

**16:30–16:45** Patrycja Orlowska-Feuer, Hanna J. Szkudlarek, Annette E. Allen, Riccardo Storchi, Marian H. Lewandowski (POLAND/UK) "Photoreceptors modulation of infra-slow oscillatory activity in the rat olivary pretectal nucleus."

**16:45–17:00** Nippe, O M, Elliott, C J H, and Chawla, S (UK) "*Drosophila melanogaster* Clock Gene Mutants Exhibit a Circadian Rhythm in Visual Contrast Response."

**17:00–17:15** Jovi Chau-Yee Wong, Gareth Banks, Alun R. Barnard, Carina A. Pothecary, Aarti Jagannath, Steven Hughes, Elizabeth S. Maywood, Russell G. Foster, and Stuart N. Peirson (UK) "Role of Cryptochromes in Retinal Responses to Light."

Room C16

#### 17:30-18:30 PLENARY LECTURE:

JOHN HOGENESCH (USA) "An Atlas of Circadian Gene Expression: Implications for Biology and Medicine."

Introduced by: Till Roenneberg (Germany)

Room C15

19:00-20:30 Posters - Odd Numbers Presented





### Tuesday August 4th, 2015

Room C16

08:30-09:20 PLENARY: JSC LECTURE

TAKASHI YOSHIMURA (Japan) "Towards Understanding the Mechanism of Seasonal Time

Measurement."

Introduced by: Sato Honma (Japan)

Room C16

#### 09:30-11:00 SYMPOSIUM 6: JSC CLOCKS AND CIRCUITS

Chairs: Sato Honma (Japan) and Yoshitaka Fukada (Japan)

**09:30–10:00** CHARLOTTE FOERSTER (Germany) "The Peptidergic Clock Network in the Brain of *Drosophila melanogaster.*"

**10:00–10:30** YOSHITAKA FUKADA (Japan) "Canonical and noncanonical E-boxes Regulate Transcriptional and Post-Transcriptional Circuits."

**10:30–11:00** MICHIHIRO MIEDA (Japan) "Cellular clocks in AVP neurons of the SCN are critical for interneuronal coupling regulating circadian behavior rhythm."

11:00-11:30 Coffee Break

**11:30–12:00** HANS PETER HERZEL (Germany) "Synchronization and Entrainment – Lessons from Oscillator Theory."

**Short Communications** 

**12:00–12:15** Cannavo Rosamaria, Bieler Jonathan, and Felix Naef (Switzerland) "Cell cycle and circadian clock: reconstructing the dynamics of two coupled oscillators."

**12:15–12:30** Adam Bradlaugh, Maite Ogueta-Gutierrez, Edgar Buhl, James Hodge, and Ralf Stanewsky (UK) "Circadian clock resetting via non-canonical Rhodopsin signalling and the GPI-anchored protein Quasimodo."

12:30–12:45 Michaela Fredrich, Elmar Christ, and Horst-Werner Korf (Germany)

"Impact of melatonin on daytime-dependent changes in cell proliferation and apoptosis in the adult murine hypothalamic-hypophyseal system."

**12:45–13:00** Keisuke Ikegam, Xiao-Hui Liao, Yuta Hoshino, Hiroko Ono, Wataru Ota, Yuka Ito, Taeko Nishiwaki-Ohkawa, Chihiro Sato, Ken Kitajima, Masayuki Iigo, Yasufumi Shigeyoshi, Masanobu Yamada, Yoshiharu Murata, Samuel Refetoff, and Takashi Yoshimura (Japan) "Functional analysis of tissue-specific glycosylation of springtime hormone TSH."

Room D7

#### 09:30-11:00 SYMPOSIUM 7: INTERNAL SYNCHRONY

Chair: Henrik Oster (Germany) Sponsored by Bioscientifica

09:30-10:00 HENRIK OSTER (Germany) "Endocrine Regulation of Circadian Physiology."

**10:00–10:30** ANDRIES KALSBEEK (Netherlands) "How the SCN Synchronizes Daily Rhythms in Peripheral Tissues."





**10:30–11:00** ROBERT DALLMANN (UK) "Real-time bioluminescence reporters of circadian rhythms and signaling pathways in solid tumors in vitro and in vivo."

11:00-11:30 Coffee Break

**Short Communications** 

**11:30–11:45** Martina Pfeffer, Julian Lang, Claudia Fischer, Helmut Wicht, and Horst-Werner Korf (Germany) "Mice, melatonin, and the maintenance of diurnal rhythms."

**11:45–12:00** Yoko Komada, Norihisa Tamura, Hideki Tanaka, Yuichi Inoue (Japan) "Social jetlag affects subjective sleepiness among school-aged children in Japan."

**12:00–12:15** Alexander C West and David A Bechtold (UK) "Measuring the physiological cost of circadian desynchrony in mammals."

**12:15–12:30** Frederico Sander Mansur Machado, Nayara Abreu Coelho Horta, Thaís Santana Rocha Cardoso, Quezia Teixeira Rodrigues, Ana Maria de Lauro Castrucci, Maristela Oliveira Poletini, Candido Celso Coimbra (Brazil) "Entrainment of body temperature and locomotor activity daily cycles by acute regular exercise."

**12:30–12:45** Alun T. L. Hughes, Rayna E. Samuels, Mino Belle, Sven Wegner and Hugh D. Piggins (UK) "Scheduled locomotor exercise improves aberrant rhythms in neural and locomotor circadian function through alteration of GABAergic activity in the SCN."

13:00-14:00 Lunch Break-Barnes Wallis Building

Room C16

#### 14:00-17:30 SYMPOSIUM 8: CIRCUITS AND EXCITABILITY

Chair: Hugh Piggins (UK)

**14:00–14:30** JENNIFER EVANS (USA) "Plasticity in the role of GABA<sub>A</sub> signaling in coupling neuronal activity in the SCN network."

**14:30–15:00** MONIKA STENGL (Germany) "Pigment-dispersing factor-dependent signalling in the circadian clock of the Madeira cockroach *Rhyparobia maderae*."

**15:00–15:30** ENOKI RYOSUKE (Japan) "Multicolor imaging of circadian rhythms in the suprachiasmatic nucleus."

15:30-16:00 Coffee Break

Short Communications

**16:00–16:15** Christine Muheim, Tina Sartorius, Robert Dallmann, Ning Gu, Johan F. Storm, Roland Dürr, Reto Huber, Peter Ruth, and Steven A. Brown (Switzerland/Norway/Germany) "A screen for potassium channels contributing to local sleep."

**16:15–16:30** Edgar Buhl, Ralf Stanewsky and James JL Hodge (UK) "Electrophysiological and optogenetic characterisation of daily and acute light effects on *Drosophila* circadian clock neurons."

**16:30–16:45** MJ Parsons, M Brancaccio, S Sethi, ES Maywood, Aarti Jagannath, Yvonne Couch, Mattéa J Finelli, NJ Smyllie, Peter L Oliver, JE Chesham, M Simon, MH Hastings, PM Nolan (UK) "Short-circuit: Characterization of a transcription factor that activates a novel circadian transcriptional axis."





**16:45–17:00** Naoto Hyasaka (Japan) "A new understanding of the mammalian circadian clock as neuroglial networks."

**17:00–17:15** Mino D. C. Belle, Beatriz Baño Otalora, and Hugh D. Piggins (UK) "SCN neurons of *cryptochrome*-deficient mice lack circadian timing in intrinsic excitability states and gating response to excitatory input."

**17:15–17:30** Jessica Rodgers, Carina Pothecary, Steven Hughes, Doron Hickey, Laurence Brown, Michelle McClements, Russell Foster, Stuart Peirson and Mark Hankins (UK) "Functional assessment of human melanopsin variants, P10L and T394I, using AAV delivery *in vivo*."

Room D7

#### 14:00-17:30 SYMPOSIUM 9: CLOCKS IN THE WILD

Chairs: Till Roenneberg (Germany) and Rosa Levandovksi (Brazil)

14:00–14:30 GISELE A. ODA (Brazil) "Skeleton Photoperiods in the Wild for Subterranean Rodents"

**14:30–15:00** LUISA PILZ (Brazil/Germany) "Enlightening the Effects of Artificial Light on Biological Rhythms".

**15:00–15:30** MARIA JULIANA LEONE (Argentina) "Time to Decide, Time to Learn, Time to Sleep: Assessing Chronobiology with Data from the Real World."

15:30-16:00 Coffee Break

**Short Communications** 

**16:00–16:15** Abhishek Prayag, Sophie Jost, Pascale Avouac, Howard Cooper, Dominique Dumortier, and Claude Gronfier (France) "Characterization of Non-Visual Photoreception in Humans."

16:15–16:30 Cátia Reis and Teresa Paiva (Portugal) "DLMO in Evening Types and Sleep Phase Delay."

**16:30–16:45** Gayline Manalang Jr., Marilou Nicolas, Martha Merrow, and Till Roenneberg (Philippines/Germany) "Chronotype and Social Jetlag among Filipinos –an Analysis of Data from the Philippine Chronobiology and Social Jetlag Survey."

**16:45–17:00** Daniela Bellicoso and Martin R. Ralph (Canada) "A Preliminary Human Factors Analysis of Chronotype."

**17:00–17:15** Cheryl Isherwood, Jonathan D. Johnston and Debra J. Skene (UK) "Human Metabolite Rhythms: Effects of Obesity and Type 2 Diabetes."

Room C16

#### 17:30-18:20 PLENARY LECTURE:

STEVE KAY (USA) "Time for Change: Chemical Biology Approaches to Circadian Therapeutics." Introduced by: Elizabeth Maywood (UK)

Room C15

19:00-20:30 Posters - Even Numbers Presented

20:30-22:00 EBRS Board Meeting





### Wednesday August 5th, 2015

Room C16

08:30-09:20 PLENARY: AXELROD LECTURE

HELENA ILLNEROVÁ (Czech Republic) "From the Pineal Gland to the SCN and Back Again." Introduced by: Alena Sumova (Czech Republic).

Room C16

#### 09:30-13:00 SYMPOSIUM 10: CLOCKS AND IMMUNITY

Chairs: Nico Cermakian (Canada) and Andrew Loudon (UK)

**09:30–10:00** NICO CERMAKIAN (Canada) "Circadian control of the adaptive immune response and cancer cell proliferation."

10:00–10:30 ANDREW LOUDON (UK) "Immunity and the circadian clock."

**10:30–11:00** ANNIE CURTIS (Ireland) "Circadian control of innate immunity in macrophages by miR-155 targeting *Bmal1*."

11:00-11:30 Coffee Break

**Short Communications** 

**11:30–11:45** Michal Dudek, Baoqiang Guo, Nan Yang, Elzbieta Borysiewicz, Jack L. Williams, Michael R. H. White, Elizabeth S. Maywood, Michael H. Hastings, Ray P. Boot-Handford, and Qing-Jun Meng (UK) "Catabolic cytokines disrupt the circadian clock in cartilage and intervertebral disc via an NFκB-dependent pathway."

**11:45–12:00** Hopwood TW, Ray, DW, Loudon, ASI, Bechtold DA, and Gibbs JE (UK) "A role for CRYPTOCHROME in the regulation of inflammatory arthritis"

**12:00–12:15** Ned Hoyle, Estere Seinkmane, and John O'Neill (UK) "Circadian Regulation of Wound Healing and Actin Dynamics."

**12:15–12:30** Elham Farshadi, Jie Yan, Pierre Leclere, Albert Goldbeter, Ines Chaves, Gijsbertus van der Horst (Netherlands/Belgium) "Impact of Bmal1 knockdown on cell cycle progression of NIH3T3 cells."

**12:30–12:45** Marie Pariollaud, Julie Gibbs, Baoqiang Guo, Nicholas Tomkinson, Dion Daniels, Danuta Mossakowska, Stuart Farrow, Yolanda Sanchez, Andrew Loudon and David Ray (UK) "A novel mechanism links inflammation to the clock through REV-ERBα protein stability."

Room F14

#### 09:30-13:00 SYMPOSIUM 11: RHYTHM ONTOGENY AND ENVIRONMENTAL INFLUENCES

Chair: Maria Canal (UK)

Sponsored by: The Company of Biologists

**09:30–10:00** MARIA CANAL (UK) "Programming of Mice Circadian and Stress Systems by Postnatal Light Environment."





10:00-10:30 ALENA SUMOVA (Czech Republic) "Maternal Entrainment of the Developing Clocks."

10:30-11:00 DOUGLAS MCMAHON (USA) "Photoperiodic Programing of Neural Function: A SAD story."

11:00-11:30 Coffee Break

Short Communications

**11:30–11:45** Outa Uryu, Ryusuke Niwa (Japan) "The *lola* and *Pdk1* are Essential for Development of the sLNvs in the Fruit Fly *Drosophila melanogaster.*"

**11:45–12:00** Cristina Sáenz de Miera, Béatrice Bothorel, Michael Birnie, Valérie Simonneaux, and David Hazlerigg (France/Norway) "Maternal Photoperiodic Programming of the Hypothalamic Control of Seasonal Reproduction in the Siberian Hamster."

**12:00–12:15** Shona H. Wood, Helen Christian, Katarzyna Miedzinska, Ben R.C. Saer, Mark Johnson, Bob Paton, Le Yu, Judith McNeilly, Julian R.E. Davis, Alan S. McNeilly, David D. Burt, and Andrew S.I. Loudon (UK) "Mechanisms Driving Circannual Rhythms in Mammals."

**12:15–12:30** Federico Tinarelli, Elena Ivanova, Ilaria Colombi, Erica barini, Laura Gasparini, Michela Chiappalone, Gavin Kelsey, and Valter Tucci (Italy) "After-Hours mice show Specific Epigenetic and Physiological Alterations."

Room D7

#### 09:30-13:00 SYMPOSIUM 12: COMPARATIVE CLOCKS

Chair: Vinod Kumar (India)

**09:30–10:00** VINOD KUMAR (India) "Timekeeping in birds: Insights into molecular regulation of photoperiodic timing of seasonal physiology in migratory songbirds."

**10:00–10:30** JOHN O'NEILL (UK) "Metabolic Oscillations in Yeast and Circatidal Cycles in *Eurydice pulchra* Share Features Conserved among Circadian Rhythms."

**10:30–11:00** BAMBOS KYRIACOU (UK) "Molecular control og biological rythms in an intertidal invertebrate, *Eurydice pulchra*."

11:00-11:30 Coffee Break

**Short Communications** 

**11:30–11:45** Devraj Singh, Sangeeta Rani, Amit Kumar Trivedi, Satchidananda Panda and Vinod Kumar (India/USA) "Annual life-history dependent circadian timing in central and peripheral tissues in a migratory blackheaded bunting (*Emberiza melanocephala*)."

**11:45–12:00** Jacob G. Holland, Moshe Nagari and Guy Bloch (Israel) "Socially-mediated circadian plasticity in the social bumblebee *Bombus terrestris.*"

**12:00–12:15** Jo B Henningsen, Vincent-Joseph Poirel, Jens D Mikkelsen, Francois Gauerand, and Valerie Simonneaux (*France/Denmark*) "The role of RFRP in seasonal reproduction: sex- and photoperiod-dependent variations."





**12:15–12:30** Magdalena Markowska, Bogdan Lewczuk, Monika Malz, Pawel M. Majewski, and Iwona Adamska (Poland) "Diurnal Profile of Catecholamines, Neuropeptides and Their Receptors in the Chicken Pineal Gland."

13:00-15:30 Free Time

Room C16

#### 15:30-16:45 SYMPOSIUM 13: HUMAN CLOCKS, SLEEP, AND METABOLISM

Chair: Andries Kalsbeek (Netherlands)

**15:30–16:00** FRANK SCHEER (USA) "Bittersweet: The Impact of the Circadian System and its Disturbance on Glucose Control and Metabolism in Humans."

16:00–16:30 DEBRA SKENE (UK) "Metabolic Profiling in Human Plasma: Diurnal and Circadian Variation."

**Short Communication** 

**16:30–16:45** Annelies Brouwer, Corina de Gier, Daniël H. van Raalte, Richard G. IJzerman, Frank J. Snoek, Aartjan T. F. Beekman, Marijke A. Bremmer (Netherlands) "Cardiometabolic Correlates of Specific Insomnia Symptoms in Type 2 Diabetes Patients."

Room C16

17:-00-18:00 EBRS AGM

**18:00–18:50** PLENARY: ARIENS KAPPERS LECTURE JOHANNA H MEIJER (Netherlands) "**An ancient clock in modern society.**" *Introduced by: Andries Kalsbeek (Netherlands)* 

**19:15–22:00** Banquet The Palace Hotel Kappers Medal Presentation





### Thursday August 6th, 2015

Room C16

08:30 - 09:20 PLENARY LECTURE:

UELI SCHIBLER (Switzerland) "The synchronization of peripheral oscillators in mammals." Introduced by: Andrew Loudon (UK)

Room C16

09:30-13:00 SYMPOSIUM 14: JSC PHYSICAL AND CHEMICAL BASES FOR CIRCADIAN PERIOD AND TEMPERATURE COMPENSATION.

Chair: Takao Kondo (Japan)

09:30-10:00 MICHAEL RUST (USA) "The Cyanobacterial Clock Functions as a Cellular Energy Sensor."

10:00-10:30 S AKIYAMA (Japan) "KaiC as Circadian Pacemaker of Cyanobacterial Circadian Clock."

**10:30–11:00** KOI ODE (Japan) "Mammalian cryptochrome 1 regulates circadian period through its co-factor pocket."

11:00-11:30 Coffee Break

**11:30–12:00** MARTHA MERROW (Germany) "Integration of the temperature environment by the developmental clock in *C. elegans.*"

**Short Communications** 

**12:00–12:15** Akanksha Bafna, Tadahiro Goda, Brandi Sharp, and Herman Wijnen (UK/USA) "Cold-induced transcription of the *period* gene is associated with temperature-dependent resetting of daily rhythms in *Drosophila.*"

**12:15–12:30** Jevons L.A., Bellantuono I., Hunt J.A., Pekovic-Vaughan V. (UK) "Investigating the potential of mechanical cues in resetting cellular circadian clocks in adult stem cells."

**12:30–12:45** Baidanoff Fernando Martin, Plano Santiago Andrés, Doctorovich Fabio, Suárez Sebastián Angel, Golombek Diego Andrés, and Chiesa Juan José. (Argentina) "Nitrergic neural communication for the synchronization of the mammalian circadian clock: a putative redox-regulation."

**12:45–13:00** Allan B. James, John, W.S. Brown, and Hugh G. Nimm (UK) "An hnRNP isoform switch links temperature perception to regulation of the *Arabidopsis* circadian clock."

Room D7

#### 09:30-13:00 SYMPOSIUM 15: EBRS/ESRS JOINT SYMPOSIUM

Chair: Debra Skene (UK)

**09:30–10:00** VLADYSLAV VYAZOVSKIY (UK) "The temporal dynamics of waking and sleep: from single neurons to behaviour." (ESRS)

**10:00–10:30** GILLES VANDEWALLE (Belgium) "Human cortical excitability and excitation/inhibition balance is set by the circadian timing system." (ESRS)

**10:30–11:00** WILLIAM WISDEN (UK) "Capturing the neuronal ensembles underlying sleep - local mechanisms of circadian and homeostatic control." (EBRS)

11:00-11:30 Coffee Break





**11:30–12:00** STEVE BROWN (Switzerland) "Genetic and epigenetic mechanisms affecting the timing of sleep." (EBRS)

Short Communications

**12:00–12:15** Ruth I. Versteeg, Dirk J. Stenvers, Eric Fliers, Mireille J. Serlie, Andries Kalsbeek, Susanne E. la Fleur, and Peter H. Bisschop (Netherlands) "Effect of ambient light intensity on glucose and lipid metabolism and appetite in humans."

**12:15–12:30** Satish K. Sen, Hélène Raingard, Stéphanie Dumont, Andries Kalsbeek, Patrick Vuillez, and Etienne Challet (France/Netherlands) "Ultradian feeding coupled with caloric restriction affects both the peripheral and central clocks in mice."

**12:30–12:45** Türkan Yurtsever, Thomas M. Schilling, Monika Kölsch, Jonathan Turner, Jobst Meyer, Hartmut Schächinger, and Andrea B Schote (Germany/Luxembourg) "The stress hormone hydrocortisone affects the expression of *Period* genes in healthy subjects."

**12:45–13:00** Victoria L. Revell, Guro Giskeødegård, Sarah K. Davies, Hector Keun and Debra J. Skene (UK) "Diurnal rhythms in the human urine metabolome during sleep and total sleep deprivation."

**13:00–13:15** Jagannath A, Sanghani H, Stephaniak J, Pilorz V, Brown L, Galione A, Churchill G, Peirson S, Vasudevan SR and Foster RG (UK) "Adenosine Receptors as Chronomodulatory Targets."

13:15-14:00 Lunch

14:00-14:50 PLENARY: STOCKGRAND LECTURE

CHRISTIAN CAJOCHEN (Switzerland): TBA

Introduced by: Jo Arendt (UK)

Room C16

## 15:00–17:30 SYMPOSIUM 16: CLOCKS, NEUROLOGY, AND PSYCHIATRY: ANIMAL MODELS AND THE REAL WORLD

Chair: Andrew N. Coogan (Ireland)

**15:00–15:15** ANDREW N. COOGAN (Ireland) "Attention Deficit Hyperactivity Disorder: Time to Pay Attention to the Clock?"

**15:15–15:40** Gi HOON SON (South Korea) "Circadian Timing System and Mood Regulation through REV-ERBα."

**15:40–16:05** KATE PORCHERET (Oxford) "Is sleep always the best medicine? Investigating the effect of sleep deprivation following an analogue traumatic event."

**16:05–16:30** DOMINIC LANDGRAF (USA) "Circadian Rhythms in Animal Models of Depression and Mania."

16:30-17:00 Coffee Break

**Short Communications** 

**17:00–17:15** Pan Wang, Yafeng Zhan, Bo Zhou, Hongxiang Yao, Yan'e Guo, Yong Liu, Xi Zhang (China) "The influence of sleep quality on intra- and inter-network connectivity in Alzheimer's disease."





**17:15–17:30** Puja K. Parekh, Angela R. Ozburn, Edgardo Falcon, Michelle M. Sidor, Sade M. Spencer, Yanhua Huan, and Colleen A. McClung (USA) "Differential roles of CLOCK and NPAS2 in synaptic plasticity and reward-related behaviour."

Room D7

#### 15:00-17:30 SYMPOSIUM 17: AROUSAL AND SLEEP

Chair: Robert Dallmann (UK)

**15:00–15:30** AMITA SEHGAL (USA) "Oxalic Acid & Diacylglycerol 36:3 are Cross-Species Markers of Sleep Debt."

15:30-16:00 ELIZABETH MAYWOOD (UK) "Circadian synchrony and sleep."

**16:00–16:30** ROLAND BRANDSTAETTER (UK) "The impact of circadian phenotype and circadian entrainment on diurnal performance in athletes."

16:30-17:00 Coffee Break

Short Communications

**17:00–17:15** Yuya Nishimoto, Shunsuke Nagashima, Eiko Masutani, Naoko Komenam, Tomoko Wakamura (Japan) "The Relationship Between Sleep Disturbances and Constipation Among Female University Students in Japan."

**17:15–17:30** Maria Panagiotou, Johanna H Meijer, Tom Deboer (Netherlands) "The effect of aging on sleep and wake episodes in mice."

Room C16

17:30-18:30 Awards and Closing Remarks





# **Posters**

Please put all posters up Sunday Night/Monday Morning.

Odd Numbered Posters to be attended Monday and Tuesday am.

Even Numbered Posters to be attended Tuesday pm and Wednesday am.

- P1. RELATIONSHIP BETWEEN CIRCADIAN TYPOLOGY / SLEEP HABITS AND THE USAGES OF PERSONAL COMPUTER AND CONVENIENCE STORE OPENED 24 HOURS IN JAPANESE STUDENTS AGED 18-40 YRS
  - Tetsuo Harada, Yumiko Yamazaki, Fujiko Tsuji, Miyo Nakade, Milada Krejci, Takahiro Kawada, Teruki Noji, Hitomi Takeuchi
- P2. THE SCN RESPONSE TO SPATIAL PATTERNS IN THE VISUAL SCENE Josh Mouland, Tim Brown, Rob Lucas
- P3. NITRERGIC NEURAL COMMUNICATION FOR THE SYNCHRONIZATION OF THE MAMMALIAN CIRCADIAN CLOCK: A PUTATIVE REDOX-REGULATION
  Baidanoff Fernando Martin1, Plano Santiago Andrés1, Doctorovich Fabio2, Suárez Sebastián Angel2, Golombek Diego Andrés.1, Chiesa Juan José.1
- P4. HIGH AMPLITUDE CIRCADIAN RHYTHM GENERATED BY THE SUPRACHIASMATIC NUCLEUS OF CLOCK MUTANT MICE Yasufumi Shigeyoshi, Mitsugu Sujino
- P5. LIVER PERIOD 2 IS NECESSARY TO REGULATE FOOD ANTICIPATION Rohit Chavan, Céline Feillet, Sara Fonseca, James E. Delorme, Takashi Okabe, Jürgen A. Ripperger, Urs Albrecht
- P6. INVESTIGATING THE POTENTIAL OF MECHANICAL CUES IN RESETTING CELLULAR CIRCADIAN CLOCKS IN ADULT STEM CELLS Jevons L.A., Bellantuono I., Hunt J.A., Pekovic-Vaughan V.
- P7. JUST BREATHE: CHRONOPHARMACOLOGY FROM MOUSE BREATH Robert Dallmann, Renato Zenobi, Steven A. Brown, Pablo Martinez-Lozano Sinues
- P8. MEASURING RHYTHMS OF TEMPERATURE, ACTIVITY AND SLEEP AS WELFARE MARKERS IN MICE.
  - Laurence A. Brown, Lindsay Benson, Sibah Hasan, Mathilde Guillaumin, Russell G. Foster and Stuart N. Peirson
- P9. CHARACTERISTICS OF THE DORSAL LATERAL GENICULATE NUCLEUS NEURONAL RESPONSES TO LIGHT
  Jeczmien Jagoda, Orlowska-Feuer Patrycja, Lewandowski Marian Henryk

- P10. ACTIVATION OF THE DORSAL LATERAL GENICULATE NUCLEUS (DLGN) NEURONS BY OREXIN A - INVIVO STUDY ON URETHANE-ANESTHETISED RATS Patrycja Orlowska-Feuer, Katarzyna Dyl, Marian Henryk Lewandowski
- P11. DIURNAL PROFILE OF INTERLEUKINS, THEIR RECEPTORS AND TOLL-LIKE RECEPTORS IN THE CHICKEN PINEAL GLAND

M. Markowska, M. Twardowska, W. Stadejek, P.M. Majewski, I. Adamska

- P12. CIRCADIAN CLOCK REGULATION OF SEROTONIN LEVELS IN HAEMOLYMPH OF THE FRESHWATER MUSSELS UNIO TUMIDUS IN SUMMER Aleksandra Skawina<sup>1</sup>, Piotr Bernatowicz<sup>2</sup>, Magdalena Markowska<sup>1</sup>, Piotr Bebas<sup>1</sup>
- P13. ROLE OF REV-ERBA IN THE PROGRAMMING OF LIPOGENIC DRIVE IN MICE Siobhan A. Ahern, Peter S. Cunningham, Laura C. Smith, David A. Bechtold
- P14. TARGETING OF THE CIRCADIAN CLOCK IN OBESITY TO IMPROVE GLUCOSE HOMEOSTASIS Peter S. Cunningham, Siobhán A. Ahern, Laura C. Smith, Travis T. Wager, David A. Bechtold
- P15. REDOX REGULATION OF MOLECULAR CLOCKS IN SKELETAL MUSCLE ADAPTIVE RESPONSES Horton N., Copple I., Park K., McArdle A., Jackson M. and Pekovic-Vaughan V.
- P16. TIME-FIXED FEEDING PREVENTS BODY WEIGHT GAIN INDUCED BY CHRONIC JET LAG IN MICE Hideaki Oike, Katsunari Ippoushi, Masuko Kobori
- P17. INVERSE EFFECT OF ACUTE BLUE AND GREEN LIGHT EXPOSURE ON ANXIETY, ADRENAL RESPONSES AND CLOCK GENE EXPRESSION Violetta Pilorz, Eric S.K. Tam, Carina A. Pothecary, Russell G. Foster, Stuart N. Peirson
- P18. EXTRACELLULAR MATRIX MECHANICS CONTROL THE ROBUSTNESS OF THE CIRCADIAN **CLOCK IN MAMMARY EPITHELIA** Nan Yang, Vanja Pekovic-Vaughan, Jack Williams, Nicole Gossan, Alun Hughes, Julia Cheung, Pengbo Wang, Safia Olabi, Charles H Streuli, Qing-Jun Meng
- P19. SEX DIFFERENCES IN THE CIRCADIAN PRODUCTION OF MELATONIN AND CORTISOL IN PLASMA AND URINE MATRICES Victoria L. Revell, Pippa J. Gunn, Benita Middleton, Sarah K. Davies, Debra J. Skene
- P20. USING OSCILLATING LIGHT TO STUDY THE CONE CONTRIBUTIONS TO NIF EFFECTS IN **HUMANS**

Tom Woelders, Emma Wams, Karl van Stiphout, Jacqueline Libert, Domien Beersma, Marijke Gordijn

- P21. RHO1-SIGNALLING IN DROSOPHILA MELANOGASTER S-LNV PACEMAKER NEURONS REGULATES CIRCADIAN BEHAVIOUR Miguel Ramírez, Neethi Rao, Herman Wijnen
- P22. EFFECTS OF LIGHT EXPOSURE DURING DAYTIME ON CLOCK GENE EXPRESSION IN HAIR FOLLICULAR AND ROOT CELLS IN HUMANS Maki Sato, Tomoko Wakamura, Takeshi Morita, Akihiko Okamoto, Makoto Akashi, Takuya Matsui, Motohiko Sato
- P23. CONTRAST AND IRRADIANCE RESPONSES IN THE MOUSE SCN Dobb, R.C., Brown, T.M., Lucas, R.J.





- P24. FOOD-ENTRAINABLE CIRCADIAN RHYTHM AND PATHOPHYSIOLOGY IN CYS414-ALA MCRY1 TRANSGENIC MICE
  - Satoshi Okano, Akira Yasui, Kiyoshi Hayasaka, Masahiko Igarashi, Osamu Nakajima
- P25. IMPACT OF SLEEP TIMING AND SCHOOL SCHEDULES ON SCHOOL PERFORMANCE Giulia Zerbini, Vincent van der Vinne, Anne Siersema, Amy Pieper, Thomas Kantermann, Roelof A. Hut, Till Roenneberg, Martha Merrow
- P26. ENTRAINMENT OF THE HUMAN CIRCADIAN TIMING SYSTEM BY MEAL TIMING Skevoulla Christou, Sophie M.T. Wehrens, Cheryl Isherwood, Benita Middleton, Michelle A. Gibbs, Debra J. Skene, Simon N. Archer, Jonathan D. Johnston
- P27. IS BROAD SPECTRUM LIGHT ASSOCIATED WITH CHANGES IN INSULIN SENSITIVITY? M AlBreiki, B Middleton, S Hampton
- P28. DISSECTION OF CIRCADIAN CIRCUITS DRIVING PULMONARY INNATE IMMUNE RESPONSES Zhenguang Zhang, Louise Ince, Ryan Vanslow, Ping Wang, Nick Phillips, Magnus Rattray, David Ray, Andrew Loudon
- P29. EXTENSIVE WHEEL RUNNING RESTORE CIRCADIAN ACTIVITY RHYTHMS AND ENHANCE COGNITIVE PERFORMANCE (ATTENUATE COGNITIVE DEFICITS) OF ARRHYTHMIC DJUNGARIAN HAMSTERS Dietmar Weinert, Lisa Müller
- P30. NOCTURNAL LIGHT EXPOSURE ACUTELY DISRUPTS GLUCOSE METABOLISM A.L. Opperhuizen, E. Foppen, R.D. Jansen, D.J. Stenvers, E. Fliers, A. Kalsbeek
- P31. THE ZFHX3SCI/+ GENE: A NOVEL CIRCADIAN CLOCK TARGET AFFECTS SLEEP AND COGNITIVE PROCESSES

Edoardo Balzani, Glenda Lassi, Silvia Maggi, Siddharth Sethi, Michael J. Parsons, Michelle Simon, Patrick M. Nolan, Valter Tucci

P32. PACAP, A DAYTIME REGULATOR OF CIRCADIAN FOOD ANTICIPATORY ACTIVITY RHYTHMS (FAA).

Jens Hannibal\*, Birgitte Georg, Jan Fahrenkrug

- P33. DIM LIGHT AT NIGHT INDUCES AN ADDITIONAL FREE RUNNING RHYTHM THAT DISTURBS SLEEP WAKE BEHAVIOUR IN RATS
  - Dirk Jan Stenvers, Rick van Dorp, Anne-Loes Opperhuizen, Eric Fliers, Jorge Mendoza, Peter H. Bisschop, Johanna H Meijer, Andries Kalsbeek, Tom Deboer
- P34. EXPRESSION OF ECTONUCLEOTIDASES IN THE PROSENCEPHALON OF MELATONIN-PROFICIENT C3H AND MELATONIN-DEFICIENT C57BL MICE: SPATIAL DISTRIBUTION AND TIME-DEPENDENT CHANGES

Homola M, Pfeffer M, Fischer C, Zimmermann H, Robson SC, Korf HW

- P35. CALORIC TIME RESTRICTION DECREASES TUMOR GROWTH AND PRESERVES THE LIVER ARCHITECTURE AND FUNCTION IN A EXPERIMENTAL MODEL OF CIRRHOSIS-HEPATOCELLULAR CANCER
  - Molina-Aguilar Christian, Guerrero Carrillo María de Jesús, Vázquez-Martínez Eva Olivia, Castro-Belio Thania and Díaz-Muñoz Mauricio.
- P36. ROLE OF HEPATIC MIRNAS IN ADAPTATION TO DAYTIME FEEDING IN MICE Ngoc-Hien Du, Marieke Hoekstra, Bulak Arpat, Mara De Matos, Paul Franken, and David Gatfield



- P37. ROLES OF THE CIRCADIAN CLOCK IN THE PATHOGENESIS OF ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD)
  Han Wang
- P38. EFFECTS OF SPECTRAL MODULATION OF NOCTURNAL LIGHT ON THE CIRCADIAN SYSTEM OF THE DIURNAL OCTODON DEGUS AND NOCTURNAL RATTUS NORVEGICUS. Bonmati-Carrion M.A., Otalora B.B., Sempere A., Madrid J.A. and Rol M.A.
- P39. RECIPROCAL REGULATION BETWEEN THE CIRCADIAN CLOCK AND HYPOXIC SIGNALING IN MAMMALS

Yaling Wu, Dingbin Tang, Na Liu, Wei Xiong, Huanwei Huang, Yang Li, Haijiao Zhao, Peihao Chen, Fengchao Wang & Eric Erquan Zhang

- P40. BEHAVIOUR AND SLEEP DISTURBANCES ASSOCIATED WITH THE CANDIDATE RISK GENE FOR NEUROPSYCHIATRIC DISEASE CACNA1C REVEALED USING ENU MUTAGENESIS Eleanor Hobbs, Valter Tucci, Glenda Lassi, Greg Joynson, Patrick Nolan and Michael Parsons
- P41. EXPLORING THE EFFECTS OF CONDITIONAL DELETION OF ZFHX3 IN THE ADULT BRAIN Ashleigh G Wilcox, Gareth Banks, Greg Joynson, Michael Parsons, Patrick Nolan
- P42. MATERNAL EXPOSURE TO CHRONIC SHIFT PHOTOPERIOD IMPAIRS ADRENAL FUNCTION IN THE ADULT OFFSPRING Spichiger C, Salazar ER, Mendez N, Halabi D, Vergara K, Alonso-Vazquez P, Seron-Ferre M, Richter HG, Torres-Farfan C.
- P43. MATERNAL CHRONODISRUPTION DURING PREGNANCY ALTERS GLUCOSE HOMEOSTASIS AND ADIPOSE TISSUE PHYSIOLOGY IN THE OFFSPRING.
  Halabi D, Vergara K, Mendez N, Spichiger C, Richter HG, Torres-Farfan C.
- P44. EFFECTS ON PLASMA CIRCADIAN RHYTHMS AND GLOBAL METHYLATION STATUS IN ADULT OFFSPRING GESTATED UNDER CHRONODISRUPTION.
  Salazar E, Azpeleta C, Spichiger C, Mendez N, Vergara K, Halabi D, Richter HG, Torres-Farfan C
- P45. INVOLVEMENT OF CIRCADIAN CLOCK MECHANISMS IN FABRY DISEASE, A GENETIC LYSOSOMAL STORAGE DISORDER Barris-Oliveira AC, Pekovic-Vaughan V, D'Almeida V
- P46. SEASONAL RHYTHMS IN HYPOTHALAMIC ANGIOGENESIS. Tyler Stevenson, Andy Welch
- P47. INFORMATION SEEKING BEHAVIOUR PREDICTS EXTENSIVE SEASONAL VARIATION IN COMMON CHILDHOOD ILLNESSES
  Kevin Bakker, Micaela Martinez-Bakker, Barbara Helm, Tyler Stevenson
- P48. THE IMPACT OF CIRCADIAN PHENOTYPE ON PREDICTED TEAM PERFORMANCE Facer-Childs, E, Brandstaetter, R
- P49. C-FOS PHOTOINDUCTION IN THE SCN INCREASED AT CT23 WHEN GLIAL CELLS POPULATION ARE REDUCED Gabriela Dominguez-Monzon, Paula Vergara, José Segovia and Raúl Aguilar-Roblero
- P50. SLEEP-WAKE ACTIGRAPHY AND CARDIOVASCULAR RESPONSES TO STRESS IN NIGHT SHIFTS WORKERS
  - S. Gorokhova, T. Prigirovskaya, G. Lazarenko, M. Buniatyan





- P51. THE METHAMPHETAMINE-SENSITIVE CIRCADIAN OSCILLATOR (MASCO) IS GENE-DOSE AND AGE-RELATED IN A KNOCKIN MOUSE MODEL OF HUNTINGTON'S DISEASE Koliane Ouk, Juliet Aungier, A. Jennifer Morton
- P52. ASSOCIATION BETWEEN KNOWLEDGE OF BIOLOGICAL RHYTHM AND NURSING CARE FOR PATIENT'S ENVIRONMENT IN HOSPITAL

Masayuki Kondo, Shunsuke Nagashima, Makoto Yamashita, Chiaki Tojo, Yuya Nishimoto, Hiroto Matsuyama, Shinji Tanaka, Tomoko Wakamura

- P53. EFFECT OF CIRCADIAN EXPRESSION OF TYROSINE HYDROXYLASE IN 6-HYDROXYDOPAMINE INDUCED DAMAGE RESPONSE OF HUMAN DOPAMINERGIC CELL Doyeon Kim, Jeongah Kim, Sung Kook Chun, Kyungjin Kim
- P54. CIRCADIAN EPITRANSCRIPTOMICS: MRNA RHYTHMS DRIVEN BY RHYTHMIC POST-TRANSCRIPTIONAL REGULATION Hikari Yoshitane, Hideki Terajima, Yoshitaka Fukada
- P55. DIURNAL RESTRICTED FEEDING SCHEDULES PROMOTES CHANGES IN BODY WEIGHT AND NF-kB PRESENCE IN LIVER TISSUE OF WISTAR RATS Ana Cristina García-Gaytán, Mauricio Díaz-Muñoz, Isabel Méndez.
- P56. EFFECT OF TRYPTOPHAN SUPPLEMENT INTAKE AT BREAKFAST ON NOCTURNAL MELATONIN SECRETION UNDER DIFFERENT LIGHT INTENSITIES IN DAYTIME IN HUMANS Shunsuke Nagashima, Makoto Yamashita, Chiaki Tojo, Masayuki Kondo, Takeshi Morita, Tomoko Wakamura
- P57. INTERNAL DESYNCHRONIZATION RESULTS IN TISSUE SPECIFIC CHANGES IN THE EXPRESSION OF CLOCK GENES IN A RAT MODEL OF SHIFT-WORK Cinthya Cordoba-Manilla, María del Carmen Basualdo, Estefanía Noemí Espitia-Bautista, Ruud M Buijs, Carolina Escobar
- P58. HYPOTHALAMIC THYROID RESPONSIVE GENE SWITCHES MEDIATE CIRCADIAN CLOCK EFFECTS ON SEASONAL REPRODUCTION IN SONGBIRDS Gaurav Majumdar, Vinod Kumar
- P59. EFFECTS OF FILTERING VISUAL SHORT WAVELENGTHS ON PHASE RESETTING AND MASKING RESPONSES
  Bojana Gladanac, James Jonkman, Robert Casper, Shadab Rahman
- P60. MICE LACKING MELANOPSIN DISPLAY DEFICITS IN SOCIAL INTERACTION Sibah Hasan, Tomasz Schneider, Thomas Vogels, Russell Foster, Stuart Peirson
- P61. USE OF RESAMPLING TO IMPROVE ACCURACY OF DATA ANALYSIS IN TIME COURSE EXPERIMENTS
  Mathias Bockwoldt, Bernd Striberny, Ines Heiland
- P62. CHARACTERIZATION OF CIRCADIAN RHYTHMS OF LIVER PER2::LUC EXPRESSION IN FREELY MOVING MICE
  Xiao-Mei Li, Mircea Dumitru, Narin Özturk, Mohammad-Djafari, Francis Lévi
- P63. HUMAN PUPILLARY LIGHT REFLEX: EFFECT OF WAVELENGTH AND PHOTON FLUX M.A. Bonmatí-Carrión, K. Hild, C. Isherwood, B. Middleton, S. Sweeney, V.L. Revell, J.A. Madrid, M. A. Rol, D. J. Skene



- P64. A RIGID CLOCK DRIVING FLEXIBLE RHYTHMS; THE MOUSE SCN DRIVES NOCTURNAL ACTIVITY PATTERNS UNDER AD LIB, BUT DIURNAL RHYTHMS DURING FOOD SCARCITY Sjaak J. Riede, Vincent van der Vinne, Patricia Tachinardy, Jamey Scheepe, Jildert Akkermann, Roelof A. Hut
- P65. VELOCITY RESPONSE-BASED MODELING OF PHOTOPERIOD-DEPENDENT SYNCHRONIZATION PATTERNS OF PACEMAKER CELLS IN SUPRACHIASMATIC NUCLEUS Mitsuyuki Nakao, Ayumi Yoshioka, Norihiro Katayama
- P66. IDENTIFICATION OF GENOME REGIONS ASSOCIATED WITH AVERAGE WEEKLY SLEEP DURATION BY USING GENOME-WIDE ASSOCIATION STUDY Maris Teder-Laving, Jaanika Moro, Evelin Mihailov, Andres Metspalu
- P67. ALTERED EXPRESSION OF THE CORE CIRCADIAN CLOCK COMPONENT PERIOD2 IN A DIURNAL RODENT MODEL OF SEASONAL AFFECTIVE DISORDER Tomoko Ikeno, Lily Yan
- P68. THE CIRCADIAN CLOCK AND PROTEIN AGGREGATION IN CELL-BASED MODELS Madeti C, Geibel M, Huaroto C, Rubbe N, Vanzo R, Merrow M
- P69. TEMPERATURE ENTRAINMENT OF THE CIRCADIAN CLOCK IN DROSOPHILA MELANOGASTER Sanne Roessingh, Ralf Stanewsky
- P70. PERIPHERAL CLOCKS REGULATE THE TRANSCRIPTIONAL RESPONSE TO GLUCOCORTICOIDS
  Giorgio Caratti, Louise Ince, Mudassar Iqbal, Louise Hunter, Magnus Rattray, Andrew Loudon, Laura Matthews, David Ray
- P71. CHRONOTYPE, SOCIAL JETLAG, AND SLEEP QUALITY: EXPLORING SYMPTOMS OF ADULT ATTENTION-DEFICIT HYPERACTIVITY DISORDER (ADHD)

  McGowan, N.M., Voinescu, B.I., Coogan, A.N.
- P72. INVESTIGATING NEURAL CORRELATES OF RHYTHM DETERIORATION IN SEASONAL ADAPTIVE BEHAVIOR IN AGING MICE M.R. Buijink, A.H.O. Olde Engberink, O. Roethler, C.B. Wit, J.H. Meijer, J.H.T. Rohling, S. Michel
- P73. EXAMINING THE EFFECTS OF DIM LIGHT-AT-NIGHT ON AFFECTIVE BEHAVIOURS IN C57BL/6 MICE
  Michael Cleary-Gaffney and Andrew N. Coogan.
- P74. PHASE CONTROL OF CELLULAR CIRCADIAN RHYTHMS BY PROJECTOR ILLUMINATIONS IN LACTUCA SATIVA L. LEAVES
  Naoki Seki, Hirokazu Fukuda
- P75. NEAT1 A LONG NON-CODING RNA INVOLVED IN PITUITARY CIRCADIAN RHYTHMS: CHARACTERIZATION OF ITS INTERACTOME
  Manon Torres, Denis Becquet, Séverine Guillen, Bénédicte Boyer, Mathias Moreno, Marie-Pierre Blanchard, Jean-Louis Franc , Anne-Marie François-Bellan
- P76. THE ROLE OF THE INTERGENICULATE LEAFLET IN SCN LIGHT RESPONSES Walmsley, L., Brown, TM.
- P77. MODEL-BASED EVALUATION OF MULTIDRUG CANCER CHRONOTHERAPY Hiroshi Inokawa, Norihiro Katayama, Mitsuyuki Nakao





- P78. THE DBHS PROTEINS NONO AND PSPC1 REGULATE SLEEP-CHARACTERISTIC NEURONAL FIRING AND MODULATE CIRCADIAN GENE EXPRESSION Andrea Spinnler, Dennis Mircsof, Koen Seignette, Steven A. Brown
- P79. INVERSE EFFECT OF ACUTE BLUE AND GREEN LIGHT EXPOSURE ON ANXIETY, ADRENAL RESPONSES AND CLOCK GENE EXPRESSION
  Violetta Pilorz, Eric S.K. Tam, Carina A. Pothecary, Russell G. Foster and Stuart N. Peirson
- P80. PHASE RESPONSE OF NASAL CLOCK BY DEXAMETHASONE: MONITORING PER2 RHYTHM USING A LUCIFERASE REPORTER Aya Honma, Yoshiko Yamada, Yuji Nakamaru, Satoshi Fukuda, Ken-ichi Honma and Sato Honma
- P81. MOUSE MEDIAL HABENULA NEURONS SHOW DAILY SENSITIVITY TO NICOTINE. Beatriz B. Otalora, Mino D.C. Belle, Hugh D. Piggins
- P82. A MUTATION IN THE SNARE PROTEIN VAMP2 UNDERLIES DEFICITS IN SLEEP AND BEHAVIOUR
  I Heise, S Hasan, G T Banks, P Potter, S Wells, R G Foster, S N Peirson, P M Nolan
- P83. THE TRANSCRIPTIONAL LANDSCAPE ASSOCIATED WITH PHOTOPERIODISM Laura M.M. Flavell, Eran Tauber
- P84. DEXAMETHASONE ALTERS CLOCK GENES EXPRESSION IN A TISSUE-DEPENDENT MANNER IN GOLDFISH (*CARASSIUS AURATUS*).
  Aida Sánchez-Bretaño, Marta Montero, Laura Vázquez, Ángel L. Alonso-Gómez, María J. Delgado, Esther Isorn\*
- P85. ARCUATE NEURONES THAT RESPOND TO ENERGY STATUS EXHIBIT ROBUST RHYTHMS IN EXCITABILITY

  Adam G. Watson, Mino D. C. Belle, David A. Bechtold, Hugh D. Piggins
- P86. NEURONAL ACTIVITY-DEPENDENT REORGANIZATION OF NUCLEAR PARASPECKLES AND DBHS PROTEINS
  Koen Seignette, Andrea Spinnler, Shiva K. Tyaqarajan, Steven. A. Brown
- P87. INVESTIGATING EXPRESSION PATTERNS AND CLOCK INTERACTIONS OF ZFHX3, A NOVEL CIRCADIAN TRANSCRIPTION FACTOR Stefania Militi, Michael J. Parsons, Chris Esapa, Helen Hilton, Ashleigh Wilcox, Patrick M. Nolan
- P88. EYES LIKE SAUCERS A NOVEL ENU MOUSE LINE WITH SUBSTANTIALLY REDUCED LIGHT RESPONSIVENESS
  Gareth Banks, Greg Joynson, Saumya Kumar, Michelle Simon, Russell G Forster, Stuart N Peirson, Patrick M. Nolan
- P89. ANALYSIS OF ECLOSION PATTERNS UNDER COMBINED LIGHT AND TEMPERATURE CYCLES IN DROSOPHILA MELANOGASTER Chihiro Ito, Kenji Tomioka
- P90. EFFECT OF SUBLETHAL DOSE OF INHIBITORS OF SOME MOLECULAR PROCESSES IN THE CIRCADIAN NEGATIVE FEEDBACK LOOP ON STABILITY OF CIRCADIAN RHYTHM Masato Nakajima, Satoshi Koinuma, Yasufumi Shigeyoshi
- P91. CONSTANT LIGHT UNCOVERS BEHAVIOURAL EFFECTS OF A MUTATION IN THE SCHIZOPHRENIA RISK GENE *DTNBP1* IN MICE.
  Nicolas Cermakian, Sanjeev K. Bhardwaj, Katarina Stojkovic, Silke Kiessling, Lalit K. Srivastava



- P92. CRYPTOCHROMES AND THEIR POSSIBLE ROLE IN CIRCADIAN RHYTHMS OF ANCIENT INSECT ORDER
  - Olga Bazalová, Jia-Hsin Huang, Yu-Hsien Lin, Yun Liu, Jan Provazník Ivo Šauman, Martin Vácha, How-Jing Lee, Doležel D
- P93. SCHEDULED FEEDING COUNTERACTS SOME METABOLIC AND CIRCADIAN CONSEQUENCES OF PRENATAL LPS ADMINISTRATION IN FEMALE RATS. Elena Velarde, Clara Azpeleta, Jose Miguel Biscaia, José Gómez, Ricardo Llorente de Miguel, Eva Marco
- P94. IMPACT OF CIRCADIAN NUCLEAR RECEPTOR REV-ERBA ON PARKINSON'S DISEASE Jeongah Kim, Doyeon Kim, Sung Kook Chun, Sooyoung Chung and Kyungjin Kim
- P95. THE DOUBLE LIFE OF PER2 INTEGRATING CIRCADIAN TIME AND SLEEP NEED MMB Hoekstra, Y Emmeneger, P Franken
- P96. THE EFFECT OF RFAMIDES ON FOOD INTAKE IN TWO DIFFERENT PHOTOPERIODIC CONDITIONS IN MALE AND FEMALE SIBERIAN HAMSTER.

  Cázarez-Márquez Fernando<sup>1, 2</sup>, Laran Chich Marie-Pierre<sup>1</sup>, Kalsbeek Andries<sup>2</sup>, Simonneaux Valérie<sup>1</sup>.
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#### **PLENARY LECTURES**

PL1. MICHAEL H. HASTINGS Introduced by: Charlotte Foerster (Germany) "Cellular and Circuit-Level Circadian Pacemaking in the Suprachiasmatic Nucleus."

Michael Hastings

Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge, UK

The suprachiasmatic nucleus (SCN) of the hypothalamus is the principal circadian clock of mammals. In common with the cell-intrinsic oscillations of subordinate circadian clocks distributed widely across the tissues of the body, the timing mechanism of the SCN revolves around an auto-regulatory transcriptional/ post-translational feedback loop (TTFL). What makes the SCN special, however, is the inter-neuronal communication, which is lacking in peripheral tissues. This circuit-level signalling sustains and synchronises the TTFL of individual SCN neurons such that the entire circuit generates high amplitude, coherent rhythms of electrical activity and gene expression that can run for many months when isolated in organotypic slice culture. This stability and robustness are a pre-requisite for a central time-keeper, and in contrast, in the absence of cellular communication, tissue-level oscillations of peripheral clocks damp progressively in culture. In this lecture I shall present new and unpublished work from studies employing various types of real-time imaging and intersectional genetic manipulations of the SCN that examine the following. First, the internal mechanisms of the mammalian TTFL. Second, how this cell-autonomous process is related to circuit-level time-keeping in the SCN. Finally, how different neuronal populations of the SCN contribute specific functions to ensemble time-keeping.

THE GWINNER LECTURE:

PL2. DAVID HAZLERIGG Introduced by: Paul Pevet (France)

"What do we Really Know About Circannual Clocks?"

David Hazlerigg

Arctic and Marine Biology, UiT – Norway's University in the Arctic, Tromsø, Norway Sponsored by: The Society for Endocrinology / Journal of Endocrinology

Ebo Gwinner was among the first to undertake a detailed characterisation of circannual rhythms – which may be defined as free-running long-term (tau ≈ 12 months) cycles of physiology and behaviour. In this lecture I will first discuss the evidence for the existence of circannual clocks and the ecological drivers that have led to their evolution. I will move on to consider mechanistic aspects of circannual timekeeping in vertebrates, using current knowledge of the mammalian system as a template. I will discuss evidence that the mammalian pars tuberalis constitutes a core circannual pacemaker, synchronised by the annual day length cycle, and modulating seasonal endocrine output through thyroid-dependent and –independent pathways. I will demonstrate that seasonal phase is established in this structure in utero, exerting effects on hypothalamic and reproductive development in the perinatal period. Finally I will discuss the extent to which we can apply the mammalian model for circannual timekeeping to other vertebrate groups.

PL3. JOHN HOGENESCH Introduced by: Till Roenneberg (Germany) "An Atlas of Circadian Gene Expression: Implications for Biology and Medicine." Ray Zhang, Nick Lahens, Heather Ballance, Michael Hughes, Ron Anafi and John B. Hogenesch

Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, 19104, USA

The circadian clock regulates daily rhythms in behavior and physiology throughout the body. Many groups have sought to characterize clock-regulated gene expression as a way to understand its tissue specific roles. However, most of these studies focused on one or two organs. Here we report a survey of the mouse from 12 separate brain regions and organs using both DNA arrays at 2 hr resolution and RNA-seq at 6 hr resolution. We find that 43% of the protein encoding transcriptome is clock regulated, including the majority of human disease genes and drug targets, including targets for 56 of the top 100 best selling drugs. Most of these transcripts peak in anticipation of dusk and dawn in transcriptional "rush hours". We also find that conserved ncRNAs are substantially more like to cycle than non-conserved ncRNAs. We describe and use a new method, Phase Set Enrichment, which is optimized to detect pathway level enrichment for periodic data. Collectively, these results highlight the importance of the circadian clock and suggest ways to leverage biological time in medicine.

#### JSC LECTURE:

PL4. TAKASHI YOSHIMURA Introduced by: Sato Honma (Japan)

"Towards Understanding the Mechanism of Seasonal Time Measurement."

Takashi Yoshimura

Institute of Transformative Bio-Molecules & Graduate School of Bioagricultural Sciences, Nagoya University, Division of Seasonal Biology, National Institute for Basic Biology, Japan

Animals living in temperate zone use changes in day length to adapt to seasonal changes in environment, but mechanisms underlying seasonal (photoperiodic) time measurement are not fully understood. Japanese quail is an excellent model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. We have demonstrated that local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating enzyme (type 2 deiodinase: DIO2) regulates photoperiodism. Functional genomics analysis in quail demonstrated that long day stimulus induces thyrotropin (thyroid stimulating hormone: TSH) production in the pars tuberalis (PT) of the pituitary gland, which triggers DIO2 expression in the ependymal cells of the MBH. In mammals, nocturnal melatonin secretion provides an endocrine signal of the photoperiod to the PT that contains melatonin receptors in high density. We have also demonstrated the involvement of TSH signaling pathway in mammals by using the TSH receptor null mice. Well known function of TSH derived from pars distalis (PD) of the pituitary gland is stimulation of thyroid gland. However, the mechanisms by which PT- and PD-TSH exert distinct functions within the body remained mystery. We found TSHs from two anatomical sources undergo different glycosylation and this tissue-specific glycosylation imparts different functions on a single hormone.

PL5. STEVE KAY Introduced by: Elizabeth Maywood (UK) "Time for Change: Chemical Biology Approaches to Circadian Therapeutics."
Steve A. Kay

Dept. Molecular and Computational Biology, University of Southern California, Los Angeles, USA

The circadian clock controls daily rhythms of physiological processes, and its impairment has been associated with numerous disorders, including metabolic disease. From a chemical screen we previously identified KL001, a small molecule that specifically stabilizes the clock protein cryptochrome (CRY) and inhibits glucagon response of gluconeogenesis. We have recently investigated the molecular mechanism of how the circadian clock, through CRY, regulates the gluconeogenesis pathway in response to fasting signals in mouse primary hepatocytes. By using KL001 in a genome-wide gene expression analysis we identified the genes whose induction by glucagon is inhibited by CRY. Since CRY is a repressor that does not bind DNA but instead asserts its effect through interaction with other TFs, we developed a bioinformatic pipeline for the quantification of TFs occupancy at the active enhancer regions of gluconeogenic genes. We found several nuclear receptors and liver specific TFs highly over-represented at CRY-repressed gluconeogenic genes. Several of these genes, including the gluconeogenesis rate-limiting enzymes Pck1 and G6pc, have regulatory regions resembling super-enhancers. Interestingly, CRY repressed eRNA expression at the super-enhancer regions, supporting the idea that it impairs proper enhancer activation. In line with these results, treatment with JQ1, a bromodomain inhibitor that specifically disrupts super-enhancers, revealed that genes targeted by CRY are significantly more susceptible to JQ1 repression of glucagon induction than genes not targeted by CRY. Altogether, these results provide testable hypotheses for the molecular mechanisms underlying CRY mediated repression of gluconeogenesis, involving a novel regulatory layer connecting the circadian clock and metabolism. It also lays the groundwork for developing novel therapeutics based on modulating CRY levels in target tissues in humans.

AXELROD LECTURE:

PL6. HELENA ILLNEROVA Introduced by: Alena Sumova (Czech Republic) "From the Pineal Gland to the SCN and Back Again."

Helena Illnerová

Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

The field of biological rhythms has developed from many springs and streams. At the beginning, it depended mostly on measuring overt rhythms, mainly rhythms of the locomotor activity, but not only them. Our Society, i.e., The European Biological Rhythms Society, developed from the European Pineal Study Group, which concentrated its attention on the rhythmic pineal gland, later on mostly on the robust rhythm in melatonin production. In comparison with the locomotor activity rhythm, this rhythm had a great advantage, namely two well defined phase markers: the evening onset of melatonin production and the morning offset. Using the rhythm in melatonin production, our laboratory demonstrated that i) following a sudden light at night, levels of the melatonin precursor serotonin increased and those of melatonin decreased instantaneously, ii) even very brief light pulses such as 1 min of light phase shifted the melatonin rhythm, iii) the evening onset and the morning offset did not necessarily phase shifted in parallel, iv) duration of the high nocturnal melatonin production depended on the photoperiod and

correlated with reproductive activity, etc. All these early results pointed to a possibility that the biological clock in the SCN driving the melatonin rhythm was complex and composed of at least two clusters of oscillators. Later on we confirmed this complexity and found that the SCN itself was modulated by the photoperiod, contained melatonin receptors and its rhythms were instantaneously reset by melatonin administration. The old results will be confronted with recent ones and unsolved questions will be discussed.

ARIENS KAPPERS LECTURE:

PL7. JOHANNA H MEIJER Introduced by: Andries Kalsbeek (Netherlands)

"An ancient clock in modern society."

Johanna H. Meijer Netherlands

#### PL8. UELI SCHIBLER

Introduced by: Andrew Loudon (UK)

"The synchronization of peripheral oscillators in mammals."

Pauline Gosselin, Gianpaolo Rando, Alan Gerber, Ivana Gotic, Flore Sinturel, Ka Yi Hui, Thomas Bollinger, Pascal Gos, Fabienne Fleury Olela, Mikhail Koksharov, Maud Demarque and Ueli Schibler

Department of Molecular Biology, University of Geneva, Switzerland

The mammalian circadian timing system consists of a central pacemaker in the brain's suprachiasmatic nucleus (SCN) and subsidiary oscillators in nearly all body cells. The SCN synchronizes peripheral clocks through a variety of systemic signals. While feeding rhythms are the most dominant Zeitgebers for most peripheral oscillators, the SCN also employs blood-borne and body temperature-dependent signals to set the phase in peripheral tissues.

In the first part of the talk I will address the issue of how the SCN and feeding rhythms synchronize circadian oscillators in the liver. To this end we engineered the RT-Biolumicorder, a device enabling us to record circadian gene expression in the liver of freely moving mice during months. Using this approach, we can readily determine the kinetics of phase shifting, a parameter that is much more sensitive to the disruption of an individual signalling pathway than the steady-state phase. The results indicate that the SCN uses both indirect pathways (depending on rest-activity cycles and feeding rhythms) and more direct (feeding-independent pathways) to synchronize liver clocks. Moreover, our studies suggest that hepatocyte clocks are strongly coupled between cells and that their synchronization immediately follows the phase of the SCN upon changing the light-dark regimen.

In the second part of my talk I will present transcriptional and posttranscriptional pathways by which cyclically active blood-borne signals, cytoskeleton dynamics, and body temperature rhythms determine the amplitude, magnitude, and phase of circadian gene expression in peripheral tissues.

STOCKGRAND LECTURE: PL9. CHRISTIAN CAJOCHEN **TBA** Christian Cajochen Switzerland

Introduced by: Jo Arendt (UK)

#### **SYMPOSIUM 1: BEYOND MELANOPSIN**

Chair: Tim Brown (UK)

#### S1. SAMER HATTAR

"Retinal Photoreceptors' Contribution to Non-Image Forming Visual Function." Melissa Simmonds, Alan Rupp, William Keenan and Samer Hattar Biology, Johns Hopkins University, USA

Three photoreceptors exist in the mammalian retina, rods, cones and ipRGCs (known as intrinsically photosensitive retinal ganglion cells). ipRGCs function both as ganglion cells and also contain the photopigment melanopsin. This allows ipRGCs to detect light intrinsically through the melanopsin phototransduction pathway and by incorporating rod/cone input through the classical retinal circuits. Despite a decade of intense research, the relative contribution of rods, cones and melanopsin phototransduction in ipRGCs to several non-image forming behaviours is both lacking and controversial. In this talk, I will advance the exciting hypothesis that light detection for non-image forming pathways employs parallel retinal circuits that mimic the more complex image forming vision. I will provide evidence that rods play a pivotal role in non-image functions in the absence of the melanopsin protein and cones play minor modulatory roles. I will also provide evidence that melanopsin phototransduction is necessary for non-image forming functions, since rods/cones fail to compensate for its absence under several physiological conditions. These results provide tantalizing evidence for the preservation of the melanopsin gene across evolution.

#### S2. STUART PEIRSON

"The Effects of Light on Learning and Memory Depend Upon Both Melanopsin and Classical Photoreceptors."

Tam S.K.E., Hughes S., Watson T.S., Hankins M.W., Foster R.G., Bannerman D.M. and Peirson S.N.

Nuffield Department of Clinical Neurosciences, University of Oxford, UK

Light exerts widespread effects on physiology and behaviour, including the regulation of circadian rhythms, hormone synthesis, pupillary constriction and sleep. These responses are accompanied by a marked but transient effect on various brain regions involved in attentional and memory processes, such as the parietal area and hippocampus. Although the effects of light on alertness and cognitive performance are well known, it is unclear whether these responses are mediated via rods and cones, or the melanopsin-expressing photosensitive retinal ganglion cells (pRGCs). Here we investigate the effect of light on short-term memory in mice using spontaneous object recognition, which provides a simple non-aversive measure of memory performance. Firstly, we show that mice lacking rods and cones are capable of recognising novel objects, demonstrating that this task was not simply visually-dependent. We then go on to demonstrate that performance is disrupted by changes in visual context, which are dependent upon classical photoreceptors but not melanopsin. Finally, and most surprisingly, we show that test performance is disrupted by changes in lighting context, which are dependent upon both melanopsin and classical photoreceptors. Our data provide the first direct evidence that the effects of light on short-term memory performance depend upon rods/cones and melanopsin, and cannot be mediated by melanopsin alone.

#### S3. ETHAN BUHR

### "Photic Entrainment of Local Circadian Clocks in the Mouse Retina by Non-Canonical Photoreceptors."

Ethan Buhr, Wendy Yue, Richard Lang, King-Wai Yau and Russell Van Gelder Department of Ophthalmology, University of Washington, USA

The mammalian retina contains autonomous molecular clocks which can be directly entrained to light:dark cycles. This photo-entrainment occurs even in situations when the SCN/behavioral phase is in an atypical phase compared to the light:dark cycle. Rods, cones, and melanopsin are not required for photo-entrainment of local circadian clocks. Analyses of the spectral sensitivity of wild-type retinas revealed sensitivity to short wavelength light <417nm. This spectral sensitivity suggests that rods, medium-wavelength sensitive cones (Opn1mw), and melanopsin (Opn4) are not sufficient for entrainment of even wild-type retinas. We analyzed the circadian light response of retinas lacking short-wavelength sensitive cones (Opn1sw) to light:dark cycles and acute light pulses and found no differences between Opn1sw-/- and wild-type retinas. These data therefore suggest the presence of a short-wavelength sensitive photoreceptor in addition to the canonical retinal photoreceptors.

#### S4. ROB LUCAS

#### "Visual information reaching the SCN."

Robert Lucas, Rachel Dobb, Josh Mouland, Lauren Walmsley, Annette Allen, Franck Martial, Riccardo Storchi, Dan Elijah and Timothy Brown Faculty of Life Sciences. University of Manchester, UK

The mammalian SCN receives both direct and indirect inputs from the retina. These pathways are typically considered to allow the clock to infer time of day according to ambient light levels (irradiance) averaged over time and space. In a series of projects combining environmental measurements with electrophysiological and behavioural recordings in laboratory mice we have explored the extent to which other visual features (spatial patterns, higher frequency temporal modulations, and colour) are encoded by the SCN and their significance for circadian resetting mechanisms.

#### Short Communications

### SC1. "Phosphorylation of rat melanopsin at Ser-381 and Ser-398 by light/dark and its importance for ipRGCs cellular Ca<sup>2+</sup> signalling."

<u>Birgitte Georg</u>, Birgitte Falktoft, Jens Hannibal, Sarah B. Kristiansen, Thomas K. Klausen, Lauge Kellermann and Jan Fahrenkrug

Department of Clinical Biochemistry, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen NV, Denmark

The secondary structure of the photoreceptor melanopsin predicts a long cytoplasmic tail with many potential phosphorylation sites. We generated phosphor-specific antibodies

against two potential phosphoserines, Ser-381 and Ser-398, having high probability of being phosphorylated and being conserved among vertebrate species. Antibody specificity was verified by immunoblotting and immunohistochemical staining of HEK-293 cells expressing rat melanopsin mutated in either Ser-381 or Ser-398 and thus unable to get phosphorylated. Using antibody recognising phospho-Ser-381 and melanopsin expressing HEK-293 cells, we demonstrated that melanopsin is phosphorylated at Ser-381 during darkness and dephosphorylated during light exposure. On the contrary, melanopsin was found to be unphosphorylated at Ser-398 in darkness and becoming phosphorylated during light exposure. The light-induced changes in phosphorylation of both Ser-381 and Ser-398 were rapid and lasted for up to at least 10 hours. In addition, we found that phosphorylation of the two was independent of each other. Furthermore, the changes in phosphorylation of melanopsin during light and darkness were confirmed in vivo by immunohistochemical staining of rat retinas taken either at the end of night or after exposure to light in the morning. By comparing the light-evoked Ca2+ response in HEK-293 cells expressing either native or mutated melanopsin, we demonstrated that abolishment of phosphorylation of either Ser-381 or Ser-398 by mutation reduced the light-induced Ca2+ response. Examination of the light-evoked Ca2+ response in Ser-381 and Ser-398 double mutants revealed additional reduction in Ca2+ response thus indicating independence of the two phosphorylations also in term of Ca<sup>2+</sup> response.

#### SC2. "The intergeniculate leaflet: Development and connectivity."

Brock, O.1, Balint, K.2, Jager, P.1, Roska, B.2 and Delogu, A.1

<sup>1</sup>Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, UK, <sup>2</sup>The Friedrich Miescher Institute (FMI), Basel, CH

Melanopsin-expressing retinal ganglion cells (aka ipRGCs) are a class of sensory neurons required to drive light-dependent behaviours and physiology. Given the broad distribution of ipRGC targets in the subcortical brain, it is yet unclear what brain regions mediate each of the ipRGC-dependent responses to light. We are particularly interested in the intergeniculate leaflet (IGL): a retinorecipient structure in the thalamus with an obvious projection to the master circadian clock at the hypothalamus. We have adopted a developmental approach to elucidate the genetic determinants that guide IGL differentiation and used this information to devise a strategy to map its direct synaptic afferents. Here, we provide a first glimpse into the range of mono-synaptic inputs to the IGL, which confirms its preferential innervation by a subset of ipRGCs and its potential role as integrator of multiple cues.

### SC3. "A novel in vitro slice preparation for studying geniculohypothalamic signalling."

<u>Lydia Hanna</u>, Michael Howarth and Timothy M. Brown Faculty of Life Sciences, University of Manchester, Manchester, UK

The suprachiasmatic nucleus (SCN), intergeniculate leaflet (IGL) and the pretectal olivary nucleus (PON) form an interconnected network of retinorecipient nuclei controlling reflex responses to environmental light. While both the IGL and PON are implicated as components of the circadian light input pathway, the cellular circuitry through which these

nuclei influence circadian responses to light are poorly understood. To better understand the organisation and properties of this circadian network, here we aimed to create an in vitro slice preparation retaining all of the key nuclei and the connections between them. Using melanopsin reporter mice (Opn4tauLacZ) to visualise retinal inputs, we determined that a 600µm thick slice preparation cut at 30 degrees off the coronal plane would preserve the optic tract (OT) and the majority of the SCN, IGL and PON intact. Multielectrode recordings confirmed that the resulting slices maintained circadian rhythmicity for >24h in vitro and an intact OT, as electrical stimulation of the chiasm evoked glutamatergic responses throughout the extended circadian system. We further determined that the geniculohypothalamic tract (GHT: linking the IGL to the SCN) was also functional in these preparations: both electrical and optogenetic stimulation of the IGL evoked GABAergic inhibitory responses in the SCN. Moreover, we found that optogenetic stimulation of GHT neurons inhibited the SCN response to OT stimulation, demonstrating functional convergence of retinohypothalamic tract (RHT) and GHT afferents on a subset of SCN neurons. In conclusion, this novel slice preparation is a valuable tool for investigating the functional connectivity of the extended circadian system.

## SC4. "Orexins/Hypocretins modulate the activity of NPY-positive and -negative neurons in the rat intergeniculate leaflet via $OX_1$ and $OX_2$ receptors."

<u>Palus K.</u>, Chrobok L. and Lewandowski M.H. <u>Poland</u>

Orexins (OXA and OXB) are two hypothalamic peptides involved in many physiological processes as regulation of sleep wake cycle, food intake and arousal. The orexinergic system of the lateral hypothalamus is considered to be a major non-specific system. One of the brain nuclei innervated by orexinergic cells is the intergeniculate leaflet of the thalamus (IGL) - the small but important structure of the mammalian biological clock. It consists of GABAergic neuronal subpopulations characterized by their specific neuropeptide synthesis. The first group of neurons produces neuropeptide Y (NPY) and the other, produces enkephalin. The aim of our study was to evaluate the electrophysiological effects of orexins on the NPY-positive and -negative subpopulations of the IGL. Moreover, the receptor type and localization was established.

Most of the investigated neurons were sensitive to the OXA administration during our *in vitro* patch clamp studies. In addition, both NPY-positive and -negative neurons were depolarized by the substance. Our data suggest the similar participation of both orexin receptors in the recorded OXA effects. It is also important to mention, that IGL neurons were either directly depolarized by OXA or the effect was network dependent. Moreover, we have observed the increase of the GABA release onto the investigated neuron after OXA application, what suggest the orexin receptors localization on the presynaptic terminals. In conclusion, our results prove the complicated and sophisticated interplay of excitatory and inhibitory modulation of IGL network by the orexinergic neurons.

[This work is supported by NCN grant: 2013/09/B/NZ4/00541]

**SYMPOSIUM 2: CLOCKS AND METABOLISM** 

Chair: David Bechtold (UK)
Sponsored by: The British Society for Neuroendocrinology

#### S5. AK REDDY

#### "Metabolic Oscillations in the Circadian Clockwork."

Akhilesh B. Reddy

University of Cambridge, Department of Clinical Neurosciencs / Biochemistry, Wellcome Trust-MRC Institute of Metabolic Science, Cambridge CB2 0QQ, UK

Much is now understood about the nature of transcriptional oscillations in multiple organisms. Until recently, however, little was known about non-transcriptional mechanisms that may contribute to circadian timekeeping. Our recent work in red blood cells and marine algae pointed towards redox oscillations playing an unanticipated role in 24 hour timekeeping. A family of proteins called the peroxiredoxins appear to be key readouts of the non-transcriptional clockwork, and their circadian oscillation is, remarkably, conserved in all phylogenetic domains, including bacteria, archaea and eukaryotes. Thus, redox mechanisms are likely to be deeply embedded within the clockwork of multiple species, in stark contrast to the lack of evolutionary conservation of transcriptional components of the clockwork. Our recent work highlights the likely importance of central carbon metabolism in modulating the transcriptional oscillator, suggesting one possible link between non-transcriptional and transcriptional clock networks.

### S6. URS ALBRECHT

### "Liver Period 2 is Necessary to Regulate Food Anticipation."

Rohit Chavan, Céline Feillet, Sara Fonseca, James E. Delorme, Takashi Okabe, Jürgen A. Ripperger and Urs Albrecht

University of Fribourg, Switzerland

Mammals can anticipate food availability recurring at a particular time of the day. This daily anticipation is independent of the suprachiasmatic nuclei (SCN) the central pacemaker of the circadian system. Therefore the question arises where the food anticipatory signal originates and what role components of the circadian clock may play. To address this question we generated tissue specific Per2 knock-out mice. A liver specific deletion of Per2 abolished food anticipation (FA), which was rescued by viral overexpression of Per2 in the liver. RNA sequencing revealed that enzymes of the ketonebody metabolism are deregulated in these animals. Timed application of  $\beta$ -hydroxybutyrate rescued the FA phenotype in liver Per2 knock-out mice. We conclude that liver Per2 is necessary for FA and that it regulates FA at least in part via modulation of  $\beta$ -hydroxybutyrate levels in the serum.

#### S7. JON JOHNSTON

"Meal timing regulates the human circadian systemMeal timing regulates the human circadian system"

Jon D. Johnston

Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK

Timed feeding is recognised as a powerful entraining stimulus for rodent circadian rhythms. Peripheral clocks appear most sensitive to food entrainment, while SCN

rhythms entrain to food timing in a hypocaloric state. Many rodent studies have used extreme restrictions on food availability to demonstrate food entrainment. Furthermore very few studies have investigated the effects of timed feeding on the human circadian system.

This talk will present data from a study testing the hypothesis that a 5-hour delay in meal times delays the phase of peripheral rhythms in humans, without altering markers of SCN rhythmicity. Data are derived from subjective questionnaires, together with analysis of serial blood samples and biopsies. Results from the study demonstrate that meal times regulate the human circadian system and can alter the phase relationship between individual circadian rhythms.

#### **Short Communications**

# SC5. "Absence of effects of Sir2 knockdown on *Drosophila melanogaster* circadian rhythms."

Sangeeta Chawla and Emma Lord
Department of Biology, University of York, York YO10 5DD, UK

The NAD+-dependent deacetylase SIRT1 can modulate mammalian circadian rhythms by deacetylating BMAL1 and PER2, which are components of the transcriptionaltranslation feedback loop with orthologs in Drosophila. Mammalian SIRT1 and the Drosophila homolog Sir2 deacetylate many conserved transcription factors, including FOXO proteins, to regulate stress resistance and energy metabolism. Here, we investigated whether Sir2 is important for sustaining robust circadian behavioral rhythms in Drosophila. We find that flies deficient in Sir2 have normal circadian rhythms of locomotor activity. In contrast, we find that Class IIa histone deacetylases (HDACs), which can also regulate FOXO deacetylation in both mammals and flies, are conserved regulators of circadian function. In mouse fibroblasts, over-expression of HDAC5 severely impairs transcriptional rhythms of core clock genes. Gene disruption of the Drosophila homolog HDAC4 leads to a loss or weakening of locomotor activity rhythms of flies and decreased period mRNA levels. RNAi-mediated knockdown of HDAC4 in Drosophila clock cells also dampens circadian function. We observe cyclical nucleocytoplasmic shuttling of HDAC5 in mouse fibroblasts that is characteristically circadian. Given that the localization of Class IIa HDACs is influenced by reactive oxygen species, Ca2+ and cAMP signals, these findings offer a mechanism by which extracellular stimuli that generate these signals can feed into the circadian timekeeping machinery.

# SC6. "Insulin resets the circadian clock in "non-metabolic" cells via induction of clock gene *PER2.*"

Priya Crosby and John O'Neill

MRC Laboratory of Molecular Biology, Cambridge, UK

The mammalian circadian clock is an approximately 24 hour rhythm that is reset by a number of physiological and environmental cues. Prominent among these is feeding time, which can reset the clock in peripheral tissue. When this occurs out of phase with other timing cues, such as light, the result is a disruption of internal timing — associated with

an increased risk of pathologies such as type II diabetes and obesity. However, the mechanism by which feeding resets the clock in peripheral tissues is not well understood. Previous work has suggested that the metabolic hormone insulin plays a role in food-resetting circadian rhythms in liver and adipose tissue, but has not been able to explain the basis of this resetting in other "non-metabolic" tissue types. Here, we show that administration of insulin at physiologically-relevant concentrations stimulates acute expression of the circadian clock gene *PER2* in a range of non-metabolic cell types. This insulin-induced increase in PER2 protein is sufficient to modulate key parameters of the cellular clock in a dose-dependent, phase-independent manner. These effects are independent of glucose availability, but are reliant on a number of components of the insulin receptor signalling pathway. Our data suggest that insulin may be sufficient to account for food-resetting of mammalian circadian rhythms.

# SC7. "Conserved circadian rhythms in intracellular [Mg<sup>2+</sup>] determine clock features and global energy metabolism."

Gerben van Ooijen

School of Biological Sciences, University of Edinburgh, UK

Circadian rhythms are a fundamental property of cellular life, regulating metabolism to match our planet's day/night cycle. A substantial knowledge gap exists between cycles of clock-controlled gene expression and the biochemical mechanisms that ultimately facilitate cell-autonomous metabolic rhythms.

We identified clear circadian rhythms in intracellular magnesium concentrations in two species that diverged more than 1 billion years ago; the unicellular marine alga *Ostreococcus* tauri and human U2OS cells. Magnesium oscillations feed back to determine the amplitude, phase, and free-running period of the transcriptional clock.

Magnesium ions are essential cofactors for ATP, the key intermediate of energy metabolism. We found that regulation of cellular energy expenditure over the daily cycle is a functional consequence of oscillating [Mg<sup>2+</sup>]. Our results therefore suggest that magnesium oscillations represent a key clock mechanism that links gene expression rhythms to metabolic rhythms in evolutionarily diverse cell types.

# SC8. "Effects of regular feeding and adrenalectomy on daily gene expression rhythms in rat white adipose tissue."

Yan Su<sup>1</sup>, Ewout Foppen<sup>2</sup>, Zhi Zhang<sup>2</sup>, Eric Fliers<sup>2</sup> and Andries Kalsbeek<sup>1,2</sup>

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In order to synchronize tissue-specific rhythms in gene and protein expression with the environmental day/night changes, the master clock in the hypothalamic suprachiasmatic nucleus (SCN) has to deliver its rhythmic information to the periphery. This rhythmic information is thought to be transmitted via hormonal, neuronal and/or behavioral pathways. The daily feeding and plasma corticosterone rhythm are considered important signals for synchronizing peripheral rhythms, because both restricted feeding and corticosterone injections can shift the phase of (clock) gene expression rhythms in the periphery. In this study, we took the opposite approach and investigated the necessity of

an intact feeding and/or adrenal hormone rhythm for the maintenance of daily clock and metabolic gene rhythms in white adipose tissue (WAT). We show that WAT clock gene rhythms are not abolished during prolonged exposure to a regular feeding schedule or adrenalectomy, whereas most of the rhythmic metabolic/ adipokine genes in WAT lost their rhythmicity. Therefore, subsequently we investigated the effect of a simultaneous disruption of these humoral and behavioral signaling pathways, by exposing animals to adrenalectomy and a regular feeding schedule. Interestingly, under these conditions all the clock genes and >90% of the rhythmic metabolic/adipokine genes in WAT lost their rhythmicity. These data indicate that corticosterone and a daily feeding rhythm are indispensable for the maintenance of daily rhythms in metabolic/adipokine gene, but not clock gene, expression in WAT. One the other hand at least one of these two signals should be present in order for WAT clock gene rhythms to be maintained.

### SC9. "RNA sequencing shows disturbed adipose tissue clock rhythms in patients with type 2 diabetes."

<u>Dirk J. Stenvers</u>, Aldo Jongejan, Sadaf Atiqi, Jeroen P. Vreijling, Edward J. Bradley, Eelkje J. Limonard, Frank Baas, Perry D. Moerland, Eric Fliers, Andries Kalsbeek and Peter H. Bisschop

Department of Endocrinology and Metabolism, Academic Medical Center (AMC), University of Amsterdam, Amsterdam, The Netherlands

Background: Adipocyte clock genes are involved in the regulation of adipocyte differentiation and lipid metabolism. Rodent studies suggest that disturbed adipocyte clock rhythms may contribute to the pathogenesis of obesity and type 2 diabetes (T2DM). We aimed to determine adipose tissue gene expression rhythms in obese human subjects with T2DM.

Methods: We included six obese males with T2DM and six age-matched healthy lean control males. For three days, all participants consumed three identical mixed meals per day at ZeitgeberTime (ZT) 0:30, 6:00 and 11:30. We obtained adipose tissue samples from abdominal subcutaneous tissue on day2 at ZT 15:30 and on day3 at ZT 0:15, 5:45 and 11:15. We performed genome-wide detection of gene expression rhythms with RNA sequencing and analysis with a generalized linear model.

Results: Patients with T2DM showed a six-fold reduction in the number of rhythmic genes (100 genes) compared to healthy subjects (611 genes). Of the rhythmic genes common to both groups (78 genes), most genes showed smaller amplitudes in the patients with type 2 diabetes. The genes with the largest between group amplitude differences included the clock genes PER1, PER3 and NR1D1, and the triglyceride-synthesizing gene DGAT2.

Conclusion: We present the first *in vivo* evidence of altered adipose clock functioning in human T2DM. Our findings agree with animal studies, and support the hypothesis that the adipocyte clock is involved in the pathophysiology of obesity and T2DM. Moreover, we identified several candidate genes that may constitute the link between the adipocyte clock and metabolic disease.

# SC10. "Metabolism and its influence on human phase of entrainment and sleep coupling."

Emma J. Wams, Igor Hoveijn, Heleen M. Rinsema, Laura van Rosmalen, Moniek Geerdink and Roelof A. Hut

Chronobiology, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Netherlands

From the seminal forced desynchrony studies of Jürgen Aschoff core body temperature rhythms are known to follow the circadian clock whereas sleep timing can be decoupled from the body's internal timing (Aschoff, 1981). Other metabolic outputs are known to be influenced by the clock, for example, heart rate and skin temperature. There are known feedback of metabolic factors on sleep timing with skin temperature influencing sleep onset timing (Raymann et al., 2008). Furthermore, despite thermoregulation not being observed to be modulated by the sleep homeostat, evidence suggests that the relationship between sleep and the clock are thermoenergetically modulated (Kräuchi et al., 2006). As well as the large variation in the phase of entrainment of humans, there is also variation in the phase relationship between sleep timing and circadian phase (as determined by melatonin profiling). It therefore follows that this phase relationship could be explained by the variation of metabolic rate between individuals. Our study of humans, conducting the unlimited activities of daily living, used ambulatory devices to monitor heart rate, core body temperature, skin temperature and activity as well as melatonin profiling. By determining the phase relationships we seek to elucidate how the relationship between chronotype (midsleep on freedays; MCTQ, Roenneberg et al., 2007), sleep onset (actigraphy and EEG) and circadian phase (melatonin profiling) are explained by the variance in these metabolic proxies. Data, currently being collected, will be presented to explain the nature of metabolic feedback and regulation of sleep and circadian system coupling.

### **SYMPOSIUM 3: TEMPORAL NICHE**

Chair: Etienne Challet (France)

#### S8. ETIENNE CHALLET

"Nocturnality Versus Diurnality: Mechanisms Within or Downstream of the Master Clock?"

**Etienne Challet** 

Dept Neurobiology of Rhythms, Institute of Cellular and Integrative Neurosciences, CNRS, University of Strasbourg, Strasbourg, France

The cerebral mechanisms underlying the differences between diurnal and nocturnal species are not well understood yet. Organization of the master clock in the suprachiasmatic nuclei (SCN), and its resetting properties to light show large similarities between nocturnal and diurnal animals. Furthermore, the phase of oscillations of circadian genes such as *Per1*, *Per2* and *Bmal1* according to the light-dark cycle is comparable in the SCN of both categories. Therefore, the current view is that the switch between diurnal and nocturnal species occurs mainly downstream of the SCN clock. Recent comparative analysis, however, indicated that the expression of the circadian gene *Clock* is respectively rhythmic and constitutive in the SCN of diurnal and nocturnal rodents, thus suggesting a transcriptional difference in the SCN. Furthermore, besides light, nonphotic stimuli, such as scheduled voluntary exercise or sleep deprivation, are also capable of shifting the master clock, providing behavioural feedback effects on the master clock. In nocturnal rodents, nonphotic behavioural cues are well known to reset

the master clock during the subjective day (resting period) and reduce light-induced phase-shifts. Of note, nonphotic behavioural cues can reset the SCN of diurnal rodents during the subjective night (resting period). Also in diurnal rodents, there is an opposite modulation (i.e., up-regulation of photic resetting at night) by behavioural cues, including sleep deprivation, caffeine injection and serotonergic receptor activation. Taken together, these findings indicate that differences between diurnal and nocturnal mammals include distinct features within the SCN clockwork and differential effects of arousal feedback to the master clock.

### S9. NICK FOULKES "When to Go Fishing...?!"

Nicholas S. Foulkes, Cristina Pagano and Daniela Vallone Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Eggenstein, Germany

Fish represent by far the largest and most diverse vertebrate group, and have successfully adapted to a myriad of different environmental niches. Furthermore, fish genetic models such as the zebrafish offer us powerful tools and approaches for exploring the basic cellular and molecular mechanisms which underpin the circadian timing system. In comparative studies involving zebrafish, medaka and blind cavefish we are investigating how environmental signals such as light and food availability are integrated by the circadian clock and thereby define the phase of activity for a given species. As well as identifying the basic mechanisms underlying this process, we also aim to understand how and why these control systems are modulated during vertebrate evolution in response to a changing environment.

#### S10. ROELOF HUT

"The Flexible Chronotype: Energy Balance and Predation Risk Determine the Circadian Phase of Entrainment."

Roelof A. Hut, Vincent van der Vinne, J. (Sjaak) Riede, Emma Wams and Violetta Pilorz Chronobiology unit, Groningen Institute for Life Sciences, University of Groningen, Netherlands

In nature, the light-dark cycle can be perceived as a predictor for the daily temperature cycle. The notion that the night is colder than the day and that sleep is associated with better thermal insulation, we derived the circadian thermoenergetics hypothesis (CTE). CTE predicts that nocturnal activity patterns are energetically costly and that nocturnal mammals will save energy by becoming diurnal. We further deduced that nocturnality only exists to avoid increased predation risk during daytime. Animals We tested this hypothesis by reducing energy intake or increasing energy expenditure. In both cases, mice indeed become diurnal while reorganising their circadian organisation in the body. As theoretically predicted, nocturnality can be reinstalled by increasing perceived predation risk. This suggests that the main circadian pacemaker (suprachiasmatic nucleus, SCN) drives slave oscillator system involved in sleep-wake regulation, which is sensitive to energy balance and anxiety. In humans, internal desynchronisation studies and correlations between chronotype and body mass indeed suggest that the duration of

the sleep-wake cycle is sensitive to sponateous variation in energy balance and metabolic rate.

#### S11. LAURA SMALE

"Temporal Niche Transitions: From Questions to Data to Speculation and Back."
Laura Smale
Michigan State University, USA

Evolutionary transitions from one temporal niche to another have been associated with a multitude of adaptive changes, most fundamentally in systems that coordinate activity and physiological functions in a dynamic way across the day/night cycle. This has included transformation of mechanisms responsible for coupling among circadian oscillators, as well as mechanisms that mediate direct effects of photic stimuli on behavior. The primary oscillator, within the suprachiasmatic nucleus, appears to be very similar with respect to its phase relative to a light-dark cycle in several nocturnal and diurnal species. Beyond the SCN, however, striking differences emerge in the patterning of oscillators when diurnal Nile grass rats are compared with nocturnal rodents, and many of these patterns are highly plastic. In addition, at least two interconnected regions that receive direct input from intrinsically photoreceptive retinal ganglion cells may play important roles in maintenance of diurnality in Nile grass rats: the intergeniculate leaflet and the olivary pretectal area. Lesions of each of these structures render the animals more nocturnal through their effects on direct responses of brain and behavior to light, as well as on endogenously driven activity patterns. In both cases the effects are phase dependent, but the actual patterns subsequent to the lesions are not the same. These data will be discussed within the broader context of issues related to how the brain has changed across evolutionary transitions from one temporal niche to another.

#### Short Communications

#### SC11. "New insights into the genetics of diurnal/nocturnal preference."

Eran Tauber and Mirko Pegoraro

Dept. of Genetics, University of Leicester, UK

Under laboratory conditions, *Drosophila* is crepuscular, showing a bi-modal activity profile. However, recent experiments in our lab indicated that high variability among individuals exist, particularly in strains that derive from different wild populations. By assembling together flies from different populations, we have generated a highly diverse population whose progeny exhibited extreme diurnal preference, including diurnal and nocturnal flies. We have used this population as a starting point for an artificial selection experiment in which we selected males that show the most extreme diurnal preference and mated them to their sisters. The response to selection was strong, and after 10 selection cycles we obtained highly diurnal (D) and nocturnal (N) strains. Another strain that was not selected and showed intermediate behaviour (crepuscular) served as a control (C). These strains provide us with a unique opportunity to understand the genetics of diurnal preference.

We have profiled gene expression in these lines using RNAseq and identified 209 differentially-expressed genes. In another set of experiments, we tested for genetic

correlation of diurnal preference with other trait phenotypes, including those related to the circadian clock, and life history traits. We also explored the visual system in the selected lines and found a significant difference in their electroretinograms (ERG).

Overall our study encompasses a broad range of approaches including quantitative genetics, microbiology and electrophysiology within a whole-organism context, and provide the first step for understanding the impact of variation in diurnal preference in a wide range of animals, including humans.

### SC12. "Search for the thermosensors involved in temperature dependent negative masking behavior in mice."

Wataru Ota<sup>1</sup>, Makiko Kashio<sup>2</sup>, Makoto Tominaga<sup>2</sup> and Takashi Yoshimura<sup>1</sup>

Graduate School of Bioagricultural Sciences, Nagoya University, <sup>2</sup>National Institute for Physiological Sciences (Okazaki Institute for Integrative Bioscience)

Adaptations to environmental changes are crucial to the survival of animals. Masking behavior is thought to be an acute adaptive response to environmental changes. However, the regulatory mechanisms of masking behavior are not well understood. Here we report that ambient temperature cycles induce negative masking behavior in mice. We were originally interested in the function of a novel UV-sensitive opsin (OPN5), which is expressed in the mouse brain. We first observed decreased locomotor activity (negative masking response) in blind mice during UV light exposure. Since the eye is believed to be the only photoreceptive organ in mammals, we examined if this response was light-dependent by injecting India ink under the scalp. The observed result suggested that the temperature changes caused by the UV light source trigger the negative masking response. Accordingly, we examined the effect of various ambient temperature cycles (24/24°C, 24/26°C, 24/28°C, 24/30°C, 24/32°C, 24/34°C) on locomotor activities under constant darkness. As a result, the increase in the masking ratio was directly proportional to the increase in temperature difference. Thus, the negative masking responses of blind mice were corroborated as temperature-dependent behavior.

Transient receptor potential channels (TRP channels) are known as the main thermosensors in mammals. Now, we are exploring the thermosensors that mediate temperature-dependent masking behavior by using several TRP channel knockout mice.

# SC13. "Sleep deprivation and caffeine treatment potentiate photic resetting of circadian clock in diurnal rodent, Sudanian grass rat (*Arvicanthis ansorgei*)."

Pawan Kumar Jha<sup>1,3,4</sup>, Hanan Bouaouda<sup>1</sup>, Sylviane Gourmelen<sup>1</sup>, Andries Kalsbeek<sup>2,3,4</sup> and Etienne Challet<sup>1,4</sup>

<sup>1</sup>Regulation of Circadian Clocks team, Institute of Cellular and Integrative Neurosciences, UPR3212, Centre National de la Recherche Scientifique (CNRS), University of Strasbourg, France, <sup>2</sup>Department of Endocrinology and Metabolism, Academic Medical Center (AMC), University of Amsterdam, The Netherlands, <sup>3</sup>Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands, <sup>4</sup>International Associated Laboratory LIA1061 Understanding the Neural Basis of Diurnality, CNRS, France and the Netherlands.

Circadian rhythms in nocturnal and diurnal mammals are primarily synchronized to local time by the light-dark cycles. However, non-photic factors, such as behavioural arousal

and metabolic cues, can also phase-shift the master clock in the suprachiasmatic nuclei (SCN) and/or reduce the synchronizing effects of light in nocturnal rodents. In diurnal rodents the role of arousal or insufficient sleep in these functions has not been studied intensively, yet. In the present study, we examined the effects of behavioural arousal (sleep deprivation by gentle handling and caffeine treatment) on the circadian pacemaker and its responses to light in the diurnal Sudanian grass rat, Arvicanthis ansorgei. Phaseshifts of locomotor activity were analysed in grass rats transferred from a light-dark cycle to constant darkness and aroused in early night (sleep deprivation from CT12 to CT16 or caffeine injection at CT14) and mid-night (sleep deprivation from CT16 to CT20 or caffeine injection at CT18). Early night sleep deprivation induced significant phase-shifts (delays). Furthermore, both sleep deprivation and caffeine treatment potentiated lightinduced phase-delays and phase-advances in response to a 30-min light pulse at CT16 and CT20, respectively. Sleep deprivation in early night potentiated light-induced c-Fos expression in the ventral SCN. Caffeine treatment in early and mid-night induced c-Fos expression in dorsal SCN and potentiated light-induced c-Fos expression in calbindin cells in the ventral SCN. These findings indicate that behavioural arousal, induced either by sleep deprivation or caffeine, potentiates light resetting of the master circadian clock in part by activating calbindin-containing neurons of SCN in diurnal rodents.

# SC14. "Relationship between locomotor activity and body temperature timing and energetic challenges under energetic challenges: a lab and field approach in a subterranean rodent."

<u>Patricia Tachinardi</u><sup>1</sup>, Øivind Tøien<sup>2</sup>, Veronica S. Valentinuzzi<sup>3</sup>, C. Loren Buck<sup>4</sup> and Gisele A. Oda<sup>1</sup>

<sup>1</sup>Instituto de Biociências, Universidade de São Paulo, Brazil, <sup>2</sup>Institute of Arctic Biology, University of Alaska Fairbanks, USA, <sup>3</sup>Centro Regional de Investigaciones Científicas y Transferencia Tecnológica (CRILAR), Argentina, <sup>4</sup>Department of Biological Science, University of Alaska Anchorage, USA

The tuco-tuco, Ctenomys aff. knighti, is a subterranean rodent from a semi-arid region of Argentina. It is nocturnal in the lab, but in the field it is seen outside its burrows, daily, during the daylight hours. Energetic challenges, which differ between field and lab, could trigger this temporal pattern of activity. In laboratory, animals are fed ad libitum and kept at a constant ambient temperature (Ta), while in nature they expend energy with foraging and face large Ta variations. The "circadian thermo-energetics hypothesis" (Hut et al., 2012) proposes that daytime activity could be a response to energetic challenges by allowing the animal to maintain lower body temperature (Tb) and rest during the coldest hours of the night, in burrows where Ta is higher and assuming postures that increase insulation. This work presents results which could validate this hypothesis for the tucotucos. First, Tb measures in outdoor enclosures showed a greater concentration of high Tb values during the day daylight hours in the enclosure than in the laboratory. In order to investigate if diurnal activity would contribute to energy savings in the tuco-tuco, surface and burrow Ta's were measured for a year. The results show that while daily surface Ta range can be over 15oC, it is less than 1oC at 40cm underground. Finally, measures of energy expenditure at different Ta were carried out in the lab and show that, bellow 26oC, energy expenditure increases with Ta, suggesting that staying in the burrow during the night would save energy with thermoregulation.

### SYMPOSIUM 4: MODELLING: FROM OSCILLATORS TO TOOLS FOR THE REAL WORLD

Chairs: Casey Diekman (USA) and Daniel Forger (USA)

S12. CASEY DIEKMAN

"Modeling Circadian Rhythmicity of Cardiac Arrhythmias."

Joseph Zaleski and Casey Diekman

New Jersey Institute of Technology, Newark, NJ USA

The cardiomyocyte circadian (~24-hour) clock influences multiple intracellular processes, including transcription and contractile function, and has recently been linked to ventricular arrhythmias in mice (Jerayaj *et al.* (2012) Nature 483:96-100). Circadian rhythms have also been observed in transient outward potassium current ( $I_{to}$ ) – a current that dominates mice action potential (AP) repolarization. We used mathematical modeling to study the dynamical mechanisms underlying secondary oscillations during the repolarization phase of the AP. These oscillations, called early afterdepolarizations (EADs), have significance because they are associated with heart failure and arrhythmias. It can be shown numerically and analytically that EADs arise from a Hopf bifurcation and that this can occur for certain ranges of the  $I_{to}$  conductance (Zhao *et al.* (2012) Cardiovascular Research 95:308-316). We investigated how variation of calcium and  $I_{Ks}$  potassium conductances affects the range over which EADs occur. This allows us to predict the role circadian regulation of currents other than  $I_{to}$  could play in cardiac activity. Finally, we compare our results on daily rhythms in EADs to existing data on the times of day that humans are most likely to suffer sudden cardiac death.

S13. OLIVIA WALCH

"A Global Assessment of Sleep Schedules Using Smartphone Data."

Olivia Walch, Amy Cochran and Daniel Forger

Department of Mathematics, University of Michigan, USA

How does the world sleep? Controlled laboratory experiments can begin to answer this question but are constrained by sample size, budget, and space. In contrast, mobile technology makes it possible to cheaply conduct large-scale studies that assess "real world" sleep, at the potential expense of greater user heterogeneity and reporting error. Here we discuss data collected through a mobile application which surveyed users about their typical sleep schedule and light. We find that women schedule more sleep than men and that users reporting they are typically exposed to outdoor light go to sleep earlier, wake earlier, and sleep more than those reporting indoor light. We note that cultural influences on sleep appear to be exerted primarily through when people choose to go to bed, even though wake time can best explain differences in scheduled amount of sleep at the individual level. We further probe these and other findings through the use of mathematical modeling.

S14. TILL ROENNEBERG "Automatic Chronotyping." Till Roenneberg

### Institute for Medical Psychology, LMU, Munich, Germany

Three clocks control our lives, the social clock organising, for example, our school and work times, the circadian clock controlling our biology, and the sun clock, which is the zeitgeber for our circadian clock. These clocks used to coincide, but have drifted apart in our recent history: the introduction of time zones separated social time and sun time and living progressively indoors has separated our circadian time from sun- and social time. Artificial light has greatly enhanced this so-called 'social jetlag', which challenges physical and mental health, sleep and cognitive performance. A quantification of these discrepancies relies on assessing individual internal time (chronotype).

Chronotyping has diverse aims: e.g., to better understand the aetiology of syndromes, to assess the outcome of interventions, to optimise medical diagnostics and therapies, or to individualise work schedules.

Several methods have been used to chronotype individuals in real life, e.g., questionnaires (MCTQ), actigraphy, and melatonin measurements. However, the first two rely on additional information (e.g., knowing work- and free days) while measuring melatonin is only accurate under certain conditions (e.g., dim light), is labour-intensive and costly.

We are currently developing an automated method for assessing internal time that solely based on actimetry (and light), independent of knowing weekly structures. This is important, e.g., for patients or people who always use alarm clocks. We also attempt to replace actual light measurements by light simulations and modelling.

#### Short Communications

### SC15. "Estimation of cellular phase response curve through a spatiotemporal pattern in plant roots."

Hirokazu Fukuda and Kazuya Ukai

Department of Mechanical Engineering, Graduate School of Engineering, Osaka Prefecture University, Japan

Plant circadian clocks entrain for environmental cycles through phase response curves (PRCs). PRC is described as a response of phase of circadian rhythm to transient stimulus. The phase response occurs essentially in each clock cell. Therefore, the modelling of entrainment requires the consideration of cell population: Plant circadian systems are composed of a large number of self-sustained cellular circadian oscillators. In previous studies, however, the individual-level responses in intact plants were ordinary investigated.

In this study, PRC was estimated from circadian rhythm of cell population in intact plant root. Plant root shows a regular circadian phase pattern, called stripe wave which travels along the primary root from basal to the root tip. The striped phase pattern can be observed spatially with high resolution using the bioluminescence of transgenic *Arabidopsis thaliana CCA1::LUC*. To observe phase response of circadian rhythm in every region of root, we imposed temperature change pulse (30 minutes, +3°C) to the root. We demonstrated that the stripe wave in roots included all phases of circadian rhythm at the same time and the PRC can be described from only one trial. In addition, we predicted and analysed the cellular response of the circadian clock to the temperature pulse using a phase oscillator model (Fukuda et al., (2012)). Finally, we have succeed to describe the cellular PRC in the root circadian clock associated with model simulations.

# SC16. "Investigating rhythmicity of gene expression in human blood and application for estimating body and sampling time."

<u>Karolina Lech</u>, Fan Liu, Katrin Ackermann, Victoria L. Revell, Debra J. Skene and Manfred Kayser

Department of Forensic Molecular Biology, Erasmus MC University Medical Centre Rotterdam, 3015 CN Rotterdam, The Netherlands

Determining a person's body time is of great value, especially for chronotherapy, as it would allow for maximizing the therapeutic effect of the medicine and minimizing its adverse effects on the patient. We investigated mRNA as source of potential biomarkers for predicting the collection-time of a single sample, which would allow for inferences of internal body time. From a set of candidate genes for which we established the expression patterns in blood samples of several individuals collected under controlled conditions at 2 h intervals for 36 h, we identified several mRNA markers with statistically significant daily expression rhythms i.e., BMAL1, HSPA1B, MKNK2, PER1, PER2, PER3, STAT3, THRA1 and TRIB1. We then used the selected mRNA markers with and without melatonin and cortisol also measured in these samples, to establish various multinomial regression models for predicting sample collection-time categories from the molecular data. In general, prediction models considering only mRNA markers provided less accurate sample collection-time estimations than those based only on hormones. Improved prediction accuracy was observed when combining three mRNA markers HSPA1B, MKNK2 and THRA1 with the two hormones melatonin and cortisol. This model was favoured by our data, showing an improved performance in predicting three sample collection-time categories: night/early morning, morning/noon, and afternoon/evening. Therefore, we demonstrate the promising value of rhythmically expressed mRNAs for estimating sample collection/body time, especially when used in combination with circadian hormones.

# SC17. "Entrainment of the mammalian circadian clock by metabolism in peripheral organs: a quantitative mathematical model."

Aurore Woller<sup>1,2</sup>, Hélène Duez<sup>1</sup>, Bart Staels<sup>1</sup> and Marc Lefranc<sup>2</sup>

<sup>1</sup>UMR 1011, "Nuclear Receptors, Cardiovascular Diseases, and Diabetes", Université Lille 2, Institut Pasteur de Lille, INSERM, Lille, France and Laboratoire de Physique des Lasers, <sup>2</sup>Atomes, Molécules, Université Lille 1, CNRS, UMR 8523, Villeneuve d'Ascq, France

To keep the time of day, circadian clocks need to be synchronized to the diurnal cycle by some environmental cue. At organismal level, daylight is the main signal driving the clock. In multicellular organisms, however, clocks in peripheral organs respond to other cues. For example, the liver clock is primarily synchronized by the fasting/feeding cycle and variations in the cellular metabolic state, as reflected by the NAD+/NADH and ATP/AMP ratios. To better understand the metabolic entrainment of peripheral circadian clocks, and more generally how the circadian and metabolic systems integrate with each other, we have constructed a mathematical model of the mammalian circadian clock incorporating the metabolic sensors SIRT1 and AMPK. This model reproduces accurately experimental clock gene expression data from mouse livers in vivo, and predicts correctly the effect of SIRT1 or AMPK loss-of-function. We used our mathematical model to investigate the response of the liver clock to various temporal patterns of AMPK

activation, mimicking the effect of a normal diet, of fasting and of a high-fat diet. Our results predict dramatic changes in NAD+ time profiles and average levels between these situations. They suggest that the night peak in NAD+ level is due to circadian rythms in NAMPT expression, while the day peak results from regulation of NAMPT by AMPK. Finally, we find that the loss of amplitude in expression rhythms observed when AMPK is depressed may be pharmacologically rescued using a REV-ERB agonist.

### SC18. "Phase organization of clock neurons and its implications for mood disorders"

<u>Forger, D.B.</u><sup>1</sup>, Myung, J.<sup>2</sup>, Belle, M.D.C<sup>3</sup>, Cochran, A.<sup>1</sup>, DeWoskin, D.<sup>1</sup>, Joshi, A.<sup>3</sup>, Stinchcombe, A.<sup>1</sup>, Walch, O.J.<sup>1</sup>, Takumi, T.<sup>2</sup> and Piggins, H.<sup>3</sup>

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Circadian (daily) timekeeping within our body regulates a surprising number of physiological processes including mood. Mood regulation is a prime example of the body's use of timekeeping information.

The site of circadian timekeeping in the brain is the suprachiasmatic nucleus (SCN). The inhibitory neurotransmitter, GABA, is contained in all SCN cells, but its role in circadian timing remains unclear. Our work studies the connection between SCN states and mood phenotypes.

Our modeling and experimental studies show how SCN neurons can signal through GABA, while simultaneously using GABA to determine time of day. We show how depolarized states of SCN neurons can be geared by the intracellular chloride concentration as a function of light duration during a day. We predict that novel depolarized neuronal states send tonic GABA signals that generate and co-ordinate circadian timekeeping. Phasic GABA is used to control the firing of action potentials and allows individual neurons to send of a variety of output signals without affecting circadian timekeeping. This highlights how a neuronal system can simultaneously code for a variety of signals with one signaling molecule.

These findings enable understanding of how the SCN encodes the balance of night and day, to signal annual daylength changes to the body, and potentially the basis of seasonal affective disorder. Our results show the relative balance of excitatory and inhibitory GABA within the SCN encodes daylength.

# SC19. "UCHRONIA: Practice-led research project at the intersection of design, chronobiology and chronosociology."

Helga Schmidt

UK

Within the last 200 years, the Western world has gone through a process of transformation from an agricultural to an urbanised 24/7 society. The shift in dominance from natural time to the mechanical clock has significantly influenced biological and social rhythms. Modern technology has led to an increasing temporal fragmentation, heralding an era of flexible time with ever more complex processes of synchronisation. The prevailing clock-based time persisted with greater precision (atomic clock), but societal

synchronisation dynamics have changed over time due to digital technologies. For instance, punctuality gave way to flexibility, which is now the decisive factor in the pace of postmodern or 'hypermodern' life. Our society is described as 'instant network society' or 'digital society', suffering from increasing time pressure and the acceleration of time. The natural rhythmicity of the human biological clock, however, conflicts with such contemporary algorithmic structures and inhumane rhythms.

My practice-based research investigates an alternative time system based on the human circadian rhythm. It explores the possibility to think outside the boundaries of clocks, and calendars. In an interdisciplinary approach, the research combines two theoretical strands. The first, chronobiology, deals with the temporality of the human body, and the second, chronosociology, investigates the principles and structures of temporal systems in societies. The practice element conceives an alternative temporal system developed and realised in the form of a biotemporal and sociotemporal architectural space. It is an original artistic visualisation and exploration of how scientific research can be translated into a lived, aesthetic experience. In distinct experiments, participants live in a homeorhythmic space, pursuing their own independent rhythm, irrespective of today's temporal organisation.

By developing a temporal utopia, termed uchronia, the project challenges thought patterns regarding the temporal structure of contemporary, technology driven life.

#### SYMPOSIUM 5: TIMEKEEPERS IN THE EYE

Chair: Mario Guido (Argentina)

S15. MARIO GUIDO

"Sensing Light and Time by Inner Retinal Cells of Birds."

Mario E. Guido, Luis P. Morera and Nicolás M. Díaz

CIQUIBIC-Department of Biological Chemistry, Facultad de Ciencias Químicas-Universidad Nacional de Córdoba-CONICET, 5000 Córdoba, Argentina

The vertebrate retina is a main component of the circadian system being responsible for photoreception of the environmental (day/night) lighting conditions and for transmitting this information to the brain, to entrain the suprachiasmatic nucleus master clock and regulate other non-image forming tasks. In birds, we found that retinal ganglion cells (RGCs) are autonomous oscillators synthesizing melatonin during the day. A subset of RGCs were shown to be intrinsically photosensitive (ipRGCs) expressing the photopigment melanopsin (Opn4). ipRGCs temporally regulate photic entrainment of daily rhythms and pupillary light reflexes even in blind GUCY1\* chickens. In non-mammalian vertebrates, two genes encode Opn4: the Xenopus (Opn4x) and the mammalian (Opn4m) orthologs. Opn4m expression is restricted to the RGC layer whereas Opn4x is found in this layer at embryo day 8 (E8), but mostly in Prox1 (+) horizontal cells (HCs) from E15 to postnatal days in control and GUCY1\* retinas. Primary cultures of Opn4x-RGCs and -HCs were found to express different circadian markers, the clock genes Bmal1, Clock, Per2 and Cry1, and the key melatonin synthesizing enzyme, arylalkylamine N-acetyltransferase (AA-NAT). Strikingly, we found that HCs do also intrinsically respond to light of different intensities and durations by increasing intracellular Ca<sup>2+</sup> levels. These observations provide the first evidence for a novel avian retinal photoreceptor type in HCs; and also suggest that Opn4-photoreceptors and endogenous clocks converge all together in these inner retinal cells to further support the circadian system and improve the temporal organization of physiology.

[Supported by ANPCyT-FONCyT (PICT 2010-647/PICT 2013-021), SECyT-UNC and CONICET.]

#### S16. GIANLUCA TOSINI

### "Circadian Organization of the Mammalian Eye."

Gianluca Tosini and Kenkichi Baba

Circadian Rhythms and Sleep Disorders Program, Neuroscience Institute, and Department of Pharmacology & Toxicology, Morehouse School of Medicine, USA

Circadian rhythms are a ubiquitous feature of living systems. The retinal circadian clock was the first extra-SCN (suprachiasmatic nucleus) circadian oscillator to be discovered in mammals and several studies have now demonstrated that many of the physiological, cellular and molecular rhythms that are present in the retina are under the control of retinal circadian clocks. Accumulating evidence suggests that dysfunction of the circadian rhythms due to genetic mutations or environmental factors may contribute to the development of many eye diseases. Experimental data also indicate that other ocular structures may contain circadian clocks that drive circadian rhythms in the eye. Indeed previous studies have reported the presence of a circadian rhythm in PER2::LUC bioluminescence in cultured mouse photoreceptors, inner retina, retinal pigment epithelium and cornea. The circadian clocks in these ocular structures are believed to drive circadian rhythmicity in photoreceptor disk shedding and phagocytosis, axial chamber length, choroidal volume, corneal curvature and cornea thickness. However, it is important to mention that the retinal photoreceptors are the only cells that can communicate the photic signals to the rest of the ocular circadian system in order to maintain the different structures in synchrony with the external light/dark cycle. In the present talk we will describe that circadian organization of the mammalian eye and the role melatonin and dopamine in the entrainment of the extra-retinal ocular circadian clocks.

#### S17. ANNETTE ALLEN

### "Melanopsin Acts as the Retina's Light Meter."

Annette E. Allen, Riccardo Storchi, Franck P. Martial, Timothy M. Brown and Robert J. Lucas

Faculty of Life Sciences, University of Manchester, UK

In bright light, mammals use a distinct photopigment (melanopsin) to measure irradiance for centrally-mediated responses such as circadian entrainment and the pupillary light reflex. We aimed to determine whether melanopsin is also used by the visual system as a signal for light adaptation. To this end we compared retinal and thalamic responses to identical visual stimuli presented using spectral compositions either approximating natural daylight ('daylight'), or selectively depleted of wavelengths to which melanopsin is most sensitive ('mel-low'). We found substantial differences in responses to a range of visual stimuli under these two conditions. These originated with changes in feature selectivity in both temporal and spatial dimensions, and resulted in a richer, more reliable encoding of natural scenes in the 'daylight' condition. These data indicate that melanopsin performs a role analogous to a photographer's light meter, providing an independent measure of irradiance that determines optimal setting for visual circuits.

#### Short Communications

### SC20. "Non-image forming light detection by melanopsin, rhodopsin and L/M cone opsin in the eyes of the blind mole rat, the *Spalax Ehrenbergi*."

Gema Esquiva Sobrino<sup>1</sup>, Aaron Avivi<sup>2</sup> and <u>Jens Hannibal</u><sup>3</sup>

<sup>1</sup>Department of Physiology, Genetics and Microbiology, University of Alicante, Alicante, Spain, <sup>2</sup>Institute of Evolution, University of Haifa, Israel, <sup>3</sup>epartment of Clinical Biochemistry, Bispebjerg Hospital, University of Copenhagen, DK2400, NV Copenhagen, Denmark

The blind mole rat, Spalax Ehrenbergi, can despite severely degenerated eyes covered by fur entrain to the daily light/dark cycle and adapt to seasonal changed due to an intact circadian timing system. The present study demonstrate that the Spalax retina contain photoreceptor layer, outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL). immunohistochemistry, the number of melanopsin bearing retinal ganglion cells (RGCs) was analyzed in details. Furthermore, it was shown that the Spalax retina is rich in rhodopsin and coneopsin bearing photoreceptor cells. Using the ganglion cell marker RBPMS, we found the Spalax eye to contain 890±62 GCL. Of these, 87 % (752±40) contain melanopsin (cell density 788 melanopsin GCL/ mm<sup>2</sup>). The remaining GCLs costore Brn3A and calretinin. The melanopsin cells were located mainly in the GCL and projected to both the IPL and the OPL with few fibres also found in the ONL. Using Ctbp2 as a marker for ribbon synapses we found that in both rod and cone pedicles of the OPL melanopsin dendrites had synaptic contact with both rhodopsin and long/middel wave (L/M) cone opsin photoreceptors in the OPL. A subset of cone bipolar cells and all photo receptor cells contain recoverin while a subset of amacrine cells contains calretinin. The calretinin expressing amacrine cells had synaptic contacts with rhodopsin containing photoreceptor cells in the OPL and in the IPL contact with melanopsin cell bodies and processes. The study demonstrate the complex retinal circuity used by the Spalax to detect light, and provide evidence for both melanopsin and a non-melanopsin projecting pathways to the brain.

# SC21. "Circadian and dopaminergic control of $Cpt-1\alpha$ expression in retina and photoreceptors."

<u>Patrick Vancura</u><sup>1</sup>, Tanja Wolloscheck<sup>1</sup>, Kenkichi Baba<sup>2</sup>, S. Anna Sargsyan<sup>3</sup>, Gianluca Tosini<sup>2</sup>, P. Michael Iuvone<sup>3</sup> and Rainer Spessert<sup>1</sup>

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The oxidative metabolism of the mammalian retina has to comply with daily changes in energy demand and its impairment contributes to diabetic retinopathy - one of the most common causes of blindness in Europe and USA. To gain a view of the regulation of the oxidative metabolism of the retina, the transcriptional control of the key regulator of mitochondrial  $\beta$ -oxidation - *carnitine palmitoyltransferase-1a* (*Cpt-1a*) - has been investigated in the present study. *Cpt-1a* expression was seen to be most prominent in

the inner segment of photoreceptors and to display a daily rhythm with elevated values during the daytime in preparations of the whole and outer retina. The cycling of  $Cpt-1\alpha$  persisted in constant darkness and was decreased in mice deficient for dopamine  $D_4$  receptors. The data of the present study suggest that daily regulation of the oxidative metabolism of the retina involves a pathway in which the clock-dependent release of dopamine and its subsequent action on  $D_4$  receptors targets  $Cpt-1\alpha$  transcription. Further studies are required to investigate the extent to which dysregulation of this circadian pathway contributes to the pathogenesis of diabetic retinopathy.

### SC22. "Photoreceptors modulation of infra-slow oscillatory activity in the rat olivary pretectal nucleus."

<u>Patrycja Orlowska-Feuer</u>, Hanna J. Szkudlarek, Annette E. Allen, Riccardo Storchi and Marian H. Lewandowski

Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland

A subpopulation of olivary pretectal nucleus (OPN) neurons discharges action potentials in an oscillatory manner, within a period of minutes. This infra-slow oscillatory activity depends on synaptic excitation originating in the contralateral retina, as it vanishes after inhibition of retinal activity. Retinal signals reach the OPN via a route created by axons of either classic retinal ganglion cells (RGCs) or intrinsically photosensitive RGCs (ipRGCs), which use melanopsin for photon capturing. Although both cell types convey light information, their physiological function differs considerably.

In the present study we used pharmacological manipulations of specific phototransduction cascades combined with extracellular single-unit recordings in urethane-anaesthetized rats to disentangle the contribution of rod-cone and melanopsin photoresponses to generation and modulation of oscillatory activity.

Our results show that under photopic conditions (bright light) ipRGCs play a major role in driving infra-slow oscillations, because blocking melanopsin phototransmission ceases or temporally disturbs oscillatory firing of the OPN neurons. On the other hand, blocking rod-cone phototransmission does not change firing patterns. Under mesopic conditions (moderate light), when melanopsin phototransmission is absent, blocking rod-cone signalling causes disturbances or even the disappearance of oscillations implying that classic photoreceptors are of greater importance under moderate light.

We provide evidence that all photoreceptors are required for the generation of oscillations in the OPN, although their roles in driving the rhythm are determined by the lighting conditions, thus consistent with their relative sensitivities. It further suggests that maintained retinal activity is crucial to observe infra-slow oscillatory activity in the OPN.

### SC23. "Drosophila melanogaster clock gene mutants exhibit a circadian rhythm in visual contrast response."

Nippe, O.M., Elliott, C.J.H. and Chawla, S. Department of Biology, University of York, York, UK

Most organisms use a molecular timekeeping mechanism centred on the so-called "clock genes", known to interact with one another in a 24-hour Transcriptional-Translational Feedback Loop (TTFL) to control circadian rhythms intracellularly. However, the

discovery of circadian rhythmicity in the oxidation state of peroxiredoxins has suggested that an alternative metabolic oscillator may govern circadian rhythms independently of gene transcription. Although circadian rhythms have been documented in the morphology of the *Drosophila* visual system, much of the underlying physiology remains unclear. It was previously found that a circadian rhythm in the visual transduction amplitude of *Drosophila* persists in some "clock" gene mutants, indicating that the rhythm may persist independently of the TTFL.

In this study the highly sensitive Steady State Visually Evoked Potential (SSVEP) assay was used to assess the visual function of the TTFL mutants  $Clk^{Jrk}st^1$  and  $per^0$  in order to determine whether a TTFL oscillator is driving oscillations in the visual contrast response of fruit flies, as well as dissect the contribution of individual neuron orders in the retina to the response. We have found that despite a complete loss of circadian rhythmicity in locomotor activity levels the  $Clk^{Jrk}st^1$  mutant exhibits robust circadian rhythms in contrast sensitivity, with a recurring peak 4 hours after anticipated light onset in the photoreceptors, lamina, and medullary neurons. We conclude that Drosophila possess a circadian rhythm in contrast sensitivity that can operate independently of clock gene transcription, and thus is likely synchronised instead by a metabolic oscillator.

### SC24. "Role of cryptochromes in retinal responses to light."

<u>Jovi Chau-Yee Wong</u><sup>1</sup>, Gareth Banks<sup>2</sup>, Alun R. Barnard<sup>1</sup>, Carina A. Pothecary<sup>1</sup>, Aarti Jagannath<sup>1</sup>, Steven Hughes<sup>1</sup>, Elizabeth S. Maywood<sup>3</sup>, Russell G. Foster<sup>1</sup> and Stuart N. Peirson<sup>1</sup>

<sup>1</sup>Nuffield Laboratory of Ophthalmology, Nuffield Department of Clinical Neurosciences, University of Oxford, <sup>2</sup>MRC Harwell, Harwell Science and Innovation Campus, Oxfordshire, UK, <sup>3</sup>MRC Laboratory of Molecular Biology, Cambridge, UK

Mammalian Cryptochromes (CRYs) are a constituent component of the core circadian clock mechanism. Recent work has suggested that CRYs may have additional physiological roles as modulators or components of the retinal clock and/or as putative light-dependent magnetoreceptors.

Reports differ in consensus on the localisation of cryptochromes in the retina. Previous work has suggested CRY2 is expressed in cones as well as the majority of cell types in the inner nuclear layer and ganglion cell layer, while convincing CRY1 immunohistochemistry has never been reported in the retina. However, many commercially available CRY antibodies do not provide specific staining, and produce a similar signal in retinae from mice lacking CRY.

Here we report a novel pattern of CRY1 and CRY2 expression in the retina using newly raised CRY antibodies that have undergone extensive validation. Furthermore, colocalization studies were performed with CRY1/2 and an extensive set of retinal cell markers to determine CRY1 expression in various retinal cell types. Colocalization data between CRY1/2 and clock proteins CLOCK and PER1, and the circadian photopigment OPN4 (melanopsin) are reported as well. Our data suggest that CRY1 is the dominant form of CRY in the mammalian retina due to its widespread expression, whereas CRY2 is undetectable.

Testing the role of CRY in the retina also requires functional assays of the retinal circadian clock. Here we demonstrate three different circadian rhythms in retinal physiology: the b-wave of photopic electroretinogram, contrast sensitivity and pupil light reflex. Whilst there are circadian rhythms of these retinal responses to light in wildtype mice, we show that these retinal circadian rhythms are abolished in Cry1-- mice. Together, these data suggest that CRY1 is an essential component of the retinal

circadian clock, whilst CRY2 appears to be non-essential. Furthermore, loss of CRY1 alone is sufficient to result in loss of retinal circadian rhythms.

### **SYMPOSIUM 6: JSC CLOCKS AND CIRCUITS**

Chairs: Sato Honma (Japan) and Yoshitaka Fukada (Japan)

#### S18. CHARLOTTE FOERSTER

"The Peptidergic Clock Network in the Brain of Drosophila melanogaster."

Charlotte Helfrich-Förster

Neurobiology and Genetics, Biocentre, Theodor-Boveri-Institute, University of Würzburg, Am Hubland, 97974 Würzburg, Germany

The circadian clock in the brain of the fruit fly *Drosophila melanogaster* consists of a network of peptidergic neurons that are mutually connected and appear to talk to each other. The involved neuropeptides are: Pigment-Dispersing Factor (PDF), IPNamide, short Neuropeptide F (sNPF), long Neuropeptide F (NPF) and Ion Transport Peptide (ITP). Each of these peptides may play a specific role in the circadian clock of the fly, but this role has so far been mainly demonstrated for PDF and recently partly for ITP. PDF is an extremely versatile component in the clock network: (1) It seems to transmit photic information from the compound eyes to the clock network, (2) it can differently alter the clock protein oscillations in specific clock neurons and (3) it works as clock output factor. I will review our current understanding of ITP and PDF function in *Drosophila's* circadian clock network with emphasis on PDF.

#### S19. YOSHITAKA FUKADA

"Canonical and Noncanonical E-boxes Regulate Transcriptional and Post-Transcriptional Circuits."

Yoshitaka Fukada

The University of Tokyo, Graduate School of Science, Japan

A heterodimer of CLOCK and BMAL1 rhythmically binds to CACGTG E-box DNA element of the target genes and drive their rhythmic transcription. We determined direct targets of CLOCK-mediated transactivation in the mouse liver by genome-wide chromatin immunoprecipitation-sequencing (ChIP-Seq) with an anti-CLOCK monoclonal antibody that we have developed for quantitative analyses of CLOCK protein. The analysis identified 7,978 in vivo CLOCK-binding sites and almost all of them exhibited circadian variation in the CLOCK occupancy at these DNA regions. The newly developed bioinformatics method, MOCCS (motif centrality analysis of ChIP-Seq), detected genome-wide distribution of previously unappreciated CLOCK-targeted E-box-like elements, such as CACGAG, CACGGG and CATGCG. All these noncanonical E-box motifs were targeted by CLOCK in a genome-wide manner (~1,000 or more sites for each motif), rather than being limited exceptions. On the other hand, deep-sequencing analysis of poly (A)+ RNA in the mouse liver revealed rhythmic modification of RNA, in addition to circadian regulation of alternative splicing and non-coding RNA formation. We found that CLOCK regulates rhythmic expression of an RNA modifying enzyme. Large populations of mRNA oscillations and the RNA modification rhythms were attenuated by deficiency of

the enzyme, supporting the importance of epitranscriptome in the formation of mRNA oscillations. Together, the data set of the ChIP-Seq and RNA-Seq revealed CLOCK-mediated circadian network and the importance of post-transcriptional regulation likely serving as a key mechanism for the entire circadian circuit and the outputs.

#### S20. MICHIHIRO MIEDA

# "Cellular clocks in AVP neurons of the SCN are critical for interneuronal coupling regulating circadian behavior rhythm."

Michihiro Mieda

Department of Molecular Neuroscience and Integrative Physiology, Faculty of Medicine, Kanazawa University, Japan

The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and entrains to the environmental light/dark cycle. It is composed of multiple types of neurons, and neuronal network properties are integral to normal function of the SCN. However, mechanisms underlying the SCN neuronal network have remained elusive.

As a first step to understand the principle of the SCN network, we generated mice in which *Bmal1*, an essential clock component, is deleted specifically in the neurons producing arginine vasopressin (AVP), one of the primary neuronal types in the SCN. These mice showed marked lengthening in the free-running period and activity time of behavior rhythms. When exposed to an abrupt 8 hr advance of the light/dark cycle, these mice reentrained faster than control mice did. In these mice, the circadian expression of genes involved in intercellular communications, including *Avp*, *Prokineticin 2*, and *Rgs16*, was drastically reduced in the dorsal SCN, where AVP neurons predominate. In slices, dorsal SCN cells showed attenuated PER2::LUC oscillation with highly variable and lengthened periods. Thus, *Bmal1*-dependent oscillators of AVP neurons may modulate the coupling of the SCN network, eventually coupling morning and evening behavioral rhythms, by regulating expression of multiple factors important for the network property of these neurons.

#### S21. HANS PETER HERZEL

### "Synchronization and Entrainment – Lessons from Oscillator Theory."

Hanspeter Herzel

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The mammalian circadian clock is a system of coupled oscillators. Synchronization of sloppy SCN neurons allows precise orchestration of peripheral clocks. Zeitgeber signals establish appropriate entrainment phases of physiological rhythms. Mathematical theory provides a framework to study oscillator properties, coupling mechanisms, phase response curves, entrainment ranges and seasonal variations of the entrainment phase. We distinguish single cell oscillators from synchronized coupled networks. Many neurons and fibroblast cells can be characterized as damped noise-driven oscillators (Westermark 2009). Such "weak oscillators" are easily synchronized by coupling (Bernard 2007). However, oscillating neuropeptides and receptor levels can interfere with intrinsic feedback loops. Consequently, varying coupling phases can induce loss of synchronization (Ananthasubramaniam 2014). The SCN, a network of synchronized

oscillators, is a "strong oscillator" with small PRCs and narrow entrainment ranges. Contrarily, lung tissue can be easily entrained even to temperature cycles of 20h and 28h (Abraham 2010).

The entrainment range as a function of period-mismatch and zeitgeber strength has been termed "Arnold tongue". Outside the Arnold tongue "splitting", subharmonics and chaos are expected. These phenomena are indeed found in rats for 22h light-dark cycles (Granada 2011) and mutant mice (Erzberger 2013).

Strong oscillators in vertebrates with narrow entrainment ranges imply highly variable entrainments phases (Granada 2013). Oscillator theory predicts how period-mismatches, zeitgeber strength, amplitudes and photoperiods govern the entrainment phase (Schmal 2015). These results are consistent with the "circadian surface" of Neurospora and temperature entrainment of SCN slices (Bordyugov 2015).

#### Short Communications

### SC25. "Cell cycle and circadian clock: reconstructing the dynamics of two coupled oscillators."

<u>Cannavo Rosamaria</u>, Bieler Jonathan and Felix Naef *EPFL*. *Switzerland* 

Circadian rhythm and cell cycle are two periodic processes having a period in the range of one day. Therefore, is reasonable to expect that, when they run in parallel in the same cell, their coupling may lead to synchronization. Previous observations of circadian variations in mitotic indices in mammalian cells and daytime-dependence of cell division in mouse liver and cultured fibroblasts led to the hypothesis that the circadian cycle might gate cell-cycle progression.

In order to better understand how the two systems interact and to characterize the directionality of a possible coupling in mammalian cells we performed a large-scale time-lapse imaging of single fibroblasts during several days. The analysis of over 10'000 single cell traces showing circadian cycles in dividing cells indicated that both oscillators tick in a synchronized state. While gating of cell division by the circadian cycle has been most studied, our data combined with stochastic modeling unexpectedly show that reverse coupling, that is the influence of the cell cycle on the circadian cycle, is predominant in NIH3T3 cells. This synchronization state is observed over a wide range of conditions probed by genetic and pharmacological perturbations. Moreover, we reconstructed a model of the phase dynamics, identifying potential interactions between the two processes. This allows us to make predictions on cell cycle events that could influence the circadian clock, such the condensation of chromosomes coinciding with transcriptional shutdown. Our findings have implications in enlightening the role of circadian clock in proliferating tissues such the epidermis, stem cells and cancer.

### SC26. "Circadian clock resetting via non-canonical Rhodopsin signalling and the GPI-anchored protein Quasimodo."

Adam Bradlaugh<sup>1</sup>, Maite Ogueta-Gutierrez<sup>1</sup>, Edgar Buhl<sup>2</sup>, James Hodge<sup>2</sup> and Ralf Stanewsky<sup>1</sup>

<sup>1</sup>Department of Cell and Developmental Biology, UCL (London, UK), <sup>2</sup>School of Physiology and Pharmacology, University of Bristol (Bristol, UK)

The synchronisation of the circadian clock to the environment is one of the most important aspects of circadian function and allows for an organism to accurately anticipate environmental change and modify its behaviour accordingly. It is perhaps not surprising then that organisms can utilise and integrate information from multiple Zeitgebers via numerous biological pathways.

In *Drosophila* the primary Zeitgeber is the rhythmic change in light throughout the day, this change in light is fed into the clock mainly by the photoreceptor Cryptochrome (Cry). Upon exposure to light Cry undergoes a conformational change that allows it to bind to and promote the degradation of the core clock protein Timeless (Tim), thus re-setting the molecular clock. As well as this, light information also reaches the clock through the compound eyes via the retinal rhodopsin/ phospholipase C pathway.

Although perturbed, flies lacking both canonical retinal photo-transduction and Cry can still re-synchronise their clocks to light, as evidenced by slow behavioural resynchronisation to shifted light dark cycles and synchronised TIM expression in some clock neurons.

We present evidence that this persistent light sensitivity is mediated by the Rhodopsins 5 & 6 and by the GPI anchored extra-cellular membrane protein Quasimodo (Qsm), independently of Cry. Our preliminary data leads us to believe that Qsm may be an upstream regulator of the Na<sup>+</sup> K<sup>+</sup> Cl<sup>-</sup> co-transportor (NKCC). In response to light, possibly signalled via Rhodopsins 5 & 6, Qsm is cleaved from the membrane. We currently investigate if this biochemical light response activates or represses NKCC. This could ultimately affect neuronal excitability, which in turn could influence Tim degradation.

## SC27. "Impact of melatonin on daytime-dependent changes in cell proliferation and apoptosis in the adult murine hypothalamic-hypophyseal system."

Michaela Fredrich, Elmar Christ and Horst-Werner Korf

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Cell proliferation and apoptosis are known to adjust neuroendocrine circuits to the photoperiod. The latter is communicated by melatonin, the hormone secreted by the pineal organ. The present study investigated daytime-dependent changes in cell proliferation and apoptosis in the adult murine neuroendocrine system and their regulation by melatonin. Adult melatonin-proficient (C3H/HeN) and melatonin-deficient (C57BI/6J) mice, as well as melatonin-proficient (C3H/HeN) mice with targeted deletion of both melatonin receptor types (MT1 and MT2) were adapted to a 12h light, 12h dark photoperiod and were sacrificed at Zeitgeber times ZT00, ZT06, ZT12 and ZT18. Immunohistochemistry for Ki67 and activated caspase-3 served to identify and quantify proliferating and apoptotic cells in the median eminence (ME), hypophyseal pars tuberalis (PT) and pars distalis (PD). Daytime-dependent changes in cell proliferation and apoptosis were found exclusively in melatonin-proficient mice with functional MTs. Cell proliferation in the ME and the PD showed daytime-dependent changes indicated by an increase at ZT12 (ME) and a decrease at ZT06 (PD). Apoptosis followed daytimedependent changes in all regions analysed, with an increase at ZT06. Proliferating and apoptotic cells were found in nearly all cell types residing in the regions analysed. Our results indicate that daytime-dependent changes in cell proliferation are counterbalanced by daytime-dependent changes in apoptosis exclusively in melatonin-proficient mice with functional MTs. Melatonin signalling appears to be crucial in both generation and timing of daytime-dependent changes in proliferation and apoptosis that serve the high rate of physiological cell turnover in the adult neuroendocrine system.

### SC28. "Functional analysis of tissue-specific glycosylation of springtime hormone TSH."

<u>Keisuke Ikegami</u><sup>1†</sup>, Xiao-Hui Liao<sup>2</sup>, Yuta Hoshino<sup>1</sup>, Hiroko Ono<sup>1</sup>, Wataru Ota<sup>1</sup>, Yuka Ito<sup>1,3</sup>, Taeko Nishiwaki-Ohkawa<sup>1,3</sup>, Chihiro Sato<sup>1</sup>, Ken Kitajima<sup>1</sup>, Masayuki Iigo<sup>4</sup>, Yasufumi Shigeyoshi<sup>5</sup>, Masanobu Yamada<sup>6</sup>, Yoshiharu Murata<sup>7</sup>, Samuel Refetoff<sup>2</sup> and Takashi Yoshimura<sup>1,3,8,9</sup>

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Thyroid-stimulating hormone (TSH: thyrotropin) is known to be a pituitary hormone. TSH synthesized in the pars distalis of pituitary gland (PD-TSH) stimulates the thyroid gland to produce thyroid hormones, whereas TSH derived from the pars tuberalis (PT) of the pituitary gland (PT-TSH, springtime hormone) acts on the hypothalamus to regulate seasonal physiology and behaviour, e.g. seasonal breeding, moulting, and migration. However, it had not been clear how these two TSHs avoid functional crosstalk and thereby maintain their distinct functions. In this study, we show that this regulation is mediated by tissue-specific glycosylations. We first found the different expression mechanism of two TSHs in mice; PD-TSH expression was regulated by hypothalamic thyrotropin-releasing hormone (TRH), while PT-TSH is independent of TRH regulation and controlled by melatonin. In addition, we also found the attenuated bioactivity of circulating PT-TSH for the thyroid gland. By mass spectrograph, we identified the different pattern of glycosylations in two TSHs. PD-TSH bound with biantennary Nglycans mainly attached by a sulfate group, whereas PT-TSH was a combination of tetraantennary and triantennary multibranched N-glycans associated with sialic acid. Furthermore circulating PT-TSH forms the complex with immunoglobulin or albumin, inducing the alteration of its bioactivity. Thus, this study demonstrates the involvement of tissue-specific glycosylation in preventing functional crosstalk between signalling molecules in vivo.

#### SYMPOSIUM 7: INTERNAL SYNCHRONY

Chair: Henrik Oster (Germany)

S22. HENRIK OSTER

"Endocrine Regulation of Circadian Physiology."

Henrik Oster

Medical Department I / University of Lubeck, Germany

Endogenous circadian clocks regulate 24-hour rhythms of physiology and behavior. In mammals a master pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) synchronizes cellular subordinate clocks in extra-SCN central and peripheral tissues with

each other via humoral, behavioral, and autonomic cues. Different Zeitgeber signals serve to synchronize central and peripheral clocks with external time. While light resets the SCN, mistimed feeding can uncouple peripheral and central oscillators, thus leading to an unhealthy state of internal desynchrony. Using mouse genetics and ex vivo approaches we study the mechanisms of circadian entrainment by external timing signals. In mice with conditional deletion of clock function in the SCN or the adrenal gland we identified a neuro-endocrine gating mechanism by which central and peripheral clocks interact to regulate circadian stress axis activity. We further identified a novel endocrine pathway of food-mediated peripheral clock resetting in the liver involving the incretin hormone oxyntomodulin.

### S23. ANDRIES KALSBEEK

"How the SCN Synchronizes Daily Rhythms in Peripheral Tissues."

Andries Kalsbeek, Yan Su, Dirk Jan Stenvers and Anne Loes Opperhuizen

Department of Endocrinology and Metabolism, Academic Medical Center (AMC),

Amsterdam, The Netherlands

In order to synchronize the tissue-specific rhythms with environmental day/night changes, the master clock in the hypothalamic suprachiasmatic nucleus (SCN) has to deliver its rhythmic information to the periphery. This information is thought to be delivered via hormonal, neuronal and behavioral pathways. Feeding rhythm, corticosterone and neural input are considered important signals for synchronizing rhythms in the liver, because restricted feeding, corticosterone and noradrenalin can shift the phase of clock gene expression rhythms in the liver. In this study, we investigated the necessity of the feeding rhythm, adrenal hormones and neural inputs for the maintenance of daily clock gene rhythms in the liver. We found that the daily rhythm of clock gene expression did not disappear after disruption of the daily rhythms of feeding (using a 6-meals-a-day feeding schedule (6M)), adrenal hormones (adrenalectomy (ADX)) or neural inputs (total hepatic denervation (HTx)). Subsequently we disrupted any two combinations of these three signals simultaneously and investigated its effect on daily clock gene rhythms in liver. We found that clock genes did not lose their daily expression rhythm under 6M+HTx and HTx+ADX conditions. However, interestingly, all clock genes lost their daily expression rhythm in the 6M+ADX animals. These data indicate that the daily feeding rhythm, adrenal hormones and neural inputs are not indispensable for the SCN to synchronize peripheral clock gene rhythms. One the other hand, at least a daily rhythm of feeding or adrenal hormones should be present in order for the SCN to synchronize clock gene expression rhythms in the liver.

### S24. ROBERT DALLMANN

"Real-time bioluminescence reporters of circadian rhythms and signaling pathways in solid tumors in vitro and in vivo."

Robert Dallmann, Ludmila Gaspar, Ermanno Moriggi and Steven A. Brown *Institute of Pharmacology and Toxicology, University of Zurich, UK* 

Circadian clocks modulate most mammalian physiological processes in a time-of-day dependent manner. In reverse, pathophysiological maladaptations like cancer can either

disrupt the temporal structure of physiology and metabolism, or are caused by such a disruption. On the mechanistic level, these processes are not fully elucidated yet.

Here, we used bioluminescence reporter assays to assay the clock status of a number of established cell lines and human primary tumors cells in vitro. We found that while some cell lines are robustly rhythmic, others are arrhythmic. We then confirmed this interesting result in cells that were obtained from patient tumor biopsies and confirmed these results. However, using exemplary arrhythmic and rhythmic mouse cancer cell lines, we found that both, clock containing and clockless tumor cell lines were rhythmic in vivo.

Subsequently, we wanted to if this "rescue" effect happens other cell signalling pathways controlling physiology and metabolism in developing tumors using reporter system developed in the lab. Again, closely related clockless and one clock containing murine colorectal adenocarcinoma lines, i.e., C51 and C26 were used. Like circadian clock function, we found that some signaling pathways exhibit a highly rhythmic pattern in vivo, but not in vitro. This is highly interesting because, it suggests that tumor physiology might be – at least – partially driven by the host clock, and, thus, giving an enticing possible rational chronotherapy. Especially the results from our *in vivo* experiments will be valuable in understanding which cellular pathways are rhythmically regulated in tumors by the host clock.

#### **Short Communications**

SC29. "Mice, melatonin, and the maintenance of diurnal rhythms."

Martina Pfeffer, Julian Lang, Claudia Fischer, Helmut Wicht and Horst-Werner Korf

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Externally applied melatonin (MT) has entraining and phase-shifting effects in animals and man. Endogenous MT has been shown to facilitate the adaption to the new light regime after a phase advance. However, the role of endogenous MT with regard to "normal" diurnal rhythms (12:12 LD) is still poorly understood. Mice with deletions in the melatoninergic system (be it MT-deficiency or the lack of MT-receptors) do not display any obvious defects in either their spontaneous (circadian) or entrained (diurnal) rhythmic behavior. However, there are subtle effects that can be detected by analyzing the periodicity of the locomotor behaviors in some detail, and we wish to report on these. Using a combination of two statistical methods (Refinetti, 2004; Wicht et al., 2014), we found that MT-deficient mice (C57BI/6J), as well as mice lacking the MT1 and 2 receptors (C3H/HeN MT1,2 KO) reproduce their daily (12:12 LD) behavioral rhythms with significantly less accuracy than mice with an intact melatoninergic system. Vice versa, rhythmic oral application (via drinking water) of melatonin in the dark period significantly increased the stability of the rhythms in C57Bl/6J-mice, while application of a melatonin MT1,2 - antagonist ("S22153", Delagrange et al., 1999) decreased the stability of the rhythms in C3H/HeN mice with intact MT-receptors. These results show that endogenous melatonin plays a role in stabilizing diurnal behavioral rhythms not only after a jet lag, but also under "normal" daily conditions. However, the adaptive role of a stable vs. a labile rhythm is still unknown.

### SC30. "Social jetlag affects subjective sleepiness among school-aged children in Japan."

Yoko Komada<sup>1</sup>, Norihisa Tamura<sup>1,2</sup>, Hideki Tanaka<sup>2</sup> and Yuichi Inoue<sup>1</sup>

<sup>1</sup>Department of Somnology, Tokyo Medical University, <sup>2</sup>Department of Psychology, Hiroshima International University, Japan

A recent systematic review and meta-analysis suggested that bedtimes were later and total sleep time was shorter in Asian adolescents than in North American and European adolescents. It is not surprising that later bedtime and chronic sleep reduction was associated with daytime sleepiness. However, the relationship between social jetlag and daytime sleepiness has not been elucidated so far. We hypothesized that social jetlag would independently effect on excessive daytime sleepiness. The Ethical Review Board of the Neuropsychiatric Research Institute in Japan approved our study protocol. We conducted surveys using the Japanese version of the Pediatric Daytime Sleepiness Scale (PDSS-J) which was modified and validated to suit school-aged children and adolescents. There were 493students aged 11- to 16- year-old (46.2% boys); 73 elementary school children, 102 junior high school students, and 318 high school students. Mid-sleep was calculated from bedtime and wakeup time on school days, and social jetlag was calculated as the difference between mid-sleep on free days and midsleep on school days. As results, age was significantly correlated with the PDSS-J score (Pearson's r = 0.53, p=0.001). Multivariate logistic regression analysis revealed that both sleep duration on school days (OR=0.74, 95%CI: 0.61-0.90, p=0.002) and social jetlag (OR=1.31, 95%CI: 1.04-1.67, p=0.002) were significantly associated with daytime sleepiness (PDSS score >=15), after adjustment for age and sex. It must be fully taken into account not only sleep loss but also social jetlag impact daytime sleepiness among school-aged children.

### SC31. "Measuring the physiological cost of circadian desynchrony in mammals." <u>Alexander C. West</u> and David A. Bechtold Faculty of Life Sciences, The University of Manchester, UK

Mammals employ a circadian clock network to anticipate fluctuations in the environment and adapt their physiology accordingly. The clockwork is responsive to environmental signals, which not only ensures synchronisation with the external environment, but also facilitates internal synchrony between brain and tissue clocks located across the body. Unfortunately, within our modern society the natural framework of perpetual and predictable environmental rhythmicity has been undermined. Epidemiological evidence clearly associates shift-working patterns with cancer, immune dysfunction and numerous metabolic problems. The adaptive and fitness value of circadian timing has been clearly demonstrated in lower organisms. However, the intrinsic value of the circadian system in mammals is less clear, and little is known of the underpinning biology that associates clock disruption with disease states. We therefore set out to define the impact of circadian misalignment in mice by imposing stable, but non-resonant (i.e. non-24hr) cycles of light and/or food. As expected, mice exhibited robust entrainment to environments that were within the limits of the circadian clock (e.g. 22.5hr to 27hr cycles). However, despite achieving stable entrainment, chronic exposure to non-resonant light/dark cycles resulted in widespread and tissue specific disruption of the molecular clockwork and downstream clock regulated transcriptional rhythms. Moreover, mice maintained in non-resonant light/dark cycles also exhibited a number of physiological consequences, including altered metabolic rate, reduced insulin sensitivity, and cardiac dysfunction. Together these studies highlight the profound impact of circadian misalignment on mammalian physiology, and provide mechanistic insight into the clock disruption-associated pathophysiology associated with circadian disruption in humans.

### SC32. "Entrainment of body temperature and locomotor activity daily cycles by acute regular exercise."

<u>Frederico Sander Mansur Machado</u>, Nayara Abreu Coelho Horta, Thaís Santana Rocha Cardoso, Quezia Teixeira Rodrigues, Ana Maria de Lauro Castrucci, Maristela Oliveira Poletini and Candido Celso Coimbra

Department of Physiology and Biophysics, ICB/UFMG, Belo Horizonte, MG, Brazil; Department of Physiology, Institute of Biosciences, USP, São Paulo, SP, Brazil

Physical activity might be an important entrainment agent for the circadian system. The effects on the circadian rhythms might be mediated by acute adjustments and chronic adaptations induced by exercise, such as HPA axis activity, changes on energy expenditure and tuning of the thermoregulatory system. Thus, we investigated if exercise training influences two major outputs of the circadian system, i.e. core temperature (T<sub>c</sub>) and locomotor activity (LA). Wistar rats, housed at a 12/12-light/dark cycle, were used in two separate experimental conditions: acute exercise and 8 weeks of exercise training. T<sub>c</sub> and LA were continuously recorded with an implantable telemetry sensor. The tail skin temperature (T<sub>sk</sub>) and oxigen consumption were taken before and after the exercise tests; food and water ingestion were measured daily. After the last exercise capacity evaluation, animals were euthanized for tissue and blood plasma harvest. Plasma corticosterone, oxigen consumption, T<sub>c</sub> and T<sub>sk</sub> were increased by exercise in an intensity-dependent manner during the light-phase (p<0.001). Interestingly, the raise in T<sub>c</sub> induced by moderate-to-intense exercise was not completely reversed to the expected levels for that moment of the day; however, this effect was withdrawn 24 hours later. Additionally, the daily cycles of T<sub>c</sub> and LA were affected by exercise training (p<0.0001). Furthermore, along with the increased exercise capacity and thermoregulatory efficiency (p<0.0001), trained animals showed higher LA in both photo and scotophase (p<0.0001). Taking these findings together, we conclude that exercise induces direct and indirect changes in the circadian timing system, reinforcing its role as an independent entraining agent.

SC33. "Scheduled locomotor exercise improves aberrant rhythms in neural and locomotor circadian function through alteration of GABAergic activity in the SCN." Alun T. L. Hughes, Rayna E. Samuels, Mino Belle, Sven Wegner and Hugh D. Piggins Faculty of Life Sciences, University of Manchester, Manchester, UK

Exogenous time cues and feedback from endogenous clock-controlled outputs act to coordinate circadian rhythms with the environment; locomotor activity is one such influence which provides feedback to the SCN. Appropriate circadian function is dependent on synchrony between thousands of autonomous cellular oscillators that comprise the SCN and is achieved largely by vasoactive intestinal polypeptide (VIP) signalling through the VPAC<sub>2</sub> receptor. Whilst VIP promotes synchrony within the SCN, GABA is currently postulated to oppose it, acting to make changes to the phase of the SCN easier to achieve. Thus, appropriate SCN function relies on a balance between these synchronising and desynchronising factors. As such, mice lacking VPAC<sub>2</sub> receptors (*Vipr2*<sup>-/-</sup>) generate low amplitude, desynchronised, SCN oscillations that result in aberrant

behavioural rhythms. We have found that a regimen of daily scheduled voluntary exercise (SVE) promotes robust ~24h rhythms in *Vipr2*-- behaviour. Here, we demonstrate that SVE significantly increases the proportion, synchrony and amplitude of rhythmic neurons in *Vipr2*-- SCN. Using voltage clamp recordings we demonstrate a significant increase in the amplitude of GABAergic events in the ventral SCN and a significant decrease in the frequency of such events in the dorsal SCN. Further, using *in vitro* PER2::LUC recordings, we demonstrate that, in contrast to non-SVE control *Vipr2*-- tissue, post-SVE *Vipr2*-- SCN show WT-like responses to blockade of GABA<sub>A</sub> signalling. We conclude that SVE promotes behavioural rhythmicity through partial restoration of SCN function in *Vipr2*-- mice. These data are consistent with SVE-mediated alterations in GABA signalling leading to long-term reorganization of SCN temporal architecture.

#### **SYMPOSIUM 8: CIRCUITS AND EXCITABILITY**

Chair: Hugh Piggins (UK)

S25. JENNIFER EVANS

"Plasticity in the Role of GABA<sub>A</sub> Signaling in Coupling Neuronal Activity in the SCN Network."

Jennifer A. Evans

Department of Biomedical Sciences, Marquette University, USA

The suprachiasmatic nucleus (SCN) regulates the function of nearly every system in the body to program daily rhythms in mammals. SCN neurons are cellular clocks that interact (i.e., couple) with one another to synchronize their activity at the network level. Nearly all SCN neurons express GABA, but the role of GABA in regulating network synchronization has been difficult to define due to the challenge of studying coupling in the context of a functional network. Using a quantitative coupling assay, we have revealed that the role of GABA<sub>A</sub> signalling in SCN synchronization is experience-dependent. Specifically, when SCN neurons are tightly coupled under standard lighting conditions, GABAA signalling acts to inhibit neuronal synchronization. In contrast, when neurons in the SCN network are desynchronized by long day photoperiods, GABAA signalling facilitates resynchronization. Because synchronization is critical for SCN clock function, understanding the bases of this neuroplasticity is of fundamental importance. The current experiments test whether plasticity in the role of GABA<sub>A</sub> signalling reflects changes in the strength and/or polarity of GABA<sub>A</sub> signalling. First, we demonstrate that exposure to long day photoperiods markedly alters SCN resetting responses to GABA. Further, we show that plasticity in GABA<sub>A</sub> signalling during SCN synchronization reflects light-induced changes in the polarity of GABA responses driven by changes in the function of chloride co-transporters. These results provide insight into the mechanisms underlying the role of GABA during SCN synchronization. As in other neuronal networks, plasticity in GABAA signalling in the SCN can modulate network activity to encode individual experiences and sculpt behavioural responses in a changing environment.

S26. MONIKA STENGL

"Pigment-Dispersing Factor-Dependent Signalling in the Circadian Clock of the Madeira Cockroach *Rhyparobia maderae*."

Monika Stengl, HongYing Wei, Hanzey Yasar, Nico Funk and Achim Werckenthin

University of Kassel, Institute for Biology, Dept. of Animal Physiology, Heinrich Plett Str. 40, 34132 Kassel, Germany

Transplantation studies located the circadian clock controlling locomotor activity rhythms of the Madeira cockroach in the accessory medulla (AME) with associated pigmentdispersing factor-immunoreactive (PDF-ir) neurons. Locomotor activity was delayed at dusk, but advanced at dawn in response to PDF-injections. Since injections of cAMP mimicked PDF effects, it was suggested that PDF signals via cAMP. Indeed, in ELISAs PDF application increased cAMP levels in cockroach optic lobes. Furthermore, cAMP levels cycled in optic lobe neuropils, with maxima at dusk and dawn. Thus, we hypothesized that PDF-ir evening oscillators release PDF at dusk while morning oscillators release PDF at dawn to control locomotor activity rhythms cAMP-dependently. In extracellular recordings of excised AME neuropils PDF and cAMP inhibited or activated action potential activity of different AME neurons. With calcium imaging and patch clamp studies we distinguished different PDF-sensitive circadian pacemaker neurons of the AME. PDF could increase intracellular calcium levels cAMP-dependently in type one pacemaker neurons, apparently via block of potassium channels. In addition, PDF could inhibit spontaneous activity of AME neurons, apparently via block of sodium channels. In type two circadian pacemaker neurons, however, PDF increased intracellular calcium concentrations adenylyl cyclase-independently. To further characterize PDF signaling, the PDF-receptor of the Madeira cockroach was cloned and expressed in a heterologous system. Current experiments test, whether the PDF-receptor of the Madeira cockroach can couple to more than one G-protein to synchronize locomotor activity rhythms with long-day photoperiods.

[Supported by DFG grants STE 531/18-1,2,3 to MS]

### S27. ENOKI RYOSUKE

"Multicolor Imaging of Circadian Rhythms in the Suprachiasmatic Nucleus."

Ryosuke Enoki<sup>1,2,4</sup>, Daisuke Ono<sup>1</sup>, Michihiro Mieda<sup>3</sup>, Sato Honma<sup>2</sup> and Ken-ichi Honma<sup>2</sup> <sup>1</sup>Photonic Bioimaging Section, Hokkaido University Graduate School of Medicine, <sup>2</sup>Department of Chronomedicine, Hokkaido University Graduate School of Medicine, <sup>3</sup>Kanazawa University, Faculty of Medicine, Department of Molecular Neuroscience & Integrative Physiology, <sup>4</sup>JST PRESTO, Japan

In mammals, most physiological and behavioral events are subjected to well-controlled daily oscillation, and these rhythms are controlled by the hypothalamic suprachiasmatic nucleus (SCN). Recent studies revealed that the SCN is a hierarchical and multi-oscillator system in which the neuronal network plays a critical role in expressing robust and coherent rhythms in physiology and behavior. To understand the mechanisms of the circadian clock at network level, one needs to visualize the SCN network at high resolution spatially and temporally. For this purpose, we developed a time-lapse fluorescence imaging system composed of a Nipkow-spinning disk confocal microscope and high sensitive CCD camera. Using a genetically encoded calcium sensor, we previously characterized the spatial and temporal patterns of circadian calcium rhythms in the SCN and found the topological specificity of the circadian calcium rhythm in the SCN and the presence of coupled regional pacemakers (enoki et al., PNAS, 2012). For better understanding of importance of calcium rhythms, we performed simultaneous recordings of multiple circadian functions (calcium, clock gene, firing) and analyzed the differences of spatial and temporal patterns of these functions in the SCN network.

#### Short Communications

SC34. "A screen for potassium channels contributing to local sleep."

<u>Christine Muheim</u><sup>1</sup>, Tina Sartorius<sup>2</sup>, Robert Dallmann<sup>1</sup>, Ning Gu<sup>3</sup>, Johan F. Storm<sup>3</sup>, Roland Dürr<sup>1</sup>, Reto Huber<sup>4</sup>, Peter Ruth<sup>2</sup> and Steven A. Brown<sup>1</sup>

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Recent work has impressively documented that sleep is not only a temporally but also a spatially distinct state (Hung et al., 2013). Therefore, local modulations in small brain regions such as thalamocortical neuronal assemblies might already be sufficient to locally alter the spectral waves characteristic of sleep. We have exploited such "local sleep" to conduct an AAVmediated RNA interference screen for cortical potassium channels important to sleep. Among the 31 channels examined, large-conductance potassium channels figured prominently among potentially important genes. One of these, Kcnma1, was characterized in more detail using a classic full-brain knockout model. Kcnma1 is a large conductance calcium dependent potassium channel (BK) with a broad expression throughout the mammalian brain. Local BK channel depletion reduces "slow wave" power in all vigilance states, thereby altering kinetics of the homeostatic sleep process. These phenotypes could be rationalized both electrophysiologically through reduced burst firing capacity and transcriptomically because of altered inhibitory synaptic composition. The reduction of slow wave power is independent of time of day and furthermore can only be restored partially under increased sleep pressure, suggesting a bona fide effect upon the sleep homeostat. By contrast, we and others (Meredith et al., 2008, Montgomery et al., 2012) have shown separately that BK activity in the SCN is necessary for circadian electrical output from SCN neurons. Because the BK channel shows this dual function, our results suggest that ion channels and in particularly BK channels can integrate circadian and homeostatic effects upon sleep directly through regulation of neuronal firing and inhibition. More generally, they demonstrate that individual ion channels can play roles specific to individual brain "signatures" of sleep.

# SC35. "Electrophysiological and optogenetic characterisation of daily and acute light effects on *Drosophila* circadian clock neurons."

Edgar Buhl, Ralf Stanewsky and James JL Hodge School of Physiology and Pharmacology, University of Bristol, University Walk, Bristol BS8 1TD. UK

Circadian clocks regulate changes in behaviour, physiology and metabolism to ensure they occur at certain times during the day allowing the organism to adapt to their environment. In *Drosophila* the clock is comprised of ~150 neurons grouped into identifiable clusters that sub-serve different circadian functions. Synchronisation of the clock to environmental light:dark cycles is achieved by visual and non-visual pathways including via *cryptochrome* (*cry*) and *quasimodo* (*qsm*) and involves the large lateral ventral neurons (I-LNv). However the mechanism by which the activity of clock neurons is synchronized to light:dark cycles is currently unknown.

We are addressing this question using whole-cell recordings as well as genetically encoded calcium, chloride and membrane potential reporter imaging of I-LNvs in whole adult brains. This has allowed us to characterise circadian changes in physiological properties as well as the acute effect of light. Our results show that in control flies the I-LNv respond differentially to blue light depending on the time of day. In early night, exposure to blue light leads to a depolarisation and an increase in firing whereas during morning these cells become less excitable. In order to determine the mechanism by which light causes these daily switches in excitability, we are characterising molecules known to interact with QSM, a protein involved in light-signalling to the clock. We find that manipulating levels of QSM, the potassium channel Shaw (Kv3.1) and the sodium potassium chloride cotransporter (NKCC) in I-LNv differentially affects circadian changes in membrane potential, spontaneous firing frequency and the blue light response.

### SC36. "Short-circuit: Characterization of a transcription factor that activates a novel circadian transcriptional axis."

M.J. Parsons<sup>1</sup>, M. Brancaccio<sup>2</sup>, S. Sethi<sup>1</sup>, E.S. Maywood<sup>2</sup>, Aarti Jagannath<sup>3</sup>, Yvonne Couch<sup>4</sup>, Mattéa J. Finelli<sup>5</sup>, N.J. Smyllie<sup>2</sup>, Peter L. Oliver<sup>6</sup>, J.E. Chesham<sup>2</sup>, M. Simon<sup>1</sup>, M.H. Hastings<sup>2</sup> and P.M. Nolan<sup>1</sup>

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Circadian rhythms are daily cycles that are controlled by a central hypothalamic pacemaker, the suprachiasmatic nucleus (SCN), that regulates both physiological and Disruptions in circadian rhythms lead to numerous pathologies, behavioral traits. including mental disorders and cognition deficits. Temporal regulation of these biological processes is managed in part by changes to the 24 hour transcriptional profile, which is governed by the interaction of transcription factors with DNA sequence motifs in gene While a handful of these circadian DNA sequence motifs have been characterized, the discovery of novel motifs will help to understand the circadian regulation of behavior. To this end, we identified a dominant mis-sense mutation in the SCN transcription factor Zfhx3, termed short circuit (Zfhx3<sup>Sci</sup>), which accelerates circadian locomotor rhythms in mice. ZFHX3 regulates transcription via predicted AT motifs in target genes and the mutant protein has a decreased ability to activate consensus AT motifs in vitro. Using RNA sequencing, we found minimal effects on core clock genes in Zfhx3<sup>Sci/+</sup> SCN, whereas the expression of neuropeptides critical for SCN intercellular signaling was significantly disturbed. Moreover, mutant ZFHX3 had a decreased ability to activate AT motifs in the promoters of these neuropeptide genes. Lentiviral transduction of SCN slices showed that the ZFHX3-mediated activation of AT motifs is circadian. Moreover, the amplitude and robustness of these oscillations was decreased in Zfhx3<sup>Sci/+</sup> SCN slices. In conclusion, by cloning Zfhx3<sup>Sci</sup> we have uncovered a new circadian transcriptional axis that determines the period and robustness of behavioral and SCN molecular rhythms.

### SC37. "A new understanding of the mammalian circadian clock as neuroglial networks."

Naoto Hyasaka

Biofunctional Imaging Lab, Immunology Frontier Research Center (IFReC), Osaka University, Japan; PRESTO, Japan Science and Technology Agency (JST), Japan

Astrocytes are known to have a multitude of functions, including control of the blood-brain barrier, induction of neuronal synapse formation, as well as regulation of the energy supply to neurons and the turnover of neurotransmitters. In addition, recent studies strongly suggest that astrocytes play a crucial role in the CNS by acting independently or by interacting with neurons. We have previously found that, unlike other cell lines, circadian rhythms in cultured astrocytes are synchronized with each other and persist as long as four weeks. We also observed that spatial distribution of astrocytes in the SCN changes in a circadian manner. These observations suggest that astrocytes in the circadian pacemaker are involved in the circadian clock machinery. To further elucidate the role of astrocytes in the circadian networks, we performed in vivo molecular dissection by generating several different astrocyte-specific knockout (KO) mice lines to distinguish the contribution of astrocytes to the clock from that of neurons. Our data demonstrated that clock/clock-related gene disruption in astrocytes in vivo resulted in abnormality in circadian rhythms, i.e., altered circadian periods in behavior, instability in circadian rhythms, desynchronized rhythms among individual cells ex vivo and in vitro. Furthermore, we identified novel candidate genes involved in driving circadian clock in astrocytes and examined their roles by cell type-specific gene manipulation both in vitro and in vivo. Taken together, our present study suggest that not only neurons, but astrocytes play a key role in the central circadian clock by regulating circadian period, rhythm stability and cellular synchronization.

# SC38. "SCN neurons of *cryptochrome*-deficient mice lack circadian timing in intrinsic excitability states and gating response to excitatory input."

Mino D. C. Belle, Beatriz Baño Otalora and Hugh D. Piggins

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Encoding and generating appropriate responses to environmental stimuli are key tasks of the nervous system. In mammals, the master circadian pacemaker in the brain's suprachiasmatic nuclei (SCN) sense and process natural variations in external light to appropriately drive circadian rhythms in physiology and behavior. Individual SCN neurons contain the molecular clockwork of which the cryptochrome (Cry1-2) and period (Per1-2) genes are important components. This intracellular clock drives changes in excitability, such that SCN neurons are more excited during the day than at night. This enables the SCN to communicate clock phase information to the rest of the brain and gates its responses to light. The absence of the molecular clockwork abolishes SCN-driven rhythms, but it is unclear how excitability of SCN neurons and their responses to neural input are affected. Using SCN slices from Per1::Luc animals lacking a functional molecular clock (Cry1-/-Cry2-/-) and their Cry1+/+Cry2+/+ littermates we demonstrate that most SCN neurons from Cry1-2-2 animals lacked overt 24h rhythms in Per1::Luc bioluminescence. Whole-cell recordings and intracellular calcium [Ca2+] imaging show that although SCN neurons from Cry1-Cry2- mice expressed all the spontaneous excitability states that characterize such cells in Cry1+/+2+/+ animals, they did not show obvious day-night differences in these fundamental measures of excitability. Additionally, we found that SCN neurons from  $Cry1^{-/-}Cry2^{-/-}$  mice lacked appropriate gating to AMPA, a glutamatergic mimic of the light-input pathway. This is the first study to demonstrate how the absence of a functioning molecular clock affects excitability of SCN neurons to influence gating to photic input.

# SC39. "Functional assessment of human melanopsin variants, P10L and T394I, using AAV delivery *in vivo.*"

<u>Jessica Rodgers</u>, Carina Pothecary, Steven Hughes, Doron Hickey, Laurence Brown, Michelle McClements, Russell Foster, Stuart Peirson and Mark Hankins Nuffield Laboratory of Ophthalmology, University of Oxford

Two single nucleotide polymorphisms (SNPs) in human melanopsin (Opn4), P10L and T394I, have been identified. These are associated with seasonal affective disorder (Roecklein et al, 2009; 2012) and attenuated pupil constriction (Roecklein et al, 2013; Lee et al, 2014) respectively. Immunocytochemistry and fluorescent calcium imaging suggest that both P10L and T394I are correctly trafficked to the plasma membrane and are able to couple to a G<sub>q/11</sub> signalling pathway, similar to wildtype melanopsin. Therefore, it is unclear from in vitro assays how P10L and T394l could be causing behavioural change. Using targeted adeno-associated viruses, we delivered human melanopsin by intravitreal injection to pRGCs in Opn4<sup>Cre</sup> knockout mice. We produced 3 viruses; 2 human melanopsin mutants, P10L and T394I, and a wildtype human melanopsin virus to use as a control. Using immunohistochemistry and qPCR, we were able to confirm all 3 viruses successfully transduce and express human melanopsin in mouse pRGCs. Opn4<sup>Cre</sup> knockout animals injected with wildtype human melanopsin show restored pupil constriction and normal post illumination pupil response to 480nm light. All three groups were screened using pupillometry to 480nm or 600nm light, and activity monitoring under a 12:12 light dark cycle. In addition, we used multielectrode-array recordings of flatmounted retinas to determine if there was any change in pRGC spike firing due to the presence of P10L or T394I. This targeted AAV approach could potentially be used to screen other human SNPs, in order to identify disease related mutations in human melanopsin.

#### **SYMPOSIUM 9: CLOCKS IN THE WILD**

Chairs: Till Roenneberg (Germany) and Rosa Levandovksi (Brazil)

S28. GISELE A. ODA

#### "Skeleton Photoperiods in the Wild for Subterranean Rodents"

Gisele A. Oda<sup>1</sup>, Veronica S. Valentinuzzi<sup>2</sup>, Danilo E.F.L. Flôres<sup>1</sup> and Milene G Janetti<sup>1</sup> Departmento de Fisiologia – Instituto de Biociências – Universidade de São Paulo, Brasil, <sup>2</sup>CRILAR-CONICET, Anillaco, La Rioja, Argentina

We have obtained, for the first time, automated recordings of daily light exposure patterns of subterranean rodents from South America, known as tuco-tucos (*Ctenomys* aff. *knighti*). These new data confirmed our previous, visual observations that they expose to light in sporadic, brief (minutes to one hour) emergences to the surface in non-uniform random times, on a daily basis. The automated data showed, however, that the degree of randomness of daily light exposure varies seasonally and revealed a new feature that

had been escaping from our visual observations: during the summer months, tuco-tucos expose to light almost regularly twice per day, during early morning (confirming observations) and during the twilight of dusk hours (which we could not see before). This corresponds grossly to the "skeleton photoperiod" pattern that is commonly used in laboratory settings, and which has shed light on photic entrainment mechanisms. However, this can also be interpreted from the point of view of a bimodal aboveground activity that has been widely studied in the ecophysiological context, which associates this peculiar pattern to masking by high temperatures of midday summer. This is a unique opportunity to integrate photic entrainment, endogenous rhythmicity and ecophysiological approaches to understand seasonal activity patterns in the wild. (FAPESP, CONICET)

#### S29. LUISA PILZ

### "Enlightening the Effects of Artificial Light on Biological Rhythms".

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Quilombos are settlements founded far from civilisation by African slaves (the Quilombolas) in Brazil. The remaining Quilombos have a wide range of electrification-status, from people that have no electricity or were only recently connected to the grid to communities living in modern conditions. Artificial light has profoundly changed human life in the last century: people have become independent in their activities of natural daylight. Quilombolas are, therefore, a unique opportunity to study the historical transition in rest-activity behaviour from the pre-electricity era until today.

We are investigating rest-activity- and sleep-patterns in the Quilombolas' daily context, with wrist actimetry and the Munich ChronoType Questionnaire. By comparing people with different levels and histories of light exposure, we aim to unveil how human sleep changed with the possibilities brought by artificial light.

The data collected so far refer mostly to Quilombolas living away from the cities. Preliminary analyses suggest that these Quilombolas show earlier sleep patterns, as expected for people who rely more on natural light and work mostly outdoors. They are also less dependent on alarm clocks: less than a third report to use them on workdays, while in the industrialised world alarm-clock usage can exceed 80%. Similar to findings in a variety of populations, young people are later chronotypes and experience the highest levels of social jetlag.

Quilombos represent a contrast to the industrialised urban societies that work and are connected in a 24/7 mode. Studying this population may help us to understand the impact of the modern lifestyles on health.

#### S30. MARIA JULIANA LEONE

"Time to Decide, Time to Learn, Time to Sleep: Assessing Chronobiology with Data from the Real World."

María Juliana Leone<sup>1,2</sup>, Diego Fernández Slezak<sup>3</sup>, Mariano Sigman<sup>2,3</sup> and Diego A. Golombek<sup>1</sup>

<sup>1</sup>Universidad Nacional de Quilmes, <sup>2</sup>Universidad Torcuato Di Tella, <sup>3</sup>Universidad de Buenos Aires. Buenos Aires, Argentina

Understanding diurnal fluctuations on human behavior in real-life situations, although sometimes difficult to assess, is a necessary task to reveal the importance and adaptive value of circadian rhythms. These variations will depend on light exposure, social cues and chronotypes. We are using different scenarios to study diurnal rhythms in natural environments.

First, we studied diurnal variations in decision-making as a function of an individual's chronotype capitalizing on a vast repository of human decisions: online chess servers. We found reliable diurnal rhythms in activity and performance which are both dependent on individual diurnal preferences. Response time fluctuates along the day; however, this oscillation is not affected by chronotypes.

In addition, we are analyzing Twitter activity, which allow us to assess changes in "natural" human communication throughout the day and other periods. Using text analysis techniques we are studying diurnal changes in mood, changes in sleepiness, light exposure, etc.

Finally, we analyzed the effect of time of day in education by assessing academic performance in young adults during their initial course at the university and comparing morning, afternoon or nocturnal classes. Our results show that the effect of chronotypes on academic performance depends on the time when the subjects received instruction. In summary, through complementary techniques we are able to assess the impact of diurnal rhythms, natural light exposure or individual preferences on human behavior in real-life situations, which allow us to infer the real impact of chronobiology in our daily life.

#### Short Communications

#### SC40. "Characterization of non-visual photoreception in humans."

<u>Abhishek Prayag</u><sup>1,2</sup>, Sophie Jost<sup>3</sup>, Pascale Avouac<sup>3</sup>, Howard Cooper<sup>1,2</sup>, Dominique Dumortier<sup>3</sup> and Claude Gronfier<sup>1,2</sup>

<sup>1</sup>Department of Chronobiology, INSERM, U846, Stem Cell and Brain Research Institute, Bron, France, <sup>2</sup>Université de Claude Bernard, Lyon-1, Lyon, France, <sup>3</sup>Housing Sciences, École Nationale des Travaux Publics de l'état, Rue Maurice Audin, Vaulx-en-Velin, France

Objective: In the retina, intrinsically photosensitive ganglion cells (ipRGCs) subtend the non-visual (NV) effects of light including on alertness and cognition. These cells do not function independently: while they can encode light, they are also modulated by cone and rod input. Our objective is to investigate which photoreceptor informs NV brain regions and contribute to elicit NV functions.

Methods: Our strategy relied on the differential spectral and spatial properties of cones vs. ipRGCs. In a within-subject design, 28 participants were exposed consecutively to 4 light stimuli of 50min each, from 19-23h. The stimulus was composed of a central white light (CWL, 7000 lux) centered on the fovea (20°) to activate cones specifically, and a peripheral light (20-220°, 300 lux) either enriched in blue (BE) for ipRGCs activation, or enriched in red (RE) to limit ipRGCs activation. The EEG was recorded continuously at 256 Hz and submitted to FFT analysis.

Results: There was no effect of RE light on the beta-band (13.5-32 Hz). Under BE conditions, beta-power rose significantly after 2min, increased further after 5min (+35%) and remained elevated until the end of light exposure.

Conclusion: These preliminary results suggest that a light stimulus presented in the peripheral visual field, at low intensity (300 lux) and with a sub-optimal polychromatic spectrum can activate the EEG in the beta-band. Given 1) the dynamics of beta-

activation mimic ipRGC activation pattern and 2) the lack of effect of the cone-directed CWL, we propose light activates the EEG through ipRGCs and not rods and cones.

### SC41. "DLMO in evening types and sleep phase delay."

Cátia Reis and Teresa Paiva

CENC - Sleep Medicine Center, Lisbon, Portugal

Dim Light Melatonin Onset (DLMO) is used with actigraphy and sleep diaries to evaluate the sleep-wake cycle.

It is currently accepted that in normal subjects DLMO is around 10pm, while the difference between DLMO and waking up (DIF) is around 14h. These values are not known for extreme evening types; furthermore most studies use young adults/adolescents.

72 patients with delayed schedules were compared with 10 normal subjects. The mean age was 39.5 (min=17; max.92) years; 51.3% were females. In all subjects saliva melatonin and sleep diaries were collected. Four subgroups were used- G1: late bedtimes with DLMO before midnight (n=16); G2: Idem but DLMO between 0am and 2am (N=24); G3: idem with DLMO after 2am (n=32). Controls had DLMO before midnight. ANOVA and correlation analysis were done.

DLMO and DIF had normal distributions. The DLMO was 10:21pm for controls; 10:56pm for G1; 0:42am for G2 and 3:27am for G3; significant differences among groups. The DIF was for G1: 12:04h; G2: 15:57h; G3:16:45h and healthy 14:43h.

The reported averages of waking up and sleep onset were respectively 08:02am/00:02am for healthy subjects; 12:22am/03:02am for G1; 09:26/00:55am for G2; 11:31am/03:27am for G3.

There were no group differences for age, notwithstanding the wide range among patients. Conclusions: 1)G1 had late schedules but relatively normal DLMO; it was considered a behavioural evening group; 2)G3 considered as sleep phase delay, differed from the others subgroups in most comparisons; 3)DIF values also varied markedly among groups ranging from 06:30 to 21:10h with the higher ranges in G3.

# SC42. "Chronotype and social jetlag among filipinos –an analysis of data from the philippine chronobiology and social jetlag survey."

Gayline Manalang Jr. <sup>1,2</sup>, Marilou Nicolas<sup>3</sup>, Martha Merrow<sup>4</sup> and Till Roenneberg<sup>4</sup>

<sup>1</sup>Center for International Health, Ludwig-Maximilians-Universität München, <sup>2</sup>College of Public Health, University of the Philippines, <sup>3</sup>College of Arts and Sciences, University of the Philippines, <sup>4</sup>Institute for Medical Psychology, Ludwig-Maximilians-Universität München

The Philippine Chronotype and Social Jetlag Survey builds on the initiative of the research group, PhilSHIFT, to promote chronobiology-based research concerning shift workers in the contact center industry. A Philippine variant of the Munich Chronotype Questionnaire (PhilMCTQ) is used in the Survey. This initiative aims to build a chronotype database of Filipinos who do regular daytime work, from which chronotype distribution and relationship to age, sex, outdoor light exposure, social jet lag, and other health factors can be investigated.

Survey data from July 2010 to December 2014 is presently being analyzed. Comparison with a matched set of German data suggests that Filipinos are generally earlier chronotypes and also experience social jetlag. While the Survey will continue in the Philippines in the coming months, this poster/short communication intends to share insights from this preliminary analysis.

### SC43. "A preliminary human factors analysis of chronotype."

Daniela Bellicoso and Martin R. Ralph
Department of Psychology, University of Toronto, Canada

Chronotype is a broadly defined term, often used interchangeably with morningnesseveningness (M-E) to describe a person's performance capabilities as they change through the day. The term implies that human beings can be characterized as "types" according to the optimal times of day at which they perform best at various activities. Selfreport instruments in circadian typology include the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) (Horne & Östberg, 1976), which provides a quantification of chronotype (circadian typology) based on preferred times for mental and physical activities, and the Munich ChronoType Questionnaire (MCTQ) (Roenneberg et al., 2003), which provides quantitative assessment of sleep timing, and is useful for determining population wide patterns of entrainment, but does not define type. Numerous arguments have been made that the MEQ and MCTQ each measure different human characteristics: MCTQ addresses circadian phase while MEQ considers circadian timing as one of many influences on human time preference. We present a preliminary analysis of the human factors that contribute to the determination of time of day preference, and argue that chronotype is determined primarily by a combination of explicitly and implicitly stored self-images of executive function.

### SC44. "Human metabolite rhythms: Effects of obesity and Type 2 diabetes (T2DM)."

<u>Cheryl Isherwood</u>, Jonathan D. Johnston and Debra J. Skene Chronobiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7XH, UK

Obesity and type two diabetes (T2DM) have been associated with increased circulating concentrations of acylcarnitines and branched chain amino acids (BCAA) (isoleucine, leucine and valine) in single time point sample studies. Investigating 24h metabolite rhythms in obesity and T2DM would help direct future study into the mechanisms behind the reported changes.

The hypothesis that weight and/or T2DM affect the concentration and rhythmicity of acylcarnitines and BCAA across a 24h day was tested. In a controlled clinical study, hourly milkshakes (06:30–21:30h) were given to 8 lean, 9 overweight (OW) and 6 OW with T2DM men, aged 45-65 years, lying .semi-recumbent, awake between 06:30–22:30h (light) and sleep between 22:30-6:30h (dark). Plasma samples (2-hourly across 24h) were analysed using quantitative targeted LC/MS metabolomics.

Two-way ANOVA identified significant increases in 24h concentration in OW and T2DM propionylcarnitine (C3) (L-OW p=0.009 and L-T2DM p<0.001), butyrylcarnitine (C4) (L-OW and L-T2DM p<0.001) and valerylcarnitine (C5) (L-OW p<0.001) compared to lean.

They displayed significant 24h cosinor rhythms in the lean, OW and T2DM (except C5 in T2DM). Weight and T2DM also increased the 24h concentrations of isoleucine (L-OW p=0.009, LT2DM P<0.001), leucine (L-OW p=0.035, L-T2DM p=0.010) and valine (L-T2DM p<0.001). All lean participants displayed significant daily BCAA rhythms, which were retained by isoleucine and valine in OW and by isoleucine in T2DM.

The increased concentrations of C3, C4 and C5, and BCAA in obesity and T2DM support previous studies. These metabolites also displayed clear daily 24h rhythms that were most evident in the lean group.

[Funded by Diabetes-UK (grant 08/0003607), EU FP7-HEALTH-2011 EuRhythDia (grant 278397), BBSRC (grants BB/I019405/1 and BB/D526853/1) and Stockgrand Ltd.]

### **SYMPOSIUM 10: CLOCKS AND IMMUNITY**

Chairs: Nico Cermakian (Canada) and Andrew Loudon (UK)

#### S31. NICO CERMAKIAN

"Circadian Control of the Adaptive Immune Response and Cancer Cell Proliferation."

Nicolas Cermakian<sup>1</sup>, Silke Kiessling<sup>1</sup>, Chloé C. Nobis<sup>1,2</sup>, Lou Beaulieu-Laroche<sup>1</sup>, Geneviève Dubeau-Laramée<sup>1</sup>, Erin E. Fortier<sup>1,2</sup>, Ian Blum<sup>1</sup>, Dominic Landgraf<sup>3</sup>, Jean-François Daudelin<sup>2</sup>, David K. Welsh<sup>3</sup>, Kai-Florian Storch<sup>1</sup> and Nathalie Labrecque<sup>2</sup> <sup>1</sup>Douglas Mental Health University Institute, McGill University, Montreal, QC, Canada, <sup>2</sup>Maisonneuve-Rosemont Hospital Research Centre, University of Montreal, Montreal, QC, Canada, <sup>3</sup>Center for Circadian Biology and Department of Psychiatry, University of California San Diego, La Jolla, CA, USA

Circadian clocks control different aspects of the immune system. However, the links between circadian rhythms and the antigen-specific immune response remain poorly understood. To test whether T cells response to antigen presentation by dendritic cells (DCs) is clock-regulated, we immunized mice with DCs loaded with a peptide antigen from ovalbumin (OVA), and measured OVA-specific T cell responses after one week. We found higher T cell responses after immunization during the middle of the day than the middle of the night, and a persistent rhythm of effector functions. Indeed, interferon gamma-producing T cells show a 24h rhythm, but interestingly, there is no rhythm for another cytokine, interleukin 2. We are presently investigating the molecular and cellular mechanisms underlying these T cell rhythms.

We also addressed the hypothesis that enhancing circadian rhythmicity in tumour cells would control their cell cycle and thereby reduce proliferation. We found that clock gene expression is suppressed in B16 melanoma cells, but dexamethasone, forskolin or heat shock induce rhythmic clock gene expression. Activation of the tumour clock induced rhythmic cell-cycle gene expression and rhythmic entry into cell-cycle phases, which resulted in fewer cells in S-phase. Consistently, B16 cell proliferation and tumour growth in vivo were slowed down. A similar effect was observed in human carcinoma HCT-116 cells. Knocking down *Bmal1* in B16 cells prevented the effects of dexamethasone on tumour growth, showing that it is mediated by the B16 clock. Thus, our data reveal that enhancing clock function might represent a novel strategy to control cancer progression.

### "Immunity and the Circadian Clock."

Andrew Loudon, Julie Gibbs, John Blaikley, Marie Pariollaud, Laura Matthews, Toryn Poolman, Louise Ince, James Zhang, Kathryn Else and David Ray Faculty of Life Sciences, University of Manchester, UK

It is widely recognized that the circadian clock controls multiple aspects of immune function. Systemic challenges to the innate immune system elicit marked variation in inflammatory responses depending on time of day. This is known to depend on the operation of circadian gating mechanism within cells of the monocycte lineage, particularly macrophages. These reside in every tissue of the body, where they engulf apoptotic cells and pathogens and produce immune effector molecules such as cytokines. Using conditional targeting approaches, we have shown that rhythmic RevErb signaling plays a crucial role in temporal control of cytokine secretion. There is however a remarkable selective response, and this depends on cell type. Within the lung, the gating mechanism regulating magnitude of inflammatory response is driven via a bronchial epithelial cellular clock, rather than resident macrophages. Glucocorticoid signaling also regulates this epithelial clockwork, which controls the rhythmic expression of a key proinflammatory epithelial derived chemokine. Critically, this depends on local rhythmic control of the glucocorticoid receptor (GR) rather than circadian adrenal corticosteroid secretion. In this way, normal physiological responses depend on resonance between a rhythmic hormone signaling pathway and cytokine responses to microbial challenges. A critical and largely unaddressed question is why cellular clocks in target tissues regulate so many features of immune function. One intriguing possibility is that immunity and energy metabolism are inextricably linked, and that rhythmic immunity may therefore emerge as a consequence of clock control of energy flux through the cell.

#### S33. ANNIE CURTIS

# "Circadian Control of Innate Immunity in Macrophages by miR-155 Targeting Bmal1."

Curtis A.M.<sup>1</sup>, Fagundes C.T.<sup>1</sup>, Yang G.<sup>2</sup>, Palsson-McDermott E.M.<sup>1</sup>,Wochal P.<sup>1</sup>, McGettrick A.F.<sup>1</sup>, Foley N.H.<sup>1</sup>, Early J.O.<sup>1</sup>, Chen L.<sup>2</sup>, Zhang H.<sup>3</sup>, Xue C.<sup>3</sup>, Geiger S.S.<sup>1</sup>, Hokamp K.<sup>4</sup>, Reilly M.P.<sup>3</sup>, Coogan A.N.<sup>5</sup>, Vigorito E.<sup>6</sup>, FitzGerald G.A.<sup>2</sup> and O'Neill L.A.<sup>1</sup> 

<sup>1</sup>School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute (TBSI), Trinity College Dublin, Dublin 2, Ireland, <sup>2</sup>Institute for Translational Medicine and Therapeutics (ITMAT), Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA, <sup>3</sup>The Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA 19104, USA, <sup>4</sup>Department of Genetics, Trinity College Dublin, Dublin 2, Ireland, <sup>5</sup>Department of Psychology, National University of Ireland, Maynooth, Ireland, <sup>6</sup>Laboratory of Lymphocyte Signaling and Development, The Babraham Institute, Babraham Research Campus, Cambridge, UK

The response to an innate immune challenge is conditioned by the time of day, but the molecular basis for this remains unclear. In myeloid cells, the induction by lipopolysaccharide (LPS) of the pro-inflammatory microRNA miR-155 is greater at ZT12 versus ZT0 and its level of induction is suppressed by BMAL1. BMAL1 in the myeloid lineage protects mice from LPS induced sepsis particularly at ZT0, correlating with suppression of a range of pro-inflammatory cytokines. This maybe due to inhibition of NF- RB as levels of phosphorylated p65 induced by LPS were lower in myeloid cells lacking BMAL1. Bmal1 has two miR-155 binding sites in its 3'-UTR and in response to LPS, miR-155 binds to these two target sites leading to suppression of BMAL1 mRNA and protein. This effect was observed in mice and in humans exposed to low dose LPS. Blocking of

miR-155 with antagomirs protected BMAL1 from LPS induced suppression and also caused a reduction in p65 phosphorylation and the pro-inflammatory cytokine TNF $\alpha$ . MiR-155 deletion gives rise to a shorter period length in mice and also ablates the circadian gating on cytokine responses to LPS. Innate immunity is therefore utilizing the control of BMAL1 by miR-155 to control the circadian inflammatory response in myeloid cells. Our findings provide insight into the temporal control of inflammation, which could have consequences for our understanding of the pathogenesis of inflammation and infectious diseases where circadian regulation is known to be important.

### **Short Communications**

# SC45. "Catabolic cytokines disrupt the circadian clock in cartilage and intervertebral disc via an NF B-dependent pathway."

Michal Dudek, Baoqiang Guo, Nan Yang, Elzbieta Borysiewicz, Jack L. Williams, Michael R. H. White, Elizabeth S. Maywood, Michael H. Hastings, Ray P. Boot-Handford and Qing-Jun Meng

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Chronic low grade inflammation that accumulates with age has been suggested to drive the early degeneration and late stage tissue destruction during the development of osteoarthritis and disc disorders. But how inflammation impacts on the circadian rhythm in cartilage and disc is still unknown. This study aims to investigate how the catabolic cytokines (IL-1 and TNFα) affect the circadian clock mechanism and the expression of clock-controlled catabolic genes within cartilage and intervertebral discs, and to examine the downstream pathways linking the cytokines to the molecular clock within the cells. Stimulation with IL-1ß severely disrupted circadian gene expression rhythms in cartilage and intervertebral discs. This effect was reversed by an anti-inflammatory drug dexamethasone, but not by other clock synchronizing agents. Circadian disruption mediated by IL-1β was accompanied by disregulated expression of endogenous clock genes and clock-controlled catabolic pathways. Mechanistically, NFkB signalling was involved in the effect of IL-1\beta on the cartilage clock partially through interference with the core Clock/BMAL1 complex. In contrast, TNFα had little impact on the circadian rhythm and clock gene expression in cartilage or intervertebral discs. Our data implicate disruption of the cell autonomous clocks in cartilage and disc as a novel aspect of the catabolic responses of cartilaginous tissues to pro-inflammatory cytokines, and elucidate an additional mechanism for how chronic joint inflammation may contribute to osteoarthritis and disc degeneration.

SC46. "A role for CRYPTOCHROME in the regulation of inflammatory arthritis" Hopwood T.W., Ray, D.W., Loudon, A.S.I., Bechtold D.A. and <u>Gibbs J.E.</u>
Faculty of Medical and Human Sciences, University of Manchester, UK

The circadian clock is critical in regulating immunity. Inflammatory cells possess endogenous timers, which regulate the amplitude of response to an immune stimulus.

Disruption of these clocks has a dramatic negative impact on the outcome of inflammatory challenge.

Rheumatoid arthritis (RA) is a chronic inflammatory disease, which exhibits strong diurnal variation in symptoms. Typically RA patients report increased joint stiffness in the early morning, corresponding with a peak in levels of circulating pro-inflammatory cytokines.

In keeping with these clinical observations, we show that mice induced with arthritis display robust circadian variation in disease. In the morning (ZT0-6) levels of circulating pro-inflammatory cytokines are heightened; paw swelling is maximal; and cytokine transcript levels within inflamed joints are highest. Conversely, arthritic joint inflammation is dampened at night, likely a consequence of actions of the repressive arm of the molecular clock (PER and CRY).

Environmental perturbations were utilised to assess the impact of circadian disruption on disease rhythmicity. Under constant light, rhythmic behaviour in mice was disrupted and peripheral organs became arrhythmic, with dampened expression of PER and CRY. In arthritic mice, this resulted in loss of rhythmicity in disease markers, with heightened inflammation throughout the day.

A causative role for CRY was confirmed through genetic and pharmacological modulation of CRY *in vitro* using joint derived cells. To conclude, we have identified an output pathway from the clock to inflammation mediated by CRY, and highlight the clock as a potential target for therapeutic intervention in RA.

### SC47. "Circadian regulation of wound healing and actin dynamics."

Ned Hoyle, Estere Seinkmane and John O'Neill

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH. United Kingdom

Dynamic regulation of actin polymerisation and depolymerisation is crucial to cell motility and crucial for many biological processes, including wound healing. Actin dynamics are reported to be circadian regulated in mouse liver and spleen *in vivo* but the underlying mechanism is unclear. Moreover, the functional consequences have not been explored at the cellular or organismal level.

We present data showing circadian rhythms of actin polymerisation in immortalised fibroblasts *in vitro*, indicating that circadian regulation of cytoskeletal dynamics occurs cell-autonomously. Using cells from arrhythmic *Cry1*-/-/*Cry2*-/- double homozygous null mice, we demonstrate that these rhythms are driven by canonical transcription-translation circadian feedback mechanisms.

We go on to show that, whilst cell motility is not circadian *per se*, there is a robust time-of-wounding effect that exerts persistent influence upon the efficacy of wound healing in cultured fibroblasts. Consistent with predictions from these *in vitro* observations, we observe that fibroblast exfiltration from wounded skin into blood clots shows the same time-of-wounding effect *ex vivo*.

Circadian regulation of wound healing and cytoskeletal dynamics may hint at wider ramifications, possibly applying an influence whenever tissue rearrangement occurs.

SC48. "Impact of Bmal1 knockdown on cell cycle progression of NIH3T3 cells." Elham Farshadi<sup>1</sup>, Jie Yan<sup>2</sup>, Pierre Leclere<sup>1</sup>, Albert Goldbeter<sup>2</sup>, Ines Chaves<sup>1</sup> and Gijsbertus van der Horst<sup>1</sup> <sup>1</sup>Department of Genetics, Erasmus University Medical center, Rotterdam, The Netherlands, <sup>2</sup>Faculté des Sciences, Université Libre de Bruxelles, Brussels, Belgium

Gating of cell division by the circadian clock has been observed in various studies. There is significant evidence that in mammals circadian rhythms affect the timing of cell division *in vivo*. Circadian clock disruption, either by light or genetic mutations, is linked to alterations in the rate of cell proliferation, apoptosis, DNA damage and metabolism, and to cancer predisposition. However, still little is known on how these two oscillators interact. In order to understand the mechanism behind the circadian gating of cell division, we have studied the impact of circadian clock deregulation on cell cycle progression.

In a recent study we used a mouse fibroblast line with fluorescent reporter genes for clock and cell cycle phase (NIH3T3<sup>3C</sup>), and identified a tight coupling between the circadian clock and the cell cycle (Feillet et al. 2014, PNAS 111:9828-33). We showed that cell division always occurs at the same circadian clock phase. Using the NIH3T3<sup>3C</sup> cells as a tool, we then analyzed timing of cell division when the circadian clock is disturbed, by knocking down *Bmal1* or *Clock*. Our data show that, in both cases, the length of the cell cycle is prolonged. In agreement with this observation, a mathematical simulation of the effect of knockdown on the coupling between cell division and the circadian clock also revealed a lengthening of the cell cycle with decreasing *Bmal1* mRNA.

The data so far indicate that, in the absence of the positive limb of the circadian clock, cell division takes longer. Ongoing experiments will delineate the mechanism behind the coupling between the two cycles.

# SC49. "A novel mechanism links inflammation to the clock through REV-ERB protein stability."

<u>Marie Pariollaud</u>, Julie Gibbs, Baoqiang Guo, Nicholas Tomkinson, Dion Daniels, Danuta Mossakowska, Stuart Farrow, Yolanda Sanchez, Andrew Loudon and David Ray *University of Manchester, Manchester, UK* 

Recently, we discovered a major role for the clock in epithelial cells regulating lung inflammation, mediated by control of neutrophil chemokine expression. In these studies we disrupted BMAL1 in the epithelial cell lineage. It is not clear whether these effects are mediated via central circadian clock disruption or a more general pleiotropic effect of BMAL1 disruption. In these new studies, we examined the role of REV-ERBα in pulmonary immunity, using *in-vivo* gene targeting, and *ex-vivo* cell biology approaches. Initial studies of *Rev-erbα* knock-out mice revealed an increase in pulmonary neutrophilia and inflammation upon nebulised lipopolysaccharide (LPS). *Ex-vivo* analysis revealed bronchial epithelial cells and macrophages both responded to selective REV-ERB ligands, with a striking different pattern depending on cell type. This raised the possibility that REV-ERB may be either pro- or anti-inflammatory depending on cell type. Moreover, by selectively deleting REV-ERBα in the mouse bronchial epithelium, we observed exaggerated inflammatory responses to LPS, and augmented CXCL5 secretion; this is strikingly similar to the effects of BMAL1 deletion in these same cells.

We next tested whether expression of REV-ERB protein is responsive to inflammatory stimuli. Using a novel monoclonal antibody, we observed a striking loss of REV-ERB protein upon pro-inflammatory challenge. Further analysis revealed this degradation was dependent on 26S proteasome and driven by ubiquitination of REV-ERB. Moreover, using a novel REV-ERB-selective ligand, ubiquitination was blocked and the protein protected from degradation.

Collectively, our results propose a new model for a central role for REV-ERBa in conferring clock control to lung inflammation.

### SYMPOSIUM 11: RHYTHM ONTOGENY AND ENVIRONMENTAL INFLUENCES

Chair: Maria Canal (UK)
Sponsored by: The Company of Biologists

#### S34. MARIA CANAL

# "Programming of Mice Circadian and Stress Systems by Postnatal Light Environment."

Maria Mercè Canal and Georgia Coleman

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The postnatal light environment that a mouse experiences during the critical first 3 postnatal weeks has long-term effects on both its circadian rhythm output and clock gene expression. Furthermore, data from our lab suggest that postnatal light may also impact the hypothalamo-pituitary-adrenal (HPA) axis, which is a key regulator of stress, but the specific effects of postnatal light experience on future stress responses and circadian rhythmicity are still unclear.

To test this, we raised mice under 24-h light-dark cycles (LD), constant light (LL) or constant dark (DD) during the first 3 postnatal weeks, and examined HPA axis and circadian function in adulthood under both basal and stressed conditions. We found that postnatal light environment has long-term effects on: a) brain neuropeptide and glucocorticoid receptor expression, b) plasma corticosterone concentration rhythm and body temperature rhythm under LD and LL environments, and c) depression-related behaviour in adult animals.

Taken together, these data suggest that, in addition to the effects on the circadian system, altered postnatal light environments also have long-term effects on the HPA axis, which may lead to a depressive phenotype later in life.

### S35. ALENA SUMOVA

### "Maternal Entrainment of the Developing Clocks."

Sumová A., Olejníková L., Polidarová L., Houdek P. and Sládek M. *Institute of Physiology, the Czech Academy of Sciences, Prague, Czech Republic* 

The mammalian circadian system develops gradually during early ontogenesis as the suprachiasmatic nuclei (SCN) begin to play a role of the central clock and entrain the periphery. Maternal cues set the phase of the developing SCN already during the prenatal period but the peripheral clocks are entrained until the weaning. Our results suggest that maternal cues drive rhythmically gene expression in the fetal SCN before the individual cellular oscillators are synchronized and exhibit rhythms at the tissue level. Previously we found that circadian phenotype of adult spontaneously hypertensive rats (SHR), which were bred from Wistar strain as rats developing pathology, differs from that of Wistar rats. In the Wistar rat x SHR cross-foster study, we demonstrate that sensitivity of the circadian clocks in the SCN and peripheral tissues to maternal cues is greatly dependent on the offspring genotype. We revealed significant differences in responses of

the developing clocks of these two closely related rat strains to the cross-foster procedure. The effect on the developing clocks was tissue dependent. The sensitivity to maternal cues was determined not only by the maternal circadian phenotype but also by the pup's genetic background. Importantly, we found that circadian phenotype of the foster mother affected the pup's circadian system also after weaning when it caused after-effects on the hepatic clock amplitude. Our results suggest that during the critical period, the developing molecular clocks are unexpectedly and robustly responsive to cues related with inappropriate maternal care.

[The study was supported by the Czech Science Foundation grant P30412G069]

S36. DOUGLAS MCMAHON

"Photoperiodic Programing of Neural Function: A SAD story."

Douglas G. McMahon

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Day length, or photoperiod, influences depression and anxiety behaviors, but the neurobiological mechanisms are not known. Serotonin is a regulator of affective behaviors and the serotonergic dorsal raphe nuclei receive light input from the circadian visual system and indirect input from the biological clock nuclei. We examined the effect of photoperiod during development and found that it can induce enduring changes in the function of mouse dorsal raphe serotonin neurons—programming their firing rate, responsiveness to noradrenergic stimulation, intrinsic electrical properties, serotonin and norepinephrine content in the midbrain, and depression/anxiety-related behavior in a melatonin receptor 1 (MT1)-dependent manner. Our results establish mechanisms by which seasonal photoperiods may dramatically and persistently alter the function of serotonin neurons. In addition previous work from our laboratory has shown that seasonal photoperiods presented during development and maturation have enduring effects on the functional properties of the biological clock and its constituent clock neurons, and on retinal light sensitivity. Taken together, these results suggest that the function of the developing biological clock, retina and raphe is informed of and modified by photoperiod.

#### Short Communications

# SC50. "The *Iola* and *Pdk1* are essential for development of the sLNvs in the fruit fly *Drosophila melanogaster.*"

Outa Uryu and Ryusuke Niwa

Faculty of Life and Environmental Sciences, University of Tsukuba, Japan

Pigment-dispersing factor (PDF) is the neuropeptide indispensable for regulating circadian rhythm in the fruit fly *Drosophila melanogaster. pdf* gene is specifically expressed in the small ventral lateral neurons (sLNvs) and large ventral lateral neurons. While it is well known that the *pdf*-positive neurons play an essential role in maintaining behavioral rhythmicity under free-running conditions, little is known about how development of the clock cells are regulated at the molecular level. Here, we found two genes that influence development of the sLNvs. These are *longitudinals lacking* (*lola*), which is a Zn-finger transcriptional factor, and *Phosphoinositide-dependent kinase* 1

(*Pdk1*). The adult brain of *Iola<sup>RNAi</sup>* and *Pdk1<sup>RNAi</sup>* flies showed abnormal development of the sLNvs. The numbers of sLNvs in either *Iola* or *Pdk1* knockdown flies decreased, and their cell sizes also became smaller. Furthermore, *Iola* and *Pdk1* knockdown flies display arrhythmic behaviour. We therefore hypothesize that, these genes are essential for development of the sLNvs in *Drosophila*.

# SC51. "Maternal photoperiodic programming of the hypothalamic control of seasonal reproduction in the Siberian hamster."

<u>Cristina Sáenz de Miera</u><sup>1,2</sup>, Béatrice Bothorel<sup>1</sup>, Michael Birnie<sup>2</sup>, Valérie Simonneaux<sup>1</sup> and David Hazlerigg<sup>2,3</sup>

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Mammals perceive seasons via the photoperiodic changes in circulating melatonin and adapt their biological function accordingly. Melatonin acts through a hypothalamic network involving thyroid stimulating hormone subunit pars subjectatis, the type 2 (Dio2) and 3 (Dio3) thyroid hormone deiodinases in the tanycytes. Maternal melatonin reaches the fetus via the placenta and is known to affect fetal photoperiodic responses. We used pregnant female Siberian hamsters (Phodopus sungorus) placed under long (LP: 16h light (L):8h dark (D)) or short (SP: 8L:16D) photoperiod until pups' weaning to investigate the programming effect of maternal melatonin on the hypothalamic mechanisms regulating seasonal physiology. At weaning, half of the offspring from both groups were transferred to intermediate photoperiod (IP) (14L:10D). LP gestated animals showed a decrease in testicular development after exposure to IP in comparison to the LP kept controls whereas offspring gestated in SP showed increased testicular development when exposed to IP as compared to the SP kept controls. TSH expression in the pars tuberalis was identical in IP exposed animals. In contrast, Dio2 expression was higher in SP than in LP gestated animals, and vice-versa for Dio3. This difference appears to arise from a different sensitivity to the TSH signal at the level of the tanycytes. This hypothesis is currently being tested by analysis of gene and protein expression and of DNA methylation in the tanycytes. Our results show that the photoperiod experienced during gestation is able to program the adult offspring hypothalamic gene expression in response to the same melatonin input.

### SC52. "Mechanisms driving circannual rhythms in mammals."

<u>Shona H. Wood</u><sup>1</sup>, Helen Christian<sup>2</sup>, Katarzyna Miedzinska<sup>3</sup>, Ben R.C. Saer<sup>1</sup>, Mark Johnson<sup>2</sup>, Bob Paton<sup>3</sup>, Le Yu<sup>3</sup>, Judith McNeilly<sup>4</sup>, Julian R.E. Davis<sup>5</sup>, Alan S. McNeilly<sup>4</sup>, David D. Burt<sup>3</sup> and Andrew S.I. Loudon<sup>1</sup>

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Free-running circannual rhythms have evolved in animals to regulate hormone cycles and time annual reproduction. Little is yet known of the mechanisms involved. In contrast, the photoperiodic relay is now relatively well understood. Here, the rhythmic melatonin signal drives a circadian controlled transcriptional co-activator (EYA3) in the pituitary pars tuberalis (PT) to drive thyroid stimulating hormone (TSH $\beta$ ) expression in specialized PT-thyrotrophs. Thyroid hormone (TH) conversion in ependymal tanycytes is stimulated, timing seasonal changes in physiology. We set out to explore whether EYA3 and the PT-thyrotroph are also involved in the generation of the circannual cycle.

We used seasonal sheep to characterise the circannual cycle, transferring animals from short (winter-like) photoperiods (SP) to long (summer-like) photoperiods (LP). This induced a rapid increase in prolactin concentrations and activation of EYA3 and TSHB protein, and suppression of chromogranin-A (CHGA) in PT-thyrotrophs. Following prolonged LP-exposure, prolactin concentrations declined and PT-thyrotrophs reverted to a SP-state dominated by CHGA expression. Remarkably, individual PT-thyrotroph cells operate as a binary signal within the tissue, and can only be either EYA3+ve/CHGA-ve (LP-like) or CHGA+ve/EYA3-ve (SP-like). No cells expressed both proteins. RNA SEQ studies revealed dynamic changes in pathways involved in cellular re-modelling and axon guidance, and anatomical analyses revealed dynamic morphological changes and tissue re-organisation, extending from the PT to the adjacent hypothalamic tanycytes. We now propose that the PT operates as a critical calendar cell, driving circannual neuroendocrine cycles, and via morphological gradients, may also re-model the adjacent hypothalamus. The key signal (EYA3) is encoded digitally, with the overall phase of the cycle determined by the numbers of expressing thyrotroph cells. There are remarkable parallels here to recent models defining vernalisation mechanisms in plants, suggesting the conservation of ancient genetic pathways driving seasonal cycles in eukaryotic organisms.

SC53. "After-hours mice show specific epigenetic and physiological alterations." Federico Tinarelli, Elena Ivanova, Ilaria Colombi, Erica Barini, Laura Gasparini, Michela Chiappalone, Gavin Kelsey and Valter Tucci

Neuroscience and Brain Technologies Department, Neurobehavioural Genetic group, Istituto Italiano di Tecnologia, Genova 16163 Italy

At present, very little is known on the role of epigenetic mechanisms in circadian clock, while transcriptional and translational positive and negative circadian loops are well characterized. This work investigated specific questions about the interaction between circadian clock alterations and DNA methylation using the After-hours (Afh) circadian mouse mutants. Recent studies demonstrated that methylation is an incredibly dynamic process in the brain, which relies on environmental variations and neuronal activity. We performed a genome-wide methylation screening for CpG Island (CGIs) in suprachiasmatic nucleus (SCN) of Afh homozygous animals and controls. Reduced representation bisulphite sequencing (RRBS) and Pyrosequencing approaches have identified a subset of 15 CGIs genetic regions presenting significant changes, greater than 10%, in DNA methylation between Afh mutants and wild-type controls. Moreover, 14 of 15 selected sequences were hypermethylated in the mutants compared to the wild type, implying an involvement of Afh mutation in hypermethylation of specific intragenic CpGs areas. Gene expression levels of RRBS targets were coherent with methylation status in SCN. Among the identified targets, we have further studied the role of Opn4 and Chat in Afh homozygous animals. Investigation of intrinsic physiological Afh neuronal

network properties *in vitro* revealed a different responsiveness to synchronization drug treatment in homozygous mutants. Overall, this work revealed that a the *Afh* mutation can modify the DNA methylation profile of genes involved in cell signalling, synaptic plasticity and transcriptional regulation processes.

### **SYMPOSIUM 12: COMPARATIVE CLOCKS**

Chair: Vinod Kumar (India)

#### S37. VINOD KUMAR

"Timekeeping in Birds: Insights Into Molecular Regulation of Photoperiodic Timing of Seasonal Physiology in Migratory Songbirds."

Vinod Kumar

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Birds seasonally switch from one life-history state (LHS) to another in order to maximize their fitness. This occurs in synchrony with the predictable seasonal change in the photoperiod that triggers a sequential change in the behaviour and physiology, adaptive for migration in several species. Thus, there are clear differences in the phenotypes between the day and seasons. Possible molecular mechanisms underlying these changes have scarcely been examined. Therefore, recent studies in my laboratory have focused on the measurement of key genes that are implicated in the regulation of photoperiodic-induced seasonal LHSs in the long distance migratory songbirds, the blackheaded (Emberiza melanocephala) and redheaded buntings (E. bruniceps). The mRNA levels of circadian clock genes and of genes that encode for proteins/ enzymes involved in the regulation of glucose and lipids showed LHS-dependent changes in their expression patterns in the brain and peripheral tissues. Along with, there were differences in the daily expression pattern of genes coding for enzymes involved in the oxidative decarboxylation of pyruvate to acetyl-CoA and oxidative phosphorylation in the TCA cycle in the hypothalamus and liver. These results on genetic regulation of physiology suggest the adaptation of oxidative phosphorylation pathway(s) at gene levels to the seasonal alternations in metabolism, and extend the idea that the transitions between life-history states are accomplished by changes at multiple regulatory levels in migratory songbirds. These findings thus promise new insights into the mechanism(s) of adaptation to seasons in higher vertebrates.

#### S38. JOHN O'NEILL

"Metabolic Oscillations in Yeast and Circatidal Cycles in *Eurydice pulchra* Share Features Conserved Among Circadian Rhythms."

John O'Neill, Helen Causton, Kevin Feeney, David Wilcockson, Michael Hastings, Charalambos Kyriacou, Simon Webster et al.

MRC Laboratory of Molecular Biology, Cambridge

Circadian rhythms allow organisms to temporally orchestrate their internal state to anticipate and/or resonate with the external environment. Although 24-hr periodicity is observed across aerobic eukaryotes, the central mechanism has been hard to dissect because few simple models exist, and known clock proteins are not conserved across

phylogenetic kingdoms. In contrast, contributions to timekeeping made by a handful of post-translational mechanisms, such as phosphorylation of clock proteins by casein kinase 1, appear conserved among phyla. These kinases have many other essential cellular functions and are better conserved in their contribution to timekeeping than any of the clock proteins they phosphorylate. Temperature compensation and metabolic rhythms are another universal feature of circadian timekeeping. Here, we use comparative chronobiology to distinguish fundamental clock mechanisms from species-specific adaptations and thereby identify features shared between the mammalian cellular clock, ultradian respiratory oscillations in budding yeast and circatidal rhythms in the crustacean Eurydice pulchra. Our data point to common mechanisms underlying all three biological rhythms and suggest two interpretations: either certain biochemical systems are simply permissive for cellular oscillations (with frequencies from hours to days) or this commonality arose via divergence from an ancestral cellular clock.

#### S39. BAMBOS KYRIACOU

# "Molecular control of biological rhythms in an intertidal invertebrate, *Eurydice pulchra*"

Lin Zhang<sup>1</sup>, David Wilcockson<sup>2,3</sup>, John O'Neil<sup>4</sup>, Kate Lee<sup>1</sup>, Simon Webster<sup>2</sup>, Michael Hastings<sup>4</sup> and Charalambos P Kyriacou<sup>1</sup>

<sup>1</sup>Dept Genetics University of Leicester, <sup>2</sup>School of Biological Sciences, Bangor University, <sup>3</sup>Institute of Biological, Environmental, and Rural Sciences, Aberystwyth University, <sup>4</sup>MRC Laboratory of Molecular Biology, Cambridge, UK

Eurydice pulchra, is an intertidal crustacean that shows tidal and circadian phenotypes in behaviour, physiology and gene expression. The 12.4 h tidal swimming behaviour is temperature compensated, free-runs, and can be entrained by vibration. The peroxiredoxin over-oxidation cycle is also tidal, as is the expression of most mitochondrially expressed genes. Circadian phenotypes include the 24 h modulation of tidal swimming, chromatophore dispersion and Eptimeless mRNA rhythms. Constant light or Epper knockdown blunts circadian but not tidal cycles, suggesting that a functional circadian oscillator is not necessary for tidal behaviour. However, somewhat surprisingly, casein kinase 1  $\epsilon/\delta$  inhibitors disrupt both circadian and tidal phenotypes, suggesting that either the two cycles may share a common inhibitor target, or that the inhibitors act promiscuously on different targets to affect the two phenotypes independently. We attempt to resolve this issue with further biochemical and genetic analyses.

#### **Short Communications**

SC54. "Annual life-history dependent circadian timing in central and peripheral tissues in a migratory blackheaded bunting (*Emberiza melanocephala*)."

Devraj Singh<sup>1</sup>, Sangeeta Rani<sup>2</sup>, Amit Kumar Trivedi<sup>1</sup>, Satchidananda Panda<sup>3</sup> and Vinod Kumar<sup>1</sup>

DST-IRHPA Center for Excellence in Biological Rhythms Research and IndoUS Center for Biological Timing, <sup>1</sup>Department of Zoology, University of Delhi, Delhi, 110 007, India, <sup>2</sup>Department of Zoology, University of Lucknow, Lucknow, 226 007, India, <sup>3</sup>Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA

How circadian clock connects to photoperiod-induced changes with seasons is poorly understood. We hypothesized phase and amplitude plasticity in circadian clock gene oscillations with photoperiod-induced change in seasonal life history states (LHSs) in seasonal species. To test this, we measured daily Per2, Cry1, Bmal1 and Clock mRNA expressions in night-migratory blackheaded buntings under short (8h light: 16h darkness, 8L:16D; short day sensitive, SD-S) and long (16L:8D) photoperiods in different phases of their migratory activity such as, only day active (long day premigratory, LD-pM), intensely night-active (long day migratory, LD-M) and again back to day active (long day refractory, LD-R). Differences in the phase and amplitude of circadian oscillations of Per2, Cry1 and Bmal1 genes, less so of the Clock, between photoperiod-induced LHSs, corresponded with seasonal shifts in the activity behaviour. Phase relationship in circadian molecular oscillations also differed between seasons in component oscillators constituting the central circadian pacemaker system (retina, pineal and hypothalamus) and between the central clock and peripheral (liver, muscle) tissues. These results for the first time show altered waveforms of clock genes expression in both, central clock and peripheral tissues, in parallel with behavioral shifts, and suggest the involvement of circadian system in photoperiodic induction of seasonal LHSs in a photoperiodic species.

### SC55. "Socially-mediated circadian plasticity in the social bumblebee *Bombus* terrestris."

Jacob G. Holland, Moshe Nagari and Guy Bloch

Department of Ecology, Evolution and Behavior/Hebrew University of Jerusalem, Israel

Insect societies often contain genetically similar individuals specialising in different roles via a division of labour. In honeybees, bumble bees, and several ant species, colony roles are associated with plasticity in circadian rhythmicity, with externally foraging workers and in-nest nurse workers exhibiting strong and weak (or absent) rhythms respectively, in both behaviour and brain clock gene expression. This role-related plasticity in circadian rhythms is maintained irrespective of photic regime, but can change during the lifetime of individuals. We studied two important aspects of plasticity in circadian rhythms 1) determining social factors which regulate chronobiological plasticity, and 2) identifying differentially regulated clock controlled molecular processes. Firstly we show that, in bumble bees, isolated individuals exhibit behavioural and molecular rhythms, but that these rhythms are significantly reduced in power by exposure to developing larvae or pupae. Thus, we reveal that interactions with various brood stages regulate circadian plasticity. Secondly, we present an ongoing study designed to produce circadian transcriptomes from individual bumble bees which vary in social environment and behavioural rhythmicity. Given the importance of circadian regulation of physiological processes in maintaining animal health, it is puzzling that some animals, such as nurse bees, can naturally show prolonged periods of around-the-clock activity during which they exhibit attenuated or absent circadian rhythms. Thus, we aim to detect putative processes for which circadian regulation still occurs, even in behaviourally arrhythmic animals.

SC56. "The role of RFRP in seasonal reproduction: sex- and photoperiod-dependent variations."

<u>Jo B. Henningsen</u><sup>1</sup>, Vincent-Joseph Poirel<sup>1</sup>, Jens D. Mikkelsen<sup>2</sup>, Francois Gauerand<sup>1</sup> and Valerie Simonneaux<sup>1</sup>

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Hypothalamic RF-(Arg-Phe) related peptides (RFRP-1 and -3) are considered to play a role in the seasonal regulation of reproduction and their expression is down-regulated in SP. RFRP-3 regulates reproductive activity; however the peptides effect depends on species and gender. This study aimed at characterising and comparing the RFRP system in male and female Syrian hamsters in LP and SP, as well as evaluating the effects of intracerebroventricular RFRP-3 administration in female Syrian hamsters.

The neuroanatomical distribution of RFRP neurons and fibers, as well as GPR147 mRNA, is similar in male and female Syrian hamsters. The number of RFRP neurons is higher in females as compared to males, and in both sexes, number of RFRP neurons is reduced in SP. GPR147 mRNA levels are higher in females than in males and are down-regulated in SP, particularly in females. Interestingly, the number of RFRP-positive fibers in the MPN/AVPV is higher only in SP-adjusted females. Finally, chronic icv administration of RFRP-3 decreases gonadal size in sexually active female hamsters, whereas in SP, RFRP-3 stimulates reproductive activity.

RFRP and its receptor GPR147 are regulated by season and their expression is down-regulated in SP in male and female Syrian hamsters. Moreover, our results suggest that the RFRP system is particularly important in females with a distinct role in the MPN/AVPV, possibly in regulation of the pre-ovulatory LH surge via kisspeptin neurons. The effect of RFRP-3 in female Syrian hamsters varies with season and interestingly, RFRP-3 potently stimulates reproductive activity in SP-adapted females despite photoinhibitory conditions.

# SC57. "Diurnal profile of catecholamines, neuropeptides and their receptors in the chicken pineal gland."

Magdalena Markowska<sup>1</sup>, Bogdan Lewczuk<sup>2</sup>, Monika Malz<sup>1</sup>, Pawel M. Majewski<sup>1</sup> and Iwona Adamska<sup>1</sup>

<sup>1</sup>Department of Animal Physiology, Faculty of Biology, University of Warsaw, Poland, <sup>2</sup>Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

Avian pineal gland is a partially autonomous component of the endogenous master clock. Its hormone, melatonin, secreted in a diurnal rhythm acts as a chemical transducer of photoperiodic information. Thus, understanding of mechanisms involved in the regulation of melatonin biosynthesis is crucial for explanation of processes synchronized with photoperiod. It has been postulated for many years that the pineal gland activity is regulated mainly by the sympathetic noradrenergic innervations. Then it was shown that vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) modify the release of melatonin, but they do not affect pineal oscillator. The aim of this study was to examine the diurnal rhythm of catecholamine metabolism and the expression of neuropeptides and their receptors in the chicken pineal gland. Sixteen-day-old chickens kept from the day of hatch in L:D 12:12 were killed by decapitation every 2h over a 24h period. Pineal glands were isolated under dim red light. Catecholamines levels were measured by HPLC and mRNA level of VIP, PACAP and their receptors were measured by RT-qPCR. The pineal content of dopamine, 3,4dihydroxy-L-phenylalanine, 3,4 dihydroxyphenylacetic acid and homovanillic acid changed rhythmically with acrophases at the end of the photophase. Noradrenaline level was relatively high and changed rhythmically with the lowest level at the and of a day. Diurnal profiles of VIP and PACAP were similar, with the acrophase at the beginning of the night whereas the mRNA level of their receptor, VPAC1, changed rhythmically with the reversed acrophase.

[Supported by National Science Centre Grant UMO-2012/07/B/NZ3/02919]

#### SYMPOSIUM 13: HUMAN CLOCKS, SLEEP, AND METABOLISM

Chair: Andries Kalsbeek (Netherlands)

#### S40. FRANK SCHEER

"Bittersweet: The Impact of the Circadian System and its Disturbance on Glucose Control and Metabolism in Humans."

Frank A.J.L. Scheer

Medical Chronobiology Program, Brigham and Women's Hospital; Division of Sleep Medicine, Harvard Medical School, USA

"A calorie is a calorie", with these words, scientists and clinicians in the field of nutrition and metabolism wave away the idea that the timing of food *per se* can influence body weight regulation. Diagnosis of diabetes is typically based on morning fasting assessments, driven by practicality, while glucose control has been shown to greatly depend on the time of day and night. Shift work is well-recognized to increase the risk for obesity, diabetes, and cardiovascular disease. However, to what degree this is dependent on changes in life style, sleep, or meal timing, and their interaction with genetics, is still unclear. Here, I will present recent findings from human studies on the influence of circadian phase, circadian misalignment, sleep, meal timing, and genetics on weight regulation, glucose control, energy expenditure, and cardiovascular risk factors. Increased understanding of the independent effects and underlying mechanisms for these cardiometabolic findings may help in the development of countermeasures against the adverse effects of shift work, as well as in the battle against the increasing prevalence of obesity and diabetes.

### S41. DEBRA SKENE

### "Metabolic Profiling in Human Plasma: Diurnal and Circadian Variation."

Debra J. Skene<sup>1</sup>, Sarah K. Davies<sup>1</sup>, Victoria L. Revell<sup>1</sup>, Benita Middleton<sup>1</sup> and Florence I. Raynaud<sup>2</sup>

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Metabolic profiling (metabolomics) offers the promise of identifying specific metabolic phenotypes associated with disrupted sleep and circadian timing that may help to elucidate the mechanisms linking circadian clock disruption, sleep deprivation and metabolic disorders. We have established untargeted and targeted reverse phase liquid chromatography/mass spectrometry (LC/MS) metabolomics to measure a range of features (250+) and metabolites (120+) in human plasma.

Initial studies have been designed to characterise the diurnal variation in metabolite levels in entrained healthy volunteers living in "real-life" controlled conditions (light/dark

cycle; wake/sleep; timed meals). Next the effect of a night of total sleep deprivation on metabolite levels and their 24 h rhythmicity was investigated. In addition, participants have been studied in a constant routine (CR) protocol to identify metabolites that exhibit intrinsic circadian rhythmicity.

Principal component analysis (PCA) revealed a clear time of day variation in the metabolome during a regular sleep/wake cycle, mean score on PC1 having a significant fit to a cosine curve (n=367 features R²(PC1)=15%; n=171 metabolites R²(PC1)=36%). Daily rhythms were observed in the majority of metabolites (n=109/171, 64%). During the 24h of wakefulness that followed, 78 of the metabolites maintained their rhythmicity, most with reduced amplitude (n=66). Under CR conditions, circadian variation was observed in the plasma features (n=259) and metabolites (n=40) with the mean score on PC2 (R²(PC2)=10%) and PC6 (R²(PC6)=3%), respectively, significantly fitting a cosine curve. Characterising the relative contribution of the circadian clock, sleep/wake, light/dark and fed/fasting state on metabolic profiling will aid future disease biomarker development. [Supported by BBSRC Grant (BB/I019405/1). DJS is a Royal Society Wolfson Research Merit Award holder]

### **Short Communication**

# SC58. "Cardiometabolic correlates of specific insomnia symptoms in type 2 diabetes patients."

<u>Annelies Brouwer</u><sup>1\*</sup>, Corina de Gier<sup>1</sup>, Daniël H. van Raalte<sup>2</sup>, Richard G. IJzerman<sup>2</sup>, Frank J. Snoek<sup>3</sup>, Aartjan T. F. Beekman<sup>1</sup> and Marijke A. Bremmer<sup>1</sup>

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Objective: Although sleep duration is well known to be associated with cardiometabolic factors, few studies have investigated specific insomnia symptoms. The objective of this study was to investigate the relationship between insomnia symptoms and cardiometabolic factors in type 2 diabetes (T2D) patients, who are at high risk for cardiovascular disease.

Methods: Patients with T2D (n=105) completed a one-week sleep diary and sleep questionnaires, and cardiometabolic measures of these patients were obtained. Cross-sectional regression analysis was performed to examine the association of sleep duration and specific insomnia symptoms with individual cardiometabolic factors and a clustered cardiometabolic risk (CCMR) score. The CCMR score incorporates standardized measures of obesity, dyslipidaemia, hypertension and hyperglycaemia.

Results: Sleep duration was associated with CCMR, triacylglycerol levels, high-density lipoprotein-cholesterol levels, and systolic blood pressure. Insomnia symptoms, namely sleep onset latency (SOL) and wakefulness after initial sleep onset (WASO), were associated with total cholesterol (P=0.013 and P=0.004) and low-density lipoprotein (LDL)-cholesterol (P=0.012 and P=0.005) levels. These associations remained significant after adjusting for potential confounders, such as depressive symptoms and sleep apnoea. The insomnia symptom of early morning awakening was not associated with CCMR and individual cardiometabolic factors. Sleep duration and specific insomnia

symptoms were not associated with glycated haemoglobin levels, body mass index, fat mass, and waist-hip ratio.

Conclusions: Sleep duration, but also specific insomnia symptoms, namely SOL and WASO, were associated cardiometabolic factors in T2D patients. Sleep duration and specific insomnia symptoms may therefore be important modifiable factors in the management of cardiovascular disease risk in T2D patients.

### SYMPOSIUM 14: JSC PHYSICAL AND CHEMICAL BASES FOR CIRCADIAN PERIOD AND TEMPERATURE COMPENSATION.

Chair: Takao Kondo (JAPAN)

S42. MICHAEL RUST

"The Cyanobacterial Clock Functions as a Cellular Energy Sensor."

Gopal K. Pattanayak, Guillaume Lambert, Connie Phong, Kevin Bernat and Michael J. Rust

Department of Molecular Genetics and Cell Biology, University of Chicago, USA

The natural function of circadian clocks is to contend with rhythmically varying external conditions. Thus, a fundamental question is how fluctuations in the external environment are biochemically coupled to the clock machinery. We show that the cyanobacterial clock machinery (KaiABC) is intimately connected to the ATP/ADP ratio available in the cell and the purified oscillator can synchronize itself to changes in nucleotides in vitro. Further, many mutants in vivo that alter the clock response also show correlated changes in energy charge and glycogen metabolism. To test the hypothesis that the cyanobacterial clock is sensing metabolism and not light directly, we used a synthetic biology approach to make *S. elongatus* capable of growth on exogenous sugar using an *E. coli* sugar transporter. Feeding these transgenic cyanobacteria with sugar supports high energy charge in the dark and blocks the resetting effect of a dark pulse. Further, in the absence of any light-dark cues, the clock in these cells will efficient entrain to rhythmic sugar feeding schedules. We conclude that the primary function of the cyanobacterial clock is to track metabolic rhythms, independent of their source.

S43. S. AKIYAMA

"KaiC as Circadian Pacemaker of Cyanobacterial Circadian Clock."

Shuji Akiyama

Research Center of Integrative Molecular Systems (CIMoS)/ Institute for Molecular Science, Japan

KaiC is a core protein of the cyanobacterial Kai oscillator, which persists without transcription–translation feedback. In the presence of KaiA and KaiB, KaiC reveals rhythmic activation/inactivation of its ATPase and autokinase/autophosphotase activities over approximately 24 h. Newer data are emerging on its slow, temperature-compensated ATPase activity, which is involved in the pacemaker for dynamic cellular events such as assembly/disassembly of the Kai components in cyanobacteria. To seek the molecular basis of the circadian timescale, we have studied the structure and function of KaiC ATPase extensively. On the basis of recent observations, we will discuss the origins of slow but ordered dynamics of the Kai oscillator.

#### S44. KOLODE

### "Mammalian Cryptochrome 1 Regulates Circadian Period Through Its Co-factor Pocket."

Koji L. Ode and Hiroki R. Ueda

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Mammalian cryptochrome 1 (CRY1) is a transcriptional repressor that closes a negative feedback loop to drive autonomous oscillation of circadian clock. It is widely accepted that the turnover rate or stability of CRY1 determines the circadian period. This study aims to investigate critical residues or domains within CRY1 for controlling the circadian period and ask whether protein stability is an only potent property of CRY1 to tune the period. By introducing mutations on the phosphorylation sites or conserved electron-transfer pathway of CRY1, many of these residues were found to be important for proper circadian period both in cultured cell and mice. Analysis of the mutation effect reemphasized close coupling between CRY1 turnover rate and the circadian period. This relationship were rigorously tested by applying small-molecule-inducible degradation of CRY1; artificially-induced degradation of CRY1 shortened the circadian period. Nevertheless, several mutants showed markedly altered circadian period without significant alteration on the CRY1 stability. These mutations are located mostly on near the p-loop and C-lid domain of CRY1, the structures of which are known to be altered by KL001, a period-lengthening compound that binds to the FAD pocket of cryptochrome. We further confirmed that residues not directly linked to phosphorylation/electrontransferring but supposed to be retain the structure of the two domain are critical for the circadian period. In summary, this study suggests that CRY1 regulates the circadian period not only through proper turnover rate as a collective dynamics of molecular population, but also through an inherent structure of individual CRY1 molecule.

### S45. MARTHA MERROW

# "Integration of the Temperature Environment by the Developmental Clock in *C. elegans.*"

Martha Merrow, Mirjam Geibel, Bala Koritala, Zheng Chen, Sandra Weber, Tanja Popp and Maria Olmedo

Institute of Medical Psychology, LMU Munich, Munich, Germany; Andalusian Center for Developmental Biology, Sevilla, Spain

We have characterised the developmental clock in *C. elegans* with respect to perturbations that are generally conserved in circadian clocks. Lithium lengthens developmental timing, similar to how it lengthens circadian free-running period. Pharmaceutical agents and heavy water that lengthen the circadian period also slow down the developmental clock. In contrast to circadian clocks, developmental clocks of worms and flies are characteristically temperature sensitive: they become slower at lower temperatures and faster at higher ones. We investigated temperature effects on developmental timing with a new, highly quantitative assay. At temperatures above 18 °C developmental timing is temperature compensated in the nematode. The duration of the molts is more compensated than is that of the larval stages, suggesting distinct timing

mechanisms. The animals fail to entrain however with structured zeitgeber cycles indicating a fundamentally different mechanism for integrating temperature into development.

#### Short Communications

SC59. "Cold-induced transcription of the period gene is associated with temperature-dependent resetting of daily rhythms in Drosophila."

Akanksha Bafna<sup>1</sup>, Tadahiro Goda<sup>2</sup>, Brandi Sharp<sup>2</sup> and Herman Wijnen<sup>1,2</sup>

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Many organisms take advantage of daily temperature cycles to synchronise their daily time keeping systems as well as associated physiological and behavioural rhythms. However, the molecular mechanisms underlying circadian temperature entrainment remain poorly understood. In a study of temperature-mediated resetting of molecular circadian oscillations in the fruit fly, Drosophila melanogaster, we noticed that the core clock gene *period* (*per*) exhibited early and prominent temperature-driven regulation. This was further confirmed in the context of temperature cycles or step-down experiments in an arrhythmic tim<sup>01</sup> background. qRT-PCR analyses of transgenic flies carrying a series of per-luciferase reporter constructs revealed that per is subject to cold-induced transcription via cis-regulatory elements residing between the transcriptional and translational start site. Further details regarding this regulation and its significance for circadian timekeeping will be discussed.

SC60. "Investigating the potential of mechanical cues in resetting cellular circadian clocks in adult stem cells."

<u>Jevons L.A.</u><sup>1</sup>, Bellantuono I.<sup>2</sup>, Hunt J.A.<sup>1</sup> and Pekovic-Vaughan V.<sup>1</sup>

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The circadian clock is an evolutionarily conserved timing mechanism in a wide array of organisms regulating our daily physiology and metabolism. Endogenous circadian clocks are entrained to oscillate with 24h periodicity, resulting in rhythmic changes in the expression of numerous genes, proteins and metabolites in a tissue-specific manner. Emerging data has indicated that circadian clocks play an important role in adult stem cell regulation, especially in their responses to external stimuli. Moreover, recent findings suggest that mechanical vibrations, in addition to light, temperature and food, act as a novel resetting cue to synchronize the circadian rhythms at the organismal level. Understanding how circadian rhythms could be reset by biophysical cues is of significant clinical value for regenerative stem cell therapies as disruption of circadian rhythms is recognised as one of the key drivers of age-related tissue dysfunction as well as increased risk of disease and drug toxicity.

Here we have tested the hypothesis that the time-scheduled cyclical mechanical stimulation has the ability to synchronise the cellular circadian clocks. We have investigated the properties of molecular clock in skeletal muscle progenitors using

mechanical stimulation protocol based on passive physical cell elongation and relaxation. Here we demonstrate rhythmic expression of several mechano-sensitive proteins around the 24h clock. Moreover, our data suggest that mechanical stimulation protocol leads to acute induction of key circadian genes, suggesting the potential of mechanical cues to reset the molecular clock. Further work will investigate whether cyclical mechanical stimuli can be used to reset dampened clocks during adult stem cell ageing.

# SC61. "Nitrergic neural communication for the synchronization of the mammalian circadian clock: a putative redox-regulation."

<u>Baidanoff Fernando Martin</u><sup>1</sup>, Plano Santiago Andrés<sup>1</sup>, Doctorovich Fabio<sup>2</sup>, Suárez Sebastián Angel<sup>2</sup>, Golombek Diego Andrés<sup>1</sup> and Chiesa Juan José<sup>1</sup>

<sup>1</sup>Laboratorio de Cronobiología, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Argentina, <sup>2</sup>INQUIMAE, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Buenos Aires, Buenos Aires, Argentina

Transduction pathways for light-induced phase advances and delays of the mammalian circadian clock in the suprachiasmatic nucleus (SCN) involve the activation of nitric oxide synthase (NOS). Nevertheless, it is still unclear how the same NOS activity can lead to the gating of one or the other pathway in the SCN neurons. Recent reports indicate that the redox state can oscillate in a circadian fashion in SCN neurons. We hypothesize that NOS enzyme would produce two different messengers depending on the redox state of neurons: gaseous nitric oxide (NO, for photic advances induced at CT18), and Snitrosylated gluthathione (GSNO, for delays at CT14). We have used different types of NO species donors in order to modulate light-induced phase changes. While both a NO and NO donor increases advances after a light-pulse, only when an S-nitrosothiol type of NO donor was used, both advances and delays were potentiated. How different type of NO species donors generate this enhancement is under study. In addition, an intracerebroventricular administration of L-N-acetyl-cysteine, a well-known anti-oxidizing agent, inhibited both photic phase changes, but generated delays when delivered without the light pulse at CT14. In summary, our data suggests that different NO species participate for delaying or advancing the clock, that changing the redox state of SCN neurons is enough to induce phase changes, and that the photically-induced second messengers produced by the NOS could be dependent on the redox state of the cells.

# SC62. "An hnRNP isoform switch links temperature perception to regulation of the *Arabidopsis* circadian clock."

Allan B. James, John, W.S. Brown and Hugh G. Nimm Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, UK

We have previously established extensive alternative splicing (AS) in *Arabidopsis* clock genes, a feature of which are dynamic changes – notably for *LATE ELONGATED HYPOCOTYL* (At*LHY*) - in AS transcripts across diurnal cycles for plants acclimated to different steady state temperatures or for plants undergoing temperature transitions. We proposed a functional link between AS and the clock, with temperature-associated AS as a mechanism involved in the operation and control of the plant circadian clock.

We have now identified a heterogeneous nuclear ribonucleoprotein (hnRNP), POLYPYRIMIDINE TRACT-BINDING PROTEIN1 (AtPTB1), as playing a role in temperature associated splicing of the 5'UTR intron 1 of AtLHY. AtPTB1, and its closely related homologues (AtPTB2 and 3), is subject to auto- and cross-regulation via AS coupled to the nonsense mediated decay pathway (NMD). By employing a mutant strain ablated for the NMD-pathway (upf1-3;pad4-1), we can estimate the extent of turnover of PTB1 under steady state conditions and for plants undergoing temperature transitions. Modest temperature transitions of 2-4°C rapidly change the ratio of PTB1 splice isoforms. Modulation of gene expression without transcriptional change may be a feature of isoform switching; therefore a PTB1 isoform switch may represent a link between perception of fluctuations in temperature to downstream gene splicing.

We are aiming to further investigate the role of *PTB*s in circadian clock function by using RNA-EMSAs to test the association of PTB1 with AtLHY mRNAs, and by employing *ptb* mutants expressing clock promoter luciferase fusions.

#### SYMPOSIUM 15: EBRS/ESRS JOINT SYMPOSIUM

Chair: Debra Skene (UK)

S46. VLADYSLAV VYAZOVSKIY

"The Temporal Dynamics of Waking and Sleep: From Single Neurons to Behaviour."

Vladyslav V. Vyazovskiy

Department of Physiology, Anatomy and Genetics, University of Oxford, UK

The temporal dynamics of sleep is manifested in the alternation of non-rapid eye movement (NREM) and REM sleep and slow homeostatic changes of EEG slow-wave activity (0.5-4Hz). Recent evidence suggests that these relatively slow and global processes are tightly linked with the dynamics of cortical activity on a much faster temporal scale. EEG slow waves during sleep are associated with the occurrence of neuronal network silent (OFF) periods, which alternate with periods of intense spiking (ON-periods). We found that both long-term and immediate preceding state history affect the dynamics of EEG slow waves and their neuronal counterparts. In turn, the temporal organisation of waking is manifested in the distribution of waking episodes across 24h, the duration of continuous waking bouts and regular shifts between behavioural states. We found recently that in the first minutes upon spontaneous awakening, cortical neurons tend to enter OFF periods more frequently. On the other hand, cortical OFF periods are also typical after sleep deprivation, even in a behaviourally awake animals. Intriguingly, even during highly active behavioural states, local cortical "sleep" can occur, and it appears to be associated with stereotypical or habitual behaviours. Moreover, we found that the quality of awake state, as reflect in the type of behaviour and corresponding patterns of local and global cortical activity predicts the duration of sustained waking bouts as well as intensity of subsequent sleep. Thus, investigating cortical network activity provides important insights into understanding the temporal organisation of waking and sleep on a global scale.

# "Human Cortical Excitability and Excitation/Inhibition Balance is Set by the Circadian Timing System."

Gilles Vandewalle

Cyclotron Research Centre, University of Liège, Belgium

Wakefulness is associated with molecular, cellular and systemic changes in human brain function which are deemed to negatively impact cognition. The wake-related progressive performance decrement is however highly non-linear. Cognitive performance remains indeed remarkably stable during a normal waking day, but undergoes abrupt degradation if wakefulness is extended into the biological night. This non-linearity reflects the influence of the circadian timing system, which counter sleep need during the day and favours sleep at night.

Whether the non-linearity in human cognitive performance across the 24h day-night cycle mirrors a circadian dynamics in neuron responsiveness is unknown. Here, we inferred cortical excitability and excitation/inhibition balances within a cortical column based on TMS-evoked EEG responses acquired in healthy young human subjects during a constant routine sleep deprivation protocol.

Results show that cortical excitability increase across 28h of continuous wakefulness but undergoes sharp local decrease and increase respectively at the end of the day and at the end of the night. This non-linear dynamics reflects a dual circadian/sleep homeostasis influence

Surprisingly excitation/inhibition balance, inferred through a data driven physiologically valid computational model, appeared to be mainly under circadian influence. Excitation/inhibition connectivity balance in across neuronal subpopulations of a cortical layer progressively decreased during the day and increased overnight. In contrast, glutamate/GABA receptor density balance increase during the day and decreased overnight.

These opposite dynamics within a cortical column may represent some of the means though which the circadian machinery copes with wakefulness extension and set the dynamics in cortical excitability, system level brain function and cognitive performance.

### S48. WILLIAM WISDEN

## "Capturing the Neuronal Ensembles Underlying Sleep - Local Mechanisms of Circadian and Homeostatic Control."

William Wisden, Xiao Yu, Zhe Zhang, Elizabeth S. Maywood, Michael H. Hastings and Nicholas P. Franks

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The hypothalamus integrates and controls basic body functions such as sleep and wakefulness, and circadian rhythms. The drive to sleep has circadian and homeostatic components that combine to trigger sleep. Beyond the master circadian clock in the suprachiasmatic nucleus, local clocks in the hypothalamus critically regulate biochemical functions – for example, we have found that histaminergic neurons, which promote wakefulness, contain their own local circadian clock under the control of BMAL1, which determines the levels of histamine and so controls sleep architecture (Yu et al., 2014). We have also been mapping the dynamic connectivity of sleep-active brain areas and linking sedative and sleep mechanisms. Ensembles of activated hypothalamic neurons can be genetically tagged (TetTagged) during sleep deprivation or sedation and then pharmacologically reactivated to test sufficiency for inducing these behaviours (Zhang et al., 2015). We have identified neuronal ensembles in the preoptic hypothalamus that are sufficient for both recovery sleep after sleep deprivation and the sedative actions of

alpha2 adrenergic agonists. The sedative state induced by certain classes of drugs and recovery sleep are driven by the same hypothalamic circuitry.

Yu X, Zecharia A, Zhang Z, Yang Q, Yustos R, Jager P, Vyssotski AL, Maywood ES, Chesham JE, Ma Y, Brickley SG, Hastings MH, Franks NP, Wisden W. (2014) Curr Biol.24:2838-44.

Zhang Z, Ferretti V, Güntan İ, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG, Yustos R, Pillidge ZE, Harding EC, Wisden W, Franks NP. (2015) Nat Neurosci. 18:553-61

#### S49. STEVE BROWN

"Genetic and Epigenetic Mechanisms Affecting the Timing of Sleep."

Steven A. Brown

Chronobiology and Sleep Research Group, Institute of Pharmacology and Toxicology, University of Zurich, Switzerland

Sleep is governed by both homeostatic and circadian mechanisms that influence its amount, intensity, and timing during the night. Although the process itself is universal among metazoans and beyond, its timing varies greatly even within a single species like Homo sapiens. Daily timing in humans is highly variable: individual people can be "larks" with very early sleep, or "owls" with much later habits. Our laboratory has applied a variety of molecular methods to understanding these differences, ranging from genomic quantitative trait mapping in primary human cells, to metabolomics using human serum, and brain epigenomic studies in murine models. Overall, we have uncovered a wide variety of mechanisms, each of which contributes either statically or dynamically to what we have termed "circadian plasticity", an ability to modify sleep timing to suit environmental needs. From mice to humans, we show that the same mechanisms are operative in setting biological clock time: genes in which human polymorphisms affect circadian phase are also important to circadian mechanism in mice, and genes identified by global RNAi screens as "clock genes" in mice are also enriched in human genetic studies of clock function. On an epigenetic level, cellular modifications that affect sleep timing also reorganize the relative phase of neurons controlling this timing. Thus, the mechanisms that we have uncovered could provide a paradigm for understanding many other forms of environment-related changes in behavior.

#### Short Communications

SC63. "Effect of ambient light intensity on glucose and lipid metabolism and appetite in humans."

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Background: Artificial light may contribute to the increasing prevalence of obesity and type 2 diabetes mellitus (T2DM). However, the acute effect of light on appetite and glucose and lipid metabolism in humans has never been investigated.

Methods: In this randomised cross-over study, 8 healthy lean men and 8 obese men with T2DM were admitted to the clinical research unit twice in balanced order with a one week interval. After a standardised mixed meal in the evening, subjects slept in darkness for 8 hours. In the morning they were exposed to either bright light (4000 lux) or dim light (10 lux) for 5 hours. Subjects consumed a mixed meal 60 minutes after lights on and blood samples were taken at regular intervals.

Results: In the lean subjects, bright light did not affect glucose concentrations, but increased fasting and postprandial plasma triglycerides compared to dim light. In patients with T2DM, bright light increased fasting and postprandial glucose and postprandial triglycerides compared to dim light. Furthermore, postprandial appetite scores were significantly higher in patients with T2DM under bright light condition. In both groups, heart rate variability analysis of continuous ECG recordings indicated higher sympathetic activity in the bright light condition.

Conclusion: Our data show that exposure to bright light in the morning increases sympathetic drive, appetite scores, and plasma glucose and triglyceride levels in patients with type 2 diabetes. These data support a role for artificial light exposure in glucose derangements in obese patients with T2DM.

# SC64. "Ultradian feeding coupled with caloric restriction affects both the peripheral and central clocks in mice."

<u>Satish K. Sen</u><sup>1,2,3</sup>, Hélène Raingard<sup>1,3</sup>, Stéphanie Dumont<sup>1,3</sup>, Andries Kalsbeek<sup>2,3</sup>, Patrick Vuillez<sup>1,3</sup> and Etienne Challet<sup>1,3</sup>

<sup>1</sup>Institute of Cellular and Integrative Neurosciences, CNRS, University of Strasbourg, Strasbourg France, <sup>2</sup>Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands, <sup>3</sup>International Associated Laboratory (LIA1061) Understanding the Neural Basis of Diurnality, CNRS France and The Netherlands

It is well known that restricted feeding (RF) during the resting period causes pronounced shifts in many peripheral clocks. Moreover, RF in combination with hypocaloric feeding impacts the master clock in the suprachiasmatic nucleus (SCN), as assessed with shifted oscillations of clock and clock-controlled genes (e.g. vasopressin). To determine if these changes on the SCN are due to metabolic cues associated with the caloric restriction, mice were challenged with an ultradian feeding schedule (1 food access every 4 h), under either isocaloric or hypocaloric conditions, thus avoiding the synchronizing effect of a single meal. Mice fed with isocaloric ultradian feeding (body mass loss<10%) remained nocturnal, but displayed a 1.5-h phase-advanced body temperature rhythm and an altered pattern of vasopressin in the SCN. Mice fed with hypocaloric ultradian feeding (body mass loss>10%) became more diurnal, hypothermic in late night, and displayed a 3.5-h advanced body temperature rhythm and an inverted pattern of SCN vasopressin. Hepatic expression of metabolic genes (Pparα, Pgc-1α, Sirt1 and Fqf21) was downregulated and/or phase-shifted by ultradian feeding. Daily variations in liver glycogen showed the same phase in mice fed ad libitum and mice on ultradian feeding. By contrast, Per2 mRNA expression was more phase-advanced in the liver of hypocaloric than isocaloric fed mice on ultradian feeding. In addition, hypocaloric ultradian feeding led to hypoglycemia and an inverted glucose rhythm, while mice on isocaloric ultradian feeding showed normoglycemia but impaired glucose rhythmicity. Hence, ultradian

feeding in mice markedly alters rhythmicity of both peripheral clocks and the master clock.

# SC65. "The stress hormone hydrocortisone affects the expression of *Period* genes in healthy subjects."

<u>Türkan Yurtsever</u><sup>1</sup>, Thomas M. Schilling<sup>2</sup>, Monika Kölsch<sup>2</sup>, Jonathan Turner<sup>3</sup>, Jobst Meyer<sup>1</sup>, Hartmut Schächinger<sup>2</sup> and Andrea B Schote<sup>1</sup>

<sup>1</sup>Institute of Psychobiology, Division of Neurobehavioral Genetics, University of Trier, Trier, Germany, <sup>2</sup>Institute of Psychobiology, Division of Clinical Psychophysiology, University of Trier, Trier, Germany, <sup>3</sup>Department of Immunology, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

The physiological stress system and the circadian clock system communicate with each other at different signaling levels. We tested the effect of escalating cortisol doses on the expression of period genes (PER1, PER2 and PER3) in healthy subjects. Furthermore, we analyzed if the glucocorticoid receptor (GR) is involved in the cortisol-mediated period gene expression. 40 subjects (50% males, 50% females) were randomly assigned to groups receiving a saline placebo solution or 3 mg, 6 mg, 12 mg, 24 mg of hydrocortisone. Blood and saliva samples were drawn every 15 minutes up to 225 minutes to measure quantitative gene expression of PER1, PER2 and PER3 and free cortisol, respectively. A potential role of the GR was determined by an ex-vivo study stimulating whole blood with hydrocortisone and RU486 (a GR antagonist). As a result, hydrocortisone produced a concentration-dependent induction of PER1 mRNA expression, whereas PER2 and PER3 showed differential expression profiles. Higher doses stimulated PER1 mRNA expression more rapidly and strongly, which remained elevated for a longer temporal duration. Moreover, when whole blood was co-treated with hydrocortisone and RU486 ex-vivo, the cortisol-dependent induction of PER1 was blocked by the GR antagonist. In conclusion, we confirmed the regulation of period genes by cortisol in-vivo and ex-vivo in whole blood. In our settings, hydrocortisone induced PER1 expression in a concentration-dependent manner. This induction seems to be mediated via the GR and leads to the assumption that PER1 might be a hypersensitive glucocorticoid-target gene. These findings might be relevant for investigations of stressdependent sleep disorders.

# SC66. "Diurnal rhythms in the human urine metabolome during sleep and total sleep deprivation."

<u>Victoria L. Revell</u><sup>1</sup>, Guro Giskeødegård<sup>2</sup>, Sarah K. Davies<sup>1,2</sup>, Hector Keun<sup>2</sup> and Debra J. Skene<sup>1</sup>

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Urine is a commonly used biofluid for metabolomics studies as it is non-invasive and easily acquired. Understanding how metabolites change during the 24h day under both sleep/wake and continual wakefulness would be beneficial for optimizing urine sample selection procedures for clinical and epidemiological metabolomics and exposome studies. Our aim was to characterise time-of-day variation and the effects of total sleep

deprivation on urinary metabolite profiles. Healthy male participants (n=15) completed an in-laboratory session consisting of an adaptation night, 24h sleep/wake cycle (8h sleep period) and 24h continual wakefulness. Environmental conditions were highly controlled with respect to light/dark, sleep, meals and posture. Urine samples were collected sequentially over defined 2-8h intervals and analysed by <sup>1</sup>H NMR spectroscopy. Cosinor analysis was used to assess diurnal rhythmicity.

Significant changes were observed with respect to both time of day and sleep deprivation in 32 quantified metabolites. Of these, 7 metabolites (22%) exhibited a cosine rhythm over at least one 24h period during the study; 5 metabolites (2-hydroxyisobutyrate, trimethylamine-N-oxide (TMAO), hippurate, xylose and trigonelline) exhibited a cosine rhythm on both days. In addition, 16 metabolites changed significantly between the sleep and sleep deprivation periods; 8 increased during sleep deprivation compared with sleep (taurine, formate, citrate, 3-indoxyl sulfate, carnitine, 3-hydroxyisobutyrate, TMAO and acetate) and 8 decreased (dimethylamine, 4-deoxythreonic acid (4-DTA), creatinine, ascorbate, 2-hydroxyisobutyrate, allantoin, 4-deoxyerythronic acid (4-DEA), 4-hydroxyphenylacetate). Our data suggest that knowledge of sampling time and sleep/wake state are both highly relevant when identifying and quantifying biomarkers in urinary metabolic profiling studies.

[BBSRC supported (BB/I019405/1)]

### SC67. "Adenosine receptors as chronomodulatory targets."

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Recent evidence strongly suggests that the molecular mechanisms underlying the circadian clock and the pathophysiology of various disorders including mood and metabolic disorders are intimately linked. Further, treating circadian disruption may restore these deficits, resulting in a beneficial effect on patient health. However, suitable tools to validate these findings in the clinical setting do not exist. We have validated safe and approved adenosine receptor ligands as potent chronomodulators and have elucidated their mode of action on the molecular clock to involve cAMP/CREB-mediated transcription of clock genes. Here we provide evidence of the efficacy of these drugs at altering core clock properties such as period length in U2OS cells. Through work both *in vitro* and *in vivo*, we show that adenosine, via these receptors, may influence molecular clock function in native conditions. We have further validated these compounds *in vivo* and demonstrate effects on phase and re-entrainment in a jet-lag protocol. Finally we show preliminary effects at modulating mood-related behaviours in mice. We suggest these compounds may be suitable tools with which to enter clinical evaluation of chronomodulators in the future.

# SYMPOSIUM 16: CLOCKS, NEUROLOGY, AND PSYCHIATRY: ANIMAL MODELS AND THE REAL WORLD

Chair: Andrew N. Coogan (Ireland)

### "Attention Deficit Hyperactivity Disorder: Time to Pay Attention to the Clock?"

Andrew N. Coogan

Maynooth University Department of Psychology, National University of Ireland, Maynooth, Maynooth, Ireland

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder with a prevalence of approximately 7% in childhood and adolescence, and 3% in adulthood. It is characterised by the core psychopathologies of impulsivity, inattention and hyperactivity. In common with other neuropsychiatric disorders, ADHD is highly comorbid with sleep disturbances and disorders. Previous studies have also demonstrated changes in circadian parameters associated with ADHD, including increased eveningness, delayed dim light melatonin onset and also a delay in the rhythm of salivary cortisol. An important question that arises is to what extent are circadian processes normally implicated in the cognitive domains that are compromised in ADHD, such as sustained attention and impulse control.I will present data examining the associations between chronotype, social jetlag, sleep and subjective and objective measures of attention and impulsivity in a general population. These data will be discussed in the light of ADHD, and how chronobiological principles might be applied to the management of this disorder.

#### S51. GI HOON SON

"Circadian Timing System and Mood Regulation Through REV-ERB."

Department of Brain & Cognitive Sciences, DGIST, Daegu 711-783, South Korea

Circadian rhythm regulates a variety of physiological and behavioral consequences in mammals. The mammalian circadian timing system is hierarchically organized: The central circadian pacemaker residing in the suprachiasmatic nucleus(SCN) of the anterior hypothalamus orchestrates numerous extra-SCN local oscillators in several regions of the brain and peripheral tissues. The molecular clock machinery has two interlocking feedback loops that drive the circadian oscillation in a cell-autonomous, self-sustainable manner. It works through transcription/translation-based auto-feedback loops and posttranslational modifications that contribute to the fine regulation of molecular circadian clockwork. The circadian nature of mood and its dysfunction in affective disorders is well recognized, but the underlying molecular mechanism remains unclear. We found that REV-ERBα impacts midbrain dopamine (DA) production and mood-related behaviors in mice. Genetic abrogation of Rev-erbα gene or pharmacological inhibition of REV-ERBα activity in the ventral midbrain induced mania-like and depressive behaviors in association of hyper-dopaminergic state. REV-ERBa represses tyrosine hydroxylase (TH, a rate-limiting step of DA synthesis) gene transcription by competition with NURR1(a nuclear receptor for DA neuronal development and maintenance) and functions driving circadian expression of DA system. Furthermore REV-ERBa represses TH gene transcription by recruiting histone deacetylase 3(HDAC3) to the promoter region resulting in suppressive histone deacetylation. In summary, the present study demonstrates the novel functional link between circadian timing system and DA-controlling mood regulation through REV-ERBa.

# "Is sleep Always the Best Medicine? Investigating the Effect of Sleep Deprivation Following an Analogue Traumatic Event."

Kate Porcheret, Dalena Van Heugten, Emily Holmes, Guy Goodwin, Russell Foster and Katharina Wulff

Nuffield Department of Clinical Neurosciences, University of Oxford, UK

Objectives: To examine the effect of sleep deprivation compared to sleep on the response to an analogue traumatic event.

Methods: Healthy subjects (aged 18-35 yrs) where shown a traumatic film before either total sleep deprivation in the sleep lab (study 1) or at home (study 2) compared to normal sleep. Polysomnographic and actigraphic recordings were conducted during the sleep/sleep deprivation period following the study film. The psychological effect of the traumatic film was assessed using the Impact of Event Scale-Revised. Memory of the study film was assessed by self-reported intrusive memory frequency (over one week following trauma film) and in study 2 visual and verbal memory recall (two days after the trauma film).

Results: In study 1 the sleep deprived group showed a lower psychological effect of the trauma film and reported fewer intrusive memories, compared to the sleep group (Porcheret et al Psychological Effect of an Analogue Traumatic Event Reduced by Sleep Deprivation, Sleep, In Press). Study 2 is ongoing. Currently a trend has been found for the sleep deprived participants reporting fewer intrusive memories compared to the sleep group. No difference is seen for visual or verbal memory recall.

Conclusion: Our findings from study 1 suggest that sleep deprivation immediately following an analogue traumatic event reduces the emotional effect and intrusive memories to that event. The tentative findings from study 2 suggest a similar finding when subjects are sleep deprived in a more naturalistic setting.

### S53. DOMINIC LANDGRAF

### "Circadian Rhythms in Animal Models of Depression and Mania."

Dominic Landgraf, Ph.D.<sup>1,2</sup> Jaimie Long, M.S.<sup>1,2</sup> and David K. Welsh and M.D., Ph.D<sup>1,2</sup> <sup>1</sup>Veterans Affairs San Diego Healthcare System, San Diego, CA 92161, <sup>2</sup>Dept. Psychiatry & Center for Circadian Biology, University of California, San Diego, La Jolla, CA 92093

Neuropsychiatric disorders like major depressive disorder and bipolar disorder are associated with disrupted circadian rhythms. To model depression and mania, we use learned helplessness and the dopamine transporter knock-down (DAT-KD) mouse, respectively. Using the PER2::LUC reporter, we identified alterations of rhythms in mood-related brain regions in mice showing depression-like behavior after undergoing learned helplessness. We found that helplessness in mice is frequently associated with arrhythmicity in a subset of brain regions implicated in mood regulation. In turn, disrupting brain rhythms by knocking-down the central clock in the SCN leads to depression-like behavior in mice.

DAT-KD mice are characterized by elevated dopamine levels and mania-like behavior. We assessed running-wheel behavior of DAT-KD mice under various lighting conditions and under the influence of the mood stabilizer valproic acid (VPA). DAT-KD mice are hyperactive, their circadian system is more sensitive to light pulses, and they show longer free-running periods in constant darkness. Chronic VPA treatment shortens circadian period in wild-type and in DAT-KD mice. Similarly, VPA shortens period in cultured organotypic mouse brain explants and in human cells from bipolar patients and control subjects. Together, our results show that depression- and mania-like behavior is associated with pronounced changes of circadian rhythms in mood-regulating brain areas

and at the behavioral level. Conversely, manipulating brain circadian rhythms makes mice more vulnerable to develop depression-like behavior, revealing circadian clocks as a casual factor in mood disorders. Furthermore, our data suggest that VPA may improve symptoms of mania by adjusting the period of the circadian clock.

Acknowledgements: We thank our collaborators: Drs. Christophe D. Proulx, Roberto Malinow, and Jared W. Young, University of California, San Diego, USA. Funded by a U.S. Veterans Affairs Merit Award (DKW).

#### Short Communications

# SC68. "The influence of sleep quality on intra- and inter-network connectivity in Alzheimer's disease."

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Objective: Alzheimer's disease (AD) is the most common cause of cognitive impairment in older individual. Sleep disturbance is a risk factor of AD and can impair daytime function by disrupting attention and brain activity. The aim of the present study is to examine the relationship between sleep quality and intra-/inter-network functional connectivity in AD patients.

Methods: Sleep quality was measured using the actigraph in AD patients and normal control (NC). And we explored functional connectivities based on previously well-defined brain areas that comprise the five key functional systems [i.e., the default mode network (DMN), dorsal attention network (DAN), control network (CON), salience network (SAL), and sensorimotor network (SMN)] in AD patients with different sleep quality.

Results: Based sleep quality of NC, there was no significant difference in cognition, but sleep quality differed between AD patients with good and bad sleep quality. Based on three levels (integrity, network and connectivity pairs) of analysis, we found that intra- and inter-network connectivity were impaired in AD patients with bad sleep quality, comparing with good sleep quality. Importantly, the poor sleep quality might impair the attention and sensorimotor functions, and impair the interaction between attention functions and other networks. But, the DMN was spared. Furthermore, lower cognitive ability was significantly associated with greater reductions in functional connectivity in all AD patients.

Conclusion: These profiles indicated that sleep quality appears to alter intra- and internetwork functions, which changes might precede clinically cognitive changes and be potential biomarkers of AD progression.

# SC69. "Differential roles of CLOCK and NPAS2 in synaptic plasticity and reward-related behaviour."

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The circadian molecular clock regulates monoaminergic systems that control reward behaviors and are relevant to addiction. We investigated how disruptions in the homologous circadian transcription factors, CLOCK and NPAS2, lead to differential physiological alterations in mesolimbic circuitry and sensitivity to drugs of abuse. Using Clock mutant mice (Clock∆19), a model of bipolar mania, we measured excitatory synaptic strength of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), an area that receives a dense dopaminergic (DA) projection from the ventral tegmental area (VTA) and is critical for motivated behavior. Clock mutant mice display an increased preference for cocaine and abnormal VTA DA signaling which may affect plasticity in target regions. In addition to reduced levels of GluA1 protein and alterations in intrinsic membrane properties, they also have reduced AMPA-mediated synaptic strength of MSNs with no change in presynaptic release of glutamate indicating a postsynaptic adaptation. These changes represent potential compensatory mechanisms as a result of increased DA transmission. We also examined the effect of viral-mediated knock down (KD) of NPAS2 in C57BL/6J mice on the conditioned response to cocaine and synaptic strength of MSNs. Both a global Npas2 mutation along with NAc-specific NPAS2 KD caused a decrease in cocaine preference and KD produced an increase in mEPSC amplitude in MSNs compared with scramble virus. The results of our study suggest an important role for circadian transcriptional mechanisms in the regulation of synaptic activity and reward-related behavior. They also highlight differential roles of two homologous circadian proteins in these important functions.

### **SYMPOSIUM 17: AROUSAL AND SLEEP**

Chair: Robert Dallmann (UK)

S54. AMITA SEHGAL

"Oxalic Acid & Diacylglycerol 36:3 are Cross-Species Markers of Sleep Debt."

Aalim M. Weljie<sup>1\*</sup>, Peter Meerlo<sup>2\*</sup>, Namni Goel<sup>3,4,\$</sup>, Arjun Sengupta<sup>1,\$</sup>, Matthew S. Kayser<sup>3,4</sup>, Ted Abel<sup>5</sup>, Morris J. Birnbaum<sup>6</sup>, David F. Dinges<sup>3,4</sup> and Amita Sehgal<sup>7,8,\$</sup>

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Sleep is an essential biological process, but little is understood about how it is regulated or what function it serves. It is becoming apparent though that loss of sleep causes neurobehavioral impairments, as well as metabolic dysfunction that may be associated with risk for weight gain, diabetes, obesity, and cardiovascular disease.

S55. ELIZABETH MAYWOOD

"Circadian Synchrony and Sleep."

Elizabeth S. Maywood, Johanna E. Chesham and Michael H. Hastings Neurobiology Division, MRC-Laboratory of Molecular Biology, Cambridge CB2 0QH, UK It is a widely accepted view that the timing and duration of sleep and wakefulness are governed by interplay between the circadian clock and the wake-dependent homeostat. Although the SCN contributes to circadian but not homeostatic control, whether it is the only circadian influence on sleep is unknown. Whereas the nature of the homeostat in unknown, the contribution of the suprachiasmatic nucleus (SCN) as the ultimate circadian timer is clear. Being retinorecipient, the SCN is able to synchronise to, and thereby predict, the cycle of light and darkness, and thus co-ordinate physiology and behaviour to the environment. Identification of the molecular basis to circadian timekeeping, however, has revealed that cellular circadian oscillators are present in most major peripheral organs as well as many regions of the brain. Using intersectional genetics we have generated a temporally chimeric mouse by targetting the floxed casein kinase  $1e^{Tau}$  allele using the Dopamine R1a Cre- expressing mice to create a mouse where the SCN is expressing 24h rhythms whereas the rest of brain (non-targetted) runs at 20h. We have looked at the consequences of this temporal chimera on the locomotor behaviour of the mouse and the sleep-wake cycle in particular.

### S56. ROLAND BRANDSTAETTER

"The Impact of Circadian Phenotype and Circadian Entrainment on Diurnal Performance in Athletes."

Brandstaetter R., Facer-Childs E., Marsh P. and Holloway D. School of Biosciences, University of Birmingham, B15 2TT Birmingham, UK

Circadian rhythms have been shown to regulate key physiological processes involved in athletic performance. Previous studies showed personal best performance of athletes in the evening across different sports. Contrary to this view, we identified peak performance times in athletes to be different between human 'owls' and 'larks', i.e. individuals with well documented genetic *and* physiological differences that result in disparities between their biological clocks and how they entrain to exogenous cues, such as the environmental light/dark cycle and social factors.

Peak performance occurred at midday in early circadian phenotypes, in the afternoon in intermediate circadian phenotypes, and in the evening in late circadian phenotypes. We found time since awakening to be the major predictor of peak performance times rather than time of day, as well as significant individual performance variations in the course of one day. Following a specific non-photic phase-shifting protocol, relevant sleep/wake parameters as well as peak performance times shifted to earlier times of day demonstrating the impact of circadian entrainment on athlete performance.

Our novel approach that combines the use of an athlete-specific chronometric test, longitudinal circadian analysis, physical performance tests, and phase-shifting interventions, allows a comprehensive analysis of the link between the circadian system and diurnal performance variation. We establish that the correct evaluation of an athlete's personal best performance requires consideration of the entrainment status of the circadian system and that optimal performance can be adjusted by re-entrainment of the circadian clock.

# SC70. "The relationship between sleep disturbances and constipation among female university students in Japan."

<u>Yuya Nishimoto</u><sup>1</sup>, Shunsuke Nagashima<sup>1</sup>, Eiko Masutani<sup>2</sup>, Naoko Komenam<sup>3</sup> and Tomoko Wakamura<sup>1</sup>

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Constipation is common among shift workers because they often experience sleep and circadian rhythm disturbances. However, these disturbances are not regarded as risk factors for constipation among the general population. The aim of this study is to demonstrate the influence of sleep and circadian rhythm disturbances on constipation among female Japanese university students.

This study consecutively enrolled 842 healthy female students (mean age: 19.5±1.8 yr). All participants filled out the Constipation Assessment Scale (CAS), and "Social-Jetlag". They also completed questionnaires about their subjective symptoms of constipation and the interval of their bowel-movements (IBM). The multivariate logistic regression analysis was performed to identify factors that are associated with constipation.

The 458 participants were segmented into two groups: the constipation (C) (CAS  $\geq$ 5, subjective constipation, IBM  $\geq$ 3 days) and healthy (H) groups (CAS <5, no symptoms of constipation, IBM  $\leq$ 2 days). There were 113 participants in the C-group and 345 participants in the H-group. The subjects in C-group slept for significantly shorter periods than those in H-group on weekdays (p=.001, t-test). "Social-jetlag" in the C-group was significantly greater than the H-group (p=.004, t-test). In the multivariate analysis, the odds ratios for sleep duration on weekdays (1h) and "social-jetlag" (1h) were 0.76 [95% CI: 0.60-0.96] and 1.31 [95% CI: 0.99–1.72], respectively. The associations detected between constipation and insufficient-sleep/social-jetlag might indicate that the disturbance of circadian rhythms has an adverse effect on gastrointestinal function. Therefore, assessments of sleep duration and circadian rhythms should be performed during attempts to prevent constipation.

### SC71. "The effect of aging on sleep and wake episodes in mice."

Maria Panagiotou, Johanna H Meijer and Tom Deboer

Department of Molecular Cell Biology, Leiden University Medical Centre, Leiden, The Netherlands

With advancing age an overall decline in circadian amplitude is observed. In addition, sleep-wake impairments emerge, predominantly sleep fragmentation. In this study, we want to investigate the effect of age on sleep and electroencephalogram (EEG) in young (6 months, n=9) and aged (18-24months, n=24) C57BL/6 mice. EEG and the electromyogram of the mice were recorded for 48h in a 12:12 L:D cycle beginning at lights onset. The second day started with a 6h sleep deprivation. The amount of vigilance states and the duration and frequency of their episodes were determined. During the light period, high NREM and REM sleep values were found in both groups compared to dark. Old animals displayed more NREM sleep and less Waking during darkness and less REM sleep at the end of the light period (t-tests, p<0.0001 after significant ANOVA). Moreover, during darkness, aged mice showed more long NREM sleep episodes (32-256s) (t-tests, p<0.0001). During light, older animals had less brief awakenings (8s) and more long (32-256s) waking episodes, whereas during darkness, they had more middle duration waking episodes (8-64s) and less of the longest ones (>2056s) (t-tests, p<0.001). Older mice showed less long REM sleep episodes (128s) during light and more

short episodes during darkness (4-16s) (t-tests, p<0.001). The increased sleep, the longer NREM sleep episodes, and the reduced brief awakenings are not only indicators of a reduced circadian amplitude, but also suggest an increased sleep pressure in the older mice. Further data analysis including EEG spectral analysis is in progress.

### **POSTERS**

# P1. RELATIONSHIP BETWEEN CIRCADIAN TYPOLOGY / SLEEP HABITS AND THE USAGES OF PERSONAL COMPUTER AND CONVENIENCE STORE OPENED 24 HOURS IN JAPANESE STUDENTS AGED 18-40 YRS

Tetsuo Harada<sup>1</sup>, Yumiko Yamazaki<sup>1</sup>, Fujiko Tsuji<sup>1</sup>, Miyo Nakade<sup>2</sup>, Milada Krejci<sup>3</sup>, Takahiro Kawada<sup>4</sup>, Teruki Noji<sup>4</sup> and Hitomi Takeuchi<sup>1</sup>

<sup>1</sup>Laboratory of Environmental Physiology, Graduate School of Integrated Arts and Sciences, Kochi University, <sup>2</sup>Faculty of Health and Nutrition, Tokai-Gakuen University, <sup>3</sup>University of Physical Education and Sport PALESTRA in Prague, <sup>4</sup>Center for Regional Collaboration, Kochi University

This study aims to make it clear the relationship of the usages of convenience stores (CS) and personal computer (PC) at night to circadian typology and sleep habits in Japanese students aged 18-40 yrs. An integrated questionnaire was administered in June-October 2003-2013 to 4564 (females: 2346, males: 2218) Japanese students attending Kochi University and two practical schools to make medical nurses and physical therapists (answer ratio: > 95%). The questionnaire included the diurnal type scales (Torsval & Ålerstedt, 1980), questions on bedtime, wakeup time in weekdays and holidays and sleep hours, questions on mental health (irritation, anger, out of control of emotion and depression), and questions on the usage of CS and PC. The students who used CS after sunset were more evening-typed and had later bedtime and later wake-up time than the other students (p<0.001). The ratio of students who used PC in 21:00-24:00 occupied 58.6% and those students were more evening-typed and showed shorter sleep hours and later bedtime and later wakeup time (p<0.025). Students who used PC in 24:00-03:00 occupied only 13.2% and those students showed later phase of diurnal rhythm (p<0.001) and lower mental health index (p<0.001). The clear relationship of night usages of CS and PC was shown to the evening-typed life style. The blue lights (with 460nm of wave length) from fluorescent lamps in convenience stores and PC might suppress melatonin secretion at night and also make the circadian phase of Japanese students delayed.

### P2. THE SCN RESPONSE TO SPATIAL PATTERNS IN THE VISUAL SCENE

Josh Mouland, Tim Brown and Rob Lucas *University of Manchester* 

We set out to describe spatial receptive field properties of neurons within the mouse SCN. We find that individual neurones have a range of receptive field sizes and types, including cells which exhibit centre surround antagonism. Further experiments have shown that many of the cells show non-linear spatial summation. Using drifting gratings we were able to record the spatiotemporal tuning of these cells. Due to the non-linear spatial summation we hypothesized that inclusion of spatial structure might alter the SCN firing rate at a given irradiance. We detected individual cells that altered their firing rate depending on whether a spatially uniform or a patterned stimuli was presented. Over the population of the SCN recorded we saw a general increase in time averaged firing rate in response to a patterned stimuli. As patterned stimuli increased the firing rate, an output of the SCN, we performed behavioural experiments to see if they had an impact on the

magnitude of light-induced phase shift in wheel running activity rhythms. We found that phase shifts were unaffected by the inclusion of spatial patterns.

# P3. NITRERGIC NEURAL COMMUNICATION FOR THE SYNCHRONIZATION OF THE MAMMALIAN CIRCADIAN CLOCK: A PUTATIVE REDOX-REGULATION

Baidanoff Fernando Martin<sup>1</sup>, Plano Santiago Andrés<sup>1</sup>, Doctorovich Fabio<sup>2</sup>, Suárez Sebastián Angel<sup>2</sup>, Golombek Diego Andrés<sup>1</sup> and Chiesa Juan José<sup>1</sup>

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Transduction pathways for light-induced phase advances and delays of the mammalian circadian clock in the suprachiasmatic nucleus (SCN) involve the activation of nitric oxide synthase (NOS). Nevertheless, it is still unclear how the same NOS activity can lead to the gating of one or the other pathway in the SCN neurons. Recent reports indicate that the redox state can oscillate in a circadian fashion in SCN neurons. We hypothesize that NOS enzyme would produce two different messengers depending on the redox state of neurons: gaseous nitric oxide (NO, for photic advances induced at CT18), and Snitrosylated gluthathione (GSNO, for delays at CT14). We have used different types of NO species donors in order to modulate light-induced phase changes. While both a NO and NO- donor increases advances after a light-pulse, only when an S-nitrosothiol type of NO donor was used, both advances and delays were potentiated. How different type of NO species donors generate this enhancement is under study. In addition, an intracerebroventricular administration of L-N-acetyl-cysteine, a well-known anti-oxidizing agent, inhibited both photic phase changes, but generated delays when delivered without the light pulse at CT14. In summary, our data suggests that different NO species participate for delaying or advancing the clock, that changing the redox state of SCN neurons is enough to induce phase changes, and that the photically-induced second messengers produced by the NOS could be dependent on the redox state of the cells.

# P4. HIGH AMPLITUDE CIRCADIAN RHYTHM GENERATED BY THE SUPRACHIASMATIC NUCLEUS OF CLOCK MUTANT MICE

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Clock mutant mice show long free-running period of about 27 hours. Previous reports demonstrated that peripheral tissues do not show apparent circadian rhythm and the suprachiasmatic nuclei (SCN) showed a circadian rhythm of low amplitude. To know the distinct characteristics of the circadian rhythm in the SCN of the clock mutant mice, we crossbred the Clock mutant mice of ICR strain with PERIOD2::LUCIFERASE knock-in mice (Yoo et al. PNAS 2004) and obtained hybrid mice. This allowed real-time monitoring of circadian rhythm by bioluminescence.

We prepared organotypic slice cultures containing the SCN and observed bioluminescence emitted from the SCN using a cool CCD camera. The cultured Clock mutant SCN from neonatal mice showed a stable circadian bioluminescence rhythm with a long free-running period. Compared with those of wild-type SCN, the bioluminescence intensity of mutant SCN decreased by half but the bioluminescence rhythm was stable

and maintained over four weeks without a medium change. Here, we show characteristics of the circadian rhythm of oscillators in the SCN of the clock mutant mice, especially with reference to the synchronizing manner.

#### P5. LIVER PERIOD 2 IS NECESSARY TO REGULATE FOOD ANTICIPATION

Rohit Chavan, Céline Feillet, Sara Fonseca, James E. Delorme, Takashi Okabe, Jürgen A. Ripperger and Urs Albrecht *University of Fribourg* 

Mammals can anticipate food availability recurring at a particular time of the day. This daily anticipation is independent of the suprachiasmatic nuclei (SCN) the central pacemaker of the circadian system. Therefore the question arises where the food anticipatory signal originates and what role components of the circadian clock may play. To address this question we generated tissue specific Per2 knock-out mice. A liver specific deletion of Per2 abolished food anticipation (FA), which was rescued by viral overexpression of Per2 in the liver. RNA sequencing revealed that enzymes of the ketonebody metabolism are deregulated in these animals. Timed application of  $\beta$ -hydroxybutyrate rescued the FA phenotype in liver Per2 knock-out mice. We conclude that liver Per2 is necessary for FA and that it regulates FA at least in part via modulation of  $\beta$ -hydroxybutyrate levels in the serum.

# P6. INVESTIGATING THE POTENTIAL OF MECHANICAL CUES IN RESETTING CELLULAR CIRCADIAN CLOCKS IN ADULT STEM CELLS

Jevons L.A.<sup>1</sup>, Bellantuono I.<sup>2</sup>, Hunt J.A.<sup>1</sup> and Pekovic-Vaughan V.<sup>1</sup>

<sup>1</sup>Institute of Ageing and Chronic Disease, Musculoskeletal Biology, University of Liverpool, UK, <sup>2</sup>Department of Human Metabolism, Academic Unit of Bone Biology, University of Sheffield, UK

The circadian clock is an evolutionarily conserved timing mechanism in a wide array of organisms regulating our daily physiology and metabolism. Endogenous circadian clocks are entrained to oscillate with 24h periodicity, resulting in rhythmic changes in the expression of numerous genes, proteins and metabolites in a tissue-specific manner. Emerging data has indicated that circadian clocks play an important role in adult stem cell regulation, especially in their responses to external stimuli. Moreover, recent findings suggest that mechanical vibrations, in addition to light, temperature and food, act as a novel resetting cue to synchronize the circadian rhythms at the organismal level. Understanding how circadian rhythms could be reset by biophysical cues is of significant clinical value for regenerative stem cell therapies as disruption of circadian rhythms is recognised as one of the key drivers of age-related tissue dysfunction as well as increased risk of disease and drug toxicity.

Here we have tested the hypothesis that the time-scheduled cyclical mechanical stimulation has the ability to synchronise the cellular circadian clocks. We have investigated the properties of molecular clock in skeletal muscle progenitors using mechanical stimulation protocol based on passive physical cell elongation and relaxation. Here we demonstrate rhythmic expression of several mechano-sensitive proteins around

the 24h clock. Moreover, our data suggest that mechanical stimulation protocol leads to acute induction of key circadian genes, suggesting the potential of mechanical cues to reset the molecular clock. Further work will investigate whether cyclical mechanical stimuli can be used to reset dampened clocks during adult stem cell ageing.

#### P7. JUST BREATHE: CHRONOPHARMACOLOGY FROM MOUSE BREATH

Robert Dallmann, Renato Zenobi, Steven A. Brown and Pablo Martinez-Lozano Sinues Institute of Pharmacology and Toxicology, University of Zurich & Department of Chemistry and Applied Biosciences ETHZ

The circadian clock is modulating drug efficacy and toxicity through multiple mechanisms and all stages of adsorption, metabolism and excretion of a drug. One of the key aspects in drug development is estimating drug exposure, both levels and time, by establishing the pharmacokinetic (PK) profile of a candidate substances. In current "brute force" drug discovery programs, hundreds of compounds are synthesized, and numerous animals are required to determine the blood PK profile of each compound *in vivo* at one time of day let alone around the 24-day. Here, we present a novel, non-invasive *in vivo* method to determine PK properties of such compounds and their metabolites from the breath of a single mouse in real-time. We then showcase this method to determine the circadian rhythm in ketamine drug metabolism, and show that it is dependent on the presence on a functioning circadian clock in hepatocytes. Furthermore, we provide some evidence that – as preciously described for human breath – mouse breath contains a sizeable number of endogenous compounds like fatty acids and other small molecules, which can be quantified.

This technique might represent an important step in reducing animal numbers and refining experiments for drug discovery programs. Also, it might open up new experimental designs for chronobiologists, e.g., where otherwise multiple blood samplings would have been necessary but practically impossible.

#### P8. MEASURING RHYTHMS OF TEMPERATURE, ACTIVITY AND SLEEP AS WELFARE MARKERS IN MICE

Laurence A. Brown, Lindsay Benson, Sibah Hasan, Mathilde Guillaumin, Russell G. Foster and Stuart N. Peirson

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There is a growing demand for standardised measures of welfare in laboratory animals to benefit the lifetime experience of animals and to improve scientific quality (NC3Rs, 2014). Despite the welfare issues associated with both genetic alteration and scientific procedures in mice, our ability to reliably assess pain, suffering, distress and lasting harm (PSDLH) in this species is poor.

We are currently exploring novel objective measures of welfare in mice, based upon characteristic changes in physiology and behaviour that are known to accompany infection, injury and disease. These responses are collectively termed 'sickness behaviour' (Tizard, 2008). Whilst hypothermia and increases in sleeping time are

recognised as markers of PSDLH in laboratory mice, they are difficult to measure non-invasively.

Here we will show that thermal imaging provides an ideal non-invasive measurement of body temperature rhythms and that passive infrared (PIR) sensors can provide accurate real-time longitudinal measures of both locomotor activity and sleep status in mice. Furthermore, by building tools using affordable microcontrollers and open-source software, this provides the necessary tools for any laboratory to incorporate these measures for their research.

Longitudinal measurements also allow the structure and stability of biological rhythms to be accurately established. Disruption of biological rhythms occurs in many diseases, and may provide an ideal indicator of health status.

NC3Rs (2014) Our Vision: 2015-2025.

Tizard I (2008) Anim Health Res Rev 9:87-99.

#### P9. CHARACTERISTICS OF THE DORSAL LATERAL GENICULATE NUCLEUS NEURONAL RESPONSES TO LIGHT

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The dorsal lateral geniculate nucleus (dLGN) is the thalamic relay nucleus that controls the major visual gateways to the cortex. The signal transmission through the dLGN is dependent on behavioural states such as sleep or arousal. The retinal afferents extending across the dLGN provide information from all photoreceptors: rods, cones and melanopsin cells. The type of neuron response to light divides cells within the mice dLGN into 'sustained', 'transient ON' and 'OFF', where only 'sustained' (melanopsin-derived) neurons track increases in the irradiance. The aim of the present study was to determine if light sensitivity and activity of the dLGN cells in the pigmented rat are correlated with the general state of the brain as assessed by electroencephalography (EEG). Our second goal was to verify whether previously reported types of light-induced responses occur in the rat dLGN. We performed in vivo extracellular studies on urethane-anaesthetized Long Evans rats combined with the EEG recordings and light stimulations. We observed a striking difference at the excitation level of the dLGN neurons correlated with the EEG synchronization/non-synchronization occurring under urethane anaesthesia. This difference was reflected in neurons sensitivity to light, which was significantly enhanced during the non-synchronized state. The mean firing rate was strongly decreased during synchronized urethane sleep components in 44% of the tested cells. Moreover, previously described types of light-induced responses was recorded within the rat dLGN. Our results suggest that the dLGN neurons in the pigmented rat transmit sensory information in a state-dependent manner, which influence their mean firing rate and light responsiveness.

[Supported by 2013/08/W/N23/00700.]

#### P10. ACTIVATION OF THE DORSAL LATERAL GENICULATE NUCLEUS (DLGN) NEURONS BY OREXIN A – INVIVO STUDY ON URETHANE-ANESTHETISED RATS

Patrycja Orlowska-Feuer, Katarzyna Dyl and Marian Henryk Lewandowski Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland The dorsal lateral geniculate nucleus (dLGN) is a retinorecipient thalamic structure convening visual information to the cortex. It was shown that its activity and sensory information processing is strongly modulated by the corticothalamic feedback. Besides, a variety of different neuromodulators regulating the sleep-wake cycle can affect activity and firing pattern of the dLGN neurons. Orexins (OX-A, OX-B) are well-known hypothalamic neuropeptides involved in the sleep mechanisms, autonomic control and arousal, which influence on the dLGN is not fully understood.

Thus, the aim of the present study was to investigate OX-A action on the dLGN neurons by means of in vivo extracellular recordings on urethane-anaesthetised Wistar rats. OX-A (200  $\mu$ M) was locally administrated into the dLGN by the pressure ejection (200 nL). Moreover, EEG signal was simultaneously recorded.

We have tested 20 neurons located within the borders of the dLGN and OX-A ejection increased the mean activity of 5 of them (from  $5.50 \pm 1.90$  Hz to  $11.65 \pm 2.74$  Hz). The effect was slow and lasted approximately 220 seconds. These neurons had an irregular or tonic firing pattern and did not respond to light stimulation. Moreover, their general activity was not modulated by changes occurring in the EEG signal (cortical synchronisation/desynchronisation).

To our knowledge this is the first study showing OX-A action on the dLGN neurons during in vivo extracellular recordings and suggesting that orexins may be involved in the modulation of the primary visual thalamic nucleus or corticothalamic loop.

[This work is supported by NSC grant OPUS V: 2013/09/B/NZ4/00541.]

#### P11. DIURNAL PROFILE OF INTERLEUKINS, THEIR RECEPTORS AND TOLL-LIKE RECEPTORS IN THE CHICKEN PINEAL GLAND

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Melatonin synthesis in the chicken pineal gland is modified by peripheral inflammatory process. Previously, we have shown that IL- 6 decreased synthesis of pineal melatonin but in the presence of increased. Theoretically, the information about the inflammatory reaction can reach the pineal gland directly through pathogens sharing pathogenassociated molecular patterns or indirectly by cytokines synthesized by peripheral leukocytes or produced within the pineal associated lymphoid tissue. Therefore, the aim of this study was to measure in the chicken pineal gland the diurnal rhythm of expression of pattern recognition receptors TLR 4, TLR 15, TLR 21, as well as IL-6 and IL-18 receptors and pro-inflammatory cytokines IL-6, IL-18 and IL1b. Sixteen-day-old chickens kept from the day of hatch in LD = 12:12 were decapitated every 2h over a 24h period. Part of animals was kept in DD conditions 24h prior the decapitation. Pineal glands were isolated under dim red light. The level of mRNA were measured by RT-qPCR. We observed the diurnal expression of all examined genes. TLR 21 expression changed rhythmically with acrophase in the light phase and the rhythm persisted in DD conditions. Among interleukins rhythmic expression was shown for IL-6 in LD photoperiod with the acrophase in the light phase. The expression of IL 6 receptor exerted reverse acrophase to the IL-6, itself. This data strongly support hypothesis that the pineal gland can perceive the information on immune status of animal both directly and indirectly.

[Supported by National Science Centre Grant UMO-2012/07/B/NZ3/02919]

P12. CIRCADIAN CLOCK REGULATION OF SEROTONIN LEVELS IN HAEMOLYMPH OF THE FRESHWATER MUSSELS *UNIO TUMIDUS* IN SUMMER Aleksandra Skawina<sup>1</sup>, Piotr Bernatowicz<sup>2</sup>, Magdalena Markowska<sup>1</sup> and Piotr Bębas<sup>1</sup>

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Light is regarded as the strongest Zeitgeber for biological clocks. Body of bivalves is usually light protected by their shell; furthermore, semi burrowing unionids live buried within the deposits, nevertheless they have the photoreceptors within their incurrent aperture. Photoreceptors drive the light information via the nervous system, mostly by its serotoninergic part. Serotonin levels in hemolymph of the marine gastropod *Aplysia* have been shown as strictly regulated by the light-dark conditions. Little is known how unionoids transfer the light signals to the effectors and what is the role of serotonin in this regulation in the context of bivalves circadian biology.

*Unio tumidus* were kept *in situ* in lake littoral in natural photoperiod (LD) (16:8h light:darkness) and constant darkness (DD) conditions in June 2014; every 4 hours haemolymph was isolated from 10 adults, and other tissues were immediately preserved in a fixative. The level of serotonin was measured by the ELISA method. The distribution of the serotonin in tissues was assessed by immunohistochemistry.

We observe an endogenous circadian rhythm (under DD conditions) of serotonin level in haemolymph of *U. tumidus*. In DD we observe low level of serotonin during the subjective day, and about double higher level in the subjective night. The results from LD conditions show strong influence of the long day light on *U. tumidus* physiology - all the levels of the serotonin, both from the light phase, and the dark phase of the day are low. No daily changes in serotonin levels in bivalve tissues were observed.

### P13. ROLE OF REV-ERB IN THE PROGRAMMING OF LIPOGENIC DRIVE IN MICE

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The circadian clock coordinates multiple behavioural and physiological processes, including energy homeostasis. Mounting evidence suggests that disruption of the clock contributes to the development of obesity and its comorbidities, including insulin resistance and type-2 diabetes. However, the causal mechanisms within this pathophysiology are not well defined. The nuclear hormone receptor, REV-ERBa, has been established as a clear link between the clockwork and metabolic processes. Here we use the Rev-erbα-/- mouse to explore clock-metabolic coupling, specifically glucose and lipid metabolism. In line with published work, Rev-erba-/- mice exhibit an obese phenotype with profound susceptibility to diet induced obesity (DIO). Increased adiposity in Rev-erbα-/- mice was associated with an upregulation in gWAT of lipogenic genes (Dgat2, Fasn) as well as Lpl, an essential gene for lipid utilization and storage. Differences in fat mobilization are observed as Rev-erbα-/- mice show a heightened insulin stimulated lipogenic drive and in the fasted state an attenuation of the lipolytic drive is observed. This indicates an increased propensity for fat accumulation in the knockout mouse. Furthermore, despite their severe obesity under D.I.O. challenge, Reverbα-/- mice remain insulin sensitive. This indicates a key role of the clock in both lipid homeostasis and suggests that the clock may be important in the development of insulin resistance in obesity.

### P14. TARGETING OF THE CIRCADIAN CLOCK IN OBESITY TO IMPROVE GLUCOSE HOMEOSTASIS

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The circadian clockwork is tightly coupled to energy metabolism at a cell, tissue and system-wide level. Growing evidence has shown that disruption of this internal timing can increase the incidence and severity of metabolic diseases, including obesity and type 2 diabetes. Here, we conduct a broad analysis of behavioural and molecular rhythms in high fat diet (HFD) fed mice to assess the impact of obesity on the clock, and examine whether strategies aimed at strengthening the molecular circadian clock can confer metabolic benefit. We demonstrate a specific and pronounced disruption of the circadian clockwork in gonadal white adipose tissue (WAT), which was not due to altered nutritional input (i.e. acute consumption of HFD), but was associated with chronic obesity, the development of obesity-related inflammation and dysregulation of the clock-coupled metabolic regulators PPARa/y. In contrast, in most other tissues examined, including subcutaneous WAT, clock gene rhythms were largely unperturbed by HFD feeding. Importantly, we further show that pharmacological targeting of the molecular circadian clock through daily administration of a novel casein kinase 1δ/ε inhibitor (PF-5006739) improved glucose tolerance in both diet-induced (DIO) and genetic (ob/ob) models of obesity. The current study further implicates circadian clock disruption in obesity and associated metabolic disturbance, and suggests that strengthening of the clock represents a novel therapeutic avenue for the treatment of the comorbidities of obesity.

### P15. REDOX REGULATION OF MOLECULAR CLOCKS IN SKELETAL MUSCLE ADAPTIVE RESPONSES

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Disruption of circadian rhythms is recognised as a major risk factor for several chronic diseases, in which altered redox regulation plays an important part. Emerging research has implicated that circadian control of redox systems is essential in the regulation of disease processes in several tissues upon environmental challenge. However, little is known about physiological redox stimuli that drive circadian regulation of cellular physiology.

Transcription factor NRF2 (nuclear factor erythroid 2 related factor 2) is the redox-sensitive master regulator of environmental stress responses in various tissues through activating a coordinated antioxidant defence program. Recent work has identified circadian transcriptional control of NRF2-mediated antioxidant protection in the lung, which determines tissue susceptibility to 'time-of-day' induction of oxidative injury. Since reactive oxygen and nitrogen species generated by contracting skeletal muscles are necessary signalling cues for their optimal function, we have utilised skeletal muscle as a model to investigate the circadian control of redox physiology. We have confirmed circadian expression of NRF2 and its tissue-specific targets in skeletal muscle cells and tissues. Using molecular, pharmacological and real-time imaging approaches, we have investigated a hypothesis that non-transcriptional circadian redox regulation of NRF2 pathway plays a role in skeletal muscle cell contractile adaptations. Moreover, we have examined whether this regulation exerts a feedback control on molecular clock during acute responses to stress. Indeed, targeted inhibition and activation of NRF2 using pharmacological agents significantly altered time-of-day responses to oxidative stimuli.

These new results provide a potential mechanism for readjusting altered redox homeostasis in age-associated muscle wasting disorders.

### P16. TIME-FIXED FEEDING PREVENTS BODY WEIGHT GAIN INDUCED BY CHRONIC JET LAG IN MICE

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Recent studies show that feeding timing alters metabolic states even though total calorie intake is same. We examined the effects of chronic jet lag on metabolism in mice. C57BL/6J mice were housed under a normal 12 h Light/Dark (LD) condition (7:00-19:00 light on) or a chronic LD shift condition; a 12 h LD cycle was advanced 6 hours twice a week continuously for three months. The mice fed ad libitum under the LD-shift condition increased the body weight and the blood glucose during intraperitoneal glucose tolerance test as compared to the control mice housed under a normal LD schedule. However, mice fed during fixed time (19:00-7:00) under the chronic LD shift schedule showed the identical levels of body weight and glucose tolerance to the control group. Our results indicate that body weight gain and impairment of glucose tolerance were induced by the abnormal feeding timing but not by the chronic LD shift. Fixed meal timing could be a way to prevent metabolic disorders caused by shift-work or irregular life-styles.

### P17. INVERSE EFFECT OF ACUTE BLUE AND GREEN LIGHT EXPOSURE ON ANXIETY, ADRENAL RESPONSES AND CLOCK GENE EXPRESSION

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Light plays a critical role in the regulation of numerous physiological and behavioural rhythms to daily solar changes by synchronising the suprachiasmatic nuclei (SCN). Apart from its role in entraining the circadian pacemaker, light has numerous other effects on physiology and behaviour e.g. nocturnal light exposure induces rapid sleep induction. Paradoxically, nocturnal light exposure can also result in elevation of adrenal corticosterone (CORT) synthesis and release. Light exerts its effects via three classes of mammalian photoreceptors: rods, cones and melanopsin-expressing photosensitive retinal ganglion cells (pRGCs). Although there is a growing progress in understanding of non-image-forming responses (NIF) to light, the contribution of different wavelengths to sleep and arousal responses remain largely unclear. Using wildtype mice with intact retinae we investigated the effect of acute monochromatic light exposure on sleep onset. light aversion, CORT secretion and immediate early gene (IEG) expression. Here we show that green (530nm) and blue (470nm) light of the same photon flux produce markedly different effects on physiology and behaviour. Surprisingly, blue light elicits attenuated sleep induction, aversive response with elevated CORT secretion and increased IEG expression in both the SCN and adrenal gland. By contrast green light elicits inverse response, with rapid sleep induction and much lower aversive, CORT and IEG responses. Using melanopsin-deficient mice we show that these arousing responses as well as sleep induction are attenuated to 530nm and enhanced to 470nm. Hence our data show for the first time wavelength dependent sleep induction and arousal regulation. These findings emphasise the importance of wavelength in physiological and behavioural responses to light.

### P18. EXTRACELLULAR MATRIX MECHANICS CONTROL THE ROBUSTNESS OF THE CIRCADIAN CLOCK IN MAMMARY EPITHELIA

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The cell-autonomous circadian clocks drive ~24 hr rhythms in fundamental biological processes that control behaviour and tissue physiology. The amplitude of circadian rhythms in multiple tissues dampens with ageing, leading to a compromised temporal control of physiology. However, our understanding of how cellular clocks within a given tissue maintain robust circadian outputs, and how this robustness is lost during ageing, remain largely unknown. Here we have combined tissue-mechanics and real-time clock imaging studies to reveal that the breast epithelial clock is regulated by the mechanochemical stiffness of the cellular microenvironment. Moreover it is controlled by the periductal extracellular matrix *in vivo*, which contributes to the dampened circadian rhythm during ageing. Mechanistically the Rho/ROCK pathway, which transduces extracellular stiffness into cells, regulates the activity of the core circadian clock complex. Importantly, genetic perturbation or age-associated disruption of self-sustained clocks compromises the self-renewal capacity of mammary epithelia. Thus our results reveal that the mammalian circadian clocks are mechano-sensitive, providing a potential mechanism for how ageing influences their robustness and function.

### P19. SEX DIFFERENCES IN THE CIRCADIAN PRODUCTION OF MELATONIN AND CORTISOL IN PLASMA AND URINE MATRICES

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Conflicting evidence exists as to whether males and females differ in circadian timing. The aim of the current study was to assess whether sex differences are present in the circadian regulation of melatonin and cortisol in plasma and urine matrices under constant routine conditions. Thirty-two healthy individuals (16 females taking the oral contraceptive pill (OCP)), aged 23.7±3.7 years (mean±SD), participated. Blood (hourly) and urine (4-hourly) samples were collected for measurement of plasma melatonin and cortisol, and urinary 6-sulfatoxymelatonin (aMT6s) and cortisol, respectively. Data from 28 individuals (14 females) showed no significant differences in the timing of plasma and urinary circadian phase markers between men and women. Females, however, produced significantly greater amounts of plasma melatonin and cortisol than males (AUC melatonin 937±104 vs. 642±47 pg/ml.h; AUC cortisol 13581±1313 vs. 7340±368 mmol/L.h, both p<0.05). Females also exhibited higher amplitude rhythms in both hormones (melatonin 43.8±5.8 vs. 29.9±2.3 pg/ml; cortisol 241.7±23.1 vs. 161.8±15.9 mmol/L, both p<0.05). By contrast, males excreted significantly more urinary cortisol than females (AUC: 520 vs. 349 mol) but urinary aMT6s levels did not differ between sexes. It was not possible to distinguish whether the observed elevated levels of plasma melatonin and cortisol in females resulted from innate sex differences or OCP effects on the synthetic and metabolic pathways of these hormones. The fact that the sex differences observed in total plasma concentrations for both hormones were not reproduced in the urinary markers challenges their use as a proxy for plasma markers, especially in OCP users.

### P20. USING OSCILLATING LIGHT TO STUDY THE CONE CONTRIBUTIONS TO NIF EFFECTS IN HUMANS

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Besides being intrinsically photosensitive, ipRGCs receive indirect synaptic input from visual photoreceptors. Here we studied the contribution of cones to non-image-forming (NIF) effects of light in humans. Recent studies suggest that in various NIF effects of light, cones contribute little in response to steady light because of rapid light adaptation. Here we tested whether light oscillating in intensity increases the NIF responses of increasing alertness and melatonin suppression in humans, with the hypothesis that cones repeatedly increase their sensitivity during the dark phases of such oscillations. From 23:40 until 1:20 AM, 23 participants were exposed to one of 5 light protocols in a within-subject design. The intensitiy of the light oscillated with a period of 1,2,4 or 8 minutes, or remained dim or constant (average oscillation intensity). Two test blocks were performed before and after the light exposure during which scores on the Karolinska Sleepiness Scale (KSS) and psychomotor vigilance task (PVT) were assessed. Saliva samples were taken throughout the test session at regular intervals. The increase in KSS score and PVT reaction times over a session did not differ between conditions (p>0.05). neither did melatonin suppression (p>0.05). Interestingly, no difference was found between any of the light conditions and dim light with respect to sleepiness (p>0.05). whereas melatonin concentrations did differ (p<0.01), supporting recent evidence that the pathways involved in these effects are divergent. Furthermore, these results suggest little involvement of cones in the alerting effect of light, but it might be that the chosen oscillations were too slow for cones to reach their full potential.

### P21. RHO1-SIGNALLING IN DROSOPHILA MELANOGASTER S-LNV PACEMAKER NEURONS REGULATES CIRCADIAN BEHAVIOUR

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Studies in the fruit fly *Drosophila melanogaster* have contributed much to our understanding of daily timekeeping. *Drosophila* circadian behaviour relies on a conserved negative feedback loop of gene expression found in a neural circuit of ~150 clock neurons. Subsets of these neurons take on specialized roles in synchronization and maintenance of circadian rhythms. In a genetic screen aimed at identifying molecular signals underlying clock-controlled behaviour, we recovered mutant genotypes affecting the gene for the small GTPase Rho1. *Rho1* mutants exhibited enhanced temperature-mediated resetting of behavioural rhythms. Moreover, selective *Rho1* knockdown in clock neurons resulted in a loss of activity:rest rhythms in constant dark conditions. Based on Rho1's critical role in regulating the dynamics of the actin cytoskeleton, we examined associations between the behavioural phenotypes and clock neuron morphology. Our observations showed that the molecular clock inside these neurons was not disrupted. However, while gross neuro-morphology remained unaffected, the daily remodelling of

the s-LN $_{v}$  axonal projections was dampened in *Rho1* knockdown flies. Several other experiments support the notion that Rho1-signalling impacts circadian behaviour preferentially in the s-LN $_{v}$ s: (1) *Rho1* knockdown in PDF (Pigment Dispersing Factor)-expressing s-LN $_{v}$ s and l-LN $_{v}$ s is sufficient to disrupt circadian behaviour in DD and (2) PDF-independent behavioural rhythms persist in *Rho1* knockdown flies.

### P22. EFFECTS OF LIGHT EXPOSURE DURING DAYTIME ON CLOCK GENE EXPRESSION IN HAIR FOLLICULAR AND ROOT CELLS IN HUMANS

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Light is the strongest synchronizer controlling circadian rhythms. The intensity and duration of light change throughout the year, thereby influencing body weights, food preferences, and melatonin secretion in humans and animals. Although the expression of clock genes has been examined in humans, it currently remains unknown whether bright light during daytime affects the expression of these genes. Therefore, we herein investigated the effects of bright light exposure during daytime on clock gene expression in the hair follicular and root cells of the human scalp. Seven healthy men participated in this study. The subjects completed 3-day experimental sessions twice in one month during which they were exposed to bright and dim light. The mRNA expression of Per1-3, Cry1-2, Rev-erb-α, Rev-erb-β, and Dec1 was analyzed using branched DNA probes. The expression of Rev-erb-α and Rev-erb-β was significantly increased in a time-dependent manner, with a peak being observed at 3 am with each light. No significant changes were observed in the expression of Per1, Per2, Per3, Cry1, Cry2, Rev-erb-α, Rev-erb-β, or Dec1 following exposure to bright light. These results suggested that bright light stimuli did not influence the expression of clock genes in humans. Long lasting bright light exposure may be required during the daytime to change the expression of clock genes in humans.

#### P23. CONTRAST AND IRRADIANCE RESPONSES IN THE MOUSE SCN

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Mammalian physiology and behaviour is regulated by light, as it exerts effects on the master circadian oscillator, the suprachiasmatic nuclei (SCN). Electrophysiological recordings have shown that ocular light exposure excites many SCN neurones. Upon exposure to a simple light pulse, the neuron response has a clear form; a sharp 'ON' excitation that relaxes to steady state firing, whose magnitude reflects irradiance of the step. The 'ON' response implies that these cells are disproportionately excited by abrupt increases in irradiance. This suggests that light therapies targeting the SCN could be made more efficient by including appropriate temporal modulations.

All tests used male *Opn1mw*<sup>R</sup> mice which have the human red cone opsin gene, enabling the isolation of contributions made by different photoreceptor classes. Mice were anaesthetised and subjected to in vivo electrophysiological recordings by means of multielectrode arrays. Light stimuli presented to the contralateral eye, stimulating

neuronal responses within the SCN, were recorded and analysed. Afterwards electrode placement was verified histologically.

We report that many SCN neurones are excited by both gradual and abrupt changes in irradiance. Such responses can be elicited by changes in light spectrum that increase the effective irradiance of either rod/melanopsin or cone photoreception in isolation. However, the biggest changes in firing are produced by stimuli targeting all photoreceptive inputs. Investigating time averaged firing for steps, sinusoids, ramps and sustained light indicated that firing can be modulated with the inclusion of temporal modulations. The functional significance of this effect was explored by assays of circadian entrainment in mice.

### P24. FOOD-ENTRAINABLE CIRCADIAN RHYTHM AND PATHOPHYSIOLOGY IN CYS414-ALA MCRY1 TRANSGENIC MICE

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In mammals, the suprachiasmatic nucleus (SCN) is the master pacemaker that generates circadian locomotor behavior. We have demonstrated that the mutant mCRY1 (Cys414 in mCRY1 being replaced with alanine) expressing transgenic (Tg) mice display unusual circadian rhythms that their locomotor free-running periods were long (about 28 hours) with rhythm splitting. In addition, they readjust immediately to the 6h-phase advance of LD cycles, indicating the possibility of some disorders in the SCN in the Tg mice. We recently examined the locomotor activity of Tg mice in cyclic restricted feeding conditions (RF). The results indicated that the SCN of Tg mice is entrainable to RF, and that, for the SCN in Tg mice RF dominates LD cycles as a zeitgeber. Our results suggest that the mutant mCRY1 affects the coupling mechanisms among neurons in the SCN, and thereby alters its sensitivity to feeding cues as well as its relation to the food-entrainable oscillator (FEO). We also found that the expression levels of Rgs2 and Rgs4 genes were elevated in the brain of Tg mice. Thus the disturbance of the signaling pathways mediated by cAMP and calcium in the brain may be involved in such alterations. In addition to the unusual circadian behaviors, we previously demonstrated that Tg mice show the symptoms of diabetes mellitus characterized by age-dependently progressive dysfunctions in pancreatic β-cells. Our recent analyses suggest that, the dedifferentiation of β-cells, along with the lowered proliferation due to the senescence-like changes in βcells, are accountable for the  $\beta$ -cell failure in Tg mice.

### P25. IMPACT OF SLEEP TIMING AND SCHOOL SCHEDULES ON SCHOOL PERFORMANCE

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Early school hours challenge the late and long sleep need in adolescents. This conflict of social and biological conditions also impacts their cognitive performance. We collected ~4.800 grades from a Dutch high school to assess how sleep timing, chronotype (Munich ChronoType Questionnaire) and time of day affect grades. Our study is the first detailed description of chronotype-dependent fluctuations in grades across a typical school day. On average, late chronotypes (F4,520.6 = 3.864, p = 0.004) and short sleepers (F4,546.6 = 4.615, p = 0.001) achieved the lowest grades. The chronotype effect varied by time of day: during the morning early chronotypes achieved significantly higher grades than late types (F2,3551 = 4.171, p = 0.016). The group difference disappeared in the afternoon. Taking test time in reference to chronotype (internal time) into account, we found that early types achieved higher grades earlier in their internal day, whereas late types showed the reversed pattern (F1,3627 = 9.656, p = 0.002). As school exams should provide chronotype-independent assessments of capabilities, school-starting times and exams should be delayed to realise equal learning environments for all chronotypes. In addition, we performed home-based studies testing the effect of increased morning light or reduced evening blue-light exposure on sleep timing in adolescents. Both interventions advanced sleep on school nights in especially late types, and might prove complementary to our proposed school system adjustments.

[Supported by STW grant P10-18/12186]

Reference: van der Vinne, Zerbini, Siersema, Pieper, Merrow, Hut, Roenneberg and Kantermann. Timing of exams affects school performance differently in early and late chronotypes. Journal of Biological Rhythms 2015

### P26. ENTRAINMENT OF THE HUMAN CIRCADIAN TIMING SYSTEM BY MEAL TIMING

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In humans, the entraining effect of environmental cues on peripheral clocks is poorly understood. Animal studies indicate that timed feeding can entrain peripheral clocks whilst under fixed light-dark (LD) cycles. We hypothesised that a delay in the timing of meals would delay the phase of peripheral clocks, but not the suprachiasmatic nuclei, in humans kept in fixed sleep-wake and LD cycles.

10 healthy male participants (age 22.9±4 years (mean±SD), BMI 23.1±2.5kg/m²) conducted a 13 day laboratory study. For the first 3 days, 3 isocaloric meals, adjusted to individual energy needs, were given at 5-h intervals, beginning 30 minutes after wake under 16:8 LD and sleep-wake conditions. Participants then completed a 37-h constant routine (CR) where serial blood samples, adipose biopsies and subjective questionnaires were taken to assess circadian gene expression, endocrine and other rhythms at baseline. Following this, participants completed 6 days where all meals were delayed by 5-h but no other parameters changed. A second 37-h CR was then completed to assess the circadian system after the delayed food intervention.

There was no difference in subjective hunger or sleepiness between each CR. Melatonin phase, estimated by 25% dim light melatonin onset (DLMO) showed no significant difference between the 2 CRs (p>0.05, paired t-test) as hypothesised. Analysis of adipose tissue clock gene expression (*PER2, PER3*) through qRT-PCR showed significant delay in acrophase times of both genes in the second CR (p<0.05, paired t-test)

Our data provide the first demonstration of food entrainment of molecular rhythms in human peripheral tissue.

### P27. IS BROAD SPECTRUM LIGHT ASSOCIATED WITH CHANGES IN INSULIN SENSITIVITY?

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Light at night is the major component for disruption of SCN function, resulting in melatonin suppression via intrinsically photosensitive retinal ganglion cells. Human Evidence from sleep deprivation (Wehrens et al 2010) and circadian misalignment (Scheer et al 2009) human studies have reported changes in insulin sensitivity. This study aimed to investigate the impact of light and/or melatonin on hormone and metabolite responses to a meal in healthy participants.

Seventeen healthy participants were randomised to a two way crossover design protocol; dim light (DL<5 lux) and bright light conditions (BL>500 lux), Clinical sessions commenced at 18:00h and finished at 06:00h the following day. Participants consumed an isocaloric meal (1066 Kcal, 38g protein, 104g CHO, 54g fat, 7g fibre), meal timings were individualised based on estimated dim light melatonin onset (DLMO). Plasma and saliva samples were collected at specific time intervals to assess glucose, insulin and melatonin levels. Three factor repeated measures ANOVA, followed by *post hoc* tests and paired Student's t-test were performed.

Salivary melatonin levels were significantly higher in the DL compared to BL conditions (p=0.005). Postprandial glucose and insulin levels were significantly greater in the BL compared to DL conditions (p = 0.02, p = 0.001) respectively.

Melatonin suppression was expected due to the light intensity. Postprandial plasma glucose levels were greater in BL than in DL despite the presence of higher insulin levels in the bright light condition these finding could be explained by changes in insulin sensitivity (Defronzo 1988, Scheer et al 2009).

### P28. DISSECTION OF CIRCADIAN CIRCUITS DRIVING PULMONARY INNATE IMMUNE RESPONSES

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Our earlier studies have revealed a key role for the epithelial cells lining the bronchioles of the lung in driving circadian immune responses to endotoxin challenge (Gibbs et al Nat Med. 2014). This led to the identification of a circadian controlled epithelial-derived chemokine (CXCL5).

In this study, to reveal transcriptomal change, circadian laser-capture dissection of bronchiolar epithelial cells was done, with samples collected every 4h in 48h for RNA SEQ analysis in *CCSP-iCre- Arntl*<sup>flox/flox</sup> and controls. Data are analyzed for circadian and differentiated expression (DE).

Core clock genes were constitutively suppressed (Nr1d1, Nr1d2, Per1, Per3, Tef, Dbp and Ciart/chrono) or elevated (Rorc, Naps2, Cry1 and E4bp4), with still rhythmic Per2 expression. In control group, 1835 genes were defined as circadian rhythmic, but remarkably in BMAL1-deficient group, 1630 genes emerged as rhythmic, of which 1343 were not detected in WT cells. Biological process analysis in the latter group showed involvement of rRNA and RNA regulation. Top 40 upregulated genes (2.5-25 fold) included CXCL5, 3, 15, IL1F9, Retnla and NLRP10. This is the first study profiling

circadian genes in targeted mutant cells within tissues and reveals diverse roles of clock in bronchiolar epithelial cell functions, prominently immune defence.

## P29. EXTENSIVE WHEEL RUNNING RESTORE CIRCADIAN ACTIVITY RHYTHMS AND ENHANCE COGNITIVE PERFORMANCE (ATTENUATE COGNITIVE DEFICITS) OF ARRHYTHMIC DJUNGARIAN HAMSTERS

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A certain number of animals in our breeding stock of Djungarian hamsters are episodically active throughout the day. Also body temperature and melatonin lack 24-h rhythms. Obviously, the SCN of these animals do not generate a circadian signal. Moreover, these so called arrhythmic (AR) hamsters have cognitive deficits. Since motor activity is believed to stabilize circadian rhythms, we investigated the effect of voluntary wheel running.

Hamsters were bred and kept under standardized housing conditions with food and water ad libitum and a 14L/10D lighting regimen. AR animals were selected according to their activity pattern obtained by means of infrared motion sensors, and transferred to cages with running wheels. After 3 weeks under standard LD conditions, motor activity was recorded for another 3 weeks in constant darkness (DD). A novel object recognition (NOR) test was performed to evaluate cognitive abilities both before and after 3 weeks of wheel availability.

The activity patterns of hamsters with low level of wheel activity (<2500 revolutions/day) were still arrhythmic. With more intense running, daily patterns with higher values in the dark were obtained. Obviously, light suppressed and dark induced motor activity. This was confirmed in experiments with light and dark pulses. In hamsters with medium activity levels (≈10,000 revolutions/day) the rhythm disappeared in DD, whereas in hamsters running most actively (>20,000 revolutions/day) it did free run (masking vs. rhythm restoration). After three weeks of extensive wheel running, AR hamsters were able to recognize the novel object in the NOR test but not so before.

### P30. NOCTURNAL LIGHT EXPOSURE ACUTELY DISRUPTS GLUCOSE METABOLISM

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Most mammals have organized their behavior and physiology in a near 24-hour rhythm which is controlled by the central circadian clock in the brain, the suprachiasmatic nucleus (SCN). With the perception of (sun)light, the SCN converts light information from the outer world to time information for the body and thereby controls numerous physiological processes including glucose metabolism. The multifactorial interplay of glucoregulatory hormones (e.g., glucose, insulin, glucagon) and organs (e.g., liver, pancreas, muscle) forms a tightly controlled mechanism enabling optimal energy supply for the brain and other organs. The SCN-controlled rhythmic secretion and plasma levels of glucose and insulin are disturbed when the SCN receives mistimed light information (i.e., light at night). Previously long term nocturnal exposure to light (i.e., LL, dimlight) has been shown to affect glucose metabolism, but in the current study animals were exposed to mistimed light information for only 1-2 hours at different times along the L/D-cycle. During light or dark exposure animals were subjected to glucose tolerance tests.

Nocturnal light exposure acutely decreased glucose tolerance with higher glucose (at ZT15) and insulin levels (at ZT21). Interestingly, diurnal exposure to darkness (at ZT3) also decreased glucose tolerance. Corticosterone levels remained unaffected suggesting that the effect on glucose levels is not caused by stress. Hepatic sympathectomy or parasympathectomy did not reverse the effects of nocturnal light suggesting that the decreased glucose tolerance is not due to increased hepatic glucose production. Underlying mechanisms of the acute disruption of glucose metabolism are currently under investigation. We expect that our experiments will contribute to a further awareness and understanding of the harmful effects of mistimed exposure to light or darkness.

### P31. THE ZFHX3SCI/+ GENE: A NOVEL CIRCADIAN CLOCK TARGET AFFECTS SLEEP AND COGNITIVE PROCESSES

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The novel circadian mouse mutant, Zfhx3Sci/+, is characterized by a shortening of the period due to a AT motif-dependent circadian axis. In the present work we demonstrate that this axis affects processes outside the canonical circadian system. We explored the gene enrichment ontology of the mutation in order to identify SCN targets and we observed a down-regulation of many genes involved in sleep and cognition. In vivo studies on sleep and cognitive performance highlighted a defect in sleep homeostasis and short-interval time perception in Zfhx3Sci/+ mice. We implemented a stochastic chemical reaction model in order to investigate how circadian clock alterations affects genetic noise in simulated neural networks. Finally, we assessed the dynamics among genetic noise, sleep and circadian rhythms in conditioning daily behaviors. For the first time we report statistical dependency between behavioral and cognitive measures with various biological processes that oscillates with the time of the day. Remarkably, the Zfhx3Sci/+ mutation changes the dynamics of how the circadian clock and sleep can influence cognitive functioning in time.

### P32. PACAP, A DAYTIME REGULATOR OF CIRCADIAN FOOD ANTICIPATORY ACTIVITY RHYTHMS (FAA)

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Light signals from intrinsically photosensitive retinal ganglion cells (ipRGCs) entrain the circadian clock and regulate negative masking. Two neurotransmitters, glutamate and PACAP, found in ipRGCs, via glutamate receptors and the specific PACAP type 1 (PAC1) receptor transmit light signals to the brain. Light entrainment is considered to occur during the twilight zones whereas light has little effects on clock phase during daytime. When animals have access to food only few hours during the resting phase at daytime, they adapt behavior to the restricted feeding paradigm (RF) and show food anticipatory activity (FAA). A recent study in mice and rat demonstrating that light has great impact on FAA in nocturnal rodents prompt us to investigate the role of PACAP signaling in the light mediated regulation of FAA. PAC1 – and PACAP deficient mice placed in running wheels were examine in a full photoperiod (FPP) of 12:12 h LD and a skeleton photoperiod (SPP) 1:11:1:12 h L:DD:L:DD at 300 and at 10 lux light intensity. Both genotypes and their wild type littermates entrained to FPP and SPP and both light

intensities. When placed in RF with access to food for 4-5 h during the subjective day a significant change in behavior was observed in PACAP and PAC1 receptor deficient mice. At FPP at 300 lux both wild type, PACAP- and PAC1 deficient mice showed similar FAA. However, when placed in SPP at 300 lux, PAC1- and PACAP deficient mice demonstrated an advanced onset of FAA with a nearly 3-fold increase in amplitude compared to wild type mice. PAC1deficient mice demonstrated the same pattern of FAA during FPP at 10 lux and in SPP at 10 lux. The present study demonstrates a role of PACAP signaling during light regulated FAA and suggests an unknown role of the melanopsin system during FAA.

### P33. DIM LIGHT AT NIGHT INDUCES AN ADDITIONAL FREE RUNNING RHYTHM THAT DISTURBS SLEEP WAKE BEHAVIOUR IN RATS

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Light pollution is ubiquitous in the present society. We aimed to investigate the effects of light at night on the suprachiasmatic nucleus (SCN), sleep-wake behaviour and energy metabolism in rats. Therefore we exposed male Wistar rats to either a regular 12:12 light (200 lux):dark (0 lux) cycle (LD) or a 12:12 light (200 lux):dim light (5 lux) cycle (LDim). Passive infrared recordings of locomotor activity revealed that LDim exposure reduced the strength of the 24hr rhythm and induced an additional free-running rhythm with a period of ~25 hrs. We sacrificed rats at ZT6 or ZT18 and we performed in situ hybridisations on SCN sections. LDim exposure evidently reduced the day-night differences in the expression of Per1 and Arntl compared to LD. EEG/EMG recordings showed that LDim exposure strongly decreased the amplitude of the daily rhythm of waking, NREM and REM sleep compared to LD. Within the NREM sleep EEG, activity in the 16-19 Hz range in particular showed a decrease in amplitude, underscoring the reduced SCN output strength. To investigate potential metabolic consequences, we placed rats in metabolic cages and performed intravenous glucose tolerance tests. LDim exposure reduced the daily rhythm of food intake and energy expenditure, but LDim did not affect body weight, adiposity or glucose tolerance. In conclusion, we show that LDim induces an additional free running rhythm that interferes with the regular 24hr rhythm. Consequently, light at night has detrimental effects on the SCN and sleep/wake behavior, emphasizing the big effects of small amounts of light.

## P34. EXPRESSION OF ECTONUCLEOTIDASES IN THE PROSENCEPHALON OF MELATONIN-PROFICIENT C3H AND MELATONIN-DEFICIENT C57BL MICE: SPATIAL DISTRIBUTION AND TIME-DEPENDENT CHANGES

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Extracellular purines (ATP, ADP and adenosine) are essential signaling molecules in the CNS involved in many physiological processes, such as neurotransmission, neuromodulation, neural protection, nociception and wakefulness. Levels of extracellular purines are regulated by enzymes located at the cell surface referred to as ectonucleotidases. At physiological extracellular pH, NTPDase1, -2 and -3, which hydrolyze ATP and ADP to AMP, and ecto-5'nucleotidase, which hydrolyzes AMP to adenosine, are considered to be the dominant ectonucleotidases in the brain. Despite some enzyme histochemical and immunocytochemical data available, our knowledge regarding the exact localization of these ectonucleotidases is still incomplete. Moreover, time-dependent changes in their expression may have profound influence on the availability of extracellular purines and thereby on purinergic signaling. Using radioactive in situ hybridization, we analyzed the spatial and temporal mRNA distribution of the enzymes NTPDase1, -2 and -3 and ecto-5'-nucleotidase in the prosencephalon of two mouse strains: melatonin-proficient C3H and melatonin-deficient C57Bl. The mRNAs of these enzymes were localized to specific brain regions, such as hippocampus, striatum, medial habenula and ventromedial hypothalamus. Interestingly, NTPDase3 expression was more widely distributed than hitherto thought. Notably, all ectonucleotidases investigated revealed prominent time-dependent expression patterns, which differed between the two mouse strains. In C3H, the mRNA expression of all four enzymes gradually increased during the day and peaked during the night. Contrarily, in C57BI, ecto-5'-nucleotidase revealed an exact opposite temporal pattern. Finally, recording of locomotor activity revealed higher daytime activity of C57BI than of C3H implying involvement of melatonin in sleep regulation. Our results indicate that the expression of ectonucleotidases varies according to time and genotype and suggest that melatonin exerts modulatory effects on purinergic signaling in the brain. These findings provide an important basis for further examination of the complexity of the purinergic system in the brain.

# P35. CALORIC TIME RESTRICTION DECREASES TUMOR GROWTH AND PRESERVES THE LIVER ARCHITECTURE AND FUNCTION IN A EXPERIMENTAL MODEL OF CIRRHOSIS-HEPATOCELLULAR CANCER

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Caloric Restriction without malnutrition ameliorates the rate of aging and prevents the development of diseases associated to chronic inflammatory disorders, such as diabetes, neurodegenerative diseases and cancer. Circadian synchronization by restricted food access also plays a relevant protective role against these maladies. In this work we used diethylnitrosamine (DEN) to induce cirrhosis (12 weeks of treatment) followed of Hepatocellular Cancer (HCC) (16 weeks of treatment) reproducing the pathological sequence reported for humans and tested the posible protective effect of Time Caloric Restriction (T-CR) in develop of both pathologies.

We had 4 groups: AL (healthy rats with *Ad-Libitum* food access). AL+DEN (rats with Cirrhosis-HCC and food *Ad-Libitum*). T-CR (healthy rats with 2h of food access, from 12:00 to 14:00 h). T-CR+DEN (rats with Cirrhosis-HCC and 2h of daily food access).

T-CR ingested 30% less food and T-CR+DEN ingested 45% less food in comparison to their corresponding control group. However, none of these groups showed malnutrition. Both groups treated with DEN showed similar weight loss (12-16%) with an evident development of cirrhosis and HCC. T-CR+DEN showed increase collagen deposits but without inflammation, regenerative process, or any architectural and cytological changes.

This group showed a significant reduction of circulating Carcinoembryonic Antigen (marker of metastasis and relapse process) as well as minor number and smaller tumors. The dual effect of Caloric Restriction and circadian synchronization promoted a protective effect in the development of tumors and better preserved the liver functions in rats with Cirrhosis-HCC by DEN treatment.

#### P36. ROLE OF HEPATIC MIRNAS IN ADAPTATION TO DAYTIME FEEDING IN MICE

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Recently, we have reported a comprehensive profiling of the rhythmic transcriptome in mouse livers depleted of miRNAs (hepatocyte-specific *Dicer* knockout) and proposed roles for miRNAs in modulating rhythmic gene expression<sup>1</sup>. In brief, we found that miRNA-mediated regulation affected as much as 30% of the rhythmic transcriptome and influenced the phase and amplitude relationships between rhythmic transcription and rhythmic mRNA accumulation. In contrast, the core clock was surprisingly resilient to miRNA loss with the exception of mild posttranscriptional upregulation in *Per2*, *Per1* and *Cry2* mRNAs. At the protein level, however, only PER2 was significantly increased. Moreover, free-running PER2::LUC rhythms measured in liver explants showed long periods in the absence of miRNAs. We concluded that the liver clock is fully functional upon miRNA loss, but that a core clock period length phenotype is masked in the entrained animal. This hypothesis prompted us to explore the role of miRNAs in the hepatic core clock under conditions when the relationship between the master clock in the SCN and the peripheral liver clocks is brought out of equilibrium such as food inversion experiments.

To this end, we have now measured hepatic PER2::LUC rhythm in living *Dicer* knockout and control animals upon restriction of feeding time. Mice were fed ad libitum before switching to daytime feeding, and PER2::LUC rhythms were recorded using the RT-Biolumicorder device<sup>2</sup>. First experiment suggested modified kinetics of phase-shifting in Dicer knockouts compared to controls. At present, we are increasing the number of analysed animals in order to precisely quantify the effect and designing experiments to pinpoint the responsible miRNA(s). Altogether, our data point to an involvement of miRNAs in regulating flexibility of the clock towards changes in entrainment conditions.

<sup>1</sup>Du NH.\*, Arpat B.\*, et al., *eLife*, 3:e02510, 2014. (\* co-author).

### P37. ROLES OF THE CIRCADIAN CLOCK IN THE PATHOGENESIS OF ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD)

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Attention-Deficit Hyperactivity Disorder (ADHD) is one of the most prevalent psychiatric disorders in children and adults. While ADHD patients often display circadian abnormalities, the mechanisms underlying circadian regulation of the pathogenesis of ADHD are unclear. Here we found zebrafish mutants for circadian gene *period1b* (*per1b*) display hyperactive-, impulsive-, attention deficit-like behaviors, low levels of dopamine and elevated levels of norepinephrine, reminiscent of human ADHD patients. We found that the circadian clock directly regulates the dopamine catabolism genes *dopamine beta* 

<sup>&</sup>lt;sup>2</sup>Saini et al., *Genes Dev.*, 2013, 27:1526.

hydroxylase (dbh) and monoamine oxidase (mao), and likely acts through genes important for the development or maintenance of dopaminergic neurons to regulate their number and organization in the ventral diencephalic posterior tuberculum (PT). We then found that Per1 knockout mice also display ADHD symptoms and reduced levels of dopamine, thereby implicating circadian roles in ADHD are highly conserved. Our studies demonstrate that disruption of a circadian clock gene elicits ADHD-like syndrome. The circadian model for ADHD sheds light on ADHD pathogenesis and opens avenues for exploring novel targets for diagnosis and therapy for this common psychiatric disorder.

# P38. EFFECTS OF SPECTRAL MODULATION OF NOCTURNAL LIGHT ON THE CIRCADIAN SYSTEM OF THE DIURNAL OCTODON DEGUS AND NOCTURNAL RATTUS NORVEGICUS

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To maintain a stable phase-relationship with the external world, the activity of the master circadian clock in the Suprachiasmatic Nuclei (SCN) is reset daily by the light/dark cycle. Although light intensity and timing are important for SCN-activity synchronisation, light spectra containing the blue-wavelength play a fundamental role. Lights with shortwavelengths are critical for visual acuity, but inappropriate exposure to blue-light at night could misalign the circadian system. Here, we evaluate the capacity of the diurnal Octodon degus and nocturnal Rattus norvegicus to synchronise to a variety of nocturnal light spectra. We determine whether blue-light can be substituted for less circadiandisruptive wavelengths. To do this, animals were exposed to a combined Red-Green-Blue LEDs (RGB) illumination for 12h during daytime. At night, they were subjected to different conditions: i)Darkness; ii) Red lights; iii) combined Red-Green LED (RG) lights; and iv) combined Red-Green-Violet LED (RGV) lights. Finally, animals were maintained in darkness before receiving a 1h RGB- or RGV-light pulse at CT16.Wheel running activity rhythms in the rats free-run after green-lights were introduced at night (RGB:RG) and became arrhythmic under RGB:RGV conditions. Interestingly, the degus remained synchronised showing a typical diurnal activity pattern. In both species, SCN c-Fos activation tended to be higher following the RGB-light pulse compared to RGV-pulse. This study shows that substituting blue-light for shorter-wavelength nocturnal illumination can synchronise the behaviour of a diurnal rodent. This could be relevant for developing lighting strategies that reduce the disruptive effects of nocturnal light in humans without compromising visual acuity.

Acknowledgments: RETICEF (RD12/0043/0011), Fundacion Seneca (19701/PD/14), AYA2011-15808-E, MINECO (SAF2013-49132-C2-1-R) and INNPACTO (IPT-2011-0833-900000) including FEDER cofounding to JAM. FPU - AP2009-1051.

### P39. RECIPROCAL REGULATION BETWEEN THE CIRCADIAN CLOCK AND HYPOXIC SIGNALING IN MAMMALS

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It is known that circadian regulation in mammals plays an important role in maintaining metabolic and physiological homeostasis. However, it is not well understood whether the circadian clock is associated with abnormalities under certain pathological conditions. Here, we report the discovery of bi-directional regulation between the clock and hypoxia, a situation that could cause catastrophic damage to the body. We show that hypoxic signals slow down the circadian cycle in a dose-dependent manner and dampen the amplitude of oscillations; the clock in turn controls the strength of responses to hypoxic stimuli both in vitro and in vivo. Further, the severe consequences caused by acute hypoxia, such as in the event of heart-attack, correlates with defects in circadian rhythms. We propose here that the clock plays an important role in fine-tuning hypoxic responses under patho-physiological conditions, and note that this could be exploited therapeutically to reduce the severity of fatal hypoxia-related diseases.

## P40. BEHAVIOUR AND SLEEP DISTURBANCES ASSOCIATED WITH THE CANDIDATE RISK GENE FOR NEUROPSYCHIATRIC DISEASE CACNA1C REVEALED USING ENU MUTAGENESIS

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Neuropsychiatric disorders such as schizophrenia and bipolar disorder have a large genetic component with symptoms including hallucinations, disturbed emotional state, cognitive deficits and sleep/circadian disturbances. Genome-wide association studies (GWAS) have identified variants in genes associated with these disorders, including CACNA1C, a voltage-gated calcium channel subunit. Risk variants in patients are associated with changes in anxiety, sleep latency and quality, and narcolepsy. While the Cacna1c null allele is homozygous lethal in mice, conditional and brain-specific knockouts show behavioural defects including increased anxiety. At MRC Harwell, we have used an ENU mutagenesis DNA archive to screen for additional Cacna1c allelic variants expressing more subtle and varied behavioural phenotypes. Using this approach, we identified two mutations: one with a missense mutation G1457D in the EF-hand region of the gene (responsible for inactivation of the channel) and one with a nonsense mutation Q1466\*, leading to a premature truncation of the protein. Mice carrying these mutations underwent a battery of phenotyping to study behaviours including anxiety and sleep. Interestingly, the lines showed opposing anxiogenic phenotypes in Open Field, and using inactivity as a correlate of sleep we have also shown that there are opposing effects on sleep latency. These results, together with further phenotyping results and functional characterisation, could validate CACNA1C as a risk gene for bipolar disorder and sleep disturbances.

### P41. EXPLORING THE EFFECTS OF CONDITIONAL DELETION OF ZFHX3 IN THE ADULT BRAIN

Ashleigh G Wilcox, Gareth Banks, Greg Joynson, Michael Parsons and Patrick Nolan

The transcription factor zinc finger homeobox 3 (Zfhx3) has been implicated in numerous processes including neural development, cardiac function and tumourigenesis; until now the role of Zfhx3 in adult brain has not been studied. However, recently a single point mutation in Zfhx3 was found to be the cause of a shortened free running period in the ENU mutant Short Circuit (Sci). This, combined with expression analysis showing highly localised expression in adult SCN, suggested that Zfhx3 has an important function in mammalian rhythm regulation. Surprisingly, the mutation does not dramatically affect core clock gene expression. Instead, binding to AT motifs in target gene promoters by Zfhx3 appears to be disrupted.

Zfhx3 is highly expressed in development; mice homozygous for developmental deletion of the gene are not viable. Intriguingly, knock-down of Zfhx3 expression using siRNA *in vivo* causes a lengthening of free running period. Therefore, to elucidate how adult Zfhx3 expression contributes to observed circadian phenotypes a variety of Cre driver lines are being employed to generate conditional knock-outs of the gene. Initial characterisation of mice crossed to a hypothalamic specific Cre - Six3, has revealed similar circadian activity to the siRNA knock-down of Zfhx3 as opposed to that of Sci.

Further behavioural analysis will be conducted on these mice in parallel with molecular characterisation. Mice expressing a tamoxifen inducible Cre to create a null Zfhx3 allele whilst preserving developmental expression are also being validated. Building on this work, further conditional mutants will be created to study spatial Zfhx3 function.

### P42. MATERNAL EXPOSURE TO CHRONIC SHIFT PHOTOPERIOD IMPAIRS ADRENAL FUNCTION IN THE ADULT OFFSPRING

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Developmental programming of disease can be determined by adverse prenatal conditions. We reported that alteration of the photoperiod along pregnancy (gestational chronodisruption) induced fetal growth restriction and affect adrenal physiology, heart morphology and long-term effect on cognitive function. Here, we explored the effects of chronic photoperiod shifting throughout pregnancy on the rat fetal and adult adrenal.

Adult female rats maintained under 12:12 light:dark photoperiod were mated and separated in two groups: control 12:12 light:dark photoperiod (LD) and chronic photoperiod shifting (CPS). At 18 days of gestation (dg), adrenal glands were collected from LD and CPS fetuses every 4-h. In a parallel cohort, dams were allowed to deliver and returned to LD conditions with their pups. LD and CPS adult (90 days-old) males were euthanized every 4-h. In the fetal and adult, we investigated clock and clock-controlled gene expression, corticosterone and aldosterone levels. In addition, behavioral studies were performed in adult.

At 18dg CPS fetal adrenal did not presented significative circadian rhythm of clock genes, stereogenic enzymes and adrenal corticosterone content. In adult CPS, the circadian rhythm of corticosterone and aldosterone was nearly opposite to LD offspring (almost 12-h phase shift). CPS adults showed altered performance in Morris water maze and Forced swim test.

Therefore, gestational chronodisruption desynchronizes the fetal adrenal clock and, remarkably, these changes carry on into adulthood, as the corticosterone phase shift with probable effects on behavioral. These alterations might increase the susceptibility of acquiring chronic diseases.

### P43. MATERNAL CHRONODISRUPTION DURING PREGNANCY ALTERS GLUCOSE HOMEOSTASIS AND ADIPOSE TISSUE PHYSIOLOGY IN THE OFFSPRING

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Epidemiological and experimental evidence supports an association between chronodisruption and incrased incidence of chronic diseases. However, the effects of maternal chronodisruption during pregnancy and the offspring remain unclear. Here, we investigated the effects of chronic photoperiod shifting (CPS) throughout gestation on the offspring's glucose homeostasis and adipose tissue physiology.

Material and Methods: Female rats (maintained in photoperiod 12:12 light:dark; n=16) were mated and separated into two groups: A) control photoperiod 12:12 (LD; n=8) and B) chronic phase shift photoperiod (CPS; n=8), simulating a shift work schedule. At birth, mothers and their pups returned to photoperiod 12:12. On day 90 of age, male rats were exposed to a high fat diet (HFD; 45% excess calories) for 12 weeks, to carry out intraperitoneal glucose tolerance test (IGTT) and insulin tolerance test (IITT). At day 200 of age, rats were euthanized to collect blood samples and fat depots (interescapular, inguinal, perigonadal, and perirenal) for histological, molecular and functional analysis.

Results: At 200 days (after 12 weeks with HFD) we observed clear changes in the content and morphology of brown adipose tissue; weight of interescapular adipose tissue was significantly decreased in the CPS group with clear differences in the histology, while the inguinal adipose tissue was significantly increased. Thus, animals gestated under CPS conditions presented a higher response to IGTT and IITT regards to LD, whit an increase of body weight without food intake increase.

Conclusion: The present findings support that chronic photoperiod shifts throughout pregnancy program impaired metabolic responses in later life.

[Funding: ANILLO ACT-1116; FONDECYT 1120938 (Chile)]

#### P44. **EFFECTS** ON **PLASMA** CIRCADIAN **RHYTHMS** AND **GLOBAL STATUS** IN ADULT OFFSPRING UNDER **METHYLATION GESTATED CHRONODISRUPTION**

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It is well established that an adverse intrauterine environment is linked with chronic disease in adult life. Here we investigated if maternal chronodisruption, that modifies fetal development, contributing to the onset of adult disease. Thus, we studied long term effect of chronic photoperiod shifting (CPS) during gestation on chronic disease biomarkers and global methylation status on several adult tissues.

Female rats were mated and separated into two groups: control photoperiod 12:12 (LD) and CPS. At birth, mothers and their pups returned to LD. At 90 days old, to investigate plasma biomarkers (glucose, cholesterol and hepatic metabolite), blood sample was taken around the clock. Tissue samples (adrenal, white adipose tissue-WAT, heart, liver,

kidney and spleen) were obtained at 08:00-h and 20:00-h and used to measure global DNA methylation.

In CPS offspring: 1.- No circadian rhythms were found for cholesterol and HDL-Cholesterol and plasma levels and circadian rhythms of aspartate aminotransferase, alanine transaminase and C3 complement factor were slight modified. 2.- kidney heart and spleen showed different global methylation status versus animals gestated in LD photoperiod. Meanwhile, WAT, kidney and adrenal, showed AM/PM differences only in the CPS group.

Taken together, these data support that gestational chronodisruption is detrimental for adult health. Preliminary, the CPS effects on methylation status could be associated with increased risk of liver damage, (cardio) metabolic and immune diseases.

[Support: ANILLO ACT-1116; FONDECYT 1120938 (Chile)]

### P45. INVOLVEMENT OF CIRCADIAN CLOCK MECHANISMS IN FABRY DISEASE, A GENETIC LYSOSOMAL STORAGE DISORDER

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Fabry disease (FD) is a rare genetic lysosomal storage disorder, inherited in an X-linked manner. In FD, the mutations in the GLA gene encoding for a lysosomal enzyme alphagalactosidase A lead to a deficiency in the enzyme activity. This results in excessive accumulation of the particular type of fat called glycosphingolipid (mainly globotriaosylceramide) inside cells and tissues, including the cells lining the blood vessels of the skin as well as the heart, kidney and nervous system. This progressive disease often starts in early childhood and affects multiple systems, thus negatively influencing patients' quality of life and life expectancy.

In order to better understand the systemic effects of this disorder, we have investigated the hypothesis that circadian clock mechanisms play a role in FD pathogenesis. We show that core clock and clock-controlled genes show altered expression, phase and rhythmicity in synchronized FD patient fibroblasts compared to healthy controls as assessed by the qPCR analyses and real-time-recording of clock::gene reporters. Moreover, the regulation of several antioxidant genes, responsive to lysosomal stress, show dampened circadian expression in FD patient cells. Furthermore, healthy control fibroblasts show a circadian variation in the alpha-galactosidase A enzyme activity, suggesting that altered circadian regulation of this enzyme may play an important role in FD. Further work will test whether timed supplementation of the recombinant alpha-galactosidase A enzyme to patient cells (used as enzyme replacement therapy in FB) can rescue their altered circadian rhythms. All together, these data suggest a novel involvement of the circadian clock mechanisms in the pathophysiology of lysosomal disorders.

#### P46. SEASONAL RHYTHMS IN HYPOTHALAMIC ANGIOGENESIS

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Blood flow in the brain is regulated in a highly precise manner; slight deviations lead to severe pathological states with significant dysfunction in physiology, behaviour and

immunity. Previous high-throughput work from two different seasonal species revealed an evolutionarily conserved, genomic switch in the expression of a single gene that regulates local hypothalamic blood flow; specifically brain-angiogenesis inhibitor (BAI). Using molecular techniques coupled with Positron Emission Topography (PET) brain imaging, we tested the hypothesis that seasonally breeding species exhibit marked rhythms in brain angiogenesis. We will present data that demonstrates short winter-like days (SD) reduce the expression of bai-1 and bai-3 in the Siberian hamster hypothalamus. This change is paralleled by an increase in hypothalamic vascularization indicated by greater CD31 immunoreactivity in SD hamsters. Timed melatonin injections are sufficient to reduce hypothalamic bai1 and bai3 expression in hamsters housed in long summer-like day (LD). PET scans of hamster brains reveal a significant increase in hypothalamic glucose uptake in SD compared to LD. We propose that the increased hypothalamic andiogenesis in SD hamsters facilitates local availability of energy resources and maintain the functional integrity of the brain during harsh winter conditions. Using a range of in vivo and in vitro methods, this presentation provides convergent data that reveals naturally occurring and dynamic changes in angiogenesis in discrete brain regions. These findings provide novel insight into the proximate mechanism by which light and hormones regulate localized blood flow in the brain.

### P47. INFORMATION SEEKING BEHAVIOUR PREDICTS EXTENSIVE SEASONAL VARIATION IN COMMON CHILDHOOD ILLNESSES

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Childhood illnesses are common across all societies and caused by a variety of viral and bacterial pathogens. However, our understanding of their population dynamics is limited to medical reports which are sparse, often in-accessible and restricted to include only clinical cases. Internet searches of common illnesses provide a novel means to acquire longitudinal time-series data-sets that can be used to track local and national seasonal incidence of illnesses. Information seeking behaviour derived from internet-based key word searches for chicken pox, scarlet fever, whooping cough, croup and hand, foot & mouth disease over a 10 year period in several countries were examined for seasonal variation. Here, we show that information seeking behaviour in humans reflects overt seasonal biology in the vast majority of childhood illnesses. We infer that the data cannot be explained by cultural or societal events and instead provide a novel indication of seasonal disease dynamics. The seasonal variation in the amplitude of information seeking behaviour predicts seasonality in illnesses, which we confirm using medical records. Interestingly, analyses of the level of information seeking behaviour over the past ten years shows significant negative trends for countries that implement government vaccination programs whereas countries that do not actively vaccinate exhibit a linear increase in information seeking behaviour. Using non-mechanistic models, internet-based information seeking behaviour can accurately predict pathogen transmission at the population level. Overall, these data and methodological approach can be used by government agencies to improve infectious disease surveillance from local to global levels.

### P48. THE IMPACT OF CIRCADIAN PHENOTYPE ON PREDICTED TEAM PERFORMANCE

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Team performance is a complex phenomenon involving numerous influencing factors across different disciplines including physiology, psychology and management. The study of biological rhythms and the impact of circadian phenotypes have not been included in this array of factors despite our knowledge of the circadian regulation of key physiological processes involved in physical and mental performance. This study involved 216 individuals from 12 different sports teams who were categorized into circadian phenotypes using the novel RBUB chronometric test. The composition of circadian phenotypes within each team was used to model predicted daily team performance profiles based on physical performance tests. Our results show that irrespective of age and gender, the composition of circadian phenotypes within teams is variable and unpredictable. Predicted peak performance ranged from 14.00 h to 21.00 h with performance levels fluctuating by up to 15% over the course of the day. We conclude that circadian phenotype can be a performance predictor in teams. Knowledge of circadian phenotype composition allows new insight and a better understanding of team performance variation in the course of a day. Our novel tools could impact on performance management and provide teams with a circadian advantage if applied

### P49. C-FOS PHOTOINDUCTION IN THE SCN INCREASED AT CT23 WHEN GLIAL CELLS POPULATION ARE REDUCED

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The circadian system is composed of the suprachiasmatic nucleus (SCN) and neuronal structures, regulating biological time measurement, entrainment to geophysical cycles, and control of circadian rhythms expression in mammals. Glial cells play an important role for support and maintain neurons. Also, astrocytes modulate different signal to neurons that are indispensable for their function, for instance, regulation of extracellular glutamate and calcium which participates in the CREB/C-Fos pathway in the SCN. We suggest that if glial cells on the SCN are removed, the clock function could be affected. We induced apoptosis of glial cells in the SCN using a glial specific adenoviral vector containing the apoptotic gene *gas1* (AdSGas1) under the GFAP (Glial fibrillary acidic protein) promoter. Subsequently, we recorded locomotion and drinking activity in continuous light (L/ L) condition. Also we determine whether glial reduction affects the C-Fos expression after light pulses at CT 23.

Our results shows that animals treated with AdSGas1 have a significant reduction in glial population of the SCN demonstrated by GFAP immunohistochemistry, and lost circadian rhythmicity when are transferred to LL conditions. Furthermore the C-Fos photoinduction increase at CT23 in animals treated AdSGas1 compared to control animal. These results suggest that the glial cells from the SCN have an important role regulating SCN circadian response to light.

[Supported by CONACyT 12858]

### P50. SLEEP-WAKE ACTIGRAPHY AND CARDIOVASCULAR RESPONSES TO STRESS IN NIGHT SHIFTS WORKERS

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Background: Night shifts increase the risk of cardiovascular diseases. The aim of the study was to research peculiarities of sleep-wake activity by actigraphy and heart rate variability (HRV) based indicators of stress in night shifts workers.

Methods: The study included 21 male railway employees who work in night shifts (mean age 45.4). All participants underwent routine clinical examination, 24 h Holter monitoring, and HRV assessment. Sleep-wake pattern and motor activity was measured with actigraphy according to bed rest protocol performed >5 days after the last night shift.

Results: All participants had alterations of sleep-wake cycle. Total time in bed increased up to 43.6%; sleep efficiency index was 34.7% to 81.9%; there was sleep fragmentation; average number of movements/changes of body position during night sleep was 81.6. Analysis of HRV-based indicators revealed eustress 10% of patients only; 90% had increased stress level, including severe distress in 40%. Moderate, working, and pronounced depletion of adaptive reserves was diagnosed in 60%, 20%, and 20% of patients, respectively. Four patients had cardiac arrhythmias (supraventricular and ventricular extrasystoles, allorhythmia, and episodes of supraventricular tachycardia).

Conclusions: Alterations of sleep-wake cycle were registered in railway employees who work in night shifts in >5 days after the last night shift. Disturbed sleep-wake cycle is associated with decreased stress resistance and adaptation reserve. This may lead to heart rhythm disorders.

## P51. THE METHAMPHETAMINE-SENSITIVE CIRCADIAN OSCILLATOR (MASCO) IS GENE-DOSE AND AGE-RELATED IN A KNOCKIN MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative genetic disorder characterised by progressive motor and cognitive deficits, as well as circadian and sleep abnormalities. The R6/2 mouse model of HD recapitulates the circadian disruption seen in HD patients. The rest-activity rhythm controlled by the suprachiasmatic nucleus disintegrates completely in R6/2 mice by 16 weeks of age. A second circadian oscillator, the methamphetamine-sensitive circadian oscillator (MASCO), is impaired even earlier, and is not evident after 8 weeks of age1. Here we used a second 'knock-in' model (Q175) of HD to study the effect of the mutation on the MASCO. We induced the MASCO by administering methamphetamine chronically at a low dose (0.005%) via the drinking water, to WT mice and mice heterozygous or homozygous for the mutation. We measured locomotor activity under constant darkness to assess MASCO function at 9, 25 and 50 weeks of age. We found that at 9 weeks, all mice expressed a MASCO, and had rest-activity rhythms with period >>24hrs. The number of mice exhibiting a MASCO declined with age. This was exacerbated by the HD mutation. At 25 weeks of age, MASCO was seen in 87% of WT mice, but only 43% of heterozygous and 17% of homozygous mice. By 50 weeks, MASCO was seen in 44% of WT mice, but only 12% of heterozygous and 9% of homozygous mice. Thus, MASCO is disrupted by both aging and the HD mutation. We suggest that dysfunction of MASCO, if present in humans, may contribute to the circadian HD phenotype.

<sup>1</sup>Cuesta M, Aungier J, Morton AJ. The methamphetamine-sensitive circadian oscillator is dysfunctional in a transgenic mouse model of Huntington's disease. Neurobiol Dis. 2012

### P52. ASSOCIATION BETWEEN KNOWLEDGE OF BIOLOGICAL RHYTHM AND NURSING CARE FOR PATIENT'S ENVIRONMENT IN HOSPITAL

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Light and ambient temperature are strong Zeitgebers controlling circadian rhythms for patient. The care based on knowledge of biological rhythm bring patient's welfare benefits. However, in nursing, it cannot be said that knowledge on biological rhythm is enough. Therefore, the aim of this study is to demonstrate the association between the knowledge of biological rhythm and the nursing care to coordinate the patient's environment. Four hundred nurses who work at several hospitals in Japan, participated in Internet investigation. They answered in 19 items about the nursing care in coordination with patient environment (light, humidity and ambient temperature) and in 15 items about knowledge of biological rhythm in hospital. On 19 item's care, participants were separated by two groups by whether paid the attention usually to that care (A) or not (B). In light environment adjustment, the score of nursing care with theoretical knowledge was significantly high in the A group who performed ceiling illumination and opening / shutting of window's curtain respectively (p=.004, p<.001). In air environment adjustment, the score of nursing care with theoretical knowledge was significantly high in the A group who performed opening / shutting of window's curtain, applying hot-water bottle or ice pillow and position of the bed (p=.015, p=.037, p=.036). It was suggested that theoretical knowledge of biological rhythm might influence the choice of effective patient care. Thus, further spread of knowledge of biological rhythm may improve patient's QOL in home care.

### P53. EFFECT OF CIRCADIAN EXPRESSION OF TYROSINE HYDROXYLASE IN 6-HYDROXYDOPAMINE INDUCED DAMAGE RESPONSE OF HUMAN DOPAMINERGIC CELL

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Tyrosine hydroxylase (TH) constitutes the rate-limiting step of dopamine production. Several studies suggested that the activity of TH is related with apoptotic cell death of dopaminergic neurons. We previously found that expression of TH in dopaminergic neurons is regulated in a circadian manner by the antagonistic competition of two nuclear receptors that link between circadian rhythm and mood regulation. Based on these findings, we postulated that circadian rhythm of TH expression is important for both survival and function in dopaminergic neurons. Using recently developed light-switchable transgene system, we established a novel in vitro model for analyzing the effect of rhythmic expression of TH in a 6-hydroxydopamine (6-OHDA) treated condition. For this purpose, human dopaminergic cell line SH-SY5Y was transfected with expression vectors

of TH that are regulated by blue light inducible promoter and its activator. We examined the rate of cell death induced by treatment of 6-OHDA with exposure to blue light in a circadian-like light-dark schedule. We also compared the effect of rhythmic and constant TH expression on the susceptibility to 6-OHDA by exposure to different light schedule. These results suggest that circadian regulation of TH has a protective role for dopaminergic neurons. Further, the effect of rhythmic expression of TH in dopaminergic neuronal degeneration may be applied to Parkinson's disease.

### P54. CIRCADIAN EPITRANSCRIPTOMICS: MRNA RHYTHMS DRIVEN BY RHYTHMIC POST-TRANSCRIPTIONAL REGULATION

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Physiological rhythms with about 24-hr period were controlled by thousands of rhythmically expressed genes, which are believed to be generated by circadian transcription via transcription-translation feedback loops. However, recent development in next-generation sequencing technology found an unexpected fact that approximately 70-80 % of rhythmic mRNAs are not rhythmic at de novo transcription levels. So, posttranscriptional regulation should be important for explaining the large part of circadian gene expression. We found circadian phosphorylation of CLOCK-BMAL1 complex, and identified its phosphorylation sites that inhibit DNA-binding ability of the complex. CLOCK-ChIP-Seq identified the genomic regions targeted by the rhythmic DNA-binding of the complex, and RNA-Seq gave us information about rhythmically expressed genes. Here, we focused on an RNA modifying enzyme, which has functional E-boxes in its intronic region and rhythmically expressed in mouse liver. The rhythmic expression of the enzyme and the RNA modification rhythms mediated by the enzyme were perturbed in Bmal1-deficient mice. Importantly, not only the RNA modification rhythms but also large populations of mRNA oscillations were attenuated by deficiency of the enzyme. Our finding supports the importance of epitranscriptome in driving circadian rhythms of mRNA amount. Furthermore, our study using the mutant mice showed that the RNA modification determines pace of circadian rhythms in both molecular oscillations and behavioral rhythms, and such a dramatic change of rhythmic transcripts should affect many physiology as outputs from circadian clock systems.

### P55. DIURNAL RESTRICTED FEEDING SCHEDULES PROMOTES CHANGES IN BODY WEIGHT AND NF-kB PRESENCE IN LIVER TISSUE OF WISTAR RATS

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Introduction: P65 of NF- $\kappa$ B is a transcription factor related with cell proliferation, apoptosis and inflammation. Aberrations of its regulation has been related to diseases like diabetes mellitus and cancer, but little is known about its regulation in physiology. Daily restricted feeding schedules (DRFS) promote changes in metabolism, liver apoptosis and proliferation but no inflammation.

Objective: To evaluate different levels of p65 regulation in physiology and how DRFS may modify it.

Materials and methods: Male Wistar rats on cycles 12/12 light/dark and free water and food access (5001 rodent diet, LabDiet) were adapted to laboratory conditions and randomly assigned to two different feeding conditions: 1) AL group: *Ad libitum* access to food and water during the 24h period. 2) DRFS group: Food availability limited to 2h daily

from 12:00 to 14:00h. After three weeks, liver tissue samples of subgroups of animals were obtained by 3h intervals, starting at 8:00 h for a 24h period. Total p65 was analyzed by Western Blot in whole tissue and nuclear extracts. Expression of mRNA p65 and target genes were analyzed by qPCR.

Results: Average daily caloric intake of DRFS was 50% of AL. DRFS had less weight gain than AL. Average liver/BW of AL was higher than DRFS (p<0.05). Daily profile of the presence of p65 and its target genes was different between DRFS and AL.

Conclusions: DRFS is related to caloric restriction and modifies metabolism and liver tissue dynamics. Total p65 NF- $\square$ B and target genes present rhythmic features and are modified under DRFS.

[Supported by grant from PAPIIT, UNAM (IA200713), México]

## P56. EFFECT OF TRYPTOPHAN SUPPLEMENT INTAKE AT BREAKFAST ON NOCTURNAL MELATONIN SECRETION UNDER DIFFERENT LIGHT INTENSITIES IN DAYTIME IN HUMANS

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Tryptophan (TRP) is an essential amino acid, and it is suggested that TRP intake at breakfast with daytime bright light exposure can increase nocturnal melatonin secretion via the serotonin pathway. However, the mechanisms are not clear. In this study, we examined the effect of tryptophan supplement intake at breakfast on nocturnal melatonin secretion under different light intensities in daytime in humans.

Twelve subjects (aged 21.3±3.0 with BMI of 21.8±2.6 (Mean ± SD)) participated in experimental sessions for 3 days under 4 conditions in random order in a laboratory. The four conditions were TRP\*Bright, Placebo\*Bright, TRP\*Dim, and Placebo\*Dim. A TRP capsule (1,000mg) was taken at breakfast, and a starch capsule (1,000mg) was used as the placebo. During the daytime (7:00-18:00), subjects were subjected to different light intensities: >5000 lux (Bright) and <50 lux (Dim). Saliva samples were collected for measuring the concentration of melatonin hormone. Dim light melatonin onsets (DLMOs) were determined before and after each condition. Changes in DLMOs were compared between the 4 conditions by ANOVA.

The changes in DLMOs were significantly different between the 4 conditions (p<.001). However, the results of multi-comparison showed that there were no significant differences between TRP and placebo (TRP\*Bright vs. Placebo\*Bright, adjusted p=.621; TRP\*Dim vs. Placebo\*Dim, adjusted p=1.000). There was no significant difference among the 4 conditions in the time course of salivary melatonin secretion (p>.05).

In the human body, only 2 to 3% of total TRP is metabolized to serotonin; thus, TRP intake at breakfast had little effect on nocturnal melatonin secretion.

## P57. INTERNAL DESYNCHRONIZATION RESULTS IN TISSUE SPECIFIC CHANGES IN THE EXPRESSION OF CLOCK GENES IN A RAT MODEL OF SHIFT-WORK

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In a model of shift-work, rats are forced to be active during their resting phase, while their biological clock remains driven by the LD cycle. As a consequence of the forced activity and inverted food intake pattern, they become arrhythmic. At the molecular level, per1 and bmal1 expression in the liver is shifted to the forced activity hours and per2 is flattened. However, the gene/tissue specific effect of the shift-work protocol on different oscillators besides the liver remains unknown. Here using the same model we investigated the effect of forced activity during the light phase on clock gene expression in different tissues and the misaligned phase relation between genes and tissues. Per1, cry1, bmal1 and clock expression were determined by RT-PCR in heart, white adipose tissue (retro-peritoneal) and skeletal muscle (gastrocnemius). The data demonstrate a tissue specific effect on the clock gene expression. In white adipose tissue, bmal1 was in antiphase when compared to the control group while per2 and cry1 acrophases were advanced. Regarding the heart and skeletal muscle, per2 maintained his acrophase at the beginning of the dark phase, in contrast, cry1 was advanced and bmal1 had a delayed acrophase in both heart and skeletal muscle. In conclusion, our results indicate a tissue dependent misaligned clock gene expression by this chrono-disruption model. [Supported by DGAPA-UNAM IG200314 and CONACyT 234456]

### P58. HYPOTHALAMIC THYROID RESPONSIVE GENE SWITCHES MEDIATE CIRCADIAN CLOCK EFFECTS ON SEASONAL REPRODUCTION IN SONGBIRDS

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In seasonal species, the gonad development cycle is initiated by increasing daylengths during the late spring and early summer. This occurs when the duration of perceived light is long enough to extend into inductive phase of the endogenous circadian photoinducible rhythm. The converse appears true of the termination of photorefractoriness in response to decreasing light periods during the autumn. We measured thyroid responsive genes (Tsh-beta, Dio2 and Dio3) known to involved in the photoperiodic induction in photosensitive and photorefracory redheaded buntings (Emberiza bruniceps) in response to an acute exposure to lengthening and short photoperiods and discovered changes in the mRNA expressions of these genes. There was an incerase in the mRNA expression of Tsh-beta and Dio2 and decrease of Dio3 in photosensitive buntings exposed to photoperiods ≥12 h per day (24 h). On the other hand, there was an increased Dio3 mRNA expression in photorefractory buntings in response to the short photoperiod (8 h light per day). These effects are mediated by circadian photoinducible rhythm as evidenced in a separate set of experiment where photosensitive buntings were exposed to photoperiod cycle of different period lengths. Buntings exposed to 11 h light periods did not respond in a 24 h day (11L:13D), but showed photoperiodic induction in a 22h day (11L:11D). A similar photoperiodic induction was found under 12 h light in 24 h day (12L:12D) but not in the 26 h day (12:14D). Thus, the thyroid responsive genes play key role in mediating the photoperiodic effects as regulated by the endogenous circadian clock system in redheaded buntings.

### P59. EFFECTS OF FILTERING VISUAL SHORT WAVELENGTHS ON PHASE RESETTING AND MASKING RESPONSES

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Ocular light exposure can elicit acute behavioural and physiologic responses, such as changes in locomotor activity (masking), as well as induce phase resetting of the central pacemaker. Both these non-image forming responses are maximally sensitive to shortwavelength (blue) light. Previous studies have shown that filtering short wavelengths from polychromatic sources can attenuate the acute responses, but the effect on phase resetting was unknown. In this current study, we examined the effects of filtering short wavelengths from polychromatic white light on circadian phase resetting, expression of light-inducible molecular markers and negative masking in rats. Free-running animals were exposed to a short 1-h or long 7-h pulse of filtered or unfiltered polychromatic white light (~100 µW/cm<sup>2</sup>) at CT16 or CT13, respectively. Phase resetting and masking were examined using behavioural locomotor activity assays. Brain tissue was collected immediately following and 2-h after the short light pulse exposure to investigate the temporal and topographical expression of immediate early-gene product c-FOS in the suprachiasmatic nucleus using quantitative immunohistochemistry. Filtering visual short wavelength less than 500 nm significantly reduced behavioural phase shifts in response to short and long duration light pulses. This behavioural response corresponded to decreased c-FOS expression in the SCN core and shell. Preliminary analysis suggests reduced negative-masking responses under filtered light as compared to unfiltered light. Collectively, these results demonstrate that filtering short wavelengths from polychromatic light can attenuate both acute and circadian phase resetting responses.

#### P60. MICE LACKING MELANOPSIN DISPLAY DEFICITS IN SOCIAL INTERACTION

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Introduction: The identification of the photopigment melanopsin (OPN4), expressed in a subset of photosensitive RGCs (pRGCs), has revealed a role of light in the regulation of broad range of non-image forming (NIF) responses to light. The pRGCs play a role in photoentrainment via SCN projections, pupillary constriction and even the regulation of mood. Interestingly, the pRGCs project also to the lateral habenula, a relay site between limbic and striatal areas, and the amygdala which is involved in regulation of emotions. As social interactions in mice are used to assess emotional processes, we have investigated the behavioral, physiological and neuroanatomical correlates of social interactions (SI) deficit in  $Opn4^{-/-}$  mice.

Methods: After establishing that singly-housed *Opn4*-/mice showed deficit in SI, WT and *Opn4*-/mice were assessed for several behavioural tests: Open Field Test, Novel Odour Recognition and Elevated Plus Maze. In a 2<sup>nd</sup> new set of animals, basal CORT was assessed. In a 3<sup>rd</sup> new set, a neuroanatomic experiment was conducted using c-FOS activation pattern in brain after SI.

Results: Analyses of anxiety-like behaviors and odour memory tests did not reveal any significant differences between the WT and  $Opn4^{-/-}$ . There was no genotype effect in CORT concentration. However, CORT was reduced by 2-3 fold in singly housed (versus co-housed) mice. CORT was 2-fold higher in females. c-FOS was differentially expressed (between genotypes) in brain area involved in decision making and motivation.

Conclusion: These preliminary data suggest that chronic isolation may disrupt normal social interaction in the  $Opn4^{-7}$ , via specific brain areas.

### P61. USE OF RESAMPLING TO IMPROVE ACCURACY OF DATA ANALYSIS IN TIME COURSE EXPERIMENTS

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Time series of expression data in higher eukaryotes are costly and only a limited number of data points can be measured as the animals have to be sacrificed at every data point. Thus, often only few replicates are available. As the data points furthermore stem from different animals, a time series of expression data does not comprise true biological replicates, but rather a random assembly of time points from a larger number of biological replicates. This high variability leads to very noisy datasets and makes the identification of rhythmic transcripts difficult and error-prone. To improve the identification of circadian regulated genes while maintaining the minimal required sample number, we used data resampling. In tests with artificial time course series, the number of false positives could be greatly reduced while maintaining the number of true positives. These findings were confirmed with several common algorithms to identify rhythmic data series: ARSER, Haystack, JTK Cycle, and Biodare FFT-NLLS. Using the resampling approach, we were able to increases the accuracy of circadian expression data analysis and showed the importance of replicates for time course data analysis. Using our approach 3-4 replicate time series are sufficient to achieve a high accuracy in the detection of oscillating transcripts. We furthermore point out that averaged datasets that are often generated in circadian datasets, are not well suited for expression data analysis. The results are, however, largely depending on the algorithm used. Thus different algorithms should be considered depending on how datasets have been generated.

### P62. CHARACTERIZATION OF CIRCADIAN RHYTHMS OF LIVER PER2::LUC EXPRESSION IN FREELY MOVING MICE

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The lack of appropriate *in vivo* recording technologies has prevented the longitudinal assessment of circadian biomarkers along the course of diseases and their treatments. Here we demonstrate the relations between the rest-activity rhythm and the circadian changes in the transcriptional activity of liver *Per2* in freely moving mice, modified with a reporter system (*Per2*::luciferase).

Methods:  $Per2^{luc}$  mice were singly housed in RT-Biolumicorder units. Animals were synchronized with LD12:12, then were kept in DD for 3 days. D-luciferin was delivered within drink water [1.5 mg/ml] (n=4) or via sc implanted Alzet pumps [90 mg/ml] (n=5) during DD. Individual mouse rest-activity and liver Per2::luc expression were recorded every minute. The period component, the dominant acrophase and the circadian amplitude of each time series were computed using a new analysis method, the Bayesian Variational Approximation (BVA).

Results: The usual circadian periodicity of the rest-activity rhythm averaged 24h, with a median BVA-acrophase at ZT20:30 [25-75% quartiles, ZT19:30 to ZT22:15] in LD12:12. In DD, the median period of activity was 24h [22h to 24h], with a BVA-acrophase at ZT15 [ZT14 to ZT16]. *Per2*::luc expression was rhythmic with a period ranging from 23h to 25h. BVA-acrophase occurred at ZT20 [ZT20 to ZT21], irrespective of luciferin delivery route. The rhythm in estimated *Per2* transcription lagged behind that in rest-activity by 5 to 6 h. No correlation was found between the dominant amplitude of rest-activity and liver *Per2* expression.

Conclusions: Liver *Per2* clock gene was rhythmically expressed along the 24-h in freely moving mice in DD, with a maximum during the second half of activity, irrespective of the route of luciferin delivery.

#### P63. HUMAN PUPILLARY LIGHT REFLEX: EFFECT OF WAVELENGTH AND PHOTON FLUX

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The pupillary light reflex (PLR) is driven by the rods, cones, and intrinsically photonsensitive retinal ganglion cells (ipRGC) which express the photopigment melanopsin. The PLR could be used to evaluate the efficiency of light sources on circadian system activation in a quick, non-invasive and inexpensive way. However, to date the PLR has not been fully characterized spectrally in a large cohort of participants at very narrow bandwidth increments. Our aims were: i) to compare 5-min short  $(\lambda_{max}437nm$  and 479nm) and long  $(\lambda_{max}627nm)$  wavelength light (alone and in combination) at two irradiances on the PLR (study A, n=13); ii) assess spectral sensitivity of the PLR to monochromatic light in the  $\lambda_{max}$ 420-500nm range (study B, n=16), nine stimuli in 10-nm increments. Minimum pupil diameter and area under the curve (AUC) during the first minute(0-60s) and last minute of light exposure (240-300s) were assessed. Isolated photoreceptor contribution was estimated by mathematical modelling. In Study A, the minimum diameter was smallest under blue light and the lowest AUC<sub>0-60</sub> (greater contraction) was found under "479+627"nm light. An irradiance dependency was observed for 437nm and 479nm light. In Study B, the lowest minimum diameter was achieved under 470nm light, with the lowest AUC<sub>0-60</sub> and AUC<sub>240-300</sub>, under  $\lambda_{max}$ 490nm and 460nm, respectively. Our data agree with Mure et al. (2009) and suggest that the sustained response is most sensitive to 460nm light.

[The work was supported by the EPSRC[grant number EP/1000992/1], RETICEF (RD12/0043/0011), AYA2011-15808-E, MINECO (SAF2013-49132-C2-1-R) and INNPACTO (IPT-2011-0833-900000) including FEDER cofounding to JAM. FPU - AP2009-1051]

### P64. A RIGID CLOCK DRIVING FLEXIBLE RHYTHMS; THE MOUSE SCN DRIVES NOCTURNAL ACTIVITY PATTERNS UNDER AD LIB, BUT DIURNAL RHYTHMS DURING FOOD SCARCITY

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The timing of daily rhythms has fitness-consequences in natural situations. Combining genetic, molecular and neuroanatomical approaches has led to the construction of a detailed blueprint of the mammalian clock. Much is known about the mechanisms by which the SCN controls output rhythms in the laboratory, however, in nature these rhythms are more diverse and adapt to various environmental circumstances.

With our recent work we have shown that energetic challenge and subjective predation risk have strong modulating effects on the phase of entrainment of mice.

During simulated food scarcity mice become predominantly day-active, but the phase of the SCN does not differ between diurnal and nocturnal mice. Here we show that mice with bilateral lesions of the SCN do not adopt diurnal circadian output rhythms. This rules out that the diurnal activity is driven by an extra-SCN circadian oscillator. Instead, it indicates that the phase relation between SCN and the output rhythms is flexible.

Additionally, we show evidence that male and female mice respond differently to simulated food scarcity. Prolonged energetic challenge induces diurnality in both sexes, but males shift more readily while the primary response of females is to increase foraging efforts.

By manipulating ecologically relevant parameters we provide novel insights into how the circadian timing system ensures the optimal timing of daily rhythms.

### P65. VELOCITY RESPONSE-BASED MODELING OF PHOTOPERIOD-DEPENDENT SYNCHRONIZATION PATTERNS OF PACEMAKER CELLS IN SUPRACHIASMATIC NUCLEUS

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How variation of day length is recognized has been attracting chronobiological researchers in the context of photic entrainment mechanism. Recently, it has been reported that the phases of circadian rhythms of neural activities and expression of clock genes in the suprachiasmatic nucleus (SCN) are distributed dependent on the day length. By spatiotemporal recording of Per1-luc in in-vitro slice culture of SCN taken from the animals reared under different LD cycles, long day (LD16:8), mid day (LD12:12), and short day (LD8:16), Inagaki et al. (2007) found, (1) The distribution of circadian phase of Per1-luc rhythm was varied dependent on the day length. (2) The anterior and posterior parts exhibited different distributions, which is exemplified by the bimodal distribution for the long day in the anterior part. These findings are modeled as a population dynamics of pacemaker network by changing the balance between diversity of photic responsiveness of individual pacemakers and synchronization inputs from the network. A velocity response curve is an intriguing entrainment mechanism mediating between the parametric and non-parametric mechanisms (Daan, 1977), which is employed to show the day-length dependent collective entrainment in the pacemaker network. The simulations disclose the condition for reproducing the findings by Inagaki et al. (2007): balance between diversity in the periods of pacemakers, appropriate photoresponsiveness which allows the day-length dependent entrainment, and coupling strength between pacemakers.

### P66. IDENTIFICATION OF GENOME REGIONS ASSOCIATED WITH AVERAGE WEEKLY SLEEP DURATION BY USING GENOME-WIDE ASSOCIATION STUDY

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The aim of the current study is to find new genetic variants influencing average sleep duration in humans.

We have used the short version of Munich Chronotype Questionnaire to obtain sleep data from individuals, who joined Estonian Genome Center, University of Tartu, during time period 2007 up to now. So far we have genotyped > 15000 individuals by using different genotyping arrays from Illumina. To find associations with average weekly sleep duration, we have conducted three separate GWA studies: 1) 3226 individuals, genotyped with HumanOmniExpress array, 2) 1124 individuals genotyped with Human370CNV and 3) 3082 individuals genotyped with HumanCoreExome-12v1.0 array. Thereafter individual studies were analysed together in meta-analysis which contained 6669 individuals. Imputation was done by programs ShapeIT v2 and IMPUTE v2.2.2 by using 1000 Genomes Phase 3 as a reference panel. For GWAS we used SNPTEST v2.5 and for meta-analysis we used METAL program.

The most strongly associated markers, which met pre-specified criteria for genome-wide significance, were identified from chromosome 2 in GWAS first cohort with individuals genotyped by HumanOmniExpress array. Meta-analysis did not identify any associated markers with genome-wide significant p-values. The markers from chr 2 intergenic region remained significant at the level of p value 8x10-7. Other interesting findings emerged from a gene in chr 8 8p11.21 and from an intergenic region in chr10 10q26.12. At the moment there are no obvious clues, how the markers from these regions could influence sleep parameters. The number of studied individuals should be increased to verify our findings.

### P67. ALTERED EXPRESSION OF THE CORE CIRCADIAN CLOCK COMPONENT PERIOD2 IN A DIURNAL RODENT MODEL OF SEASONAL AFFECTIVE DISORDER

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Disruptions in circadian rhythms are often associated with seasonal affective disorder (SAD). However, the actual functioning of the circadian clocks in SAD remains unknown. The present study utilized a diurnal rodent model of SAD (Arvicanthis niloticus) to explore how the circadian system is affected in winter-like lighting conditions that elicit depression-like behaviors. The daily rhythms of a core clock component, PERIOD2 were examined in the suprachiasmatic nucleus (SCN) and extra-SCN brain regions, i.e. the hippocampus and the striatum. A significant day-night difference in PERIOD2 immunoreactivity (PER2-ir) was observed in the SCN of the animals housed in summerlike bright-light-dark conditions (BLD), as well as the SAD model housed in winter-like dim-light-dark conditions (DLD). The daily variations of PER2-ir in the hippocampus and the striatum were also observed in the BLD group, but the oscillation patterns were altered in the DLD group. Similar disturbances in the day-night differences were observed in the expression of Per2 mRNA in the hippocampus and the striatum of the DLD group. These results revealed that in this SAD model, the time-keeping function of the SCN remained intact, but the circadian oscillations in extra-SCN regions were disrupted. The results provide insights into the neuropathology of SAD.

### P68. THE CIRCADIAN CLOCK AND PROTEIN AGGREGATION IN CELL-BASED MODELS

Madeti C., Geibel M., Huaroto C., Rubbe N., Vanzo R. and Merrow M.

Neurodegenerative diseases like Alzheimer or Huntington's disease are increasingly prevalent in western populations as they become older. They often lead to disrupted sleep-wake cycles which begs the question if this disruption is upstream or downstream of the pathology. A Dutch study furthermore suggests that increasing the amplitude of the zeitgeber in dementia patients interferes with disease progression.

Neurodegenerative diseases are characterised on the molecular level by altered protein aggregation. A link between senescence and protein aggregation has also been proposed in *E. coli* (Maisonneuve et al.). We have hypothesised that the circadian clock could affect the process of protein aggregation (or vice-versa) and thus play a role in neurodegeneration.

We find that senescence in mammalian cells impacts circadian phase and amplitude. Insoluble protein content, a marker for aging and protein aggregation, is also distinctly effected by circadian entrainment: senescent cells in temperature cycles accumulated more insoluble content compared to cells kept in constant conditions. Thus, protein aggregation has an impact on the clock and the state of the clock has an impact on protein aggregation.

### P69. TEMPERATURE ENTRAINMENT OF THE CIRCADIAN CLOCK IN DROSOPHILA MELANOGASTER

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Cell and Developmental Biology/University College London

To maintain synchrony with the environment, circadian clocks adjust to various cycling sensory cues including environmental temperature cycles. We investigate the neuronal and molecular mechanisms of temperature entrainment, focusing on the (potential) role of thermosensitive TRP channels therein.

trpA1 was chosen as a candidate, remaining from a screen for the involvement of TRP channels in temperature entrainment. Locomotor activity of different trpA1 loss-of-function mutant alleles was measured during both cold and warm temperature cycles (16:25°C and 20:29°C, respectively). We observed that the locomotor activity of the mutants resynchronized to shifted temperature cycles, with the same kinetics as observed for wild-type flies. Contrary to published work implicating trpA1 in synchronization of the Drosophila clock to temperature cycles, we conclude that trpA1 is not essential for this trait. Although trpA1 mutants can still entrain to temperature cycles, we did observe abnormal 'siesta' behaviour during 20:29°C temperature cycles. In flies lacking TRPA1, activity levels don't drop to trough levels in the middle of the warm phase ('siesta'), like they normally do in wild-type flies.

pyrexia (pyx) is another thermo-TRP identified in the same screen. PYX has been implicated in entrainment to cold temperature cycles (16:20°C). We are currently investigating the molecular basis of this behavioural phenotype by visualizing the cycling of the clock protein PER in clock neurons of pyx null mutants. pyx is expressed in the cap cells of chordotonal organs, where it could possibly exert its role in temperature synchronization.

### P70. PERIPHERAL CLOCKS REGULATE THE TRANSCRIPTIONAL RESPONSE TO GLUCOCORTICOIDS

Giorgio Caratti, Louise Ince, Mudassar Iqbal, Louise Hunter, Magnus Rattray, Andrew Loudon, Laura Matthews and David Ray

Glucocorticoids (Gc) are endogenous steroid hormones with potent anti-inflammatory and metabolic actions. Synthetic Gc are important drugs for treating inflammatory disorders, with over 1% of the UK population currently on an oral Gc prescription. Gc exert their cellular effects through binding and activating the glucocorticoid receptor (GR), a ligand activated transcription factor. Recent evidence suggests that GR binding across the genome exhibits plasticity, and is remodelled in a cell-type and context dependent manner. We now know that GR requires pre-programed, open chromatin in order to mediate transcriptional activity. Additional evidence suggests that peripheral tissues undergo cyclic changes in chromatin architecture in line with their circadian phase.

In light of this, we investigate whether there is time of day variation in the response to synthetic Gc.

C57BL/6 mice were given IP dexamethasone at either Zeitgeber Time (ZT) 6 (6 hours after lights-on; day) or ZT18 (6 hours after lights off; night) and culled 2 hours later. Liver and lungs were collected and analysed by RNA-seq. As expected, a minimal overlap is seen between the lung and liver Gc-dependent transcriptomes, reflecting a highly tissue-specific response. Interestingly, there was also a significant time of day variation in the dexamethasone response. We identify striking differences in the liver, with 1709 Gc regulated genes during the day and only 209 genes at night, and 128 genes common to both times. This contrasts the lung, where we observe 555 Gc regulated genes during the day, and 522 at night, of which 325 genes were common to both times.

Gene ontology analysis reveals enrichment for genes regulating metabolic pathways, specifically glucose and lipid metabolism only at ZT6 in the liver. Genes linked to anti-inflammatory terms, specifically cytokines and cytokine receptors are regulated at both time points in both tissues.

We next focused on liver, and compared our data set with open access ChIP-seq data sets in mouse liver for GR and core clock transcription factors. 85% of the genes identified by the RNA-seq have a GR binding site as annotated by HOMER software, indicating that the majority of the response is due to direct GR regulation of transcription, not downstream effects of other GR targets. Comparison of GR ChIP-seq with circadian transcription factor ChIP-seq reveals significant co-binding between GR and Cry1 (2385 co-binding events within 120 bp), but not BMAL1, CLOCK, Per1 or Per2. In support of this, we find that 48% of the genes regulated by Gc at ZT6, are also regulated by Cry1. Given that previous reports indicate that Cry1 mediates repression of GR activity, and Cry1 expression peaks at ZT18, we propose a potential mechanism for the differential Gc effect seen between the ZT6 and ZT18, whereby Cry1 represses Gc effects at ZT18.

In summary, we now demonstrate tissue specific remodelling of Gc responses dependent on timing of administration. This raises the possibility that time-of-day dosing may be implemented to eliminate deleterious metabolic side effects in some organs, while maintaining the beneficial anti-inflammatory actions in others. Further study, into the molecular mechanisms underlying our observations will benefit the vast number of patients currently undergoing Gc therapy.

### P71. CHRONOTYPE, SOCIAL JETLAG, AND SLEEP QUALITY: EXPLORING SYMPTOMS OF ADULT ATTENTION-DEFICIT HYPERACTIVITY DISORDER (ADHD)

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There is substantial evidence pointing to disturbed circadian clock function and late circadian typology in ADHD. Where often eveningness has been associated with

individual symptoms such as impulsivity and inattention it is seldom the relationship between chronotype and day-to-day environment is examined. We hypothesise that factors such as social jetlag and sleep quality may too have a meaningful impact on symptoms of ADHD.

A study sample of 335 participants (74.1% female) were recruited among university students from Ireland and Romania. Participants had a mean age of 24.67±7.53 (range 18-58) and were absent of any physiological/psychological illness or engaged in shiftwork. We measured chronotype parameters (MSFsc, social jetlag in hours) using the Munich Chronotype Questionnaire (MCTQ) and sleep quality using the Pittsburgh Sleep Quality Inventory (PSQI). Participants also completed the adult ADHD Self-Report Scale (ASRS-v1.1), Barratt's Impulsivity Scale (BIS-11), and Cognitive Failures Questionnaire (CFQ) to assess domains of attention and impulsivity.

Our main findings report that respondents with high-risk of ADHD accumulated greater social jetlag compared to low-risk respondents, F(1, 333)=7.66, P<.05,  $\eta_p^2$ =.02, and also had greater sleep disturbances, F(1, 333)=13.82, P<.001,  $\eta_p^2$ =.04. Differences in chronotype between groups were not found.

We suggest that social jetlag and sleep quality may be important predictors of symptom severity in ADHD and might mediate the relationship between circadian typology and the disorder which other studies have found. Our findings highlight the deleterious health consequences of living against the clock and underscore the importance of the circadian involvement in disorders such as ADHD.

#### P72. INVESTIGATING NEURAL CORRELATES OF RHYTHM DETERIORATION IN SEASONAL ADAPTIVE BEHAVIOR IN AGING MICE

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Aging is often accompanied by fragmentation or loss of circadian rhythmicity of behavior in mice, which is suggested to be a consequence of reduced synchronization of rhythms in electrical activity in neurons of the suprachiasmatic nucleus (SCN). The SCN is essential for coordinating circadian rhythms in physiology and behavior, but can also respond to day length. Exposure to long-day photoperiods result in a wider phase distribution of single-cell oscillations in electrical activity and clock-gene expression when compared to short-day photoperiods. It is thought that this contributes to the adaptation to seasonal changes in the environment. In this study we investigated to what extent the behavioral response to different photoperiods is affected by aging and if this is reflected in gene expression rhythms in the SCN.

Two-year old mice, with a genetically modified clock gene (PERIOD 2; PER2::LUC) were assessed in behavioral and gene expression rhythms. Behavior was recorded with passive infrared sensors and gene expression with bioluminescence imaging. Prior to starting *in vitro* recordings of gene expression in the SCN, mice were exposed to either a long (Light:Dark; LD 16h:8h), or short (LD 8h:16h) photoperiod.

We show that old mice are also less capable of adjusting their circadian behavior to photoperiodic changes compared to young mice. Similar to findings in young mice, PER2::LUC gene expression in the SCN responds to longer photoperiods with an increased peak time distribution. However, the degree of phase distribution seems to reflect the extent of behavioral deficits, suggesting a correlation between SCN response and behavior.

### P73. EXAMINING THE EFFECTS OF DIM LIGHT-AT-NIGHT ON AFFECTIVE BEHAVIOURS IN C57BL/6 MICE

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Rates of major depression have increased substantially in recent years, although it is not currently clear what the factors behind such increases are. Environmental factors may be important, and it has recently been postulated that dim nocturnal light may contribute to depression symptoms in humans and in rodents. Sex is also a very important factor in affective disorders, with prevalence rates of major depression twice as high in females than in males. We set out to test the hypothesis that dim-light would interfere with the circadian rhythm of C57BI/6 mice and induce depressive-like behaviours and that there would be sex-specific differences. Animals were either singly or group housed for three weeks where locomotor activity was measured and then tested on a range of tests of emotional behaviours. Animals were subsequently placed into either 12 h light: 12h dim nocturnal light (~5 lux) cycle or back in a 12:12 light dark condition and retested on the behavioural battery after three weeks. Brains of the same animals were used to measure stem cell proliferation in the dentate gyrus using the biomarker Ki-67. Exposure to dim light-at-night did not lead to significant circadian disruption nor to significant changes in any of the parameters examined, and no sex-dependent effects were detected. Levels of Ki-67 were significantly decreased in the dentate gyrus of light-at-night animals. These results indicate that species and strain differences may be important in assessing potential impact of dim nocturnal light on circadian and affective systems in rodents.

# P74. PHASE CONTROL OF CELLULAR CIRCADIAN RHYTHMS BY PROJECTOR ILLUMINATIONS IN *LACTUCA SATIVA* L. LEAVES

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The fully-controlled plant factory is a facility which produces plants in enclosed environment and can be controlled light source, temperature, moisture, CO<sub>2</sub>, and some environmental conditions. There is a problem in the lighting cost. The improvement of lighting efficiency is an important subject of technological research and development in the fully-controlled plant factory.

The circadian rhythms in the plant and optimal irradiation of laser to the plant have been studied to reduce the cultivation cost. In this study, we experiment on local control of circadian rhythms in the leaf of genetically modified lettuces *AtCCA1::LUC* using an LCD projector or a laser projector. To apply imitation initial conditions to the lettuce leaves, illumination with a set of star-shaped patterns, a bright star within a dark rectangle and its inverted image, was applied using a projector connected to a computer in order to control illumination spatiotemporally. Bioluminescence of detached lettuce leaves was measured with a highly sensitive cooled CCD camera. These leaves showed circadian oscillations of bioluminescence under continuous dark conditions. The star-shaped pattern region remained for at least two days, though the intensity of bioluminescence rapidly decreased in time.

Our results indicated that the circadian rhythm can be controlled with star-shaped initial conditions by spatiotemporal illumination using the LCD projector and the low-power laser projector.

#### P75. NEAT1 A LONG NON-CODING RNA INVOLVED IN PITUITARY CIRCADIAN RHYTHMS: CHARACTERIZATION OF ITS INTERACTOME

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Post-transcriptional regulation appears increasingly essential to the circadian system functioning. Paraspeckles are nuclear bodies that control gene expression through a post-transcriptional mechanism based on nuclear retention of RNA containing in their 3'UTR region inverted repeats of Alu sequences (IRAlu).

Paraspeckles are form around the long non-coding RNA, NEAT1, together with numerous RNA-binding proteins including NONO, SFPQ, PSPC1 and RBM14. We found that all these key components of paraspeckles including Neat1 displayed a circadian expression pattern in pituitary cells.

In this study, we asked whether IRAlu elements inserted in 3'-UTR of a reporter mRNA may allow for its circadian retention within the nucleus by paraspeckles. The single antisense AluSp, or the IRAluSp element cloned from the 3'-UTR of Nicolin1 gene were inserted each between the 3'-end of EGFP cDNA and a polyadenylation signal in the expression vector pEGFP-C1. These constructs were stably transfected into pituitary GH4C1 cells. Then we monitored by real-time video-microscopy EGFP expression and determined by RTqPCR the nuclear and cytoplasmic distribution of EGFP mRNA over a circadian period.

We also aimed to identify the endogenous RNA targets of paraspeckles in GH4C1 cells. We developed an approach used to analyze RNA targets of the main component of paraspeckles, Neat1. With this approach which employed capture antisens oligonucleotides designed to specifically hybridize to Neat1, Neat1 is enriched together with its targets that are identified by RNA sequencing. Analysis of the 3'-UTR sequences of these RNAs shows that others structural elements than IRAlus can mediate the binding of RNA to the paraspeckles.

# P76. THE ROLE OF THE INTERGENICULATE LEAFLET IN SCN LIGHT RESPONSES

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Information about both the spectral composition ('colour') and amount ('brightness') of external illumination form important temporal cues used by the circadian system to synchronise to the environment. Although the suprachiasmatic nucleus (SCN) master pacemaker is known to receive direct retinal input, intergeniculate leaflet (IGL) cells forming the geniculohypothalamic tract (GHT) are also believed to contribute to photic entrainment. However, the sensory influences of these IGL inputs to the SCN are poorly understood. To investigate this issue, we performed In vivo electrophysiological recordings from the SCN of anaesthetised mice in conjunction with electrical stimulation or pharmacological inhibition (via local muscimol injection) of GHT activity.

Electrical stimulation of the thalamus evoked inhibitory responses in a subset of visually sensitive SCN neurons, including both colour and brightness sensitive units, suggesting that GHT inputs directly contact both classes of SCN cells. Moreover, we found that, at the population level, inhibition of the ipsilateral thalamus enhanced spontaneous firing and SCN responses to stimulation of the contralateral retina. We also found that inhibiting GHT inputs preferentially enhanced UV-cone mediated responses within the SCN,

altering chromatic sensitivity across the population. By contrast, SCN population responses evoked by stimulation of the ipsilateral retina were unaffected by GHT inhibition. Together, these data indicate that GHT inputs to visually sensitive SCN neurons convey specific features about the visual environment – on average these appear to be tonically active in darkness and are preferentially excited by contralateral retinal pathways originating with UV-biased cones.

#### P77. MODEL-BASED EVALUATION OF MULTIDRUG CANCER CHRONOTHERAPY

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The circadian time-dependency of drug treatment has been considered to come from interactions between the cell cycle and the circadian rhythm (Fu and Lee, 2003). In this study, we aimed to evaluate the chronotherapeutic effect by means of simulating proliferation dynamics of cell cycle mutually interacting with the circadian rhythm under influences of drug treatments. Here we constructed a regulatory model of cell cycle using a framework of stochastic automaton. During the cell cycle process, a cell is subject to DNA damage with a certain probability. Then the damaged cell dies or returns to the cell cycle, causing a phase response in the cellular circadian clock system. In G1/S and G2/M checkpoints, the cell cycle progression is restrained by the influences of the circadian rhythm. In the simulations, a cancer cell is characterized by the prolonged or shortened duration of G1 phase of the cell cycle (Rew et al., 1991). We simulated proliferation dynamics of 10,000 cells population treated by two types of drugs: one increases death rate of a cell in S phase and the other in G1 phase. As a result, we found that mutually antiphase administrations, morning and evening, of the S and G1 phase-specific drugs maximizes the damage to the cancer cells with a long G1 phase and minimizes the death of the normal cells, which maximizes the therapeutic value of treatment. The circadian time-dependent therapeutic value is due to difference in the circadian progressions of population cell cycles between the normal and cancer cells.

# P78. THE DBHS PROTEINS NONO AND PSPC1 REGULATE SLEEP-CHARACTERISTIC NEURONAL FIRING AND MODULATE CIRCADIAN GENE EXPRESSION

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The DBHS family of RNA-binding proteins, including NONO and PSPC1, are multifunctional predominantly nuclear factors that we have previously identified as auxiliary factors of the circadian clock, where they interact with PERIOD proteins as transcriptional cofactors. In neurons, they have been found to modify synaptic morphology, and are reorganized upon neuronal activity (See poster of Koen Seignette). Therefore they might link circadian gating to physiology and behavior. The goal of this project is to evaluate the importance of the DBHS proteins for EEG structure during sleep and wake.

Disruption of either factor causes significant differences in EEG spectral power density in all three vigilance states, mainly in the delta and alpha frequencies. Spectral differences might stem from alterations in synaptic structure, as DBHS proteins are involved in activity-dependent aggregation of the neuronal scaffolding protein gephyrin and regulate the abundance of synaptic RNA and inhibitory GABA receptors. In addition, homeostatic

rebound after sleep deprivation is lacking in PSPC1-/- but not NONO-/- pointing to specific roles of the individual family members.

Overall, we propose that regulation of inhibitory and excitatory synaptic structure and function via the DBHS family of proteins may play an important role in the diurnal regulation of sleep.

# P79. PHASE RESPONSE OF NASAL CLOCK BY DEXAMETHASONE : MONITORING PER2 RHYTHM USING A LUCIFERASE REPORTER

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The symptoms of allergic rhinitis (AR) show marked circadian changes and they are under the control of the circadian clock. However, little information is so far available as to the clock mechanisms in the nasal mucosa. We investigated the expression rhythm of clock gene Per2 in the mouse nasal mucosa using a luciferase reporter. In addition, we demonstrate the effects of dexamethasone (DEX) to the nasal clock for the first time, since glucocorticoids are widely used as the topical treatment for AR, therefore.

We cultured nasal mucosa of male PER2::LUC knock-in mice and measured bioluminescence levels. DEX was applied in cultured media at 4 different circadian phases, or injected intraperitoneally at one of 4 phases then effects on bioluminescence rhythms were analysed.

Mouse nasal mucosa exhibited a robust circadian rhythm in PER2 levels with the peak in the early subjective night. DEX phase-dependently shifted the circadian phase of nasal mucosa, with the maximal phase delay at subjective dusk both in ex vivo and in vivo experiments.

The phase-shifts of PER2 rhythm due to DEX were observed around the peak phase of serum glucocorticoids, suggesting that endogenous glucocorticoids regulate the peripheral oscillator of the mouse nasal mucosa. Our results imply that the best time of topical steroid treatment would be when no phase-shift occurs by DEX, from the viewpoint of circadian physiology. In the case of diurnal humans, it would be the early evening.

## P80. CIRCADIAN PHASE RESPONSE TO DIFFERENT LIGHT STIMULUS DURATIONS

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The circadian system is capable of entraining to new environmental conditions. Pittendrigh and Takamura (1989) hypothesized that light sensitivity of the circadian system would decrease with latitude, to compensate for longer light exposures during summer at high latitudes. To test this hypothesis, we investigated the circadian response of single light pulses with different durations applied at different time points. Here, we measured the phase response of two *Nasonia vitripennis* populations, one from northern (Oulu, Finland 65° N) and one from southern Europe (Corsica, France 42° N) to light pulses of three different durations (1 hr, 8 hr and 16 hr) applied at 2 hr time intervals.

Plotting the results in a phase response curve with the phase shift ( $\Delta \phi$ ) against the mid time of light pulse, we see for the long light pulses (8 hr and 16 hr) a strong response with phase shifts of several hours that decrease under shorter light pulses (1 hr). Long light pulses cause phase advances during the first half of the active phase and phase delays in both the second half of the active phase as during the rest phase; overall there is an increase in phase delays with longer light pulses.

A short light pulse (1 hr) causes different responses in the two populations. The southern population shows strongly reduced response whereas the northern population shows large advances in the first half of the active phase and second half of the rest phase and delays in the second half of the active phase and first half of the rest phase. Our results thus disprove the hypothesis that light sensitivity decreases with latitude.

#### P81. MOUSE MEDIAL HABENULA NEURONS SHOW DAILY SENSITIVITY TO NICOTINE

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In mammals, the master circadian clock is located in the suprachiasmatic nuclei (SCN). Here daily rhythms are generated through the activity of core-clock genes, such as cryptochrome (Cry1-2) driving 24h changes in SCN neuronal output. The discovery that clock genes also oscillate in many other brain areas has led to interest in the functional role of these extra-SCN oscillators. One such oscillator is localised in the epithalamic habenula (Hb). Both major divisions of the Hb, the medial (MHb) and lateral Hb, appear to contain intrinsic semi-autonomous clocks and exhibit daily changes in electrical activity. The MHb is implicated in addiction to substances of abuse, such as nicotine, but it is not known whether MHb neurons show daily changes in sensitivity to nicotine. Here, using in vitro extracellular multi-electrode arrays (MEAs) we simultaneously measure extracellular electrical signals at multiples sites across Hb slices. We show that MHb neurons significantly increase the discharge of action potentials from early to late day. Importantly, we demonstrate that when challenged with nicotine, MHb neurons show greater responsiveness to this drug during the latter part of the day. Preliminary MEA recordings from mice lacking the Cry genes indicate that this daily change in nicotine sensitivity appears to require a functional local molecular clock. This places the MHb as a potential key interface between circadian processes and addiction. We are now performing calcium imaging and voltage-clamp recordings to understand the basis of this daily alteration in nicotine sensitivity. These results could reveal strategies for nicotine addiction interventions/treatments.

#### P82. A MUTATION IN THE SNARE PROTEIN VAMP2 UNDERLIES DEFICITS IN SLEEP AND BEHAVIOUR

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Timing, depth, and duration of sleep are controlled by interactions between the circadian system and homeostatic mechanisms (referred to as the durations of prior wakefulness). Sleep is essential and sleep deprivation affects attention, learning and memory, executive functions, and emotions. The regulation of the sleep/wake cycle is complex and a variety

of factors have already been shown to be associated with this regulation, including neurotransmitter systems, transcription factors, and ion channels. Currently most studies in mice require the use of electroencephalogram (EEG) recordings which are invasive, difficult to perform and analyse, and cost and time intensive.

Here, we use a novel, non-invasive video-recording approach to monitor and score sleep behaviour in mice as a simple hierarchical-based approach to identify new genes involved in sleep regulation. One of these ENU mouse lines identified in the video-tracking screen was confirmed to have a missense mutation in the core SNARE protein VAMP2 (vesicle-associated membrane protein 2).

The original phenotype of reduced amounts of sleep was confirmed by EEG recordings and gave further information about changes in the different sleep stages. Also, homozygous animals appear to have disturbed innate behaviour and a potential attention deficit whereas overall memory recollection is functional.

## P83. THE TRANSCRIPTIONAL LANDSCAPE ASSOCIATED WITH PHOTOPERIODISM

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Nasonia vitripennis is an emerging insect model, with great potential in the field of seasonal biological rhythms. Nasonia possess a robust and easy to assess response to photoperiod: by exposing adult females to a critical number of short winter-like days, the resulting progeny will remain in a cold-tolerant reversible larval state termed 'diapause'. As we expect this response to be underpinned by changes in gene expression between long (LD) and short (SD) day exposed wasps, we employed RNA sequencing (RNAseq) to profile the wasp transcriptome. We extracted total RNA from the heads of LD and SDentrained wasps (3 technical replicates, containing ~170 pooled heads each) to obtain a photoperiod-specific transcriptome. This sequence data was analysed using the Tuxedo suite of bioinformatics tools. This highlighted 61 transcripts as being significantly differentially expressed, including interesting candidates such as a vasopressin orthologue, known to be photoperiodically regulated in mammals. Another gene, showing a substantial fold change, was a member of the dehydrogenase/reductase SDR family member 11, which have been implicated in hormone and steroid metabolism, including in the regulation of juvenile hormone in mosquitos. Utilising RNAi technique, we knocked down SDR-11 in the wasp by injecting gene-specific dsRNA into wasp pupae. Their ability to respond to photoperiod (compared to a control group injected with dsRNA against GFP) was assessed. We found that the target-injected wasps showed significantly higher levels of diapause, suggesting a causal role for this gene in the photoperiodic response.

#### P84. DEXAMETHASONE ALTERS CLOCK GENES EXPRESSION IN A TISSUE-DEPENDENT MANNER IN GOLDFISH (CARASSIUS AURATUS)

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It has been reported that glucocorticoids induce *per1* expression in mammals, but it is unknown if that also occurs in other vertebrates. Recently, we have demonstrated that glucocorticoids induce *per1a* and *per1b* expression while repress *bmal1* and *clock1a* in

the liver of a teleost (goldfish, Carassius auratus) in vitro. The aim of the present study was to investigate if such alterations by glucocorticoids in clock genes expression in vitro also occurred in vivo, and if other organs different from the liver could be targeted. To this purpose, goldfish were maintained under 12L:12D photoperiod and daily fed at ZT 2 (1% bw). The day of the experiment fasted animals were injected intraperitoneally with saline (n=14) or dexamethasone (1 μg/g; n= 14) at ZT 2, and were sacrificed at 3 and 8 h postinjection (n= 7 per experimental group). Hypothalamus, pituitary, retina and liver were sampled to analyze clock genes expression by real-time quantitative PCR. In all the tissues studied per1 expression was induced by one dexamethasone injection. This induction was earlier in the retina and pituitary, and remained at 8 h post-injection, excepting in the retina. Moreover, in the liver, but not in the other studied-tissues, dexamethasone repressed bmal1a and clock1a at 8 h post injection. In overall, present results show the role of dexamethasone in vivo as a regulator of some central and peripheral oscillators in goldfish in a tissue dependent manner, and support a relevance of glucocorticoids to maintain an internal synchrony of the circadian system in vertebrates.

[Supported by the Spanish MINECO, AGL2013-46448-C3-2-R]

### P85. ARCUATE NEURONES THAT RESPOND TO ENERGY STATUS EXHIBIT ROBUST RHYTHMS IN EXCITABILITY

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The arcuate nuclei of the hypothalamus (ARC) integrate metabolic cues of a central and peripheral origin. Core circadian clock genes/proteins such as Per1/2/PER2 are expressed in the ARC and show circadian variation in their expression, however it is unclear whether such molecular clock oscillations in this brain region drive alterations in membrane excitability. Here, performed in vitro targeted we whole-cell electrophysiological recordings in acute adult brain slices harvested from a reporter mouse, in which the yellow fluorescent protein derivative, Venus, reports the activity of the Per1 promoter (mPer1::Venus). Putative Per1 expression (Per1::Venus positive), distinguished by Venus epifluorescence, was observed in a population of ARC neurones that also show daily variations in several measures of excitability, such as resting membrane potential, input resistance and spontaneous firing rate. These measures were significantly lower during the day compared to the night, with a larger proportion of Per1::Venus positive ARC neurones firing action potentials at elevated frequencies during the night period. Further, challenging these neurones with leptin and orexin evoked decreases and increases in excitability respectively, demonstrating that these cells were able to sense energetic signals. Collectively, this study suggests that neurones in the ARC provide a key interface between circadian timing and energy homeostasis.

# P86. **NEURONAL ACTIVITY-DEPENDENT REORGANIZATION OF NUCLEAR PARASPECKLES AND DBHS PROTEINS**

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Paraspeckles are a relatively new class of nuclear bodies found in many types of mammalian cells. They are typically observed as multiple distinct foci within the cell nucleus located adjacent to another type of nuclear body called splicing speckles.

Paraspeckles are recruited and assembled by the long non-coding RNA (IncRNA) NEAT1 (MENε/β) via interactions with the DBHS family of RNA-binding proteins (PSPC1, NONO and SFPQ), which have been previously shown to interact with the circadian clock protein PERIOD and to modulate clock-dependent transcriptional repression. In possibly separate functions, they have also been implicated in the regulation of gene expression through protein sequestration and nuclear retention of hyper A-to-I edited RNAs, as well as being members of RNA transport granules in neurons. Using rat primary hippocampal cultures and immunofluorescence for DBHS proteins we show that neuronal paraspeckles are dynamic in nature and reorganize upon neuronal firing induced by KCI depolarization or mGluR5 activation. Furthermore, neuronal activity induction in vivo using mouse models for epilepsy and sleep deprivation revealed changes in PSPC1 protein level and paraspeckle organization, suggesting a possible link between paraspeckles and sleep regulation. Consistent with this hypothesis, DBHS mutant mice show reduced baseline cortical slow-wave activity and impaired rebound after sleep deprivation (See poster of Andrea Spinnler). Together, these results implicate nuclear paraspeckles and DBHS proteins in dynamic adaptations of neuronal function possibly important in both sleep- and circadian-dependent contexts.

## P87. INVESTIGATING EXPRESSION PATTERNS AND CLOCK INTERACTIONS OF ZFHX3, A NOVEL CIRCADIAN TRANSCRIPTION FACTOR

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Recently, we showed that mutations in ZFHX3 affect circadian outputs including wheel running activity in constant conditions and molecular oscillations in SCN slices. Although the protein is highly expressed in adult SCN, little else is known about its expression pattern in adults. Here, we used a custom generated antibody to investigate expression in adult brain in addition to SCN, in peripheral tissues and in cell lines. Moreover, we looked at its subcellular localisation and at whether protein levels oscillate as a function of circadian time. Finally, as ZFHX3 is a large transcription factor believed to be involved in transcriptional complexes, we were interested in investigating whether this transcription factor could interact with known clock factors. In tissues, ZFHX3 is mainly expressed in the embryonic brain, but we found that it maintains discrete patterns of expression in adult brain nuclei in addition to SCN and in adult lung. We also found that ZFHX3 is expressed in all cell lines analysed, with a higher level of expression in NIH3T3 and SHY5Y cells (in vitro neuronal model). Using immunofluorescence, ZFHX3 is localised in the nucleus, which is in agreement with its role as a transcription factor. However, a small amount of ZFHX3 is also present in the cytoplasm. We are currently investigating whether ZFHX3 levels oscillate in adult SCN and lung. Given its role in setting clock speed, we performed immunoprecipitation studies to determine whether ZFHX3 interacts with other clock components. Initial studies indicate that ZFHX3 can interact with PER2, CRY1 and CRY2.

# P88. EYES LIKE SAUCERS – A NOVEL ENU MOUSE LINE WITH SUBSTANTIALLY REDUCED LIGHT RESPONSIVENESS

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We are conducting forward genetic screens in ENU mutagenized mice to identify novel circadian and light responsive phenotypes and the genes underlying them. One of the phenodeviants identified in these screens shows a variety of attenuated responses to light, including poor entrainment or free running in light-dark conditions, and a short free running period in constant light conditions. This mouse line was designated Eyes Like Saucers (ELS). Further phenotyping of the ELS line revealed that these animals do not respond to phase shifting light pulses and have a substantially reduced pupillary light response. Histological examination revealed no retinal defects in these animals suggesting that these phenotypes are not due to retinal degeneration. Additionally, these phenotypes are supressed when the ELS line is crossed onto the sighted-C3H inbred mouse strain, demonstrating that these phenotypes are modified by genetic background. Genetic mapping identified the critical region for these phenotypes upon chromosome 6 (98 to 148Mb). Next generation sequencing of this region identified two high confidence coding sequence mutations in phenodeviant animals: a T to C missense mutation in exon 12 of the gene encoding the synaptic protein ERC1 and an A to C missense mutation in exon 8 of the gene encoding Ubquitin Specific Proteinase 18. Through continued breeding we intend to segregate these two mutations, and thus identify which of these two genes are causative for these phenotypes. Following this, molecular characterisation of the causative mutation will illuminate the pathways by which light signals are processed by the brain.

### P89. ANALYSIS OF ECLOSION PATTERNS UNDER COMBINED LIGHT AND TEMPERATURE CYCLES IN DROSOPHILA MELANOGASTER

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The timing of eclosion (emergence of an adult from the pupal case) is controlled by circadian clocks in many insect species. In 1957, Pittendrigh and Bruce proposed the two-oscillator model of the circadian system including a light sensitive master A-oscillator and a temperature sensitive slave B-oscillator that regulates the timing of eclosion in Drosophila pseudoobscura. In the present study, we have experimentally re-examined the hypothesis using combinations of light-dark (12h light, 12h dark; LD) cycles and temperature (12h thermos-phase of 28°C, 12h cryo-phase of 20°C; TC) cycles with various phase relationships in *Drosophila melanogaster*. Interestingly, phases of eclosion changed depending on the phase relationships between LD and TC cycles. The peak time of eclosion occurred around light on when the light phase and thermo-phase corresponded. The eclosion peak became slightly broader when the phase angle of TC cycles was changed by 90°C. The eclosion phase, however, was expanded throughout light phase when the cryo-phase corresponds to light phase. Further shifts of the thermophase by 6h caused an abrupt change of the eclosion phase from light on to temperature rise. Our results confirmed that a light sensitive oscillator and a temperature sensitive oscillator are involved also in the eclosion timing of *D. melanogaster*. Additionally, the results suggest that relative strengths of LD cycles and TC cycles as Zeitgebers in this study were almost equivalent. Based on these results, we will discuss features of the Aoscillator and the B-oscillator.

# P90. EFFECT OF SUBLETHAL DOSE OF INHIBITORS OF SOME MOLECULAR PROCESSES IN THE CIRCADIAN NEGATIVE FEEDBACK LOOP ON STABILITY OF CIRCADIAN RHYTHM

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Robustness or stability is an important feature of circadian rhythms. Weak rhythms are commonly observed in old people and patients with some health problem (Nakamura et al., J Neurosci, 2011; Jagannath et al., Curr Opin Neurobiol., 2013). Thus, it is expected that regeneration of robust rhythm improve therapeutic efficiency and health conditions. Moreover, reduced amplitude due to mutation in a clock gene or desynchrony of cellular clocks affected the speed of entrainment (An et al., PNAS, 2013; Vitaterna et al., PNAS, 2006). These results also suggest that understanding of mechanism of stability is useful for regulating circadian rhythms.

To find which molecular processes modulate the stability of the circadian rhythm, we examined the effect of sublethal dose of inhibitors for molecular processes in the circadian negative feedback loop in Rat-1 fibroblast. The proteasome inhibitor MG132 had little or no effect on the cellular rhythm. SP600125, an inhibitor of the phosphorylation of PERIOD proteins destabilized the cellular rhythm. In contrast, the translation inhibitor cycloheximide appeared to stabilize the cellular rhythm. Moreover, inhibition of translation also reduced magnitude of phase shift. These findings suggest that circadian oscillations could be more robust by optimizing parameters of molecular processes in the primary negative feedback loop, and the rhythm stability was closely related with phase stability against stimuli.

#### P91. CONSTANT LIGHT UNCOVERS BEHAVIOURAL EFFECTS OF A MUTATION IN THE SCHIZOPHRENIA RISK GENE *DTNBP1* IN MICE

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Various psychiatric disorders, including schizophrenia, are comorbid with sleep and circadian rhythm disruptions. To understand the links between circadian rhythms and schizophrenia, we analysed wheel-running behaviour of Sandy mice, which have a lossof-function mutation in the schizophrenia risk gene Dtnbp1, and exhibit several behavioural features of schizophrenia. While rhythms of Sandy mice were mainly normal under light-dark conditions (LD) or in constant darkness (DD), they had a significantly longer free-running period under constant light (LL) compared to wild-type (WT) littermates. The mutant mice also had a higher subjective day/subjective night ratio of activity under LL, indicating a lower amplitude, and a lower precision of their onsets of activity under all three lighting conditions. These observations are reminiscent of the circadian disruptions observed in schizophrenia patients. This prompted us to assess schizophrenia-relevant behavioural abnormalities in Sandy mice upon circadian rhythm alteration by LL treatment. Spontaneous locomotor activity, prepulse inhibition (PPI) of acoustic startle and anxiety-like behaviour were assessed under baseline LD conditions, then in LL, and then again in LD. Under LL, the Sandy mice showed significantly increased spontaneous locomotion and PPI deficits compared to WT mice. Strikingly, these behavioural deficits persisted even after mice were returned in LD. While LL led to increased anxiety-like behaviour in WT animals that was fully reversed after 3 weeks in LD, this effect was not observed in Sandy mutants. Overall, these results suggest that Dtnbp1 deficiency may lead to increased vulnerability to schizophrenia under environmental conditions where circadian rhythms are altered.

### P92. CRYPTOCHROMES AND THEIR POSSIBLE ROLE IN CIRCADIAN RHYTHMS OF ANCIENT INSECT ORDER

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Circadian genes are remarkably conserved between insects and mammals with only a few but important differences. While mammals have only CRYPTOCHROME (CRY) types which function as a transcriptional repressors (mammalian-CRY-type), fruit fly contains only one CRY(drosophila-CRY-type) functioning as a light receptor. Non-drosophila insects then contain all possible combinations of CRYs, either only mammalian-type, only drosophila-type or both. Our analysis of ancient insect order, cockroaches, revealed possible different clock architecture between the German cockroach (*Blatella germanica*) which contains both mammalian and drosophila-type CRYs and the American cockroach (*Periplaneta americana*) where only mammalian CRY was identified. Both CRY putative interacting partners, TIMELESS and PERIOD were identified in *Blatella* and *Periplaneta* transcriptomes. Role of these genes in regulating circadian activity was explored functionally by RNA-interference in both species. The results of our findings are summarized in this poster.

# P93. SCHEDULED FEEDING COUNTERACTS SOME METABOLIC AND CIRCADIAN CONSEQUENCES OF PRENATAL LPS ADMINISTRATION IN FEMALE RATS

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Prenatal inflammation is commonly used for the investigation of developmental psychiatric disorders such as schizophrenia or autism. One of the most common approaches, LPS injection to the dams, might induce disruptions on the offspring circadian system. Therefore, feeding time arises as a potential strategy to counteract some of the long-term effects of prenatal LPS administration. We assessed metabolic and circadian parameters in the female offspring of Sprague Dawley rats administered with LPS (1mg/kg, sc) or saline (Sal) during the second half of pregnancy (gd 11 to 21). From weaning to sacrifice (adulthood), females were fed ad libitum (AL) or a scheduled feeding (SF) regime (restricted temporal access for 5 hours after lights off). AL animals consumed more food than SF during the first 3 weeks; thereafter no differences were found between AL and SF animals. Only on the last weeks (8 and 9) did LPS-AL animals decrease their food intake. Additionally, LPS-AL weighted less than Sal-AL but LPS-AL exhibited the higher percentage of subcutaneous adipose tissue. Notably, SF decreased body weight, although among SF animals no differences were found between treatments (Sal vs. LPS). Temperature rhythms were food entrained in both SF groups, and acrophases advanced respect AL groups (ZT17 for SF vs. ZT19 for AL), with LPS-AL animals displaying the highest amplitude. Present results suggest that SF may counteract some of the disruptions described following prenatal LPS administration, and therefore could be a potential strategy to ameliorate some symptoms related to neuropsychiatric disorder associated with maternal immune activation.

## P94. IMPACT OF CIRCADIAN NUCLEAR RECEPTOR REV-ERB ON PARKINSON'S DISEASE

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Parkinson's disease (PD) is a common neurodegenerative disorder that is characterized by the degeneration of dopaminergic neurons in the substantia nigra and dopamine depletion in the striatum. Although the motor dysfunctions are primary symptoms of PD, non-motor symptoms also markedly impaired quality of life. Among a broad spectrum of non-motor symptoms in PD, sleep disorders and depression are suggested to be related to the alteration in circadian clock function. Interestingly, we previously reported that circadian nuclear receptor REV-ERBα links the molecular circadian clock with dopamine biosynthesis of dopaminergic neurons in ventral midbrain and mood regulation. Based on this close connection between dopamine production and circadian clock, we hypothesized that progress of both motor dysfunctions and non-motor symptoms in PD are associated with disruption of circadian rhythm. We investigated motor activity and mood-related behaviors in striatal 6-hydroxydopamine (6-OHDA) injected mice which are widely used for animal model of PD. Injection of 6-OHDA induced anxiety-like and depression-like phenotypes in mice besides their motor deficits. We also examined the effect of 6-OHDA on the circadian oscillation of TH and REV-ERBα in ventral midbrain and compared with daily oscillation patterns of body temperature and voluntary activity. Furthermore, we investigated pharmacological modulation of REV-ERBα and its impacts on progress of PD-like symptoms in 6-OHDA-injected mice. In conclusion, the present study suggests the potential role of Rev-erba as a novel molecular target in PD.

#### P95. THE DOUBLE LIFE OF PER2 – INTEGRATING CIRCADIAN TIME AND SLEEP NEED

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Sleep-wake rhythms are orchestrated by the interaction of a homeostatic and a circadian process. While the SCN are required for circadian rhythms in overt behaviors, at the cellular level, clock genes, such as *Period2* (*Per2*), engage in transcriptional/translational feedback loops resulting in rhythmic gene expression with periods of ca. 24h. Accumulating evidence demonstrates that these clock genes, besides their role in circadian rhythm generation, also play a key role in sleep homeostasis; e.g., central PER2 increases during enforced wakefulness and decreases during recovery sleep (Curie *et al.*, SLEEP 2015). Whether clock genes also increase during spontaneous wakefulness and how these changes depend on circadian phase could, however, not be studied due to lack of appropriate methodology.

Saini and colleagues (Genes Dev 2013) developed a device allowing us to measure PER2 and activity at 1min resolution in a freely behaving mouse, along with EEG recordings.

As expected, PER2 oscillated in circadian fashion in brain and periphery increasing during the active phase. This waking-associated increase could also be observed at the

level of individual waking bouts. As the increase depended on circadian phase, PER2 seems to integrate circadian time as well as time-spent-awake. Moreover, the magnitude and slope of this change was tissue specific with central PER2 levels responding more quickly.

Our findings show that behavioral state feedbacks onto the molecular clock, a finding that should be kept in mind when using clock-gene rhythms as state variables of the circadian clock.

#### P96. THE EFFECT OF RFAMIDES ON FOOD INTAKE IN TWO DIFFERENT PHOTOPERIODIC CONDITIONS IN MALE AND FEMALE SIBERIAN HAMSTER

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Kisspeptin and RFRP-3 are two peptides that control the GnRH neurons and modify the reproduction activity. It has been demonstrated that photoperiod via melatonin synchronizes reproductive and metabolic activities within seasons. The photoperiodic regulation of reproduction involves an action of kisspeptin and RFRP on GnRH neurons. Interestingly acute central injections of kisspeptin or RFRP3 increase c-FOS in some areas of the mediobasal hypothalamus (PVN, DMH, and ARC) that are involved in bodyweight regulation and feeding behavior. On the other hand, other metabolic factors like leptin and glucose are also strongly involved in the regulation of reproductive activity at different steps of life history and recent data suggest that both RF-amide peptides may be involved in these processes. The aim of this project is to delineate how RFRP is involved in the metabolic seasonal programming of male and female siberian hamsters. We will analyze if RFRP-3 has a role in the food intake behavior in the two photoperiods that could reflect the changes in the body weight associated to the seasonal changes, and we will delineate the central mechanisms involved in the observed effect of RFRP. Preliminary results indicate a sex-dependant effect of central administration of RFRP on food intake.

# P97. SELF-SUSTAINED, CLOCK-GENE-CONTROLLED, COMPLEX I ACETYLATION-DEPENDENT ULTRADIAN RHYTHMIC ACTIVITY OF THE MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION SYSTEM

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Physiology and behavior of living beings show circadian rhythms entrained by a central timekeeper present in the hypothalamic suprachiasmatic nuclei. Nevertheless, virtually all peripheral tissues hold autonomous molecular oscillators constituted essentially by circuits of gene expression that are organized in negative feed-back loops. Accumulating

evidence reveals that cell metabolism is rhythmically controlled by cell-intrinsic molecular clocks and the specific pathways involved are being elucidated. Here, we show that *in vitro*-synchronized cultured cells exhibit ultradian oscillation in mitochondrial respiratory activity, which is independent on the cell type tested, the protocol of synchronization used and the carbon source in the medium. We demonstrate that the rhythmic respiratory activity is associated to oscillation in cellular NAD content and clock-genes-dependent expression of NAMPT and Sirtuins 1/3 and is traceable back to the reversible acetylation of a single subunit of the mitochondrial respiratory chain Complex I. Finally, pharmacological inhibition of the mitochondrial oxidative phosphorylation resulted in dramatic deregulation of rhythmic clock-gene expression. Our findings provide evidence for a new interlocked transcriptional-enzymatic feedback loop controlling the molecular interplay between cellular bioenergetics and the molecular clockwork.

# P98. RFRP-3 AND KISSPEPTIN AS NEW REGULATORS OF ENERGY BALANCE: LESSONS FROM THE SEASONS

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Jerboas are desert rodents displaying strong seasonal variations. In spring they are sexually active with a low food intake while in autumn when the natural conditions are unfavourable; they shut down their reproductive axis but increase their food intake. The purpose of this study was to investigate the involvement of RFRP-3 and kisspeptin, two neuropeptides known as central regulators of reproduction, in the regulation of energy balance.

We analysed the effect of acute intracerebroventricular injections of kisspeptin and RFRP-3 on the 24h food intake during spring and autumn, and on the expression of genes coding for neuropeptides regulating energy balance (NPY, POMC).

In spring, kisspeptin administration inhibited the food intake during the 1-3h period post-injection and reduced it for the next 2 hours. In contrast, kisspeptin had no effect on food intake in autumn. Kisspeptin injections increased the expression of the POMC encoding gene in the two seasons but had no effect on the NPY gene expression. In contrast, kisspeptin displayed a differential effect on the RFRP-3 gene expression, decreasing it in spring, and having no effect on its expression in autumn. Interestingly, we found that RFRP-3 injections induced a significant increase in food intake and the NPY encoding gene, while it decreased the POMC gene expression in both seasons.

These results indicate that kisspeptin and RFRP-3 regulate Jerboa's food intake in an opposite direction. This work reveals the strong involvement of these two peptides in the regulation of two crucial systems that ensure body homeostasis, reproduction and energy balance.

Key words: Kisspeptin, RFRP-3, POMC, NPY, Jerboa, reproduction, energy balance.

# P99. RETROSPECTIVE ANALYSIS OF SERUM FERRITIN, ALAT AND GAMMAGT REPORTED TO THE TYPOLOGY "MORNINGNESS / EVENINGNESS" ASSESSED USING THE MEQ QUESTIONNAIRE

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Materials and methods: We have a 6846 patient database (2854 women and 3892 men) integrating typology Morningness / eveningness evaluated using MEQ questionnaire. Inside this base 583 patients had ferritin assay, ALT 571 and 583 Gamma GT. Withdrawals for bioassays ferritin ALT and GAMMA GT were carried out in the usual way between 7am and 9am. Assays were reported by ANOVA with groups determined by the MEQ survey (DET, MET, NT, MMT, DMT).

Results: It is identified significant differences ferritin levels (p <0.05) ALAT (p < 0.05) and gammaGT (p <0.05) in relation to the type (DM, MM, M, MS, DE) and sex.

Discussion: The differences depending on the type and carried out in a fixed time slot can be directly related to the typology or to a phase effect in relation to circadian variations of the observed parameters.

In any case, it seems necessary to take into account the typology of the subject in terms of Morningness / eveningness for the interpretation of the normality of these biological parameters

#### P100. MECHANISMS OF LIGHT REGULATED GENE EXPRESSION

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One of the remarkable properties of peripheral clocks in teleosts is that they are entrained by direct exposure to light. Furthermore, in most fish cells and tissues, light induces the expression of a broad range of genes including clock genes as well as many elements of the DNA repair machinery. This photic response is mediated by widely-expressed photoreceptors as well as light regulated transcriptional control mechanisms. We have shown that the D-box serves as a primary light responsive enhancer promoter element via its binding to members of the PAR bZip transcription factor family TEF, DBP and HLF. In turn, MAPK signalling acts as a negative regulator of light-induced gene expression. This contrasts with the situation in mammals where peripheral clocks are not entrained directly by light, the D-box represents a clock output enhancer and MAPK signalling acts as a positive regulator of clock gene expression. We aim to explore in more detail D-box regulation and function in fish as well as to gain an understanding of how and why these mechanisms have been modified during vertebrate evolution.

## P101. GEOGRAPHIC VARIABILITY OF CIRCADIAN CLOCK IN THE LINDEN BUG, PYRRHOCORIS APTERUS

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Animal species which live along the wide range of latitudes have to adapt to different climates to optimize their survival and reproduction. While the particular strategy for surviving adverse conditions differs including migration, seasonal morphs or dormancy, the initial trigger in many organisms is the photoperiod. The seasonal modulation of the environment is increased at latitudes closer to poles. In some animals this might lead to latitudinal differences in their internal timing systems.

Nearly all studies concerning the latitudinal cline of circadian clock were focused on various *Drosophila* species. Although they proved latitudinal trends in amplitude, phase

and free-running period, the study of circadian systems adapted to different latitudes is still at its beginning. The linden bug *Pyrrhocoris apterus* (Heteroptera) is phylogenetically distant from the most common insect models. It exhibits a robust reproductive diapause induced by shortening photoperiod. We have established a wide collection of field-lines originating from localities of different latitudes. We focused on determination of free running periods (FRP) and analysis of circadian phenotypes of our geographic strains. Populations from collected individuals were tested under constant conditions (constant darkness – DD) and showed great variability in their circadian phenotype. Lines originating from north of Europe (where in summer days are longer) show long FRPs, while bugs from Central Europe have shorter FRPs. Surprisingly, the most southern populations (Spain, Israel) show longer FRPs. This trend of lengthening FRP in northern populations of *P. apterus* is in contrast with previous studies performed on *Drosophila littoralis*, where circadian period lengthen southwards.

#### P102. THE CIRCADIAN CLOCK IN THE OLIVE FRUIT FLY BACTROCERA OLEAE

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Bactrocera oleae, the olive fruit fly, is a member of the Tephritidae family and the most important pest of cultivated olives causing extensive agricultural and economic damage. The Sterile Insect Technique (SIT) is a biological method to suppress reproduction in a wild pest population by a large-area release of an excess number of sterile insects of the same species (Knipling, 1955). Altered diurnal mating rhythms between mass-reared strains and insects in the wild have been one of the restricting factors of successful SIT applications in the past. In *Bactrocera*, the daily rhythm of mating activity is controlled by the endogenous circadian clock (Tychsen and Fletcher, 1971) and differences in mating time were shown to have a genetic basis (Smith, 1979).

Therefore, we aim to identify and characterize the olive fly circadian oscillator at genetic, anatomical and behavioral levels. We isolated major clock gene homologs based on their known *Drosophila* and *Bactrocera* counterparts, namely, *period* (*per*), *cycle* (*cyc*), *clock* (*clk*) and the circadian photoreceptor encoding *cryptochrome* (*cry*) and investigated their temporal mRNA expression profiles using real-time quantitative PCR (qPCR). This was followed by a comparative study between different olive fly strains. Furthermore, antibodies targeting different epitopes of *B. oleae* PER and CRY were tested along with antibodies detecting *Drosophila* clock (-associated) proteins on olive fly whole mount brains using immunohistochemistry. We also started to monitor locomotor activity rhythms, a robust and reliable output of the circadian clock, using a commercially available automated system. Regarding SIT, entrainment of mass-reared flies to the local environmental conditions prior to release (e.g., photoperiod and temperature) might improve their fitness and performance in the field.

# P103. ROLE OF THE MAMMALIAN-TYPE CRYPTOCHROME IN CIRCADIAN BEHAVIOUR OF THE HYMENOPTERAN NASONIA VITRIPENNIS

Marcela Buricova, Nathaniel Davies, Giorgio Fedele and Eran Tauber Department of Genetics, University of Leicester Accumulating evidence suggest that the circadian clock of *Drosophila* is not representative of most insects. In hymenopteran insects such as the honeybee or the Jewel wasp (*Nasonia*) a mammalian type of CRYPTOCHROME (CRY2) is present. Rather than being a photoreceptor (like the Drosophila CRY1), its function is thought to be a transcriptional repressor. However as both the light sensitive TIMELESS and CRY1 are not present in *Nasonia*, it is possible the CRY2 serves as a photoreceptor in this model. Here, we used dsRNAi to knockdown CRY2, and tested the impact on circadian function.

Under DD, the free-run period in the knockdown wasps was similar to controls, in both males and females. However, under LL (unlike Drosophila, the wasp is rhythmic in LL), females (but not males) with silenced CRY2 showed a significantly longer period (24.1±0.19) compared to control (23.4±0.25). This significant extension of the free run in CRY knockdown females was also observed in experiments using dim blue light.

We have also characterised the light phase response curve (PRC) of the wasp. Both wild-type males and females show an intriguing PRC, with peak sensitivity at Zt 22-23. When we tested the light response of CRY2-RNAi males at Zt23, we found that the phase advance was significantly attenuated, suggesting that the *Nasonia* CRY2 contributes to light input of the clock.

Nasonia is an emerging model organism that rivals *Drosophila*. We are currently exploring the molecular details underlying the circadian and photoperiodic system of this fascinating insect.

### P104. THE NEUROANATOMY AND FUNCTION OF THE CIRCADIAN CLOCK IN LARVAE OF THE FRUIT FLY CHYMOMYZA COSTATA

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Larvae of the holarctic fruit fly Chymomyza costata are highly seasonal and enter diapause under short day photoperiods. Induction of diapause requires the circadian clock gene timeless, but neither involvement of further clock components nor the role of a functional circadian timer in this process is known yet. Distribution of several clock components such as Cryptochrome (CRY), Period (PER) and Pigment dispersing factor (PDF) were investigated in photoperiodically sensitive larvae under long- and short-day conditions. Intense CRY staining was detected in a bilateral cluster of 5 neurons in the ventral lateral brain region (precursors for the s-LNvs). An identical set of cells also displayed PER-immunoreactivity while only 4 of them were PDF-positive. An additional cluster of weakly stained CRY-positive neurons was found in the dorsolateral brain region (precursors for the I-LNvs and/or LNds). Around the clock analysis revealed a daily oscillation in the intensity of the CRY staining under light/dark conditions. The different pattern in the intensity of CRY staying within the particular s-LNvs observed in diapausing and non-diapausing larvae suggest a role of s-LNvs and CRY in mediating diapause induction. Clock function was also tested by measuring larval locomotor activity. The larvae show diurnal activity patterns under both long- and short-day photocycles. However, activity cycles disappear in constant darkness, indicating that these rhythms are not generated by the clock endogenously. Thus, photoperiodically sensitive larvae of C. costata possess a simply structured, but probably not fully functional clock that is capable in reading seasonal photoperiodic cues.

## P105. DIURNAL VARIATIONS ON THE SPEED AND QUALITY OF HUMAN DECISIONS

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Human behavior and physiology exhibit diurnal fluctuation. These rhythms are entrained by light and social cues, with vast individual differences in the phase of the entrainment referred as an individual's chronotype - ranging in a continuum between early larks and late owls. Whether diurnal preferences determine decision making in real-life situations has both practical and theoretical implications. However, answering this question has remained elusive due because of the difficulty of rating precisely the quality of a decision in real-life scenarios. Here we investigate diurnal variations in decision-making as a function of an individual's chronotype capitalizing on a vast repository of human decisions: web-based chess servers. In a chess game, every player has to make around 40 decisions using a finite time budget and both the time and quality of each decision can be accurately determined. We found reliable diurnal rhythms both in performance and response time, the two main properties of decisions. Performance fluctuations depended both on the interaction between daytime and diurnal preferences, with higher performance during the preferred time of each chronotype. Instead, response times show a marked diurnal variation, which is independent of chronotypes, with all individuals making faster decisions from noon to evening. Our results show diurnal rhythms in human behavior and cognitive function under real life conditions, finding changes in decision making processes relaying both in daytime and individual chronotypes.

#### P106. ALTERATION OF CLOCK GENE EXPRESSION BY MITOMYCIN C IN FIBROBLAST

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Circadian rhythm of physiological function is regulated by clock gene, which is a component of molecular clock. It is known that clock gene regulates timing of cell division through regulating the expression of cell cycle-related genes. Some antitumor drugs exert their effect by cell cycle arrest. Therefore, the alteration of clock gene could correlate with effects of antitumor drug. Mitomycin C (MMC), originally developed as the antitumor drug, has been applied in ophthalmic surgery of glaucoma or pterygium to suppress growth of fibroblasts. In this study, we investigated the influence of MMC on clock gene expression in fibroblasts to clarify molecular link between molecular clock and cell cycle arrest effect of MMC.

In NIH3T3 treated with 40  $\mu$ g/mL MMC for 24 h, the mRNA levels of Per1, Dbp, and Reverbα significantly increased. In NIH3T3 washed MMC out after 3 h incubation, the expression levels of these clock genes significantly increased at 24 h after the MMC administration in culture medium. Although the bioluminescence oscillated with an approximately 24 h period length after dexamethasone treatment in primary fibroblasts from Per2::Luc knock in mice, the oscillation of bioluminescence was markedly damped in primary fibroblasts treated with MMC for 3 h.

These results suggest that MMC could impair circadian clock system in the fibroblasts through altering clock gene expression. It is reported that increase of Per1 expression

could induce apoptosis. Taken together, MMC induced Per1 expression might contribute to cell cycle arrest in the fibroblasts.

### P107. DAYTIME RESTRICTED FEEDING PROMOTES LIPID CATABOLISM IN SERUM AND LIVER

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A daytime restricted feeding (DRF) promotes metabolic and chronobiological adaptations to optimize the biochemical handling of nutrients before to mealtime. For example, lipolytic release of free fatty acids and production of ketone bodies are largely increased. The levels of triacylglycerols (TAG) are reduced, whereas the role of lipoproteins is very important in the transport of lipids from liver to extra hepatic tissues. The energetic status active enzymes like the AMPK and Sirt1, AMPK and Sirt1 acts over the PGC-1  $\square$  and starting the transcription of CPT-1 enzyme coactivates PPAR the mitochondrial oxidation of fatty acid. The aim of this project was to determine parameters associated to oxidation of fatty acids in liver and transport of lipids in serum in rats with temporal restricted feeding. Control group: animals fed ad-libitum. Experimental group with restricted is (from 12:00 to 14:00 h). At the end of 3 weeks, the groups animals were sacrificed at 3 h intervals, starting at 08:00 h, to complete a 24 h cycle. The results showed in liver: The presence of AMPK present cons DRF group showed a peak (2:00 h). The presence of Sirt1 presents a peak before mealtime (11:00) in DRF group. The formation of [14C]-CO2 from [14C]-palmitic acid present an increase (average of ~63%) in DRF group. In serum: the ketone bodies present two peaks (11:00 and 2:00 h). The cholesterol and LDL present an increase (average of ~25 and 50% respectively). The TAG and VLDL present the same profile with a decrease of ~50% before mealtime in the DRF group. Conclusion: Using a DRF protocol, we have promoted a change in the lipid profile and his regulators (catabolism) in liver and serum, which are interpreted as an adaptation of the body's rheostat that occurs

P108. **INTERPLAY BETWEEN HEPATITIS C VIRUS AND THE CIRCADIAN CLOCK** Xiaodong Zhuang<sup>1</sup>, Ke Hu<sup>1</sup>, Ben Saer<sup>2</sup>, Peter Balfe<sup>1</sup>, Janet Lord<sup>3</sup>, Andrew Loudon<sup>2</sup> and Jane Mckeating<sup>1</sup>

during to streamline the processing of nutrients during temporal food restriction.

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The recognition that the circadian rhythm, 24 hour oscillation cycle, underlies many pathological conditions provides a temporal dimension to our appreciation of whole body and tissue homeostasis. The Liver is one of the most circadian oscillating organs in the human body. Recent evidence shows that hepatic metabolic functions are regulated by molecular clockwork ticking through translational-transcriptional feedback loops, operated by a set of clock genes. Disruption of these biologic clocks has been associated with a variety of disorders including fatty liver disease, obesity and diabetes. HCV infects approximately 3% of the worlds population and is the leading cause of liver disease, yet our understanding of the interplay between HCV and the circadian clock is unexplored.

Bioinformatic analysis of published microarray data from normal (n=24) and HCV (n=61) infected liver biopsies identified significant changes in key circadian regulators in the infected liver, suggesting that HCV perturbs the hepatocellular circadian cycle. HCV infection of synchronized hepatocytes in vitro confirms that infection disrupts rhythmic cycling of the clock genes, providing a new pathway for HCV to perturb cellular metabolism. Importantly, we observed a circadian pattern in the transcription of the four major HCV entry receptors: CD81, SR-BI, Claudin-1 and Occludin along with miR122 in synchronized hepatoma cells. Using HCVcc and lentiviral pseudotypes expressing HCV glycoproteins we demonstrate that HCV entry and genome replication is circadian regulated. These findings provide an exciting opportunity to understand how HCV interacts with circadian pathways and the impact this will have on anti-viral therapy and HCC pathogenesis.

#### P109. ABERRANT EXPRESSION OF CIRCADIAN CLOCK GENES IN DENERVATED MOUSE SKELETAL MUSCLE

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The central circadian clock in the suprachiasmatic nucleus of the hypothalamus synchronizes peripheral clocks through neural and humoral signals in most mammalian tissues. We analyzed the effects of unilateral sciatic denervation on the expression of circadian clock- and clock-controlled genes in the gastrocnemius muscles of mice on days 0, 3, 7, 9, 11, 14 and 28 after denervation to assess the regulation mechanism of the circadian clock in skeletal muscle. Sciatic denervation did not affect systemic circadian rhythms since core body temperature, corticosterone secretion, and hepatic clock gene expression remained intact. Expression levels of most circadian clock-related genes such as Arntl, Per1, Rora, Nr1d1 and Dbp were reduced in accordance with the extent of muscle atrophy, although circadian Per2 expression was significantly augmented (Day 28). The circadian expression of Arntl (Days 7 and 28) and Dbp (Day 28) was phase advanced in denervated muscle. The mRNA expression of Clock was significantly increased in denervated muscle on Day 3 when the severe atrophy was absent, and it was not affected by atrophic progression for 28 days. Sciatic denervation did not affect the expression of these genes in the contralateral muscle (Days 7 and 28). suggesting that humoral changes were not involved in denervation-induced muscle clock disruption. Our findings revealed that sciatic denervation disrupts the circadian expression of clock and clock-controlled genes either directly or indirectly via muscle atrophy in the gastrocnemius muscles of mice in a gene-specific manner.

#### P110. ROLE OF CRYPTOCHROMES IN RETINAL RESPONSES TO LIGHT

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Mammalian Cryptochromes (CRYs) are a constituent component of the core circadian clock mechanism. Recent work has suggested that CRYs may have additional physiological roles as modulators or components of the retinal clock and/or as putative light-dependent magnetoreceptors.

Reports differ in consensus on the localisation of cryptochromes in the retina. Previous work has suggested CRY2 is expressed in cones as well as the majority of cell types in the inner nuclear layer and ganglion cell layer, while convincing CRY1 immunohistochemistry has never been reported in the retina. However, many commercially available CRY antibodies do not provide specific staining, and produce a similar signal in retinae from mice lacking CRY.

Here we report a novel pattern of CRY1 and CRY2 expression in the retina using newly raised CRY antibodies that have undergone extensive validation. Furthermore, colocalization studies were performed with CRY1/2 and an extensive set of retinal cell markers to determine CRY1 expression in various retinal cell types. Colocalization data between CRY1/2 and clock proteins CLOCK and PER1, and the circadian photopigment OPN4 (melanopsin) are reported as well. Our data suggest that CRY1 is the dominant form of CRY in the mammalian retina due to its widespread expression, whereas CRY2 is undetectable.

Testing the role of CRY in the retina also requires functional assays of the retinal circadian clock. Here we demonstrate three different circadian rhythms in retinal physiology: the b-wave of photopic electroretinogram, contrast sensitivity and pupil light reflex. Whilst there are circadian rhythms of these retinal responses to light in wildtype mice, we show that these retinal circadian rhythms are abolished in Cry1-/- mice. Together, these data suggest that CRY1 is an essential component of the retinal circadian clock, whilst CRY2 appears to be non-essential. Furthermore, loss of CRY1 alone is sufficient to result in loss of retinal circadian rhythms.

# P111. ONSET OF THE LEAST ACTIVE PERIOD (SLEEP ONSET) IS DELAYED IN WINTER COMPARED TO SUMMER AMONG DAYTIME WORKERS AT THE ARTICLE CIRCLE

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Daytime workers usually show earlier sleep onset during workdays compared to days off. Our aim was to investigate if this pattern was stable across seasons at extreme natural light conditions.

20 daytime workers were analysed at an iron ore mine located in Kiruna, Sweden (67° 51' N, 20° 13' E), North of the Arctic Circle. Data were collected in December of 2013 and June 2014. Workers had a flexitime work schedule. Workers wore actigraphs and filled in sleep diaries for 7 consecutive days at both seasons.

For each 24-hour period the starting point and mean level of activity of 1-min epochs were defined for the most active 10h (M10) and the least active 5h period (L5). A repeated mixed model analysis was performed using the factors season and day.

The results for L5 demonstrated a seasonal effect (P=0.0350), day effect (P=0.0001) and interaction (P=0.0055). This was mainly due to a delay of L5 on days off in winter but not in summer. M 10 showed a similar seasonal trend (season; P=0.0588) but in winter the difference between work days and days became non significant. Mean activity within M10 and L5 did not differ between seasons.

The dependency of electric light and lack of circadian resetting daylight in winter might delay circadian phase and rest/activity cycles but do not influence activity levels. [Support: CAPES/STINT (project 004/12)]

#### P112. SOCIAL JETLAG, CHRONOTYPE, PERSONALITY AND GLYCAEMIC CONTROL IN TYPE II DIABETES

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Circadian rhythms are endogenously generated daily cycles that may be influenced by external cues such as light, and such rhythms are important in the temporal regulation of metabolism. One expression of inter-individual differences in circadian rhythms is the expression of chronotypes, in which individuals may exhibit differences in diurnal preferences (eg. morningness of eveningness) for certain activities. Further, given the societal demands of working schedules there may be a misalignment between internal circadian time and externally imposed time cues, a phenomenon which has been termed "social jetlag". The aim of this study was to investigate the impact of chronotype on glycaemic control in type II diabetes, and to investigate if any effects might be mediated through social jetlag or personality domains. The Munich Chronotype Questionnaire was administered to outpatients at the diabetes centre in Connolly Hospital, Dublin (n=120). The Big Five Inventory was also administered to assess personality type. Clinical measures were also obtained, specifically Hba1c levels as a measure of glycaemic control. There was a moderate positive correlation between the mid-sleep and Hba1c (r = 0.237, p=0.009). There was also a positive medium correlation between Hba1c levels and social jetlag was revealed (r = 0.344, p < 0.001), as was a correlation found between the neuroticism domain of the Big Five and Hba1c levels (r=0.267, p=0.007). Partial correlation reveals that controlling for neuroticism does not affect the relationship of social jetlag and Hba1c levels, suggesting that the influence of social jetlag and personality domains on glycaemic control are independent of each other.

#### P113. ENABLING ENTRAINMENT TO NON-24H LIGHT CYCLES WITH SIMPLE LIGHT MANIPULATIONS

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Under natural conditions, a stable circadian system is presumed to be advantageous, but in shift work or after time zone travel, a more flexible clock could be beneficial. In hamsters, green dim light at night (<0.1 lux) and rhythm bifurcation (i.e. a divided activity pattern in 24 h light:dark:light:dark cycles) have been demonstrated to enhance the range and speed of entrainment. Here we tested the entrainment of wheel-running and body temperature rhythms in C57Bl/6J mice exposed to dim night-time illumination and bifurcation in non-24 cycles well beyond conventional limits of entrainment (e.g.,18, 21, 30 and 36 h).

Mice with implanted telemeters were entrained to LDim19:5 or to LDimLDim7:5:7:5 to induce rhythm bifurcation. The photophases were progressively shortened and lengthened, respectively, to reach LDim13:5 over 8 weeks. The majority of mice in both groups entrained wheel-running behaviour, with almost all activity in the dim lit scotophases. Body temperature, in contrast, was not fully entrained and exhibited a free-

running component larger than 24 hours. We can conclude that bifurcation with green light and green dim alone permit running-wheel entrainment to extreme T-cycles. Follow up studies will determine their separate contributions to behavioural entrainment in LD13:5. We propose that facilitation of behavioural entrainment to extreme light cycles involves decoupling of multiple oscillator systems. These findings could lead to promising therapies that allow faster adaptation to new environments.

#### P114. AFTER-HOURS MICE SHOW SPECIFIC EPIGENETIC AND PHYSIOLOGICAL ALTERATIONS

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At present, very little is known on the role of epigenetic mechanisms in circadian clock, while transcriptional and translational positive and negative circadian loops are well characterized. This work investigated specific questions about the interaction between circadian clock alterations and DNA methylation using the After-hours (Afh) circadian mouse mutants. Recent studies demonstrated that methylation is an incredibly dynamic process in the brain, which relies on environmental variations and neuronal activity. We performed a genome-wide methylation screening for CpG Island (CGIs) in suprachiasmatic nucleus (SCN) of Afh homozygous animals and controls. Reduced representation bisulphite sequencing (RRBS) and Pyrosequencing approaches have identified a subset of 15 CGIs genetic regions presenting significant changes, greater than 10%, in DNA methylation between Afh mutants and wild-type controls. Moreover, 14 of 15 selected sequences were hypermethylated in the mutants compared to the wild type, implying an involvement of Afh mutation in hypermethylation of specific intragenic CpGs areas. Gene expression levels of RRBS targets were coherent with methylation status in SCN. Among the identified targets, we have further studied the role of Opn4 and Chat in Afh homozygous animals. Investigation of intrinsic physiological Afh neuronal network properties in vitro revealed a different responsiveness to synchronization drug treatment in homozygous mutants. Overall, this work revealed that a the Afh mutation can modify the DNA methylation profile of genes involved in cell signalling, synaptic plasticity and transcriptional regulation processes.

### P115. SEARCH FOR THE THERMOSENSORS INVOLVED IN TEMPERATURE DEPENDENT NEGATIVE MASKING BEHAVIOR IN MICE

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Adaptations to environmental changes are crucial to the survival of animals. Masking behavior is thought to be an acute adaptive response to environmental changes. However, the regulatory mechanisms of masking behavior are not well understood. Here we report that ambient temperature cycles induce negative masking behavior in mice. We were originally interested in the function of a novel UV-sensitive opsin (OPN5), which is expressed in the mouse brain. We first observed decreased locomotor activity (negative masking response) in blind mice during UV light exposure. Since the eye is believed to be the only photoreceptive organ in mammals, we examined if this response was light-dependent by injecting India ink under the scalp. The observed result suggested that the temperature changes caused by the UV light source trigger the negative masking

response. Accordingly, we examined the effect of various ambient temperature cycles (24/24°C, 24/26°C, 24/28°C, 24/30°C, 24/32°C, 24/34°C) on locomotor activities under constant darkness. As a result, the increase in the masking ratio was directly proportional to the increase in temperature difference. Thus, the negative masking responses of blind mice were corroborated as temperature-dependent behavior.

Transient receptor potential channels (TRP channels) are known as the main thermosensors in mammals. Now, we are exploring the thermosensors that mediate temperature-dependent masking behavior by using several TRP channel knockout mice.

#### P116. CIRCADIAN AND DOPAMINERGIC CONTROL OF CPT-1 EXPRESSION IN RETINA AND PHOTORECEPTORS

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The oxidative metabolism of the mammalian retina has to comply with daily changes in energy demand and its impairment contributes to diabetic retinopathy - one of the most common causes of blindness in Europe and USA. To gain a view of the regulation of the oxidative metabolism of the retina, the transcriptional control of the key regulator of mitochondrial  $\beta$ -oxidation - carnitine palmitoyltransferase- $1\alpha$  (Cpt- $1\alpha$ ) - has been investigated in the present study. Cpt- $1\alpha$  expression was seen to be most prominent in the inner segment of photoreceptors and to display a daily rhythm with elevated values during the daytime in preparations of the whole and outer retina. The cycling of Cpt- $1\alpha$  persisted in constant darkness and was decreased in mice deficient for dopamine  $D_4$  receptors. The data of the present study suggest that daily regulation of the oxidative metabolism of the retina involves a pathway in which the clock-dependent release of dopamine and its subsequent action on  $D_4$  receptors targets Cpt- $1\alpha$  transcription. Further studies are required to investigate the extent to which dysregulation of this circadian pathway contributes to the pathogenesis of diabetic retinopathy.

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