Program

TUESDAY, JUNE 5, 2012

11:00  Registration Opens

13:00-13:30  Opening Ceremony

13:30-14:15  Plenary Lecture 1
Chairperson: John Wingfield
Understanding the role of gonadotropin-inhibitory hormone (GnIH) in avian reproduction
George Bentley

14:15-15:55  Symposium 1
Receptors and cellular signal transduction
Chairpersons: George E. Bentley, Gregoy Y. Bedecarrats
14:15-14:40 [S1-1] Adiponectin signaling in chicken growth and reproduction
Ramesh Ramachandran, Gilbert L. Hendricks III, Regina Vaslats-Trounken, and Jill A. Hadley
14:40-15:05 [S1-2] Growth hormone signaling in the chick neural retina
Marie-Laure Baudet, Esmond J. Banders, and Steve Harvey
15:05-15:30 [S1-3] Multiple crosstalk signalling pathways may be utilized by chicken GnRH receptors
Nerine T. Joseph and Gregoy Y. Bedecarrats
15:30-15:55 [S1-4] Gonadotropin-inhibitory hormone (GnIH) receptor and cell signaling
Takayoshi Ubuka, You-Lee Son, George E. Bentley, Robert P. Millar, and Kazuyoshi Tsutsui

15:55-16:25  Coffee Break

16:25-18:45  Symposium 2
Neuroendocrine function and hormones
Chairpersons: Mary Ann Ottinger, Mohamed El Halawani, Kazuyoshi Tsutsui
16:25-16:50 [S2-1] Discovery of gonadotropin-inhibitory hormone (GnIH) and advancement of avian neuroendocrinology
Kazuyoshi Tsutsui, George E. Bentley, Takayoshi Ubuka, Yasuaki Tobei, You-Lee Son, Tithi Chiewchuch, Gregoy Bedecarrats, Peter J. Sharp, and John C. Wingfield
Mary Ann Ottinger, Tiffany Carro, Meredith Bohannon, Leah Carpenter, and Karen M. Dean
17:15-17:40 [S2-3] The ‘thunder chicken’ as a potential model for understanding the neuroendocrine control of non-vocal mating displays
Andrew N. Iwaniuk, Jeremy R. Corfield, and Karen M. Dean
17:40-18:05 [S2-4] Photoreceptive oscillators within neurons of the prenammillary nucleus (PMM) drive seasonal reproduction in temperate zone birds
Mohamed El Halawani, Sunantha Kosonsiriluk, Laura Mauro, and Yupaporn Chaiseha
18:05-18:30 [S2-5] Neuroendocrine regulation of stress in birds with an emphasis on vasotocin receptors
Wayne J. Kuenzel, Seong W. Kang, and Alexander Jurkevich
18:30-18:45 [O2-1] Effects of thyroid disrupting pesticides on hypothalamo-pituitary-testicular axis of a tropical bird, Amandava amandava
Banalata Mohanty, Surya Prakash Pandey, Shamael Aamir, and Kazuyoshi Tsutsui

19:15  Get Together Party

WEDNESDAY, JUNE 6, 2012

08:00-08:45  Plenary Lecture 2
Chairperson: Jacques Balthazart
Maternal effects in quail and finches: behavior and hormones
Elizabeth Adkins-Regan

08:45-09:00  Coffee Break

09:00-11:35  Symposium 3
Hormones, brain plasticity and behavior
Chairperson: Jacques Balthazart
09:00-09:25 [S3-1] Modulation of testosterone-dependent male sexual behavior and the associated neuroplasticity
Thierry D. Charlier, Aurore L. Seredynski, Neville-Andrew Niessen, and Jacques Balthazart
09:25-09:50 [S3-2] Developmental plasticity of the zebra finch (Taeniopygia guttata) song control system—the effects of steroid receptor coactivators and early developmental stress
Laura L. Carruth and Mahin Shahbazi
09:50-10:15 [S3-3] Seasonal plasticity in the neuroendocrine control of songbird reproduction
Tyler John Stevenson

19:15  Get Together Party
Program

10:15-10:40 [S3-4]  
Estradiol-regulated engagement of non-genomic response drives long-term optimization of auditory coding and discrimination ........................................ 13  
Raphael Pinaud

10:40-11:05 [S3-5]  
Song complexity and brain plasticity in Bengalese finches ..................... 14  
Kazuo Okano, Miki Takahashi, Hiroko Kagawa, and Kent Suzuki

11:05-11:20 [O3-1]  
Examining a pathway for hormone-mediated maternal effects: Yolk testosterone affects androgen receptor expression and testosterone production in the domestic chick .......................................................... 14  
Toq G. G. Groothuis, Kristina A. Pfannkuche, Ilse M. Weltes, Bernd Riedstra, C. Wolf, and Manfred Gahr

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Blocking testosterone does not decrease territorial aggression, but changes the emphasis of vocal behaviors during simulated territorial intrusions in male black redstarts (Phoenicurus ochruros) .................... 15  
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11:40-13:40 Group Photo, Lunch & Poster Presentation (Odd numbers)

13:45-15:25 Symposium 4  
Presence and action of hormones in the brain  
Chairpersons: Barney Schlinger  
Steve Harvey

13:45-14:10 [S4-1]  
Growth hormone and neurogenesis ...................................................... 16  
Carlos Arámburo and Steve Harvey

14:10-14:35 [S4-2]  
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15:25-16:00 Coffee Break

16:00-17:55 Symposium 5  
Germ cell biology and sex determination  
Chairpersons: Jae Yong Han  
Takashi Yoshimura

16:00-16:25 [S5-1]  
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18:15-19:00 Council Meeting

THURSDAY, JUNE 7, 2012

08:00-08:45 Plenary Lecture 3  
Chairperson: Peter Sharp  
Avian circadian organization: Birdsong as an input and an output of the biological clock ................................. 4  
Vincent M. Cassone

08:45-10:15 Symposium 6  
Photoperiodism and biological rhythms in birds  
Chairpersons: Chandra Mohini Chaturvedi  
Vincent M. Cassone  
Vinod Kumar

08:45-09:10 [S6-1]  
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10:00-10:15 [S6-4]  
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<td>10:30-12:00</td>
<td>Symposium 7</td>
<td>Multiple actions of thyroid hormones</td>
<td>Veerle M. Darras, Eddy Decuyperere</td>
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<td>Modulating thyroid hormones secretion to improve thermotolerance in</td>
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<td>[S7-1]</td>
<td>Transfer of methimazole into the eggs of laying hens and effects on</td>
<td>Stijn Gaysens, Edward Balt, Grażyna Chwałko, Evelyne Daule, Stijn L.J. Van Herck, and Veerle M. Darras</td>
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<td>17:30-21:00</td>
<td>Cormorant Fishing Tour</td>
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<td>Special Lecture</td>
<td>Why did the chicken “lose its KISS”?</td>
<td>Robert P Millar</td>
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<td>Symposium 8</td>
<td>Stress responses in a changing world</td>
<td>John Cockrem</td>
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<td>Allostasis, resilience and coping with a changing world</td>
<td>John C. Wingfield</td>
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<td>Functions and significance of the stress response in a changing world</td>
<td>Frédéric Angelier</td>
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<td>John Cockrem</td>
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<td>Symposium 9</td>
<td>Life on the move: Hormones and stages of migration</td>
<td>Marilyn Ramenofsky</td>
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<td>Migratory feeding and fattening: A neuroendocrine perspective</td>
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<td>Fuel metabolism and its regulation in migrating birds</td>
<td>Susanne Janni-Eiermann</td>
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<td>Endocrinology of the migratory traits of a long-distance migrant in comparison with a nonmigratory congener</td>
<td>Marilyn Ramenofsky and Zoltán Németh</td>
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<td>The migration-breeding transition: Integrating behavior, stress physiology and experience from a long-term data set</td>
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<td>Relationships between glucocorticoids levels and fitness: What are the predictions?</td>
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<td>The stress of parenthood – the role of glucocorticoids in mediating reproductive investment</td>
<td>Lukas Jenni</td>
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<td>Update on ghrelin biology in birds</td>
<td>Hiroyuki Kaiya, Kenji Kanagawa, and Mikiya Miyazato</td>
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<td>Hormonal control of growth and development</td>
<td>Tom Porter</td>
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<td>10:30-12:05</td>
<td>Symposium 11</td>
<td>Integrating immune-endocrine interactions into life history theory</td>
<td>Kirk C. Klausing, Kate Buchanan</td>
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<td>Scott A. MacDougall-Shackleton, Kim L. Schmidt, Ainsley Furlonger, and Elizabeth A. MacDougall-Shackleton</td>
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<td>Symposium 11</td>
<td>Physiological mechanisms of range expansion in the house sparrow</td>
<td>David Costantini</td>
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<td>Glucocorticoids and redox physiology in birds</td>
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<td>18:00-19:20</td>
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<td>Cross-talk between avian leukocytic cytokines and the endocrine system</td>
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<td>Bursal growth Hormone (GH) can inhibit apoptosis by the PI3K/Akt pathway</td>
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**Saturday, June 9, 2012**
Program

11:35-11:50 [S13-4]
Central regulation of food intake in avian models of hypo- and hyperphagia
Mark A. Cline, Brandon A. Newmyer, and Paul B. Siegel

11:50-12:05 [O13-1]
Hypothalamic AGRP levels are elevated during natural anorexia
Ian C Dewi, Peter W Wilson, Tom V Smulders, Rick B D’Eath, and Timothy Boswell

12:05-13:30 Lunch

13:30-15:45 Symposium 14
Endocrine adaptations to environmental constraints
Chairpersons: Peter J. Sharp
Pierre Deviche

13:30-14:00 [S14-1]
Behavioural neuroendocrinology: Insights from studies on free living birds
Simone L. Meddle

14:00-14:30 [S14-2]
The hypothalamic-pituitary-gonadal axis in a rapidly changing environment: Acute stress modulation of plasma testosterone in free-ranging male birds
Pierre Deviche, Sisi Gao, Scott Davies, Peter J. Sharp, and Alistair Dawson

14:30-15:00 [S14-3]
Gene expression underlying the "decision" to initiate egg-laying
Nicole Perfito, Kristin Hornick, Sophie Nguyen, and George Bentley

15:00-15:30 [S14-4]
The influence of temperature on endocrinology and timing of reproduction in great tits (Parus major)
Samuel P. Caro, Sonja V. Schaper, Alistair Dawson, Peter J. Sharp, Philipp Gienapp, and Marcel E. Visser

15:30-15:45 [O14-1]
Genetic selection for egg testosterone content in Japanese quail: The effect of cross-line male exchange
Monika Okuliarova, Zuzana Kankova, Peter Skrobanek, and Michal Zeman

15:45-16:10 Coffee Break

16:10-18:30 Symposium 15
Ecological and evolutionary perspectives
Chairpersons: Alistair Dawson
Ellen D. Ketterson

16:10-16:35 [S15-1]
Hormones as mediators of life history trade-offs
Jenny Q. Ouyang, Peter J. Sharp, Michael Quetting, and Michaela Hau

16:35-17:00 [S15-2]
Responses of associated hormonal, behavioral, and morphological traits following colonization of a novel environment
Jonathan W. Atwell, Gonçalo C. Cardoso, Danielle J. Whittaker, and Ellen D. Ketterson

17:00-17:25 [S15-3]
Orchestrating trade-offs between reproduction and survival in opportunists
Thomas P. Hahn, Heather E. Watts, Kathleen R. Brazeal, Elizabeth M. Schultz, Nicole Perfito, and Jamie M. Coman

17:25-17:50 [S15-4]
Stress and cognition: Does corticosterone underlie the detrimental effects of stress on vocal learning?
Katharine L. Buchanan, Stefan Leitner, Joseph L. Woodgate, and Andrew T.D. Bennett

17:50-18:15 [S15-5]
The effect of latitude on photoperiodic control of gonadal maturation and molt
Alistair Dawson

18:15-18:30 [O15-1]
Corticosterone dynamics linked to personality in artificially selected and wild great tits (Parus major)
Alejandro T. Baugh, Michaela Hau, Sonja V. Schaper, John F. Cockrem, Piet de Goede, and Kees van Oers

18:30-19:00 Closing Ceremony

19:00-21:00 Banquet Dinner
Poster Presentation

**Poster Category 1**
Receptors and cellular signal transduction

**P01** In ovo leptin administration inhibits chorioallantoic membrane angiogenesis in female chicken embryos through the STAT3-mediated VEGF pathway
Lanli Su, Kaiqing Rao, Feng Guo, Xiaoyue Li, Abdelkareem A. Ahmed, Yingdong Ni, Roland Grossmann, and Ruqian Zhao

**P02** Characterization of serotonin receptor 6 (HTR6), serotonin receptor 7 (HTR7a) and a novel serotonin receptor 7-like receptor (HTR7b) in chickens: cDNA cloning, tissue expression and functional analysis
Jianhui Yue, Fanglian Li, Wen Fan, Yajun Wang, and Juan Li

**P03** Leptin regulates Pit-1-dependent chicken GH gene expression in mammalian cells
Daisuke Murase, Susumu Atomura, and Takeshi Ohkubo

**P04** Effect of chicken STAT5b mutant on prolactin signal transduction
Susumu Atomura, Hiromi Adachi, Daisuke Murase, and Takeshi Ohkubo

**P05** Ghrelin and leptin control chicken ovarian functions via protein kinases
Alexander V. Sirotkin and Roland Grossmann

**Poster Category 2**
Neuroendocrine function and hormones

**P06** Gonadotropin-inhibitory hormone (GnIH) in the zebra finch: Peptide isolation, cDNA cloning and brain distribution
Yasuko Tobari, Norio Iijima, Kenta Tsuchekawa, Tomohiro Osugi, Hitoshi Ozawa, and Kazuyoshi Tsutsui

**P07** TonEBP mediates the effects of hyperosmolality on arginine vasotocin gene expression in the hypothalamus of chick (Gallus domesticus)
 Noboru Saito, Saya Nozawa, Mariko Fuji, and Nicoletta Aste

**P08** Identification of vasotocin 4 receptor (VT4R) antagonists by homology modeling / docking analysis and primary anterior pituitary cell culture
Seong W. Kang, Srinivas Jayanthi, T.K. Suresh Kumar, and Wayne J. Kuenzel

**P09** Effects of repeated injection of kisspeptin-10 on the initiation of egg laying in juvenile quails
Yanbing Huang, Yunqi Xiao, Jing Wu, Fuyu Qian, Yingdong Ni, Roland Grossmann, and Ruqian Zhao

**P10** Endocrine factors and AMPKa1 are involved in the spread of hatch and subsequent neonatal performance of broiler chicks
Yufeng Wang, Yue Li, Elis Willems, Hilde Willemsen, Lies Franssens, Astrid Koppelenol, Xiaoquan Guo, Eddy Decuyper, Johan Buyse, and Nadia Everaert

**P11** GnIH in the hypothalamus of the Indian weaver bird
Shahie Malik, Amrita Srivastava, Neerja Trivedi, M. Arshad, and Vinod Kumar

**P12** Expression, cellular distribution, effects and heterogeneity of growth hormone (GH) in the chicken reproductive tract
Marcia Luna, Carlos G. Martinez, Marisesa Ahumada, Martha Carranza, and Carlos Aramburo

**P13** Cloning and characterization of prolactin regulatory element binding (PREB) in chicken and turkey
Gen Hiyama, Norio Kansaku, Rosanne McQuaid, and David Zadworny

**P14** The dopaminergic-PRL system involvement in rearing behavior of gallinaceous birds
Duangsuda Chokchaloemwong, Orn-anong Chaiyachet, Nattiya Prakobsaeng, Natagarn Sartsongkorn, Sunantha Kosonsinluk, Mohamed El Halawani, and Yupaporn Chaisiha

**P15** Effect of dioxin and polychlorinated biphenyls on estradiol secretion by chicken ovarian follicles
Andrzej Sechman, Piotr Antos, Anna Hrabia, and Agnieszka Grzegorzewska

**Poster Category 3**
Hormones, brain plasticity and behavior

**P16** Arginine-vasotocin (AVT)-mRNA expression is sensitive to testosterone and estradiol in the bed nucleus of the stria terminalis of adult female Japanese quail
Nicoletta Aste, Maya Kagami, Emiko Sakamoto, and Noboru Saito

**P17** Arginine-vasotocin (AVT)-expression is female-biased and associated with aromatase in the bed nucleus of the stria terminalis of developing Japanese quail
Nicoletta Aste, Naoki Yoshioka, Emiko Sakamoto, and Noboru Saito

**P18** Selection for high egg testosterone content up-regulates expression of brain-derived neurotrophic factor (BDNF) and reelin in the pallium and cerebellum of female but not male Japanese quail
Michal Zeman, Barbora Vikova, Lubor Kostal, Peter Celen, and Monika Okuliarova

**P19** Sex differences in preferential response for a mate’s call in the budgerigar, a monogamous parrot
Hiroko Eda-Fujiwara, Yuka Hata, Madoka Inanuma, Aiko Watanabe, Ryohei Satoh, and Takenori Miyamoto

**P20** Context-dependent calling behavior in the Bengalese finch
Midori Oda, Tetsu Okumura, and Hiro-aki Takeuchi

**P21** Expression of Exon-3 and Exon-4 skip splicing isofrom of PRL mRNA in the bird anterior pituitary gland
Norio Kansaku, Gen Hiyama, and David Zadworny
**Poster Category 5**
**Germ cell biology and sex determination**

[P22] Expression of mRNA for gene related to fatty acid oxidation in germline chimeric chicken embryos
Hiroki Furuta, Yoshiyuki Ohta, and Tatsuyuki Yoshida

[P23] Analysis of cell cycle regulation during quail embryo development
Shusei Mizushima, Tomohiro Sasamani, Norio Kancaku, Tamao Ono, and Kyoshi Shimada

[P24] Novel methodology of ICSI-assisted embryo development toward ex ovo hatching in quail
Kyung Soo Kang, Shusei Mizushima, Tamao Ono, Xiumei Jin, Min Yoo, Mi Hyeon Kim, Jae Yong Han, and Kyoshi Shimada

[P25] Fertilization and developmental capability of quail oocytes after ovulation
Min Yoo, Shusei Mizushima, Tamao Ono, Kyung Soo Kang, Xiumei Jin, Mi Hyeon Kim, Jae Yong Han, and Kyoshi Shimada

**Poster Category 6**
**Photoperiodism and biological rhythms in birds**

[P26] Expression of gonadotropin-inhibitory hormone (GnIH) in the ovary and shell gland of photorefractory Japanese quail
Rashmi Srivastava and Chandra Mohini Chaturvedi

[P27] The search for the deep brain photoreceptors regulating seasonal reproduction
Yusuke Nakane, Yasuhiro Kamei, Sho-ichi Higashi, Shosei Yoshida, and Takashi Yoshimura

[P28] Involvement of the circadian clock in the crowing of roosters
Tsuyoshi Shimmura and Takashi Yoshimura

[P29] Role of light intensity in induction of circadian and seasonal responses in the Indian weaver bird (Ploceus philippinus)
Rohit Kumar Pandey and Sanjay Kumar Bhardwaj

[P30] Mechanism of seasonal change in quail testicular size
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Understanding the role of gonadotropin-inhibitory hormone (GnIH) in avian reproduction

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The mechanisms underlying avian photoperiodic responses have been studied for almost a century. Two critical aspects of the response of the reproductive system to changing day length have been the subjects of particular interest: photostimulation and photorefractoriness. Photostimulation occurs upon the transfer of birds from short, winter-like day lengths to long, spring-like day lengths and involves activation of the hypothalamo-pituitary-gonadal (HPG) axis. Photorefractoriness occurs after prolonged exposure to long days and involves a termination of reproductive processes. Both of these processes appear to be dependent on the presence of thyroid hormones to some degree, but the actual mechanisms involved in the onset of photostimulation and photorefractoriness have remained somewhat enigmatic. The discovery of gonadotropin-inhibitory hormone (GnIH) by Tsutsui and colleagues generated a great deal of excitement because of the potential for a direct role of GnIH in regulating the photoperiodic response via interaction with the hypothalamic gonadotropin-releasing hormone (GnRH) system. It now appears that GnIH regulates reproduction within the breeding season in response to a variety of cues, rather than terminating reproduction per se. Thyroid hormones, melatonin and GnIH all respond to photoperiod, but the relative impact each of these hormones on the timing of reproduction remains unclear. Further, very little attention has been paid to the female photoperiodic response, which in many avian species is qualitatively different from that of males. I will discuss the current state of knowledge of the role of GnIH in avian reproduction, and will highlight not only recent advances in our understanding of the mechanistic regulation of the HPG axis in response to changing day length in male and female birds, but also the challenges that remain.

Maternal effects in quail and zebra finches: Behavior and hormones

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Maternal effects (more broadly, parental effects) are influences of parents on offspring occurring through pathways other than inherited DNA. In birds, two of the important routes for such transmission are parental behavior and non-DNA egg constituents. Characteristics of offspring phenotypes subject to parental effects include behavior and endocrine function. Parental effects on avian behavior have long been known to be important (as in sexual imprinting, for example). Yolk hormone mediated maternal effects are a more recent idea that is now a focus of attention by behavioral ecologists as well as avian endocrinologists. Research from the Adkins-Regan lab has used Japanese quail and zebra finches to explore parental effects mediated through parental behavior and maternal yolk hormones. In Japanese quail, embryonic and exogenous sex steroids have well established and dramatic effects on sexual differentiation of behavior during a critical period in ovo. Maternal yolk hormones decline rapidly during incubation, but there remains the possibility that they could influence later sexual phenotypes in more subtle ways, a hypothesis that has recently been tested. Social interactions and learning (Pavlovian context conditioning) influence egg investment in ways that are likely to alter offspring phenotype. Zebra finches have biparental care. In this species, single-parent rearing (removal of one parent) has striking effects on later mate preferences of the offspring, with interesting asymmetries when male removal and female removal are compared. In addition, female removal alters not only later behavior, but also the response of the hypothalamic-pituitary-adrenal (HPA) axis to an environmental stressor (isolation), as indicated by plasma corticosterone. Birds raised by fathers only have lower levels of mRNA for both glucocorticoid receptors in brain tissue as adults. Thus in both species there is evidence that one generation influences the behavioral or endocrine phenotype of the next through routes other than transmission of DNA. Additional research will be required to understand the potential adaptive significance of these permanent effects on offspring mediated by yolk hormones and parental behavior.
[PL3] Avian circadian organization: Birdsong as an input and an output of the biological clock
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As both a photoreceptor and pacemaker in the avian circadian clock system, the pineal gland is crucial for maintaining and synchronizing overt circadian rhythms in processes such as locomotor activity and body temperature through its circadian secretion of the pineal hormone melatonin. In addition to receptor presence in circadian and visual system structures, high affinity melatonin binding and receptor mRNA are present in the song control system of male oscine passeriform birds. Similar to locomotor activity, both singing and calling behavior are regulated on a circadian basis by the central clock system through pineal melatonin, since these behaviors free-run with a circadian period and since pinealectomy abolishes them in constant environmental conditions. Further, rhythmic melatonin administration restores their rhythmicity. However, the rates by which these behaviors became arrhythmic and the rates of their entrainment to rhythmic melatonin administration differed among locomotor activity, singing and calling under constant dim light and constant bright light. Overall, the data suggests a role for pineal melatonin in regulating circadian oscillations of avian vocalizations in addition to locomotor activity. Conversely, locomotor rhythms in zebra finches entrain to the rhythmic presence of conspecific, context- and dose-dependent vocalization. Rhythmic presentation of male song for 1 hr every day at the same time of day has no effect on the circadian patterns of either male or female birds. However, 2 hr and 4 hr of male birdsong entrain locomotor patterns of male birds, but not female birds. The picture that emerges is that the circadian clock of birds synchronizes a rich fabric of behaviors and physiological processes, and that these are sensitive to different environmental cues.

[PL4] Update on ghrelin biology in birds
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Do you have heard the hormone called “ghrelin” so far? More than a decade has passed since ghrelin was discovered in 1999. Ghrelin is the peptide hormone isolated from stomach extracts as an endogenous ligand for the orphan G-protein coupled receptor (Ghrelin R1a). The most striking feature is that the third amino acid (serine or threonine) is modified by a medium chain fatty acid (mainly n-octanoic acid), and the modification is essential for ghrelin binding to the receptor and for eliciting its biological activities. Ghrelin was originally considered as a growth hormone (GH)-releasing peptide, but diverse physiological actions including appetite-regulating activity have been revealed by a large number of studies on basic and medical fields. Ghrelin is present not only in mammals but also in non-mammals, of course in birds, where chicken is the first species published in 2002, and exerts species-specific actions. Especially, ghrelin activities are unique in birds when compared to those in other vertebrates, e.g., anorectic in chickens vs. orexigenic in rodents. In my review paper (Gen. Comp. Endocrinol., 163: 33-38, 2009), I gave some fundamental points of ghrelin study in birds that were not disclosed: (1) Is endogenous ghrelin an anorexigenic hormone in birds? (2) What sites within the brain are specifically targeted by ghrelin in birds? (3) What are responses to exogenous ghrelin administered peripherally? (4) What enzyme are involved in the post-translational acylation? (5) What fatty acids are used to modify ghrelin, and how do they affect ghrelin’s function in birds? (6) What is the function(s) of des-acyl ghrelin? (7) Is obestatin produced from preproghrelin and what role, if any, does it have in birds? (8) What are the roles of the ghrelin receptor identified in birds? (9) How might ghrelin function in wild birds that exhibit behaviors retained in domesticated poultry. Thereafter, some answers were obtained by recent progresses of ghrelin research including birds. In this lecture, I would like to introduce the accumulated research findings of ghrelin not only in birds but also in mammals, with mixed comparative biological knowledge, and to discuss what is the physiological significance of ghrelin in birds. Lastly, since ghrelin is a hormone that is still relatively new, hoping that the researchers, who listen to this lecture, enter actively into this field to clarify the mysterious ghrelin world in birds.
[S1-1] Adiponectin signaling in chicken growth and reproduction
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Adiponectin, a 30 kilodalton adipokine hormone, improves carbohydrate and lipida metabolism in humans and rodent animal models by activating two distinct transmembrane receptors (Adipor1 and Adipor2) that are widely expressed in various tissues in the chicken. We found that chicken adiponectin is a unique heavy molecular weight isoform that is larger than 669 kDa mass. As recombinant mammalian adiponectin was inactive in chicken cells, we developed prokaryotic and eukaryotic expression systems that robustly secretes recombinant chicken adiponectin (rcADN). In vitro studies using hepatocytes and ovarian follicular cells revealed that rcADN increased the abundance of phosphorylated adenosine monophosphate-activated protein kinase and phosphorylated acetyl Coenzyme A carboxylase abundance as well as increased glucose uptake. Furthermore, rcADN treatment lead to a dose-dependent increase in phospho-Erk 1/2 abundance and decrease in the abundance of APPL1, an adaptor protein involved in adiponectin signaling, suggesting increased turnover and utilization of APPL1 due to rcADN treatment. Co-immunoprecipitation studies using antibodies against human APPL1, chicken Adipor1, or Adipor2 revealed that rcADN treatment increased association of APPL1 with AdipoR1 but not with AdipoR2. These data suggest that the ubiquitously expressed adiponectin receptors (AdipoR1 and AdipoR2) could possibly utilize different signaling mechanisms to exert pleiotropic effects on metabolism.

[S1-2] Growth hormone signaling in the chick neural retina
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Growth hormone (GH) and its receptor are expressed in the retinal ganglion cells (RGCs) of the chick neural retina in early embryogenesis, at a time when GH promotes RGC survival during developmental waves of apoptosis. This anti-apoptotic pathway involves a reduction in Akt concentrations but increased Akt-phos levels. It also involves suppression of caspase-3 and caspase-9 activity and increased PARP-1 cleavage. This pathway also involves the activation of cysitosolic tyrosine kinases (Trks) and extracellular-signal related kinases (ERKs), which activate a CAMP response element binding protein (CREB), which is able to initiate transcription of pro or antiapoptotic genes. The expression of AIF (apoptosis inducing factor) and caspase-3 are, for instance, suppressed by GH action, although other genes involved in apoptotic signaling (bcl-2, bci-x, bid and inhibitor of apoptosis protein-l) are unaffected. These antiapoptotic actions of GH may also be mediated, in part, by insulin-like-growth factor-l (IGF-l), since IGF-l is induced by GH in the neural retina and IGF-l also promotes cell survival. The antiapoptotic actions of GH are likely mediated through its receptor, but the antiapoptotic actions of a “small” GH variant that lacks motifs essential for GHR activation, suggests the pathway involved may be more complex.

Special Lecture

Why did the chicken “lose its KiSS”?
Robert P Millar
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Diverse external and internal environmental factors are integrated in the hypothalamus to regulate the reproductive system. This is mediated through the secretion of GnRH into the portal system to stimulate pituitary gonadotropin secretion which in turn regulates gonadal function. This system is highly conserved throughout vertebrates and its disruption leads to a failure of reproductive development and adulthood infertility. In most vertebrates there are three GnRHs and cognate receptors which have arisen through gene duplications. These GnRHs and receptors have been recruited to serve a variety of functions, including reproductive behaviours, but the essential function of one of these and its receptor in gonadotropin regulation is totally conserved. The general dogma is that gene duplication increases in more complex animals to provide for finer regulation of homeostasis. However, intriguingly there has been progressive loss (or silencing) of GnRH and receptor genes during evolution from fish through amphibians to reptiles, birds and mammals. The reasons for this remain unclear but in some mammals it appears that two different forms of GnRH are able to bind to a single receptor (the other having been silenced) to recruit different intracellular signaling. We have termed this “Ligand-induced-Selective-Silencing” (LiSSS). The phenomenon has now been described for a number of receptors and dubbed “ligand-biased signaling” and provides exciting new approaches to more selective drug development.

Extraordinarily, the elimination or silencing (inactivating mutations) of genes also extends to highly conserved signaling systems regulating GnRH. Kisspeptin and its cognate receptor, GPR54, were recently found to be essential for progress through puberty and adult reproduction by the discovery of inactivating mutations in the ligand or receptor genes. A large body of research has shown that Kisspeptin directly activates GnRH neurons to induce GnRH secretion. Moreover, Kisspeptin neurons have been found to be major mediators of steroid feedback, stress, inflammatory factors and metabolic regulators of reproduction, including leptin effects, whereas GnRH neurons are not capable of directly responding to these regulators. The Kisspeptin regulation of reproduction is conserved throughout vertebrate evolution indicating that it master-minds the integration of diverse external and internal environmental inputs into the reproductive system. Astonishingly, the Kisspeptin gene, like leptin has been eliminated from the bird genome. To paraphrase the well used question “Why did the chicken cross the road?” we might pose the question “Why did the chicken lose its Kiss?” What are the evolutionary directives that have driven this? How is GnRH secretion regulated in birds in the absence of Kisspeptin? How is negative and positive feedback mediated in the absence of this system? Has there been positive selection for the removal of the Kisspeptin system? Is there another Kisspeptin equivalent system operative in birds? What does this tell us about bird reproductive endocrinology?
Gonadotropin releasing hormone (GnRH) plays a pivotal role in the regulation of reproductive functions through activation of its corresponding receptor (GnRH-R). Activation of GnRH-Rs by GnRH initiates a cascade of intracellular transduction pathways that results in the synthesis and release of gonadotrophins. In the chicken, two GnRH-R subtypes (type I and III) coincide with two endogenous GnRH ligands. Understanding the GnRH systems in an avian species provides a unique insight into the recruitment of GnRH systems throughout evolution as fewer GnRH ligand and GnRH receptor genes are retained in the genomes of mammals and birds compared to protochordates, fish, amphibians and reptiles. Loss of a GnRH ligand and receptor isoforms may be correlated with the evolution of complexity of reproductive physiology and there is some evidence that GnRH systems exhibit species-specific recruitment of receptor subtypes and ligands within different tissues. We have examined if the loss or retention of specific receptor subtypes in various species is correlated with the activation of particular signal transduction pathways. In that context, our findings describe the signal transduction pathways activated by chicken GnRH receptors and attempt to identify differing signal transduction pathways activated by the two receptors attributable to gonadotropin gene transcription.

Gonadotropin-inhibitory hormone (GnIH) was originally identified in the Japanese quail (Coturnix japonica) as a hypothalamic neuropeptide inhibitor of gonadotropin synthesis and release. The GnIH precursor encodes one GnIH and two GnIH-related peptides (GnIH-RP-1 and GnIH-RP-2) that share the same C-terminal LPXRFamide (X = L or Q) motif. The receptor for GnIH is the G protein-coupled receptor 147 (GPR147). The crude membrane fraction of COS-7 cells transfected with GPR147 cDNA showed specific binding GnIH and GnIH-RPs in a concentration-dependent manner. Scatchard plot analysis revealed one high-affinity binding site. Neurons, and GnRH neurons expressed GPR147 in birds and mammals. Accordingly, GnIH may inhibit gonadotropin synthesis and release by decreasing the activity of GnRH neurons as well as directly acting on gonadotropes.

Identification of novel neurohormones that regulate gonadotropin secretion is essential for the progress of reproductive neuroendocrinology. In the avian median eminence, Kobayashi attempted to isolate neurosecretory granules which contained some unknown hormones influencing pituitary hormone release. This line of research led to the independent discovery of gonadotropin-releasing hormone (GnRH) in the chicken in 1982, by the two groups of Millar and Miyamoto. The isolated neurohormone was called chicken GnRH-I. Subsequently, chicken GnRH-II was isolated. However, a neurohormone inhibiting gonadotropin secretion was unknown for a long time. In 2000, we discovered gonadotropin-inhibitory hormone (GnIH) in the quail brain. We now know that GnIH is not the sole hypothalamic regulatory neurohormone controlling avian reproduction. GnIH acts on gonadotropes in the pituitary and on GnRH neurons in the hypothalamus via the GnIH receptor GPR147. GnIH decreases gonadotropin synthesis and release, inhibiting gonadal development and maintenance. GnIH also acts at the level of the gonads as an autocrine/paracrine regulator of steroidogenesis and gametogenesis. Furthermore, melatonin stimulates the expression and release of GnIH via melatonin receptor expressed by GnIH neurons. GnIH expression also depends on stress conditions. The discovery of GnIH has fundamentally changed our understanding of hypothalamic control of avian reproduction.

Environmental chemicals include an array of compounds. Most studies in birds have focused on toxicology, with little attention to non-lethal effects from endocrine disrupting chemicals (EDCs). We investigated effects of EDCs in captive Japanese quail and wild songbirds. Embryonic exposure to single EDCs affected neural and thyroid systems, immune response, morphology, and behavior. Because wild birds are exposed to multiple EDCs, effects of embryonic exposure to complex mixtures of polychlorinated biphenyls (PCBs) were tested. We found impacts on endocrine systems and on heart morphology. However, our results also showed predicted toxicity (Toxic Equivalency; TEQ) was not predictive of observed effects. This indicates that the TEQ, based on comparative activation of the aryl hydrocarbon receptor (AHR) is insufficient in predicting endocrine impacts from EDCs. We are developing another approach and have termed it an Endocrine Disruption Index (EDI) to incorporate diverse data into the potency estimate that considers endocrine and neuroendocrine effects. The EDI is intended to complement other indices, and focus on endocrine disruption beyond AHR-mediated effects for a comparative assessment of non-lethal EDC impacts.
[S2-3] The 'thunder chicken' as a potential model for understanding the neuroendocrine control of non-vocal mating displays

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Courtship displays incorporate a diverse array of vocalizations and movements in birds. Although the neuroendocrine control of vocalizations has been a major focus of studies on the neural and hormonal control of avian courtship, far less is known about how the nervous and endocrine systems specifically modulate motor displays. One species that could prove to be a useful model to better understand these motor displays is the Ruffed Grouse (Bonasa umbellus), a galliform species endemic to North America. Unlike all other galliforms (e.g., quail, pheasant), the Ruffed Grouse has a very small vocal repertoire and does not incorporate vocalizations into its courtship display. Instead, the male performs a series of beats and flips in a rapid series of rhythmic movements producing a low frequency (<100Hz) ‘drumming’ sound. The drumming logs are used repeatedly throughout the year and provide an ideal spot to regularly collect fecal samples for hormone analysis, conduct behavioural experiments and trap birds for anatomical studies. Here, we present the results of three years of field research on this unique species, including individual differences in drumming displays, diel and seasonal variation in the display, the distribution of aromatase expression in the brain and possible hormonal and environmental correlates of variation in the drumming display. By providing insight into the neuroendocrine control of this unique, non-vocal courtship display, we aim to provide a better understanding of the proximate and ultimate mechanisms responsible for the diversity of courtship displays exhibited by birds.

[S2-4] Photoreceptive oscillators within neurons of the preemamillary nucleus (PMM) drive seasonal reproduction in temperate zone birds

Mohamed El Halawani1, Sunantha Kosornsri1, Laura Mauro2, and Yupaporn Chaisa2
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The pathway for light transmission regulating the reproductive neuroendocrine system in temperate zone birds remains elusive. Based on the evidence provided from our studies with female turkeys, it is suggested that the circadian clock regulating reproductive seasonality is located in putatively photosensitive dopamine-melatonin (DA-MEL) neurons residing in the preemamillary nucleus (PMM) of the caudal hypothalamus. Melanopsin is expressed by these neurons; a known photopigment which mediates light information pertaining to the entrainment of the clock. Exposure to a gonad stimulatory photoperiod enhances the activity of the DAergic system within DA-MEL neurons. DAergic activity encoding the light information is transmitted to the pars tuberalis (PT), where β-thyroid-stimulating hormone (TSHβ) cells reside, and induces the release of TSH. TSH stimulates tanyctyes lining the base of the third ventricle and activates type 2 deiodinase (Dio2) in the ependymal which enhances triiodothyronine (T3) synthesis. T3 facilitates the release of GnRH-I which stimulates LH/FSH release and gonad recrudescence. These data taken together with the findings that clock genes are rhythmically expressed in the PMM where DA-MEL neurons are localized imply that endogenous oscillators containing photoreceptors within DA-MEL neurons are important in regulating the DA and MEL rhythms that drive the circadian cycle controlling seasonal reproduction.

[S2-5] Neuroendocrine regulation of stress in birds with an emphasis on vasotocin receptors

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Vasotocin (VT) and corticotropin releasing hormone (CRH) act via four vasotocin receptors (VTRs) and two CRH receptors in mediating neuroendocrine functions. Of the four VTRs, the VT2R and VT4R are localized primarily in corticотropes and involved in the stress response. Using immunohistochemistry as a stressor, it was found that CRH1R and VT4R are down regulated while CRH2 and VT2R are up regulated based upon significant changes in transcript levels. Utilizing Fos protein as an indicator of activated cells, three neuronal groups (sub-region of accumbens, lateral hypothalamic area, lateral septum) were found to be activated by acute stress but found not significantly different from controls after repetitive stress. The paraventricular nucleus was found activated after acute as well as chronic stress. The nucleus of the hippocampal commissure was found significantly activated by acute stress and inactivated by chronic stress, however, the latter was not significantly different (p > 0.05). Immunohistochemistry showed that the VT4R was located in the cephalic lobe of the anterior pituitary and in neurons of the paraventricular nucleus, supraoptic nucleus and motor nuclei such as the oculomotor complex. The VT4R likewise immunostained glia in the brain, particularly the organum vasculosum of the lamina terminalis and median eminence.

[O2-1] Effects of thyroid disrupting pesticides on hypothalamic-pituitary-testicular axis of a tropical bird, Amadinda amandava

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Effects of two contemporary use pesticides, Mancozeb (dithiocarbamate) and Imidacloprid (neonicotenoid), on hypothalamic-pituitary-testicular axis of a tropical wild bird, Red Mani (Amadinda amandava) were elucidated. Both the pesticides were either established or suspected as thyroid disruptors. In wild, birds are vulnerable to pesticide exposure through dietary intake from fields. Hence, exposure was given through food for one month during the breeding season. Four groups (six/group) of male birds were maintained: Group I and Group II received 0.5% LD50 mg/kg body weight/day of Mancozeb and Imidacloprid respectively, while Group III exposed to mixture of both the pesticides. Group IV of control birds received same food without pesticides. Weight analysis and histopathology of thyroid, immunohistochemistry of pituitary TSH and assays of thyroid hormones (T3, T4) revealed disturbance of thyroid axis. Testicular regression was distinct as reflected in significant reduction in weight and tubules diameter, spermatogenic arrest was evident. Plasma levels of reproduction related hormones (PRL, LH, and testosterone) were significantly reduced. GnrH-immunoreactivity was more prominent in hypothalamus of exposed groups. Disruptions were more significant in the mixture pesticides group than either of the individual exposure suggesting the cumulative effects.
[S3-1] Modulation of testosterone-dependent male sexual behavior and the associated neuroplasticity
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Testosterone modulates the transcription of a multitude of genes and ultimately influences numerous aspects of reproductive behaviors. Our research investigates how one single steroid, testosterone, is able to trigger this vast number of physiological and behavioral responses. Testosterone potency can be changed locally via aromatization into 17β-estradiol which then activates estrogen receptors of the alpha and beta sub-types. We demonstrated that the independent activation of either receptor activates male sexual behavior in Japanese quail. We are currently investigating the underlying transcriptional changes in the hypothalamus-preoptic area (HPOA), the brain region that mediates the activation of male copulatory behavior by testosterone. In addition, several studies suggest that the specificity of testosterone action on target gene transcription is related to the recruitment of specific steroid receptor coactivators. We demonstrated that the specific down-regulation of the coactivators SRC-1 or SRC-2 in the HPOA by antisense techniques significantly inhibits steroid-dependent male-typical copulatory behavior and the underlying neuroplasticity. In conclusion, our results demonstrate that the interplay between several steroid receptors and their coactivators plays a key role in the control of steroid-dependent male sexual behavior and the associated neuroplasticity in quail.

[S3-2] Developmental plasticity of the zebra finch (Taeniopygia guttata) song control system—the effects of steroid receptor coactivators and early developmental stress
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Robust sex differences in the zebra finch song control system (SCS) are evident 10 days after hatch and may be influenced by endocrinological factors, such as the action of steroid receptor coactivators or exposure to early developmental stress. We are investigating two lines of research to understand the mechanisms involved in plasticity of the SCS during early life history. First, there are sex differences in the expression of two coactivators, RPL7 and SRC-1, across development of the SCS. Coactivators enhance the transcriptional activity of the steroid receptors with which they associate modulating the development of sex-specific brain morphologies and behaviors. RPL-7 and SRC-1-immunoreactive neurons were localized in the brains of male and female birds during early life through adulthood and distribution included the SCS as well as other steroid sensitive brain regions. Expression of both coactivators was sexually dimorphic suggesting a role for coactivators in the maintenance of behaviors including singing. Second, glucocorticoid receptor-like immunoreactive-neurons were localized in the brains of male birds 10 days after hatch and in adulthood. Males exposed to early corticosterone treatment exhibited a reduction in song complexity and decreased HVC size in adulthood. Together, these results suggest multiple endocrine mechanisms influencing developmental plasticity of the SCS.

[S3-3] Seasonal plasticity in the neuroendocrine control of songbird reproduction
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The neural integration of several environmental cues converges on discrete hypothalamic neurons in order to regulate seasonal reproduction. Gonadotropin-releasing hormone 1 (GnRH1) is a key regulator of the reproductive neuroendocrine system in seasonally breeding vertebrate species. Recent developments in songbirds have suggested that GnRH1 neurons exhibit far greater plasticity at the cellular and molecular levels than previously thought. We will present data collected from different songbirds illustrating that GnRH1 mRNA and protein is regulated by photoperiodic experience. Furthermore, I will show that discrete sub-populations of GnRH1 neurons in the preoptic area (POA) exhibit functional topography that is associated with photoperiodic experience and the social environment. A cDNA microarray study will also be presented that revealed the POA exhibited variation in distinct functional biological categories that included thyroid hormone receptor activity, epigenetic and angiogenetic processes. This dataset provides new insights into other neuromodulatory systems, in addition to GnRH1, that are involved in the neuroendocrine control of songbird reproduction. Overall, this presentation will illustrate that the GnRH1 neuronal system is highly dynamic, a predictor of reproductive state, and provide new directions for investigating how the GnRH1 system regulates seasonal reproduction.

[S3-4] Estradiol-regulated engagement of non-genomic response drives long-term optimization of auditory coding and discrimination
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Recent findings revealed that 17β-estradiol (E2) produced by central auditory neurons in the zebra finch brain is both necessary and sufficient to drive gene expression programs required for auditory learning. The mechanisms underlying these genomic responses, however, remain unknown. In this talk, I will present novel results that shed light onto the mechanistic bases and functional consequences of E2’s modulation of plasticity-associated gene expression. Quantitative proteomic analyses will demonstrate the identity of biochemical and protein regulatory cascades induced by E2 and driven by sensory experience in the auditory forebrain of awake animals. In-vivo pharmacology and biochemical assays will demonstrate that E2 drives plasticity-associated genes via a non-genomic mechanism that involves regulation of kinase signaling. Finally, we will demonstrate the functional relevance of the activation of this E2-driven non-genomic signaling cascade. Neurophysiological recordings coupled to intracerebral pharmacological manipulations and information theoretical metrics will reveal that E2-mediated activation of kinase signaling is required for the engagement of long-lasting changes in the information capacity and discrimination of auditory neurons, previously reported by us to be driven by locally-generated E2. Overall our results reveal that E2 produced by central auditory neurons drives the long-term optimization of auditory coding and discrimination via a non-genomic mechanism. Work supported by NIH (R01-DC-010181) and NSF (1064684).
[O3-2] Blocking testosterone does not decrease territorial aggression, but changes the emphasis of vocal behaviors during simulated territorial intrusions in male black redstarts (Phoenicurus ochruros)

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Particularly in species that defend territories during breeding and outside the breeding season the degree to which testosterone facilitates territorial behavior is still little understood. Here we suggest that species that defend territories for extended periods of time and also independent of reproduction may have lost the direct regulation of territoriality by androgens. We treated territorial male black redstarts with an anti-androgen and an aromatase inhibitor or a placebo and challenged the males with simulated territorial intrusions. In spring and in fall, both treatment groups increased their vocal performance in response to the territorial challenge. These changes in song structure seem to depend partly on testosterone or oestradiol, because both males that were implanted with the blockers and males that were challenged during non-breeding did not show the full structural change of their song. However, the territorial response as a whole was not reduced by the treatment, but it changed the emphasis of the territorial response: blocker-implanted males invested most into non-vocal behaviors, whereas placebo-treated males put most effort into their vocal response. These data suggest that overall territoriality may be decoupled from testosterone in black redstarts. However, high levels of testosterone during breeding may facilitate context-dependent changes in song structure.

[O3-1] Examining a pathway for hormone-mediated maternal effects: Yolk testosterone affects androgen receptor expression and testosterone production in the domestic chick

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Maternal testosterone in avian eggs can induce both short- and long-term changes in physiology and behaviour of the offspring, including androgen-sensitive traits. However, how the effects of maternal hormones are mediated remains unknown. We studied the possibilities that maternal androgens affect endogenous androgen production and/or androgen receptor (AR) densities in the brain influencing the sensitivity to androgens. Testosterone within the physiological range or vehicle only was injected into the egg yolk of unincubated chicken eggs and AR mRNA expression in different brain nuclei as well as plasma testosterone levels were measured in two week old chicks that had hatched from these eggs. AR mRNA expression as well as plasma testosterone levels were significantly lower in chicks hatched from testosterone treated eggs. The same effect on AR expression was also found in embryos just before hatching, although plasma T levels during embryonic development were not affected by the treatment. We are currently studying the possibility that the suppression of AR expression is caused by DNA silencing. We are also studying the possibility that embryos convert yolk T into conjugated androgens to facilitate uptake, masking elevation of plasma T levels in an ordinaryRIA.

[O3-5] Song complexity and brain plasticity in Bengalese finches

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Bengalese finches (BFs) are a species of songbirds with complex song sequence. BFs were domesticated for 250 years form of wild white-rumped munias (WRMs) of China. Unlike BF songs, song sequence in WRMs is simple and fixed. We aimed to find factors related with the strain differences in songs. First, we cross-fostered eggs of BFs and WRMs to see the extent in which song complexity was genetically determined. BF chicks learned WRM songs but WRMs had difficulty in learning BF songs, suggesting degenerated constraints for species-specificity in BFs. Next, we conducted a field work to find ecological correlates of song complexity. We located 3 colonies of WRMs in Taiwan. WRM has a sympatric related species, spotted munias. Among the 3 colonies, mixed colony ratios of WRMs with the spotted munias are correlated with song simplicity, suggesting that mixed colony requires higher degree of song simplicity to presumably to avoid cross breeding. We then measured corticosterone levels in feces and found WRM has higher concentration than BF. We also measured levels of glutamate receptors in the brain and found higher concentrations in BF. Results suggest relaxed selection might account for a part of reasons why songs became complex in BFs.

[O3-4] Examining a pathway for hormone-mediated maternal effects: Yolk testosterone affects androgen receptor expression and testosterone production in the domestic chick

Ton G.G. Groothuis1, Kristina A. Pfannkuche1, Ilse M. Weites1, Bernd Riedstra1, C. Wolf2, and Manfred Gahr2
1Behavioral Biology, University of Groningen, The Netherlands, 2Department for Behavioural Neurobiology, Max-Planck-Institute for Ornithology, Seewiesen, Germany
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Maternal testosterone in avian eggs can induce both short- and long-term changes in physiology and behaviour of the offspring, including androgen-sensitive traits. However, how the effects of maternal hormones are mediated remains unknown. We studied the possibilities that maternal androgens affect endogenous androgen production and/or androgen receptor (AR) densities in the brain influencing the sensitivity to androgens. Testosterone within the physiological range or vehicle only was injected into the egg yolk of unincubated chicken eggs and AR mRNA expression in different brain nuclei as well as plasma testosterone levels were measured in two week old chicks that had hatched from these eggs. AR mRNA expression as well as plasma testosterone levels were significantly lower in chicks hatched from testosterone treated eggs. The same effect on AR expression was also found in embryos just before hatching, although plasma T levels during embryonic development were not affected by the treatment. We are currently studying the possibility that the suppression of AR expression is caused by DNA silencing. We are also studying the possibility that embryos convert yolk T into conjugated androgens to facilitate uptake, masking elevation of plasma T levels in an ordinary RIA.
Growth hormone (GH) expression has been described in the brain and neural tissues in several vertebrate species. In birds, GH mRNA and GH immunoreactivity, and changes in their expression, have been shown in several central and peripheral nervous system locations, both during embryonic and post-natal development. Roles for GH in neural ontogeny are well established in the regulation of brain growth and in neuronal differentiation and function, including neuroprotection and neurogenesis. Neural GH shows molecular heterogeneity, with a 15 kDa isoform being predominant. In the cerebellum, GH is mainly expressed in Purkinje cells and in the granular layer. Cerebellar GH increases after a hypoxic-ischemic injury, and seems to be neuroprotective through anti-apoptotic mechanisms involving caspase-3, p-AKT and Bcl-2 pathways. The neural retina is also a site of GH expression, particularly within the retinal ganglion cells (RGCs). There, a second truncated GH mRNA (small chicken GH, scGH) coding for a 16-kDa protein was found. Retinal GH may be involved in axon growth and synaptogenesis during development of the visual system in early embryogenesis. The widespread presence of GH, GH-receptor (GHR), and GH-response gene (GHR)-1 mRNA in the brain of early chick embryos suggest autocrine/paracrine roles for GH in neural function. Supported by PAPIIT-UNAM and CONACYT, Mexico, and by NSERC, Canada.

Sex-steroids are recognized for their dramatic impact on avian brain and behavior. A widely-held perception is that these molecules are derived from peripheral sources and lack the spatial and temporal specificity ascribed to classical neuromodulatory systems. Our labs explore the idea that estradiol can function as a classic neuromodulator, even neurotransmitter, in the avian brain. With a focus on songbirds, we will present evidence for regulated pre-synaptic estradiol synthesis and functional post-synaptic estradiol actions. Our results on neuroestradiol, together with that from other labs, meets all the criteria for a neuromodulator, even neurotransmitter, and shifts our perception of estradiol as exclusively a peripheral reproductive signal to a signaling system intrinsic to the brain itself. We apply the term “synaptocrine” to describe this form of neuromodulation.

As evidenced by their phylogenetic distributions, components of social organization change vary rapidly over evolutionary time, and thus behavioral variables such as territoriality, mating system and grouping (flocking) are prone to repeated divergence and convergence. Given this, the complexity of relevant neural mechanisms, we cannot assume that evolutionary convergence in social structure has been produced by convergent modifications to the same neural characters. However, using five estrildid finch species that differ selectively in their species-typical group sizes (all biparental and monogamous) we have demonstrated that neural motivational systems evolve in predictable ways in relation to flocking and territoriality. These systems include nonapeptide circuits that encode social valence (positive-negative) and promote social behaviors. Nonapeptide and dopamine systems exhibit functional and anatomical properties that are biased towards gregarious species, and experimental reductions of nonapeptide signaling by antisense oligonucleotides and receptor antagonism significantly decrease preferred group sizes in the gregarious zebra finch. Recent studies in emberizid sparrows demonstrate that seasonal flocking is associated with seasonal plasticity of the same nonapeptide circuits that influence grouping in finches. Combined, these findings suggest that selection on species-typical group size may reliably target the same neural motivation systems when a given social structure evolves independently.
Avian species are regarded as an important alternative biomodel over mammals due to their unique embryological, physiological and evolutionary nature. Avian transgenesis can be applied for experimental animal models as well as animal bioreactors. Technologies for producing transgenic birds have been improved over the last few decades. Notably, approaches with germline chimeras that are produced by transferring genetically modified germ cells have been constantly reported. Primordial germ cells (PGCs) are the precursors of functional gametes that differentiate processes in animal species. Avian PGCs are initially localized to the central zone of the area pellucida in stage X embryos and finally migrate into the genital ridge via circulatory system. Transplantation of PGCs isolated from various developmental stages to recipient embryos generates germline chimeras and transgenic chickens. Additionally, PGCs can be applied for restoring the endangered bird species through generating the interspecies germline chimeric bird. Engineered avian biomodel will suggest us novel application perspectives for agricultural, ecological, industrial and biomedical fields.

**[S5-1] Characterization and genetic modification of avian primordial germ cells**
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Cheng Qi, and Zandong Li

There have been numerous studies on the characteristics and the migration patterns of mammalian, avian and amphibian Primordial germ cells (PGCs); however, the study of reptilian PGCs has been neglected. Thus, we chose Trionyx sinensis as our experimental animals, and we identified their PGCs and traced the migration of the PGCs. We produced the chimeras between turtle and Peking duck to study the survival and development of the PGCs in the heterologous embryos. We found the turtle PGCs were positive for PAS staining and expressed the vasa gene. Although they were able to transfer from the anterior crescent to the gonad via the blood, similar to avian PGCs, their migration into the gonad through the vascular system occurred over a longer developmental time. Additionally, we microinjected the E21 gonadal cells labeled with PKH26 into the blood vessels of E7 embryos to trace their locations, and we observed the fluorescent germ cells concentrated at the gonad region after 24 h of incubation. The donor duck-derived cells were also detected in a variety of organs in the developing turtle, especially in the gonads. This study demonstrates that Trionyx sinensis and avian PGCs share the same migration pattern, which includes inter-organizational migration and blood migration.

**[S5-2] The production of Peking duck - Trionyx sinensis chimeras and the migration of circulating PGCs in Trionyx sinensis embryo**

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The molecular mechanism of sex determining is unclear in birds. Here we show a new Z-linked gene, CHH, involved in gonadal differentiation in chicken. The mRNA was highly expressed in early embryonic gonads of male, and the expression timing slightly precede that of SOX9. It was drastically increased and achieved a peak at Day 8.5 (stage 35), and disappeared before hatching. Two-colored in situ hybridization showed that CHH was colocalized with SOX9, suggesting CHH expressed in Sertoli cells. The CHH protein was also expressed in medulla of male gonads at Day 6.5-8.5 (stage 30-35). Furthermore, in masculinized-ZW embryos treated aromatase inhibitor, the CHH expression was induced in the embryonic gonads. Gonadal expressions in early embryos have not been detected in mouse and human. Thus it was indicated that CHH functioned in sex differentiation at early stage of gonadogenesis, and this function was specific in chicken (might be in birds). We searched in the 5’-flanking region of CHH, resulting that NR5A1 (Ad4BP/SF-1) binding site was detected in chicken, not in mouse and human. The significant increase of the activity was detected by the reporter gene assay, suggesting birds-specific transcriptional regulation of CHH by NR5A1.

**[S5-3] Studies on oocyte activation, embryonic development and gene transduction by intracytoplasmic sperm injection (ICSI) method in birds**
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Intracytoplasmic sperm injection (ICSI) is recognized as a routine assisted-reproductive technology in mammals. However, in birds, it has been conducted recently. This paper reviews our results obtained in quail eggs. The oocyte was retrieved from the infundibulum or magnum and quail sperm was injected. The oocyte was cultured for 24 hr in CO2 incubator. The fertilization and development was assessed by DAPI staining and stereomicroscopic observation. The fertilization rate was ~15% with development of stage VI. To investigate oocyte activation mechanism, effects of calcium, its agonist and inhibitor are studied, suggesting that calcium is essential to activate oocyte for fertilization. To improve the fertility rate PLNeta cRNA and IP3 were injected with a sperm, increasing up to 45% and 92%, respectively. Finally, gene transfer was attempted using Triton X-100 (TX-100) treated sperm. The sperm was injected with PLCzeta cRNA and enhanced green fluorescent protein (EGFP) gene in quail oocyte. The treatment induced blastoderm development (44%) of the oocytes and 86% of oocytes showed fluorescent emission. PCR analysis detected GFP fragments in 50% of GFP-expressing blastoderm. Our ICSI method is the first step toward the production of transgenic birds.

**[S5-4] CHH is a new gene involved in the gonadal differentiation of chicken**

Fang Yuan, Zhiyi Wan, Xiaoxue Yu, Qiang Shao, Cheng Qi, and Zandong Li

There have been studies on the characteristics and the migration patterns of mammalian, avian and amphibian Primordial germ cells (PGCs); however, the study of reptilian PGCs has been neglected. Thus, we chose Trionyx sinensis as our experimental animals, and we identified their PGCs and traced the migration of the PGCs. We produced the chimeras between turtle and Peking duck to study the survival and development of the PGCs in the heterologous embryos. We found the turtle PGCs were positive for PAS staining and expressed the vasa gene. Although they were able to transfer from the anterior crescent to the gonad via the blood, similar to avian PGCs, their migration into the gonad through the vascular system occurred over a longer developmental time. Additionally, we microinjected the E21 gonadal cells labeled with PKH26 into the blood vessels of E7 embryos to trace their locations, and we observed the fluorescent germ cells concentrated at the gonad region after 24 h of incubation. The donor duck-derived cells were also detected in a variety of organs in the developing turtle, especially in the gonads. This study demonstrates that Trionyx sinensis and avian PGCs share the same migration pattern, which includes inter-organizational migration and blood migration.

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The molecular mechanism of sex determining is unclear in birds. Here we show a new Z-linked gene, CHH, involved in gonadal differentiation in chicken. The mRNA was highly expressed in early embryonic gonads of male, and the expression timing slightly preceded that of SOX9. It was drastically increased and achieved a peak at Day 8.5 (stage 35), and disappeared before hatching. Two-colored in situ hybridization showed that CHH was colocalized with SOX9, suggesting CHH expressed in Sertoli cells. The CHH protein was also expressed in medulla of male gonads at Day 6.5-8.5 (stage 30-35). Furthermore, in masculinized-ZW embryos treated aromatase inhibitor, the CHH expression was induced in the embryonic gonads. Gonadal expressions in early embryos have not been detected in mouse and human. Thus it was indicated that CHH functioned in sex differentiation at early stage of gonadogenesis, and this function was specific in chicken (might be in birds). We searched in the 5’-flanking region of CHH, resulting that NR5A1 (Ad4BP/SF-1) binding site was detected in chicken, not in mouse and human. The significant increase of the activity was detected by the reporter gene assay, suggesting birds-specific transcriptional regulation of CHH by NR5A1.
[O5-1] Genomic sequence and analysis of Japanese quail by using the chicken genome sequence as a framework

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The Japanese quail (Coturnix japonica) has been a good model organism in a range of scientific disciplines including development, behavior, physiology, and genetics. This species is also economically important as an agricultural species producing eggs and meat. However, the genome sequence of this species is not currently available. In this study, we have tried to decode the genome sequence of the Japanese quail using only next-generation sequencing (NGS). We obtained 86 Gb of sequence data in total, which were used for assembly of the Japanese quail draft genome sequence. To improve the quality of the resulting assembly, we developed a process to assist with the de novo assembly analysis by aligning the sequence reads of the Japanese quail against the reference genome of the chicken (Gallus gallus), a closely related species to the Japanese quail. After the iterative aligning process, the Japanese quail sequence reads could cover almost 80% of the chicken genome. This study demonstrates the feasibility of aligning and assembling strategy to sequence and analyze Japanese quail genome using NGS. The resultant draft genome sequence would provide valuable information about quail-specific sequences including copy number variation and microsatellite regions.

[O5-2] Reproductive regulation in birds: Role of photoperiod and temporal synergism of neural oscillations

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Many experimental studies have reported the importance of temporal phase relation of circadian neural oscillations in the gonadal development of seasonally breeding birds and poultry species Japanese quail. It has been established that administration of dopamine precursor (L-DOPA) at specific hour after the administration of serotonin precursor (5-HTP), over a period of 10-13 days induces long term effect on the development of avian gonad. In Red heading bunting, Emberiza bruniceps; Indian weaver bird, Ploceus philippinus; Spotted munia, Lonchura punchalata and Japanese quail, Coturnix coturnix japonica, administration of 5-HTP and L-DOPA at the interval of 8 hours (8-hr relation) suppresses and if given 12 hours apart (12-hr relation) stimulates gonadal development while all other relationships (0, 4, 16 and 20-hr) were found to be more or less ineffective. The presentation will highlight that temporal synergism of neural oscillations may modulate the gonadal development throughout the year in non-photoperiodic birds but not during the photorefractory phase in those species which develop absolute-photorefractoriness. However, relative-photorefractoriness may be terminated by specific phase relation (12-hr) of neural oscillations. It is concluded that, in addition to PTM, birds can detect the alteration in the circadian phase relation of neural oscillations to modulate gonadal activity.

[O5-2] Melatonin regulates diurnal changes in locomotor activity by regulating 7α-hydroxypregnenolone synthesis in quail

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Melatonin regulates diurnal changes in locomotor activity in vertebrates, but the molecular mechanism for this neurohormonal regulation of behavior is poorly understood. We previously identified 7α-hydroxypregnenolone, a novel avian neurosteroid stimulating locomotor activity, in quail brain. Here we show that 7α-hydroxypregnenolone mediates melatonin action on diurnal locomotor rhythms in quail. In this study, we first demonstrated diurnal changes in 7α-hydroxypregnenolone synthesis in quail. 7α-Hydroxypregnenolone synthesis and locomotor activity in males were much higher than in females. This sex difference in 7α-hydroxypregnenolone synthesis corresponded to the sex difference in locomotion. Subsequently, we showed that only males exhibited marked diurnal changes in 7α-hydroxypregnenolone synthesis, and these changes occurred in parallel with changes in locomotor activity. Finally, we identified melatonin as a key component of the mechanism regulating 7α-hydroxypregnenolone synthesis. Increased synthesis of 7α-hydroxypregnenolone occurred in males in vivo after melatonin removal via pinealectomy and orbital enucleation (Px plus Ex). Conversely, decreased synthesis of this neurosteroid occurred after melatonin administration to Px plus Ex males. This study demonstrates that melatonin regulates synthesis of 7α-hydroxypregnenolone, a key factor for induction of locomotor activity, thus inducing diurnal locomotor changes in male birds.
In most animals that live outside the tropic regions, reproduction is under photoperiodic control. The Japanese quail (Coturnix japonica) is an excellent model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. Recent studies revealed that central thyroid hormone (TH) activation acts within the mediobasal hypothalamus to regulate seasonal gonadal development. However, the mechanisms that underlie seasonal gonadal regression remain unclear. Short day/low temperature (SL) stimuli induced drastic testicular regression by apoptosis, which coincided with a decrease in serum testosterone and an increase in serum T₃. Temporal gene expression analysis revealed shut-off of the luteinizing hormone (LH)-dependent steroidogenesis pathway and activation of T₃ response genes involved in amphibian metamorphosis. Administration of T₃ mimicked effects of SL conditions on gene expression, apoptosis and testicular weight. We propose that TH plays dual roles in the regulation of seasonal reproduction. That is, central action induces seasonal testicular development, while peripheral action mediates seasonal testicular regression. We conclude that birds utilize T₃ not only for adaptive thermoregulation but also for testicular regression in fall or winter.

Avian breeding cycles are regulated by endogenous rhythms. Here we present the data showing that phonologies linked with breeding, viz. cycles of gonadal growth and regression and molt, in subtropical spotted munia (Lonchura punctulata) exhibit circannual rhythms in aperiodic environments. Two long term studies were performed. In the first study, birds were subjected to continuous light (LL), 12 h light per day (12L:12D) and alternating days of light and darkness (24L:24D, LL/DD) for more than two years. A group on NDL served as control. Measurement of gonads and molt of primary wing feathers at regular intervals showed that birds underwent annual cycles in gonads and molt, irrespective of the external lighting conditions, although there were group and sex differences. It is suggested that annual breeding cycles in spotted munia is regulated by the self-sustained circannual rhythms. Both sexes appear to adopt independent strategies, but females possibly share a greater role in defining the reproductive season in relation to the environment. Funded by DST-IRHPA Center for Excellence grant (IS/SO/LU-02/2005)

In free-living birds thyroid hormones (HTs) are of primary importance for seasonal female reproduction. However, in domesticated birds, such as chickens, the role of THs in the ovarian function is not fully elucidated. For the last decade effects of triiodothyronine (T₃) on the chicken ovarian function have been investigated in our laboratory. The in vitro experiments revealed that T₃ administration decreases ovarian weight and plasma LH and estradiol (E2) levels. These data are consistent with results of in vivo experiments which showed that T₃ reduces basal and LH-induced E2 secretion by white prehierarchical and yellow preovulatory follicles (F3-F1). On the other hand, these experiments established that T₃ enhances basal and LH-induced progesterone (P₄) production from F3-F1 follicles. These effects were associated with mRNA expression of steroidogenic enzymes and cAMP synthesis in theca and granulosa cells. Moreover, our recent studies have showed that, in addition to nuclear receptors, chicken ovarian follicles express mRNA of α and β integrin plasma membrane receptor, indicating nongenomic action of THs in the hen ovary. Collectively, these data reveal that THs are potent modulators of chicken ovarian steroidogenesis and can directly affect follicular growth and maturation. Supported by grants: N N311 006436 and DS-3243/KNIEZ.

**References**

Symposium 7
Multiple actions of thyroid hormones

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Thyroid hormones (THs) are important regulators of neurodevelopment and correct timing of TH action in different regions and cell types is vital. TH availability is regulated by TH transport across the cell membrane and intracellular (in)activation. Our experiments during brain development showed increasing expression of the TH activating type 2 deiodinase over time. The inactivating type 3 deiodinase showed high expression during the earliest stages of neurodevelopment and its expression pattern is probably linked to protection of specific areas from too early TH signalling. MCT8 and OATP1C1 are considered the main TH transporters in the brain. We showed that both are expressed during early development. OATP1C1 expression was highest at the blood-brain-barrier (BBB) and MCT8 was found throughout the brain, predominantly in the gray matter. The spatiotemporal variable expression pattern of the deiodinases and transporters during development indicates that TH availability is strictly regulated. This is consistent with adult mammalian data, where TH uptake into the brain is facilitated by OATP1C1 at the BBB, the THs are activated in glial cells by D2 and taken up in neurons by MCT8. Combination of our localisation studies with identification of the (precursor) cell types will help us to elucidate the exact role of THs in neurodevelopment.

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Transport and metabolism of thyroid hormone during chicken brain development

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[O7-1] Transfer of methimazole into the eggs of laying hens and effects on thyroid function in hens and embryos

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Methimazole (MMI) inhibits thyroid hormone synthesis and is often used to treat hyperthyroidism. We treated broiler breeders with MMI during 16 weeks and investigated MMI levels and thyroid function in hens and their offspring. At 16 weeks maternal MMI levels were 5-10 times higher in thyroid compared to liver and brain. Thyroid weight was increased 15-fold, with concomitant increases in thyroid peroxidase, thyroglobulin, sodium/iodide transporter, thyroid transcription factor and thyrotropin receptor expression, as well as in T4/T3 ratio. While only T3 and not T4 was strongly decreased in plasma, liver and kidney, both hormones were decreased in brain, accompanied by increased type 2 deiodinase activity. MMI levels measured from 8-16 weeks of treatment were double in egg white and yolk compared to maternal plasma and embryonic tissue levels were also higher than in hens. T4 content in egg yolk was decreased by 70%, T3 by 50%. In 6-day-old embryos, T4 and T3 levels in trunk were not affected but T3 levels in head were strongly decreased. In 14- and 18-day-old embryos plasma T4 and T3 were decreased while in brain again only T3 was decreased. We conclude that maternal transfer of MMI disturbs embryonic thyroid hormone availability throughout development.

[O7-3] Multiple actions of thyroid hormones

Symposium 7

|O7-1| Transfer of methimazole into the eggs of laying hens and effects on thyroid function in hens and embryos
|O7-3| Multiple actions of thyroid hormones

Symposium 8
Stress responses in a changing world

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A potentially serious outcome of global climate change is the increase in frequency and intensity of extreme weather events. Additionally, environmental perturbations such as human disturbance, invasive species, social disruption and pollution indicate that bird populations worldwide face major challenges in coping with stress. Responses to one or more environmental perturbations incur energetic costs in addition to those of the normal life cycle such as breeding, migrating etc. The concept of allostatics provides a framework to integrate energetic demand and wear and tear of daily and seasonal routines (the predictable life cycle) with perturbations of the environment including disease, aging and social status. The concept is particularly attractive because it allows a framework to assess the challenges faced in changing social and physical environments at the individual level because no single organism experiences the environment in exactly the same way as another. The reactive scope of the mediators of coping mechanisms, such as the adrenocortical response to acute stress, also vary on seasonal, daily, habitat and individual bases. Understanding these regulatory mechanisms will be critical to ameliorating the effects of global change in general.

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Animals live in a changing world, which is characterized by rapid, unpredictable and intense modifications to their environments. Ecological studies have demonstrated that some individuals show a drastic reduction of their performances in response to this changing environment whereas others seem able to cope with it well. Therefore, all individuals are not equal in this changing world, and it appears crucial for conservation purposes to determine what enables this ability to cope with change. Here, I will show that stress physiology can help to answer this question for several reasons. First, environmental changes induce the activation of stress mechanisms (HPA stress response and hormonal changes). Second, these stress mechanisms mediate the behavioral and physiological responses of individuals to energetic and stressful challenges. These responses can improve immediate survival, but also entail costs because they redirect the allocation of resources toward survival at the expense of other activities. Last but not least, there are substantial variations in these stress responses between individuals. This variability is essential to consider because it can help us understand what strategies individuals adopt when environmental challenges occur. It may therefore provide a functional basis for inter-individual differences in the ability to cope with a changing world.

[O8-2] Functions and significance of the stress response in a changing world

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[O8-1] Allostasis, resilience and coping with a changing world

Symposium 8
Stress responses in a changing world

|S8-1| Allostasis, resilience and coping with a changing world
|S8-2| Functions and significance of the stress response in a changing world
[O8-1] Corticosterone and fitness: Effects of developmental stress and context
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In the recent years, there has been growing interest in how glucocorticoids mediate fitness. Two non-mutually exclusive hypotheses, the “Cort-fitness” hypothesis by Bonier et al and the “Cort-condition” hypothesis by Breuner and Hahn, posit that baseline corticosterone and/or the amplitude of adrenocortical responses should relate to fitness. However, such relationships between corticosterone and fitness-related traits will likely depend on the developmental environment and context. To explore how developmental stress and context alter this relationship, we manipulated egg incubation temperature (36.2, 37.4, 38.4°C) and examined the relationships between prenatal stress, stress physiology (adrenocortical responses, responses to ACTH and dexamethasone), reproductive performance, and survival in captive zebra finches (Taeniopygia guttata). Suboptimal incubation temperature had no effect on reproductive performance but lowered survival. Stress physiology did not correlate with survival but significantly correlated with several measures of reproductive performance in males. Males with higher nesting baseline corticosterone fed offspring at a higher rate compared to those with lower baseline corticosterone. Males with higher baseline corticosterone also had higher survival, suggesting that baseline corticosterone may influence individual fitness.

[O8-3] Corticosterone responses and personality: Individual variation and the ability to cope with environmental change
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There is considerable variation between birds in their responses to the same stressor, and corticosterone responses of individual birds may be quite different from the mean response for a group of birds. Individual birds also have characteristic patterns of behaviour to cope with demands from their environment, and stress coping styles (personalities) are coherent sets of behavioural and physiological responses to common challenges faced by birds. It is proposed that birds with low corticosterone responses and proactive personalities are likely to be more successful (have greater fitness) in constant or predictable conditions, whilst birds with reactive personalities and high corticosterone responses will be more successful in changing or unpredictable conditions. The relationship between corticosterone responses and fitness thus depends on the prevailing environmental conditions, so birds with either low or high corticosterone responses can have the greatest fitness and be most successful, but in different situations. The model provides an explanation for the existence of variation in corticosterone responses in birds, and suggests that there is no optimum or “best” corticosterone response for all conditions, and also suggests that the ability of birds to cope with environmental change will be related to both their corticosterone responses and to their personality.

[S9-1] Migratory feeding and fattening: A neuroendocrine perspective
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Photoperiodic cues establish a temporal window within which birds express a suite of physiological changes to adapt to the demands of migratory flight. These include the deposition of fat, brought about primarily through increased food intake or hyperphagia. Spring migratory fattening occurs as a physiological cascade induced by changes in daylength, and the photoperiodic integration in the brain is likely to share pathways identified for the control of reproduction. The neuroendocrine pathways regulating the control of appetite in the avian hypothalamus are beginning to be understood and show evolutionary conservation with the circuitry established in mammals. Neuropeptide Y and melanocortin system peptides, interacting with metabolic hormones, coordinate behavioural and physiological responses to lost energy stores, helping to maintain body mass within homeostatic limits. These mechanisms are particularly relevant to cycles of feeding and fasting during migration. However, studies in European quail suggest that there is not a simple relationship between seasonal migratory hyperphagia and the expression of hypothalamic genes promoting appetite. Spring migratory fattening and hyperphagia are associated with an increase in a ‘sliding set point’ for body mass that is altered seasonally, but the molecular neuroanatomical basis of this phenomenon remains to be elucidated.

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During migratory flight birds rely on their fuel stores. Up to now little is known about the hormonal regulation of the energy metabolism during migration and findings so far were often conflicting. During the last years the glucocorticoid hormone corticosterone was discussed as a candidate which might orchestrate the energetic needs during migration. For the first time we measured plasma corticosterone levels in actively flying migratory birds: in red knots, Calidris canutus, flying up to 10 h in a wind tunnel and in free-ranging passerines caught out of migratory flight. We compared the plasma corticosterone concentrations of birds with different migration strategies, namely long-distance, short-distance and irruptive migrants. During migratory flight slightly elevated corticosterone concentrations seem to promote the mobilisation of the energy stores. A strong increase in corticosterone when fat reserves are near exhaustion triggers an increase in the catabolism of protein and probably a change in behaviour. The results will be discussed in comparison with literature data about the energy metabolism during endurance exercise of mammals and humans.

[S8-1] Fuel metabolism and its regulation in migrating birds
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During the last years the glucocorticoid hormone corticosterone was discussed as a candidate which might orchestrate the energetic needs during migration. For the first time we measured plasma corticosterone levels in actively flying migratory birds: in red knots, Calidris canutus, flying up to 10 h in a wind tunnel and in free-ranging passerines caught out of migratory flight. We compared the plasma corticosterone concentrations of birds with different migration strategies, namely long-distance, short-distance and irruptive migrants. During migratory flight slightly elevated corticosterone concentrations seem to promote the mobilisation of the energy stores. A strong increase in corticosterone when fat reserves are near exhaustion triggers an increase in the catabolism of protein and probably a change in behaviour. The results will be discussed in comparison with literature data about the energy metabolism during endurance exercise of mammals and humans.
[S9-3] Endocrinology of the migratory traits of a long-distance migrant in comparison with a nonmigratory congener

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Yearly, migrants travel between locations for breeding and overwintering taking advantage of seasonal resources that secure survival and increase fitness. Thus migrants express seasonal changes in behavior and physiology that anticipate and respond to the cyclical demands of the environment. The endocrine mechanisms associated with alterations of behavior and physiology throughout the migratory life cycle are poorly understood. To identify traits associated with migration, we compared two subspecies of White-crowned Sparrow: long distance migrant, Zonotrichia leucophrys gambelii and resident, Z. l. nuttalli. Migrants were sampled at wintering (Davis, CA) and breeding (Fairbanks, AK) sites during departure, arrival and throughout the overwintering stage. Commensurate stages were selected for residents residing along the California Coast. We present seasonal profiles for androgen (testosterone, 5α-dihydrotestosterone) and corticosterone in relation to morphological, histological and metabolic features of each stage. To date, migrant profiles for all variables diverge most prominently from those of the residents during migratory stages that serve to compress the duration of the breeding stage for Gambelii in comparison with Nuttalli. Thus, Z. l. nuttalli has reduced the number of life history stages per year, lost the capacity to express migratory characteristics and the endocrine support for features that are prominent among Gambelii.

[S9-4] The migration-breeding transition: Integrating behavior, stress physiology and experience from a long-term data set

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Birds transitioning from migration to breeding undergo major morphological, physiological, and behavioral changes. The timing of this transition is determined by both external (environmental, social) and internal (physiology, sex, age) factors. Long-distance migrants can face highly variable conditions on arrival at the breeding site, and need to be flexible in how rapidly they initiate breeding once there. The mountain white-crowned sparrow migrates from Mexico up to sub-alpine habitat across the mountains of Western North America. Populations breeding in the Sierra may initiate breeding immediately upon arrival, or have to delay breeding up to a month or more given local snowpack and temperature conditions. I have studied the arrival biology of the mountain white-crowned sparrow for the past 15 years, evaluating the role of stress physiology in behavioral transitions during unpredictable events in this highly flexible species. Recent work has focused on individual movement patterns using automated telemetry. I am now evaluating the opposing roles of glucocorticoids and androgens in these early behavioral decisions by evaluating both external and internal factors which regulate the transition from migration into breeding.

[S10-1] Relationships between glucocorticoids levels and fitness: What are the predictions?

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An often asked question is whether and how glucocorticoids are related to fitness measures. This question is particularly interesting for conservation biologists and people interested in animal welfare. When environmental conditions deteriorate, it has often been observed that glucocorticoid levels increase, and fitness decreases. From this it was concluded that there should be a correlation between glucocorticoid levels and fitness (the cort-fitness hypothesis). However, empirical findings are mixed and the hypothesis is not supported by many studies. In this presentation I will first show that individual variation in reaction norms may mask the relationship between glucocorticoids and fitness. Therefore, we need more studies on within-individual relationships between glucocorticoids and fitness and more experimental approaches. Second, I will show that different hypotheses produce different predictions about the glucocorticoid-fitness relationship. As a consequence we need to focus on the underlying mechanisms of how stress may affect fitness components. Finally I will present recommendations for the use of glucocorticoid levels in conservation biology as a measure of condition or health.

[S10-2] The stress of parenthood – the role of glucocorticoids in mediating reproductive investment

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Glucocorticoid hormones have widely been considered to have a suppressive effect on reproduction, instead favoring allocation of resources towards self-maintenance and survival. However, evidence from a broad array of taxa demonstrates that high baseline glucocorticoids often coincide with increased reproductive effort, and might in fact be necessary to meet the energetic demands of reproduction and parental care. I will present findings from my work with free-ranging Tree Swallows (Tachycineta bicolor) showing that 1) baseline glucocorticoids and reproductive effort are often positively correlated, 2) experimentally increased reproductive demand can elicit increases in glucocorticoids, and 3) experimentally increased glucocorticoids can cause increases in reproductive effort. Given these and other similar findings, a view of glucocorticoids as promoters of adaptive and context-dependent allocation of resources towards all energetic demands, associated with both reproduction and survival, might be more accurate and could advance our understanding of relationships between glucocorticoids and fitness.
[S10-3] HPA axis regulation, survival, and reproduction in free-living sparrows: Functional relationships or developmental correlates?
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Recently, there has been growing interest in studies relating HPA function to fitness. The CORT-fitness and CORT-condition hypotheses predict that baseline and/or stress-induced levels of glucocorticoids should correlate with fitness. In addition, fitness-related sexually selected traits (e.g., melanin-based ornaments) may also be correlated to HPA function via common underlying biosynthetic pathways, or because HPA function and ornaments are both organized by early-life stressors (developmentally correlated traits). We address these hypotheses in a free-living population of song sparrows (Melospiza melodia). In two independent studies we have found that song complexity (a sexually selected) is correlated to stress reactivity: individuals with more complex songs exhibit less CORT increase following restraint. This effect may result from early environment influencing both stress reactivity and song. Suppression of CORT by dexamethasone was also correlated to measures of body condition and immune function, and females paired to males with higher stress-induced levels of corticosterone initiated egg-laying later. Finally, stress reactivity was predictive of overwinter survival in one year, although not in another. Thus, the relationship between HPA function and fitness likely varies temporally and by context. Some of these traits may be functionally related to HPA regulation, while others are likely developmental correlates.

[S10-4] Glucocorticoids and fitness measures in long-lived birds
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Breeding at the right time is a key-component of fitness, and requires flexible physiological responses to environmental conditions but the underlying mechanisms are poorly understood. Since elevated levels of corticosterone (CORT) can suppress reproduction, this hormone appears to be a good candidate for mediating the decision to breed and when to start egg-laying. Here, I will investigate the role of CORT in reproductive decisions in long-lived seabirds. Individual variations in baseline but not in stress-induced CORT levels were a good predictor of the laying date. Females but not males bearing high pre-laying baseline CORT levels showed a reduced ability to release Luteinizing hormone (LH) and were more likely to skip breeding. Further, an experimental reduction of baseline CORT in females led to a significant advancement of egg laying and a higher breeding success. In males however, this treatment resulted in a higher probability to skip breeding. In these long-lived birds, age had a strong effect on reproductive decisions whereas baseline CORT levels were independent of age. On the other hand, young and very old birds showed higher stress-induced CORT levels than middle-aged ones. I will then discuss possible mechanisms linking CORT (baseline and stress-induced levels), reproductive decisions and fitness.

[O10-1] Stress, personality, and success: Intriguing links between corticosterone and behavioral phenotype in Florida scrub-jays
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Recently, numerous studies have revealed links between endocrine and behavioral phenotypes. In our long-term study of free-living Florida scrub-jays (Aphelocoma coerulescens), we have examined the relationship between corticosterone (CORT) and ‘personality’. Our finding that baseline CORT levels of nestlings were positively correlated with fearfulness eight months later (see Schoech et al. 2009, GCE 163:201-207), inspired follow-up studies. These yielded intriguing findings, such as tame individuals in our population have significantly lower baseline CORT levels than less tame individuals (approach distance of researchers). Further, adults with higher stress-induced CORT levels (integrated CORT) were far less likely to use a feeder than were individuals with lesser CORT responses. Similarly, in tests in which jays must approach a novel object or cross a ring to access a desired food item (peanuts), more stress responsive individuals (i.e., higher integrated CORT levels) were less likely to complete both tasks. We have also found positive relationships between baseline and integrated CORT levels. Finally, the magnitude of the CORT response to a stressor is repeatable throughout an individual’s life and it can predict general life span. Ongoing and future research will manipulate nestling CORT levels to determine whether developmental CORT ‘programs’ future behavioral and physiological phenotypes.

[O10-2] Seasonal changes in brain and peripheral intracellular glucocorticoid receptors in wild house sparrows
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Glucocorticoid hormones (GCs) help animals successfully cope with their environment. There is wide seasonal variation in GCs, which often falls into predictable patterns that occur across a number of different vertebrate taxa. One way to better understand seasonal variation in GCs is by looking downstream of the hormones to their receptors in different target tissues. This study used radioligand binding assays to quantify intracellular GC receptors in brain and peripheral tissues of wild house sparrows. We captured sparrows in eastern Massachusetts during early winter, late winter, early breeding, mid breeding, late breeding, and autumn molt. Whole-brain concentrations of the glucocorticoid receptor (GR) were higher in the early breeding period compared to other times of year. Spleens from birds in the early breeding period had more MR and a trend towards more GR compared to spleens in the late breeding period. There was no seasonal change in testes GR or MR. Animals in unpredictable environments may use supplementary cues such as food availability and weather to help time the onset of breeding. For house sparrows coping with the vagaries of spring in New England, these cues could be mediated in part through the mechanism of glucocorticoid action on different target tissues.
Integrating immune-endocrine interactions into life history theory

[S11-1] Eco-endo-immunology in the dark-eyed junco
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Broadly distributed songbirds, particularly those that migrate, encounter a wide range of pathogens. Both pathogen exposure and energy available for immune responses are expected to co-vary negatively with latitude and elevation. Dark-eyed juncos (Junco hyemalis) are found throughout North America and occupy a range of seasonal environments. Some populations migrate long distances from high to lower latitudes to overwinter; other populations occupy moderate climates year-round and are entirely sedentary. Juncos wintering in the eastern North America vary in immune function by latitude with more robust responses in milder climates. In coastal southwestern North America, juncos have recently adopted a sedentary habit, occupy a mild climate year-round, and exhibit enhanced immune function as compared to a nearby montane population. The complexity of the junco system, with variation among subspecies, populations, sexes, and individuals in phenology and migratory behavior provides a challenge and an opportunity to study the interaction of the endocrine and immune systems with respect to climate and disease exposure. By comparing populations in the field and in ‘common gardens’, we are studying how season, sex, migration, disease environment, and climate interact as reflected in endocrine and immune responses.

[S11-2] Glucocorticoid production and function in lymphoid organs: Of mice and birds
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In birds and mice, extra-adrenal glucocorticoid synthesis occurs in several tissues, including lymphoid organs of the immune system. The regulation and functions of glucocorticoids synthesized in the immune system (“immunosteroids”) are unclear. We are studying both birds and mice to better understand these potentially critical signaling molecules. In developing zebra finches, glucocorticoid concentrations are higher in lymphoid organs (thymus, bursa of Fabricius, spleen) than in plasma. Remarkably, while avian adrenals secrete corticosterone, lymphoid organs contain more cortisol than corticosterone. Moreover, while corticosterone and cortisol have similar affinities for cytosolic binding sites in primary lymphoid organs, only cortisol binds to membrane sites in the bursa of Fabricius. Thus, locally-produced cortisol may signal through a different pathway than circulating corticosterone. We are conducting similar work in mice, in which lymphocyte development is well characterized. In developing mice, both corticosterone and cortisol levels are higher in lymphoid organs (thymus, spleen) than in plasma. Glucocorticoid precursors, including progesterone, are also higher in lymphoid organs than in plasma. The similarity between zebra finches and mice suggests an important, evolutionarily conserved role for glucocorticoids in lymphocyte development. Parallel studies in birds and mice are extremely useful because they utilize the complementary advantages of each species.

[S11-3] Glucocorticoids and redox physiology in birds
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Vertebrates translate environmentally stressful stimuli into secretions of glucocorticoids that, through a cascade mechanism, activate the physiological stress response. A number of physiological and behavioral mechanisms are activated to protect the organism from allostatic failure and to promote self-maintenance and survival. Prolonged high secretion of glucocorticoids may, however, have detrimental consequences for the organism: a state of chronic stress has been linked with an increase in susceptibility to cardiovascular and metabolic diseases, and, possibly, to a decrease in survival probabilities and Darwinian fitness in natural vertebrate populations. In this communication, I will highlight that oxidative stress may be one possible mechanism for the costs of chronic stress and I will focus on the role of glucocorticoids as modulators of oxidative stress in vertebrates, with particular attention to birds. To this end, I will present results from experimental studies and a meta-analysis to describe the genomic (e.g., gene expression) and non-genomic (e.g., immune response) mechanisms through which glucocorticoids may impact on redox state, and to infer on potential functional consequences for the organism.

[S11-4] Physiological mechanisms of range expansion in the house sparrow
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Introduced species are one of the largest threats to biodiversity and ecosystem functioning, yet whether particular traits dispose some species to be better invaders than others remains understudied, especially for vertebrates. Here, we asked whether certain factors are related to the spread of an introduced bird, the house sparrow, across Kenya. Introduced to the port city of Mombasa about 60 years ago, we find that sparrow behavior, physiology, epigenetics, and genetics vary in an intelligible manner as a function of age of populations. Young populations (at the range edge near the Ugandan border) tend to be very exploratory, release abundant corticosterone in response to stressors, and survey robustly for bacterial infections. Other traits, such as genetic and epigenetic variation, inducibility of immune defenses (i.e., cytokine responses to simulated infection), and parasite burden (i.e., avian malaria), also differ among populations, but in more complex ways. Collectively though, patterns support the hypothesis that animal invasions are somewhat deterministic, as some traits and/or trait combinations appear to facilitate range expansion. molecules that regulate seasonal reproduction in birds.
The immune system senses challenges from pathogens, orchestrates protective cellular and humoral responses, and adjusts the endocrine milieu to support self-maintenance instead of growth or reproduction. In mammals, the dominant cytokines that influence the endocrine system are tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1) and IL-6. Growing male Japanese quail (Coturnix japonica) were used to examine the effects of avian cytokines on stress, growth and reproductive-related hormones. Consistent with results in chickens and turkeys, TNF-α could not be found at either the protein or mRNA levels in quail. We used the ligand-binding domain of the chicken IL-1 type-I receptor (sIL-1R) to neutralize IL-1 activity in vivo. sIL-1R was produced in Pichia pastoris and was previously shown to block IL-1 activity in vitro (Poultry Sci 74: 160). Co-injection of sIL-1R Quail with S. typhimurium LPS revealed that IL-1 contributes 72% and 81% to the increase in corticosterone and ACTH, and 51 and 66% to the decrease in growth hormone and testosterone induced by LPS. IL-6 likely contributes much of the remainder of these changes because plasma IL-6 levels increase following LPS challenge and injection of IL-6 causes changes in the levels of each of the hormones that are similar to those caused by IL-1. Thus, IL-1 and IL-6, but not TNF-α, are important regulators of stress, growth and sexual development during an immune response. These cytokines appear to mediate the tradeoff between protection and self-maintenance in quail.

Bursal growth Hormone (GH) can inhibit apoptosis by the PI3K/Akt pathway
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GH has many effects on the immune system. Previously, our group described evidence about its participation in B cell differentiation and maturation in bursa of Fabricius (BF). We have shown that GH is produced in the BF and suggest that it may act as an autocrine and/or paracrine modulator. The time course of mRNA and GH expression in the BF suggests that GH may be involved in development and involution of the BF, since GH is known to be present mainly in B lymphocytes and epithelial cells. In addition, as GH is anti-apoptotic in other tissues, we assessed the possibility that GH promotes cell survival in the BF. This work was focused on determining the mechanism by which GH can inhibit apoptosis of B cells and if the PI3K/Akt pathway is activated. Bursal cultures were treated with different GH concentrations (0.1-100nM). The results showed that 10nM GH treatment significantly increased viability (+A16.7±0.67%) compared with the control. Caspase-3 activity decreased to 40-20% in these cultures. Akt phosphorylation and Bcl2 expression were increased with 10nM GH. When a PI3K/Akt pathway inhibitor (10 μM wortmannin) was added, it blocked GH effects. Together, these data indicated that GH antiapoptotic effects are mediated by PI3K/Akt.

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In birds, the importance of growth hormone (GH) in growth is unclear. Ontogenetic changes in circulating GH concentrations are inversely related to age and body weight and positively related to weight gain, suggesting a causal relationship. GH concentrations in fast and slow growing strains are, however, poorly correlated with growth rate, as is the experimental suppression or enhancement of growth rate. Similarly, while growth is suppressed by GH immunoneutralization or hypophysectomy, it is not abolished and only partially restored by GH treatment. Exogenous GH is similarly only minimally effective in inducing growth in pituitary intact birds, possibly reflecting a lack of IGF-1 production or a downregulation of tissue GH receptors (GHRHs). GH dysfunctions can result in dwarfism, although this does not impair hatching weight. Embryonic growth is also independent of pituitary GH as it continues after decapitation. It also occurs prior to the development of the pituitary somatotrophs and the presence of GH in circulation (in the last third of incubation). Embryonic growth is not, however, in the absence of GH, as it is abundantly expressed in many extrapituitary tissues in which it may have autocrine or paracrine roles. The role of GH in avian growth is therefore uncertain.

Molecular characterization of the receptors for chicken GHRH, PrRP and their related peptides: An emerging evolutionary history
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Growth hormone-releasing hormone (GHRH) and its structurally related peptides, including GHRH-like peptide, PACAP, VIP, PHI, secretin, glucagon and GLP1 belong to the glucagon superfamily. Prolactin-releasing peptide (PrRP) and its structurally related peptide, C-RFamide peptide (C-RFa), belong to the RFamide peptide family. These peptides are reported to play critical roles in many physiological processes in vertebrates, including the regulation of pituitary functions, glucose homeostasis, food intake and gastrointestinal activity, and their actions are mediated by specific G protein-coupled receptors. In contrast to the extensive studies in mammals, the identity and functionality of the receptors for these bioactive peptides remain largely unknown in birds. In our recent studies, using chicken as an experimental model, we identified and functionally characterized the receptors for these peptides, including two receptors for GHRH, a receptor (GCG2R) for the novel glucagon (GCG2), and three receptors for C-RFa. Molecular characterization of these receptors establishes a basis to elucidate the roles of these peptides in birds. Meanwhile, identification of the novel receptors for GHRH, PrRP and their related peptides including GHRH-R2, GHRH-LPR, GCG2R, PrPR-R2, and C-RFaR in chickens, in contrast to their absence in mammals, provides important clues to the evolutionary history of these ligand-receptor pairs in vertebrates.
[O12-1] Modulation of chicken muscle protein metabolism by n-3 PUFA in relation to insulin sensitivity
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The n-3 polyunsaturated fatty acids (PUFA) are crucial for health, normal development and organ functioning in vertebrates. Their effects as regulators of lipid and glucose metabolism are well documented. They also seem to modulate protein metabolism, especially by acting on insulin sensitivity. Our aim was to investigate the role of n-3 PUFA (precursor linolenic acid ALA or Long Chain LC-PUFA) on growth and protein metabolism in chickens, by focusing on their potential function as co-regulators of insulin anabolic signaling cascade. Ross male broilers were divided into 3 dietary treatments. Diets were isoproteic (22% CP), isoenergetric (3000 kcalME/kg) and had similar lipid supply (6%) with different lipid sources: oleic sunflower oil rich in monounsaturated fatty acid 18:1 (control group), fish oil rich in LC-PUFA, and rapeseed and linseed oils providing ALA. At 21 days, we compared the effect of ALA or LC-enriched diets on growth and body composition. We next studied insulin sensitivity in the Pectoralis major muscle of chickens submitted to intravenous insulin injection or not. Our data indicated that only ALA-enriched diet may improve insulin sensitivity, with a higher activation of the insulin-induced Akt/S6K1/S6 pathway involved in mRNA translation into proteins, thereby potentially increasing muscle protein synthesis and growth.

[O12-2] Light spectra affect growth in meat type birds both in ovo and post hatch. Broilers and turkeys photostimulated in ovo with green light, gained more weight than birds incubated under dark conditions. We found that in ovo photostimulation stimulated proliferation and differentiation of satellite cells promoting effect on muscle fibers uniformity in early and post-hatch period. How does in ovo photostimulation affect post-hatch muscle growth? We didn’t detect any effect of green light on cultured myoblasts derived from standard E17 embryos and 3-day-old chicks. We suggest that green light indirectly affects myoblast proliferation by activating the endocrine system via the retinal or extra-retinal photoreceptors. We showed higher expression of GH receptor mRNA in satellite cells derived from green light photostimulated chicks plasma GH levels and IGF-I levels in muscle tissue, relative to the dark chicks in early and post-hatch. Combinations of in ovo and post-hatch green light photostimulation to broilers and turkeys did not cause synergetic effect on growth. We found that in ovo green light photostimulation suppresses green and red opsins gene expression three days before hatching, while red light enhances their expression. This down-regulation lasted up to 9 days post hatch, suggesting a possible epigenetic effect.

[O12-3] The effect of monochromatic photostimulation on growth and development of meat type birds
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Light spectra affect growth in meat type birds both in ovo and post hatch. Broilers and turkeys photostimulated in ovo with green light, gained more weight than birds incubated under dark conditions. We found that in ovo photostimulation stimulated proliferation and differentiation of satellite cells promoting effect on muscle fibers uniformity in early and post-hatch period. How does in ovo photostimulation affect post-hatch muscle growth? We didn’t detect any effect of green light on cultured myoblasts derived from standard E17 embryos and 3-day-old chicks. We suggest that green light indirectly affects myoblast proliferation by activating the endocrine system via the retinal or extra-retinal photoreceptors. We showed higher expression of GH receptor mRNA in satellite cells derived from green light photostimulated chicks plasma GH levels and IGF-I levels in muscle tissue, relative to the dark chicks in early and post-hatch. Combinations of in ovo and post-hatch green light photostimulation to broilers and turkeys did not cause synergetic effect on growth. We found that in ovo green light photostimulation suppresses green and red opsins gene expression three days before hatching, while red light enhances their expression. This down-regulation lasted up to 9 days post hatch, suggesting a possible epigenetic effect.

[O13-1] The endocrine control of energy homeostasis in chickens
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Several hypothalamic nuclei expressing (an)orexigenic neuropeptides play key roles in regulation of appetite and energy expenditure. In avian species, the Neuropeptide Y (NPY) neurons in the infundibular nucleus co-express Agouti Related Peptide (AgRP) and stimulate food intake. On the other hand, infundibular pro-opiomelanocortin (POMC) and Cocaine and Amphetamine-Related Transcript (CART) have anorexigenic properties. Candidate second-order neurons include those that express orexins and Melanin-concentrating hormone (MCH) in specific hypothalamic nuclei and these peptides might also be involved in appetite regulation. The role of hypothalamic AMPK (5'-AMP-activated protein kinase) in energy homeostasis in avian species needs to be established. We have therefore initiated a series of studies in order to investigate the effects of fasting and refeeding on hypothalamic phosphorylated AMPKα levels on AMPK protein levels and on (an)orexigenic neuropeptide gene expression. Taken together, the parallel increases in hypothalamic p-AMPKα levels with upregulation of first-order AgRP and NPY and second-order MCH and orexin gene expression due to fasting, followed by a return to normal levels after re-feeding, suggest that the appetite-regulating effect of AMPK is at least in part mediated by orexigenic neuropeptides (NPY, AgRP, MCH and orexins) rather than anorexigenic pathways (POMC and CART).

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The hypothalamus integrates peripheral signals to regulate food intake and energy metabolism. However not all genes that regulate food intake and metabolism are known. Studies were undertaken to identify new candidate genes that may function in these processes. Hypothalamic gene expression was characterized in fat and lean genetic lines of chickens. Differences in hypothalamic mRNA levels for nine genes involved in glucose metabolism were identified, suggesting that differences in glycolysis and glucose sensing within the hypothalamus might alter the set point for subsequent body composition. Hypothalamic gene expression was also analyzed in fed and fasted chicks to elucidate genes and pathways regulated by feeding and fasting. Pathway analysis predicted a network of genes involved in neuropeptide/neurotransmitter signaling. To confirm the functionality of this predicted gene network, hypothalamic neurons from fed and fasted birds did culture in the presence of neuropeptide Y, somatostatin, n-melanocyte stimulating hormone, norepinephrine, and L-phospho-serine, and mRNA levels for target genes were measured. Results confirmed functional relationships among members of the predicted gene network that were dependent upon the nutritional state of the donor animals (fed vs. fasted). The genes identified may function in the hypothalamus to control feed intake and energy metabolism in birds.
Several neuropeptides possessing the RFamide motif at their C-termini (designated RFamide peptides) have been characterized in the hypothalamus of a variety of vertebrates. Among the RFamide peptide groups, however, only LPXRFAamide peptides, including gonadotropin-inhibitory hormone (GnIH), have been characterized in the avian brain. We sought for the presence of other RFamide peptides in the avian hypothalamus. We identified a cDNA encoding an RFamide peptide orthologous to 26RFa in the hypothalamus of the Japanese quail. The deduced quail 26RFa precursor consisted of 120 amino acid residues, encoding one RFamide peptide with 27 amino acids. Furthermore, mass spectrometry analysis revealed the presence of a peptide exhibiting the mass of mature 26RFa, indicating that the peptide is actually produced from the precursor in the diencephalon. 26RFa-producing cell bodies were localized in the anterior hypothalamic nucleus in the brain. Synthetic 26RFa increased intracellular Ca²⁺ concentration in HEK293T cells transfected with the chicken G protein-coupled receptor, GPR103. Intracerebroventricular injection of 26RFa in broiler chicks stimulated feeding behavior. These data provide the first evidence for the occurrence of the peptide 26RFa in the avian hypothalamus and indicate that this peptide exerts orexigenic activity.

Because chickens are members of the closest taxonomic outgroup to mammals, using them to study appetite regulation is not only advantageous for understanding avian physiology, but also to better the understanding of evolutionary aspects of appetite regulation across species. Compared to other non-mammalian vertebrates, much is known about food intake regulatory mechanisms in class aves, especially so in chickens. A major focus of our group is to study appetite in unique chicken models resulting from long-term divergent selection for high (HWS) or low (LWS) body weight. Members of the LWS line are hypophagic and some exhibit anorexia whereas all members of the HWS line are compulsive eaters that become obese. We have tested many neuropeptides in these lines including melanocortins, leptin, neuropeptides S, AF and Y, corticotrophin-releasing factor, galanin, calcitonin, calcitonin gene-related peptide, beta-endorphin, prolactin-releasing peptide and ghrelin. For some of these neuropeptides, response thresholds and magnitudes differ between lines. For others no differences in response exist, and in some cases one line does not respond. Such results demonstrate genetic variation in neuropeptide-induced appetite-related responses and suggest implications to other species.

In food restricted chickens, we have demonstrated that AGRP gene expression in the basal hypothalamus is increased and that it differs between birds at the same body weight but with a different recent history of food availability. In contrast, POMC levels remained unchanged. To put this into perspective we have examined the expression of AGRP and POMC in situations of natural anorexia in chickens using real-time PCR. Two anorexia models were examined: stress (cages to pen transfer) and incubation behaviour. Because these reductions in food intake were ‘voluntary’ we hypothesised that AGRP levels might be low or unchanged, concomitant with a reduction in drive to eat. Unexpectedly, in both situations, AGRP mRNA levels in the basal hypothalamus were significantly greater compared to control animals. In the case of the stress induced anorexia POMC expression was significantly increased. Collectively, these findings suggest that AGRP expression is a valuable integrated measure of hunger state when feed restriction is enforced, but in natural anorexias there remains a potential orexigenic drive which must be countered by anorectic factors. In the case of stress this could be from increased POMC expression but in the case of incubation the inhibitor remains unknown. 

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Investigations into the neural mechanisms underlying complex male aggressive and reproductive behaviour in free-living wild birds are important as they provide a necessary compliment to captive studies; particularly since the context-dependency of complex behaviours is now recognized. We now have evidence that neuroendocrine adaptations underlie the unique behaviour required to maximize survival and reproductive success. Arctic-breeding birds, such as the white-crowned sparrow (Zonotrichia leucophrys gambelli), adapt their behaviour to optimise reproductive success in a very short breeding season. These birds are behaviourally insensitive to experimentally elevated testosterone and this testosterone insensitivity must require rapid and dynamic changes in the neuroendocrine system. Studies quantifying changes in the expression of aromatase, androgen and oestrogen receptors in the white-crowned sparrow brain have revealed that oestrogen-dependent rapid transitions in aggressive behaviour are mediated by differences in neural oestrogen synthesis. Such a mechanism appears to also exist in lower latitude breeding birds such as the highly aggressive song sparrow (Melospiza melodia morpha). Song sparrows are aggressive year round, except during molt, and changes in aromatase expression in specific brain regions (such as the ventromedial nucleus of the hypothalamus) are correlated with aggression suggesting that there is also oestrogen-dependent regulation of seasonal aggression in this species.


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Plasma testosterone (T) in male birds can be rapidly (i.e., within minutes to hours) modulated by environmental factors, in particular acute stressors, but the effects of these stressors and their underlying mechanisms of action are poorly characterized. In free-ranging adult male Rufous-winged Sparrows, *Zonotrichia carolinensis*, and other songbird species, 10-30 minutes of capture and restraint stress decreases plasma T by 50-90%. In Rufous-winged Sparrows, this decrease persists for at least six hours after birds are released on their breeding territory and is not associated with a change in plasma luteinizing hormone (LH). Furthermore, acute stress does not alter the plasma LH response to N-methyl-D,L-aspartate or gonadotropin-releasing hormone administration, or the plasma T response to a LH injection. These results suggest that the effects of acute stress are mediated directly at the testicular level, and not at the hypothalamic or pituitary gland level. Additionally, stressors decrease their plasma T in response to acute stress relatively more when their baseline plasma T is high than low, indicating that the stress response is baseline plasma T-related. These data inform on the factors that control plasma T in free-ranging birds and help account for naturally occurring individual variation in plasma hormone levels.

**[S14-3] Gene expression underlying the “decision” to initiate egg-laying**

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The ‘decision’ by female birds to begin egg-laying is a pivotal physiological time point in reproduction. Once rapid final maturation of follicles begins, including the incorporation of yolk, the female is committed physiologically to a major energetic investment. While decades’ worth of ecological data correlate lay dates in the field with environmental variables (e.g., temperature), and experimental work demonstrates advancement or delay of lay dates, we understand very little about the physiological mechanisms regulating this major life-history transition. We identified key genes in the hypothalamus, anterior pituitary gland, liver and developing ovarian follicles involved in activation of the reproductive axis and measured their expression in female European starlings (*Sturnus vulgaris*) exposed to natural changes in photoperiod in outdoor aviaries. We manipulated social information by depriving one group of direct physical contact with males, but not visual and auditory contact, while another group was housed together with males. Females without males had not begun to incorporate yolk into follicles at the same time that controls were actively yolking and beginning to lay eggs. Introduction of males to previously delayed females stimulated rapid follicular growth and ovulation within 8 days. These data will help to illuminate how this important physiological transition is orchestrated.

**[S14-4] The influence of temperature on endocrinology and timing of reproduction in great tits (Parus major)**

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While many bird species advance their seasonal timing in response to global warming, we know little about the causal effect of temperature. Since 1999, we perform experiments in climate-controlled aviaries to investigate how temperature affects reproductive development, timing of egg laying and onset of molt in male and female great tits. We use both natural and artificial temperature patterns to identify the temperature characteristics that matter for the birds. Our results show that temperature has a direct, causal, effect on timing of reproduction, and in particular, that it is the pattern of increase rather than the absolute temperature that birds use. Surprisingly, the pre-breeding increase of luteinizing hormone, prolactin and gonadal size were not affected by temperature. The timing of reproductive development did not correlate with the onset of laying, suggesting that these stages are fine-tuned by different factors. Yet, we found similarities between siblings in the timing of both the onset of reproduction and the underlying mechanisms. This demonstrates that there is heritable genetic variation on which natural selection can act, but given that the neuroendocrine system does not seem to be regulated by moderate temperature changes, birds might become constrained in their advancement of breeding under climate change.
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Variability of maternal hormone levels in eggs is controlled by the mother and environment, she experiences. Recently, we showed strong genetic effects on androgen transfer into the egg through the selection of high (HET) and low (LET) egg testosterone lines of Japanese quail. As females may invest more resources in their eggs sired by high-quality males, it is possible that HET males contribute to elevated yolk androgen deposition of their female partners. Inspecting the differential allocation hypothesis, we examined, whether LET females can increase their yolk testosterone deposition when paired with HET males. Eggs were collected from females housed with males of the same line (MATCH pairs), without males and then after male switching between lines (CROSS pairs). Exchange of males did not affect line differences in yolk testosterone but females of both lines in CROSS pairs laid eggs containing lower testosterone concentrations than females in MATCH pairs. We conclude that high egg testosterone content in the HET line is not primed by the phenotypic quality of HET males. Decreased yolk testosterone in CROSS pairs indicates that the social manipulation was perceived by females as a disturbance factor with possible negative consequences.

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[S15-1] Hormones as mediators of life history trade-offs
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Hormones coordinate many aspects of life history strategies and are critically involved in the environmental adaptation of complex traits. However, there is a need to investigate individual variation and plasticity in physiological traits to achieve a better understanding of the magnitude, mechanisms and functional significance of the flexibility in endocrine systems. We monitored individual great tits, Parus major, over a period of three years and measured their corticosterone and prolactin levels, reproductive fitness and local survival. We show that high prolactin levels are an indication of close proximity to lay, baseline corticosterone concentrations are positively correlated with parental feeding rates, and high body condition correlated with high local survival rates. Furthermore, we experimentally elevated baseline corticosterone levels two weeks before egg-laying to test if corticosterone casually regulates reproductive decisions and confirmed the correlative results. We will discuss the results in the context of the mechanisms that govern the expression of phenotypic variance.

[S15-2] Responses of associated hormonal, behavioral, and morphological traits following colonization of a novel environment
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Because organisms are not random assemblages of traits, efforts to understand evolutionary and developmental changes in response to novel and variable environments must consider the underpinnings and implications of trait correlations. Hormonal systems are uniquely situated to play a key role in constraining or facilitating phenotypic responses for suites of traits. We examined how associated hormonal, behavioral, and morphological characteristics have diverged following a unique colonization event, in which a typically montane forest-breeding songbird, the dark-eyed junco (Junco hyemalis), recently became established in a novel urban and climatically mild habitat in coastal San Diego, California. We used field studies and a common garden experiment to evaluate responses of both HPG and HPA endocrine axes. We found evidence for reduced peak, but seasonally prolonged, testosterone expression in the colonist population, accompanied by changes in extra-pair sexual behavior, territorial aggression, parental care, and plumage ornamentation. Also, we found that the colonist birds exhibited attenuated corticosterone responses to stress, along with correlated changes in boldness and tameness behaviors. Common garden studies indicate both plasticity and genetic or early developmental changes have occurred. Together, these studies provide unique evidence addressing the role of hormonally mediated suites of traits in adaptation and diversification.
### [S15-3] Orchestrating trade-offs between reproduction and survival in opportunists

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Birds coordinate life cycle events with changing environmental conditions. For many this leads to seasonal changes in investment in reproduction relative to survival-enhancing processes such as plumage molt and immune function, and in endocrine activity (HPG and HPA axes) that orchestrates these changes. In a few cases, however, unpredictable conditions favor opportunistic schedules. Opportunism is assumed to result from selection favoring an ability to exploit rare and unpredictable favorable conditions (“best of a bad situation” hypothesis). We suggest an alternative: Species that are good at locating favorable conditions may breed opportunistically not because they must, but because they can (“cream skimming” hypothesis). This hypothesis predicts endocrine mechanisms and life history investment trade-offs that differ from those based on the conventional view. “Cream skinners” should be more likely to invest in survival-enhancing processes (molt, immune function, HPA responsiveness) at the expense of current reproduction. Taxa making the “best of a bad situation” should invest in reproduction at the expense of survival when a reproductive opportunity arises and maintain an ability to initiate breeding at any time. We present data indicating that some opportunists may match the conventional “best of a bad situation” scenario while others may be better categorized as “cream skinners.”

### [S15-4] Stress and cognition: Does corticosterone underlie the detrimental effects of stress on vocal learning?

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The development of the songbird brain and consequent song production is known to exhibit considerable plasticity in relation to environmental conditions. Whilst stimulation enables neural development and complex cognitive abilities to develop, environmental challenges cause stunting during periods of neural growth and reduce the capacity of animals to perform complex cognitive tasks. Vocal learning in birds offers a unique chance to test the mechanisms underlying these effects, as the neural substrates controlling song production are well defined. Here, we have tested the contribution of sex and genetic background to the ability to withstand the effects of environmental stress. Using a partial cross fostering experiment in combination with a food restriction regime we show that male zebra finches are more robust to the effects of environmental stress than females. Our results also show that corticosterone may mediate the detrimental effects on growth and development. For the first time we estimate the true heritability of the song system. We also suggest that exposure and response to early developmental stress may have long term consequences for lifetime fitness. Song has evolved as a condition-dependent indicator of fitness and that mechanisms favouring stress-resistance to protect the brain will be favoured.

### [S15-5] The effect of latitude on photoperiodic control of gonadal maturation and molt

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Birds use the annual cycle in photoperiod to time the endocrine changes necessary to orchestrate the key life history stages. Obviously, changes in photoperiod differ at different latitudes. Field data show that within species, breeding tends to start earlier at lower latitudes but that molt appears to occur at a similar time. Molt can be used as a surrogate for the end of breeding and the onset of photorefractoriness. Experimental studies with birds held under annual cycle in photoperiod simulating different latitudes confirm the pattern of earlier gonadal maturation at lower latitudes but similar times for gonadal regression and molt. This indicates that the timing of photorefractoriness is not dependent on absolute photoperiod or even the rate of change in photoperiod. This also implies that there may not be a fundamental difference between species showing absolute and relative photorefractoriness. However, earlier maturation and breeding may simply be the consequence of longer ambient photoperiods at lower latitudes before the equinox, when most gonadal maturation occurs. Thus genetic differences in responses to photoperiods in populations at different latitudes are not required to explain earlier breeding at lower latitudes.

### [O15-1] Corticosterone dynamics linked to personality in artificially selected and wild great tits (Parus major)


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A central hypothesis in the study of animal personality posits that individual differences in behavior co-vary with the reactivity of the hypothalamic-pituitary-adrenal axis. We used great tits (*Parus major*) artificially selected for divergent personalities (‘fast-bold’ and ‘slow-shy’ explorers) to examine the relationship between personality and plasma corticosterone (CORT). We found support for the prediction that behaviorally inhibited birds (slow-shy) would exhibit elevated stress induced CORT, providing evidence of correlated selection on the HPA axis. Next, using natural variation in personality seen in the wild we examined the temporal dynamics of circulating CORT. Here we predicted that behaviorally inhibited birds would exhibit a CORT response characterized by a rapid onset and a prolonged duration. On separate occasions, we captured wild birds from a study population in the Netherlands for the measurement of personality (in the lab) and plasma CORT (in the field) at baseline (<3 min) and stress-induced time points (30 and 90 min). We found support for both of our predictions thereby providing evidence for a link between animal personality and the initiation and termination phases of the endocrine stress response. Together our results suggest both indirect and potentially direct roles for glucocorticoids as physiological mechanisms contributing to personality variation.
Abstracts
(Poster Presentation)
In ovo leptin administration inhibits chorioallantoic membrane angiogenesis in female chicken embryos through the STAT3-mediated VEGF pathway

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Previous studies indicate that in ovo leptin administration affects embryonic development and hatch weight in the chicken. To test the hypothesis that leptin affects embryonic growth through modifying chorioallantoic membrane (CAM) angiogenesis, we injected 0.5 μg of recombinant murine leptin into the albumen of fertilized eggs before incubation. On embryonic day 12 (E12), the number and the area of blood vessels on CAM were measured and expression of genes involved in angiogenesis was quantitated. Leptin in ovo administration decreased the total area of blood vessels and the number of small-sized capillaries on CAM of E12 female chicken embryos, which coincided with significantly decreased embryo weight on E12 and hatching weight. Vascular endothelial growth factor (VEGF) and inducible and endothelial nitric oxide synthases (iNOS and eNOS) were all down-regulated in CAM both at mRNA and protein/activity levels with reduced nitric oxide (NO) concentration in chorioallantoic fluid of female embryos. Furthermore, signal transducer and activator of transcription-3 (STAT3) was found to be diminished, associated with decreased binding of STAT3 to VEGF promoter, in CAM of leptin-treated E12 female embryos. These data suggest that in ovo leptin administration affects CAM angiogenesis and embryo growth in female chicken embryos, probably through STAT3-mediated VEGF/NO pathways.

Characterization of serotonin receptor 6 (HTR6), serotonin receptor 7 (HTR7a) and a novel serotonin receptor 7-like receptor (HTR7b) in chickens: cDNA cloning, tissue expression and functional analysis

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Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter and plays a wide range of roles in both central nervous system and peripheral tissues of mammals, including the regulation of behavior, energy balance, and gastrointestinal activity. The diverse actions of serotonin are reported to be mediated by multiple serotonin receptors. In contrast to the extensive studies on the roles of 5-HT in mammals, investigation on the roles of 5-HT in birds was impeded by inadequate information on their receptors. In this study, we cloned the full-length cDNAs encoding serotonin receptor 6 (HTR6) (433 a.a.), serotonin receptor 7 (named HTR7a in this study) (425 a.a.) and a novel HTR7-like receptor (named HTR7b) (426 a.a.) from adult chicken brain. Using a pGIL3-CRE-luciferase reporter system, we demonstrated that HTR6, HTR7a and HTR7b expressed in CHO cells could be activated by 5-HT treatment, implying that the three receptors can act as the receptors for serotonin and are functionally coupled to intracellular cAMP-PKA signaling pathway. RT-PCR assay revealed that HTR6, HTR7a and HTR7b are widely expressed in nearly all chicken tissues examined including various brain regions and gastrointestinal tract. The results from present study will contribute to our better understanding of the roles of 5-HT in birds.

Leptin regulates Pit-1α-dependent chicken GH gene expression in mammalian cells

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Leptin regulates not only feeding and energy homeostasis but also pituitary functions including GH synthesis in mammals. Although existence of leptin is controversial in birds, leptin receptor is expressed in the caudal lobe of anterior pituitary gland consisted mainly with somatotroph in chickens. To elucidate whether leptin-like protein might modulate GH gene expression in chicken pituitary gland, we analyze leptin-dependent activation of chicken GH (chGH) promoter in CHO-K1 cells stably expressing chLEPR. Leptin increased the chGH promoter activity under existence of chPit-1α. However, the promoter activation by leptin didn’t associate with STAT-binding elements in the chGH gene or activation of STAT3. Therefore, we analyzed effect of several kinase inhibitors on leptin-dependent chGH promoter activation. PI3K and p38/44MAPK inhibitors partially, but JAK2 and CK2 inhibitors completely suppressed leptin-induced luciferase activity. It was recently reported that CK2 directly activates JAK2. We found that CK2 inhibitor blocked leptin-dependent activation of chGH gene, but didn’t affect on JAK2 phosphorylation. Present results indicate that leptin may regulate GH gene expression with Pit-1α, and that is modulated by several kinases that activated by leptin. Especially CK2 may be a key kinase in regulating GH gene expression by JAK2-dependent and - independent mechanisms.

Effect of chicken STAT5b mutant on prolactin signal transduction

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STAT5 is a transcription factor which is activated by cytokines including prolactin (PRL) or growth hormone. During a process analyzing signal transduction pathway via avian cytokine receptors, we cloned a mutant STAT5b cDNA from white leghorn. The mutant chicken STAT5b (chSTAT5b) has four amino acid mutations including Arg430Cys in DNA-binding domain and Phe757Ser in transcription activation domain. Therefore, we investigated effect of mutant chSTAT5b on PRL-dependent signal transduction pathway. Both wild-type and mutated chSTAT5b were phosphorylated by PRL in CHO-K1 cells transiently expressed chicken PRL receptor. However, mutant chSTAT5b didn’t activate STAT responsive reporter vector after PRL stimulation, whereas wild-type chSTAT5b did. Site direct mutagenetic analysis of chSTAT5b showed that Phe757Ser was indispensable for PRL-induced transactivation of the target gene. In addition, mutation of Arg430Cys might modify binding affinity against STAT binding element, but not directly associated with the loss of function of the mutated chSTAT5b. These results indicates that the chSTAT5b mutant is not able to transduce PRL signal into nuclei and that was caused by one missense mutation in the transactivation domain in chSTAT5b.
Recent studies demonstrate that leptin and ghrelin are not only mediating nutritional effects of various physiological processes, but also play a role in the regulation of reproduction. In an in vitro setup we used ovarian tissue obtained from White Leghorn hens in their peak period of egg laying (24-28 weeks of age). The aim was to investigate the actions of these two peptides on basic ovarian functions and to elucidate the underlying intracellular mechanisms e.g. effects on protein kinase (PKs; protein kinase A; MAP kinase; CDC2 kinase). Moreover, the effects of recombinant human and chicken ghrelin were compared. Both ghrelin and leptin were strong stimulators of ovarian cell functions (promoter of proliferation, inhibitor of apoptosis and stimulator of steroid hormone synthesis and release). The use of pharmacological antagonists of the above PKs confirmed that leptin and ghrelin action on ovarian cells is mediated via PKs. Similar effects of human and chicken ghrelin were observed. Our data clearly demonstrate the important role of leptin and ghrelin in the physiological regulation of chicken ovarian cell functions.

Two novel Arg-Phe-NH2 (RFa) peptides, GnIH and kisspeptin are neuropeptides that appear critical in the regulation of the reproductive neuroendocrine axis. GnIH was first identified in avian brain, however, kisspeptin has not been identified in birds. To determine biochemically the presence of GnIH and kisspeptin in the zebra finch, the present study was conducted to isolate these two peptides from zebra finch brain. Peptides were isolated by immunoaffinity purification and only one peptide was characterized by mass spectrometry. This peptide was confirmed to be a 12-amino acid sequence from zebra finch brain. Peptides were isolated by immunoaffinity purification and only one peptide was identified in avian brain, however, kisspeptin has not been identified in birds. To determine biochemically the presence of GnIH and kisspeptin in the zebra finch, the present study was conducted to isolate these two peptides from zebra finch brain. Peptides were isolated by immunoaffinity purification and only one peptide was characterized by mass spectrometry. This peptide was confirmed to be a 12-amino acid sequence from zebra finch brain. Peptides were isolated by immunoaffinity purification and only one peptide was identified in avian brain, however, kisspeptin has not been identified in birds.

TonEBP mediates the effects of hyperosmolality on arginine vasotocin gene expression in the hypothalamus of chick (Gallus domesticus)
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Arginine vasotocin (AVT) expressed mainly in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus is known to act as an antidiuretic hormone in chicken. AVT and its gene expression are stimulated by hyperosmolality through unknown mechanisms. In this study, we examined the role of the transcription factor hyper-tonicity enhancer binding protein (TonEBP) in the regulation of the AVT gene. The results showed that TonEBP mRNA expression levels increased at 1 hr after salt-loading treatment in the hypothalamus and that this increase preceded that of AVT mRNA expression. Intracerebroventricular injections of TonEBP antisense oligonucleotide before the salt-loading treatment inhibited the increase in AVT gene expression. Electrophoretic mobility shift assay demonstrated that TonEBP bound to tonicity-responsive enhancer element (TonE) motif in the chicken AVT promoter. TonEBP-immunoreactive neurons localized in PVN and SON. These results, all together, suggest that the transcription factor TonEBP may be involved in the regulation of AVT gene expression in response to hyperosmotic environment in chicken.
Our previous studies indicated differential expression pattern of CRH and AVT receptor subtypes by stress and importance of vasotocinergic regulation of the stress response. To identify possible antagonists for VT4R, a 3D structural model for VT4R was built using a SYBYL platform and subjected to multiple steps of energy minimization with the explicit inclusion of water molecules. The modeled structure of VT4R was validated by PROCHECK analysis. In silico screening for antagonists of VT4R using molecular docking analysis showed that the VT4R antagonists, SR-49059, OPC-21268, H-5350, H-6722 were bound to the receptor with high affinity. The antagonists showed a common binding interface with vasotocin on the VT4R receptor. In chicken primary anterior pituitary (AP) cells, dose and time dependent effects of CRH and AVT treatments resulted in maximum stimulation (173 % increase) of hRNA pro-opiomelanocortin (POMC) at 0.1 nM CRH and 1 nM AVT for 6 hr incubation. Pretreatment of AP cells with each antagonist significantly reduced POMC hRNA expression 55% (SR-49059), 39% (OPC-21268), 35% (H-5350), 44% (H-6722) compared to CRH/AVT induced hRNA (p < 0.05). Results indicate that tested compounds should be effective avian VT4R antagonists as well as possible anti-stress compounds in avian species.

In order to investigate the effects of kisspeptin on the initiation of egg laying in birds, juvenile female quails were employed and subjected to a consecutive injection with saline (control, Con), 10 nmol (low dosage, L) or 100 nmol (high dosage, H) kisspeptin-10 (Kp-10) dissolved in 300 μL saline for each bird via intraperitoneal (i.p.) once daily for three weeks. The egg laying rate of quails in L and H group was notably increased compared to the counterparts in Con group, which paralleled an earlier ovarian growth and an increase in circulating E2 concentration. In hypothalamus, gonadotropin-releasing hormone-I (GnRH-I) mRNA expression was markedly up-regulated, while gonadotropin-inhibiting hormone (GnIH) mRNA expression was down-regulated by high dosage of Kp-10. In pituitary, the expression of GnRH receptor type II (GnRH-R-II) but not type I (GnRH-R-I) mRNA was significantly up-regulated by 100 nmol Kp-10 administration. Moreover, compared with the Con group, follicle-stimulating hormone (FSH) gene expression in pituitary was significantly decreased in L and H group, while luteinizing hormone (LH) mRNA expression was significantly increased in H but not in L group. These results indicate that repeated peripheral Kp-10 injection can advance the reproduction of female quails through regulating the activities of HPG axis.

During incubation, there is a spread in hatching time of 30 to 48 hours for late versus early hatchers. Previous reports have shown that spread of hatch resulted in chicks of different quality. The aim of the present study was to investigate the effects of spread of hatch on broiler post-hatch performance up to day 5 and its potential mechanisms. According to the hatching time, newly hatched broiler chicks were divided into early, middle and late groups. All chicks had access to feed immediately after hatch. The late hatchers had increased feed intake and relative growth up to day 5 compared to their early hatched counterparts. Intriguingly, the hypothalamic gene expressions of NPY, AMPK were divided into early, middle and late groups. All chicks had access to feed immediately after hatch. The late hatchers had increased feed intake and relative growth up to day 5 compared to their early hatched counterparts. Furthermore, in comparison with the early hatchers, the hatching plasma T3 level in late hatchers was lower. Taken together, the spread of hatch influences post-hatch appetite and growth at least until day 5. The differences in T3 level and hypothalamic gene expression at hatch may have contributed to the distinct post-hatch performance.

In the last decade, a gonadotropin inhibitory peptide (GnIH) has been implicated in regulation of annual reproductive cycle in birds, which is suggested acting by inhibiting the gonadal development via decrease in the gonadotropin synthesis and release. We proposed that in long day breeding subtropical Indian weaver birds (Ploceus philippinus), photoperiodically regulated gonadal growth-regression cycle involves the GnIH. As the first step, we identified and characterized the cDNA encoding GnIH in the hypothalamus of sexually mature male weaver birds. First, the total RNA from the brain was extracted using tri reagent, and then cDNA was prepared. The amplicon was amplified, partially cloned and sequenced. The sequence was then compared with GnIH sequences from other species. Thereafter, GnIH transcript was localised in the hypothalamus by in situ hybridisation using DIG-labelled antisense RNA probe; controls were performed using the DIG-labelled sense RNA probe. GnIH mRNA was found solely in the paraventricular nucleus (PVN). We are currently investigating the expression of GnIH under short- and long day conditions and under natural day lengths (NDL) and in continuous light (LL). We anticipate that the results obtained will enable us better understand the underlying mechanisms involved in the control of seasonal breeding in passerine birds breeding at our latitudes. Supported by both individual and Center for Excellence grants from the Department of Science and Technology, Govt. of India.
It is known that GH plays a role in the control of both female and male reproductive tract development. Acting as an endocrine, paracrine and/or autocrine regulator, GH influences proliferation, differentiation and function of reproductive tissues. We studied GH mRNA and GH protein local expression in both the chicken testis and in ovary, and found it is distributed mainly in germinal and Leydig cells, and in follicular granulosa cells (GC), respectively. GH immunoreactivity was located in the cytoplasm and within the nucleus. We also found co-localization of GH receptor (GHR) in the same locations. Locally expressed GH was found to be heterogeneous, with a 17 kDa isoform being predominant. Both testicular and follicular cells in primary cultures were able to synthesize and release GH to the culture medium. Addition of GH (0.1, 1.0, 10 nM) stimulated progesterone production in cultured GCs in a dose-dependent manner (1.5, 2.9, 5.4 times, respectively). This effect was mediated by regulating the expression of cytochrome P450ccc mRNA. GH (10 and 1 nM) also stimulated cell proliferation in both testicular cell (70%) and GC cultures (78%) as determined by 3H-thymidine incorporation, respectively. GH immunoneutralization inhibited these effects. Data suggest local GH may have important autocrine/paracrine effects. Supported by PAPIIT-DGAPA UNAM and CONACYT, Mexico.

Cloning and characterization of prolactin regulatory element binding (PREB) in chicken and turkey
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Prolactin regulatory element binding protein (PREB) is a transcriptional factor that regulates prolactin (PRL) promoter activity in mammals. Although PREB gene is well conserved in a wide range of animals, there is no information about PREB in aves. Thus, the objective of this study was to characterize the PREB gene in chicken and turkey. Cloning of PREB genomic DNA and cDNA indicated that both chicken and turkey PREB gene is comprised of 9 exons and 8 introns and encodes a 411 amino acid protein which include 3 regions of WD-repeat domains and a transmembrane helix. Since these structural features are very similar to mammalian PREB, the function of PREB may be common or similar between aves and mammals. In turkey embryonic anterior pituitary gland, the changes of PREB mRNA level were highly correlated with the level of PRL mRNA during late embryonic stages. Moreover PREB mRNA levels were clearly increased by the short term incubation with VIP or cAMP, coincident with increases of PRL mRNA. These results suggest that the VIP stimulated transcription of the PRL gene is most likely mediated by PREB via cAMP in turkey embryo.

The dopaminergic-DAGergic system involvement in rearing behavior of gallinaceous birds
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The dopaminergic (DAergic) system plays an important role in incubation behavior of birds via the regulation of prolactin (PRL) secretion. However, the role of the DA/PRL system in rearing behavior has not been elucidated. The purpose of this study was to investigate the interrelationship between the DA/PRL system and rearing behavior in gallinaceous birds (the chicken). Incubating native Thai hens were divided into two groups. Hens in the first group were allowed to care for their chicks (rearing hens; R). In the second group, chicks were removed from the hens immediately after hatching (non-rearing hens; NR). The number of DA neurons (a marker for DAergic activity) in the ni along with PRL levels were significantly higher in R as compared to NR, 14 days after chicks were hatched (P<0.05). However, there is no significant change in DAergic activity in the ML. This study showed a novel finding for the involvement of DA/PRL system in rearing behavior of the gallinaceous birds. Supported by The Royal Golden Jubilee Ph.D. Program; #PHD/0097/2549 (YC/DC).

Effect of dioxin and polychlorinated biphenyls on estradiol secretion by chicken ovarian follicles
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Our previous studies showed AhR receptor expression in chicken ovarian follicles suggesting that dioxin and polychlorinated biphenyls (PCBs) may affect their function. The present experiment was conducted to compare the in vitro effects of TCDD, PCB126 and PCB153 on estradiol (E2) secretion by chicken ovarian follicles. Small (SWF) and large (LWF) white and three the largest preovulatory follicles (F3,F1) were isolated from the hen ovary. White follicles and fragments of theca layer of F3-F1 follicles were incubated with TCDD, PCB126 (1, 10 or 100 nM) or PCB153 (1, 10 or 100 µM), ovine LH (10 ng/ml) and LH with TCDD or PCBs. TCDD decreased basal and LH-stimulated E2 secretion from white and yellow follicles. PCB126 diminished E2 secretion only by SWF, however, it elevated LH-stimulated E2 release by while follicles and decreased by F3 and F2 ones. PCB153 increased E2 secretion in white as well as in F3 follicles, and augmented LH-stimulated E2 secretion from these follicles. These results indicate that: (i) dioxin and PCBs affect both basal and LH-stimulated estradiol production by white and yellow follicles of the laying hen, (ii) the effect of these xenobiotics depends on their concentration and developmental stage of the follicle. Supported by grant N N303 561 339 (2010-2013)
[P16] Arginine-vasotocin (AVT)-mRNA expression is sensitive to testosterone and estradiol in the bed nucleus of the stria terminalis of adult female Japanese quail

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In Japanese quail, the AVT-immunoreactivity of the medial part of the bed nucleus of the stria terminalis (BSTM) is more abundant in males than in females and it is stimulated by testosterone only in males. This differential sensitivity is the result of exposure to estradiol during an embryonic critical period when steroids differentiate permanently the brain. AVT-mRNA expression is also sexually dimorphic in quail BSTM but the differential effect of testosterone on it has not been investigated yet. In an attempt to elucidate the cellular mechanisms that mediate the differentiating effects of estradiol on the quail AVT-neural system, this study analyzed the effect of gonadectomy followed by a single injection of vehicle or gonadal steroids on AVT-mRNA expression in the BSTM. Results showed that the number and intensity of hybridization of AVT-mRNA expressing neurons were increased by testosterone in a similar way in the two sexes and that the effects of testosterone in females were largely mediated by estradiol. Injections with 5α-DHT had no effects in both the sexes. Neither testosterone nor estradiol increased AVT-immunoreactivity in females. This study indicates that the insensitivity of females AVT-immunoreactivity is not totally reflected at transcriptional level.

[P17] Arginine-vasotocin (AVT)-expression is female-biased and associated with aromatase in the bed nucleus of the stria terminalis of developing Japanese quail

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AVT-immunoreactivity is more abundant in the medial part of the bed nucleus of the stria terminalis (BSTM) of male than female quail. The BSTM of males contains AVT-immunoreactive somata and fibers whereas that of females has only AVT-immunoreactive fibers. In the BSTM of male galliforms, vasotocinergic neurons are associated with those containing aromatase (the enzyme converting testosterone into estradiol). The physiological meaning of this association is not known, but it may consist in coupling hormonal signals to display of social behaviors. Contrary to adults, developing females show more aromatase producing neurons than males in the BSTM. Therefore, we wondered whether a similar female-biased sex-dimorphism would characterize the embryonic vasotocinergic system of the BSTM. This study showed female-quail had more AVT- immunoreactivity neurons and more overall AVT-immunoreactivity (measured as fractional area occupied by AVT-immunoreactive structures) from E9 to E15. Treatment of male embryos with estradiol resulted in a partial demasculinization of AVT-expression. All the AVT- immunoreactivity neurons contained aromatase. After hatch, sex-dimorphism was only observed in adult quail and it was male-biased. This study shows quail embryos may represent a useful model for studying vasotocinergic neurons and their hormonal regulation in female quail.

[P18] Selection for high egg testosterone content up regulates expression of brain-derived neurotrophic factor (BDNF) and reelin in the pallium and cerebellum of female but not male Japanese quail

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Maternal effects manifested via maternal hormones can represent an important source of adaptive phenotypic plasticity. We have studied effects of maternal androgens using genetic lines of Japanese quail selected divergently for high and low egg testosterone (HET vs LET) content. The strong genetic variance in the egg androgen content (h2=0.42) was found and young of both lines differed in their growth rate and other physiological and behavioural traits. In this study we measured expression of BDNF and reelin in the pallium and cerebellum of young (3-week-old) and mature males and females of the F1 generation. Expression was assessed by real time PCR with GAPDH as the house keeping gene. Mature HET females exhibited higher brain BDNF and reelin mRNA than LET females, with no line differences in adult males. In young, higher expression of BDNF in pallium of HET than LET line and no change of reelin were found. The presence of BDNF and reelin in both structures suggests their physiological role in the mature brain especially in females. Females hatched from eggs with higher egg testosterone may have higher adult brain plasticity and may better cope with changing environmental conditions. Supported by APVV 0047-10 and VEGA 1/0686/12.

[P19] Sex differences in preferential response for a mate’s call in the budgerigar, a monogamous parrot

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Vocal communication between both sexes of a pair in the budgerigar (Melopsittacus undulatus) involves a variety of vocalizations including the contact call which has been suggested to function to maintain the pair bond. Both sexes of a pair respond by calling to their mates’ calls more than to calls from other birds. We examined how long the preferential response to mate call is retained after long-time separation from their mate in male and female budgerigars. Males were paired with a female for 5 weeks, and subsequently separated (without auditory/visual stimuli from the mate) for 5 weeks. We then placed each subject in a sound attenuation chamber, exposed the subject to two different stimuli (call of its mate and call of an unfamiliar bird of the opposite sex), and counted the number of calls by the subject in response to the call stimuli. Females called significantly more in response to mate call than to unfamiliar call. On the contrary, males did not show preference to a mate’s call. Thus, the preferential response to mate call is retained in females for 5 weeks after separation from their mate, but not in males.
[P20] Context-dependent calling behavior in the Bengalese finch
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Like other birds, Bengalese finches (Lonchura striata var. domestica) emit several types of calls (such as alarm, distance, distress, begging, and courship calls). Among them, courship calls are most frequently observed, and these may have an important role in social interactions. In this study, we focused on the following 2 questions: How many types of calls are present in the Bengalese finch’s call repertoire? If several types of calls are present, does the Bengalese finch produce them in a context-dependent manner? We observed the calling behavior of Bengalese finches with or without presentation of movies of other males or females. We also evaluated the influence of other conspecific birds’ calls on calling behavior by multivariate analysis of variance (MANOVA). This statistical analysis was performed on the 4 phonetic parameters (sound pressure level, first and second formant frequencies, and duration) of Bengalese finch’s call to test for 4 sets of social context with a conspecific male or female bird. Our results show that Bengalese finches have the ability to discriminate between the sexes of their species and that they produce calls in a context-dependent manner. We also established that our experimental setup can be used to alter a bird’s calling behavior.

[P21] Expression of Exon-3 and Exon4 skip splicing isoform of PRL mRNA in the bird anterior pituitary gland
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PRL and GH belong to the same hormone gene family. The expression and presence of alternative splicing forms of GH mRNA were reported in several mammalian species. However, it is unknown whether similar alternative splicing forms of PRL mRNA exist in mammals or birds. Accordingly, this study aimed to examine the possibility of presence of alternative splicing forms of PRL in the anterior pituitary gland of Birds. Total RNA was extracted from anterior pituitary gland of turkey, Japanese quail and duck. One micro gram of total RNA was used for RT-PCR. Sense primers were designed in exon2 and exon3. On the other hand, antisense primers were in exon 4 and exon5. Primers in exon2 and exon4 were used for exon3-skip whereas primers in exon3 and exon5 were used for exon4-skip isoforms, respectively. PCR products were separated by electrophoresis and the part of gel of estimated size of exon-3 or exon-4 skip splicing form was used for second PCR. Since exon-3 and exon-4 of bird PRL contain the putative glycosylation site and important amino acids for receptor binding, PRL isoforms lacking exon-3 and exon-4 may have different physiological activity and different binding activity against PRL receptor, respectively.

[P22] Expression of mRNA for gene related to fatty acid oxidation in germline chimeric chicken embryos
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Primordial germ cells (PGCs) are ancestors of germ cells and transmit parental genetic information to their offspring. PGCs transfer between embryonic blood vessels is a common technique for establishment of germ line chimeric chickens. The germ line chimeric chickens has been investigated by progeny test, and it have been obtained donor derived offspring. PGCs of white leghorn Maria line hens embryos as a donor were transferred white leghorn Laura line hens embryos as a recipient. Donor PGCs which were taken out from blood vessels of embryos at stage 12-15 were transferred into blood vessels of recipient embryos at the same stage. The hatchability of germline chimeric chickens produced by transfer of exogenous PGCs was 30%, respectively. The muscles of chicks in the eggs with transferred PGCs were removed after 20 days of incubation. A cDNA was prepared from the total RNA. The expression of gene related to fatty acid oxidation as an energy source for hatching in the manipulated embryos was investigated using real-time PCR analysis. The expression of this gene was low in manipulated embryos.tched, pinealectomized quail. Thus, epsin 5 is proposed to be one of the deep brain photoreceptive molecules that regulate seasonal reproduction in birds.

[P23] Analysis of cell cycle regulation during quail embry development
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The period that is required to complete one cell cycle is shorter in embryos than that in somatic cells, and the cleaved blastomeres can enter the next M-phase without any growth in volume. These differences between embryonic and somatic cell cycle attribute to shortened G1 phase in mammalian embryo, but the underlying molecular mechanism remained unclear in birds. We first isolated cDNA clone of quail retinoblastoma (qRb), which function in suppression of G1/S phase transition by its binding to transcription factor E2F, and examined the presence of gene product of qRb during quail oocyte development. qRb mRNA was detected in all the stages of germinal discs (I to X), whereas qRb protein was downregulated between the stages VI and IX. However, induction of cyclin D protein, which phosphorylates and inactivates Rb protein, in pronuclear stage of quail oocyte by microinjecting its cRNA inhibited their first cleavage, significantly. On the other hand, treatment of α-amanitin, RNA polymerase inhibitor, did not inhibit cleavage of quail oocyte until stage V. These results indicate that maternal mRNA-derived factor might be involved in progression of G1/S phase during early cleavage stage in quail oocyte, while E2F protein activity is inactivated by qRb binding.
[P24] Novel methodology of ICSI-assisted embryo development toward ex ovo hatching in quail
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In 1988, Perry succeeded in culturing the fertilized ovum and hatching chicks, contributing to recent avian biotechnology, but no one has been successful in hatching chicks with unfertilized ovum. This study was attempted to hatch chicks of quail using intracytoplasmic sperm injection (ICSI) into unfertilized eggs in culture system. Quail were kept under conventional conditions, egg laying time was recorded by video camera and quail relatively laying in long sequence were used. Birds were killed 70-120min after previous oviposition and oocytes were collected from the oviduct. A single sperm was injected with IP3 into oocytes and oocytes were incubated at 41.5°C under 5% CO2 incubator for 24h in10g quail egg shell, transferred to 14g egg shell and cultured. Among total 104 eggs, about 80% of embryos developed to H&H stages before 3, 13% to stages between 3 and 20, and 8% developed to over stage 20. One of the embryo survived up to H&H stage 39 at 11days culture. Since hatching normally occurs about 17days of incubation in quail, hatching chicks with our ICSI method is expected near future and this method with unfertilized egg in culture system contributes to production of transgenic bird and conservation of endangered avian species.

[P25] Fertilization and developmental capability of quail oocytes after ovulation
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Natural fertilization in birds occurs shortly after ovulation in infundibulum. Although fertilization of oocytes from magnum has been demonstrated using intracytoplasmic sperm injection (ICSI), no study has observed fertilization of oocytes from isthmus and beyond. This study was conducted to determine maintenance of fertilization competency of quail oocyte after ovulation. A single sperm was injected with inositol trisphosphate (IP3) to oocytes collected at (1) 30-90, (2) 200-240 (3) 290-360 (4) 530-600 min and (5) 23-24 hr after ovulation. Each oocyte was cultured in DMEM under a CO2 incubation with inositol trisphosphate (IP3) to oocytes collected at (1) 30-90, (2) 200-240 (3) 290-360 (4) 530-600 min and (5) 23-24 hr after ovulation. Each oocyte was cultured in DMEM under a CO2 incubation for 24 hr. Using stereomicroscope, oocytes were observed and the embryos were stained with 4.6-diamidino-2-phenylindole (DAPI) to observe cell division. The oocytes collected 30-90 min after ovulation has the highest rates of fertilization (60%) and development beyond Stage III (16.7%). In other groups, fertilization and development rates are less than 36.4% and 0%, respectively. These results indicate that the competency of fertilization of quail oocyte is maintained for all hours after ovulation although the fertility and development rates significantly decreases 90 min after ovulation. This is the first report to demonstrate fertilization capability of the freshly laid eggs which could save birds from being sacrifice for collecting eggs.

[P26] Expression of gonadotropin-inhibitory hormone (GnIH) in the ovary and shell gland of photorefractory Japanese quail
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Gonadotropin-inhibitory hormone (GnIH) is a neuropeptide via its receptors (GnIHR) localized on hypothalamic GnRH or pituitary gonadotropin secreting cells plays a negative role on the avian HPG/reproductive axis. In the present study, we have demonstrated the expression of immunoreactive GnIH (ir-GnIH) in the ovary and shell gland of photorefractory and photosensitive quail. Using GnIHR-specific antibody, ir-GnIH was observed specifically in the interfollicular spaces of the ovary, outside the theca and granulosa layer and also detected in both the myometrial and endometrial layers of the shell gland of photorefractory quail, but not in the photosensitive quail. These observations indicate that GnIH is likely to be involved in the ovarian follicular development as well as in egg-laying and absence of GnIH in the reproductively active photosensitive quail suggests the inhibitory role of GnIH even at peripheral level. Present finding suggests that GnIH is an important mediator of reproductive function not only at central level (neuro-endocrine release) but may also act at peripheral level (gonad and accessory sex organ) possibly through paracrine action. Further, studies are required to monitor the gene expression of GnIH at these peripheral sites to strengthen the above suggestion.

[P27] The search for the deep brain photoreceptors regulating seasonal reproduction
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The deep brain photoreceptors that lie outside the retina and the pineal organ enable avian species to detect light for regulating photoperiodic responses such as seasonal reproduction. Little is known, however, about the identity of these photoreceptors. Recently, we demonstrated that Opsin 5 (OPN5) is one of the deep brain photoreceptive molecules in the Japanese quail (Coturnix japonica), which is an excellent model for studying seasonal reproduction because of its rapid and robust photoperiodic response. However, it appears reasonable to conclude that multiple photoreceptors are involved in the seasonal reproduction because the expressions of other opsins have been reported in the avian brain. Therefore, we produced the action spectrum for the photoperiodic response of eye-patched, pinealectomized quails by determining the TSHB mRNA expression, which is the master control gene for seasonal reproduction. Our results showed that short-wavelength lights including the peak sensitivity of OPN5 as well as long-wavelength lights trigger the photoperiodic response. Furthermore, expression analysis of opsin genes revealed that some opsin genes (OPN4m, OPN4x) were expressed in the quail brain. Thus, it is likely that multiple photoreceptive molecules participate in the regulation of seasonal reproduction in birds.
[P28] Involvement of the circadian clock in the crowing of roosters
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The crowing of roosters has been used for informing people of the start of the morning since ancient times. In the present study, we aimed to clarify the mechanism underlying the crowing in the morning phenomenon of roosters. Crowing was observed before lighting during photo-entrainment and free-run with a period <24 h under constant dim light conditions. Although crowing was induced by light and sound stimuli, the induction of crowing was observed specifically around the start time of the subjective day when either stimulus was presented. These results may indicate that crowing of roosters is controlled by the circadian clock. In groups, the highest-ranked rooster was observed to crow first in the day followed by lower-ranked roosters in descending order of social rank. Moreover, strongly positive correlations between the times of crowing of the highest-ranked rooster and lower-ranked roosters were found. Therefore, these findings suggest that the crowing of the highest-ranked rooster behaves as an “alarm clock” in groups and lower-ranked roosters start crowing in the day according to the time and their own ranks.

[P29] Role of light intensity in induction of circadian and seasonal responses in the Indian weaver bird (Ploceus philippinus)
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Several previous investigations of Indian weaver birds (Ploceus philippinus) have shown that Indian weaver birds are a photoperiodic species, but that they lack an “absolute” refractoriness. In this respect, they appear to differ from widely investigated other passerine species. Herewith, we report results from our investigations, which centered mainly on the hypothesis whether light intensity plays any role in modifying the responsiveness to day lengths. Our hypothesis stems from the observation that at this latitude, the reproduction in weaverbirds actually coincides with monsoons when day length is long but the daytime light intensity can undergo changes of several orders of magnitude, although this is not highly predictable. We performed several experiments. In one experiment, weaver birds were initially exposed to an equinox photoperiod (12 h light per day; 12L – 20 lux, 12D – 0.5 lux) and then at intervals about 2 weeks the combinations of light illumination in L and D phases were changed to 20:2 lux, 20:5 lux, 20:10 lux, 20:20 lux, 20:40 lux and 20:100 lux. Using activity and testicular size as markers, we investigated whether the intensity of illumination is key determinant of the day and night in photosensitive weaverbirds. In further series of experiments, we have analyzed the role of light intensity in photoperiodic induction of testicular response in photosensitive birds. Birds were exposed to several photoperiods, ranging from near threshold to long photoperiods, at varying light intensity and to stimulatory photoperiods at several light intensities. Light intensity did not modulate the critical day length, i.e. photoperiods shorter than critical day length remained non-inductive. On the other hand, light intensity within a range appeared to modulate the effects of stimulatory photoperiods. Taken together, it appears that light intensity modulates the photoperiodic effects, but does not compensate for the length of photoperiod.

[P30] Mechanism of seasonal change in quail testicular size
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Birds are known to drastically change their gonadal size depending on the season. Studies in the Japanese quail especially have revealed the mechanisms underlying this regulation of seasonal reproduction. Previous studies have revealed that the activation of thyroid hormone (TH) in the hypothalamus induces seasonal gonadal development, but the detailed mechanisms of seasonal gonadal regression remain unclear. It is known that rapid testicular regression in quail requires short day/low temperature (SL) stimuli. We found that drastic testicular regression was induced by apoptosis of germ cells following an increase in serum T3 (activated TH) levels during the SL condition. It is well established that TH is involved in amphibian metamorphosis by inducing apoptosis, for example, in the tail. Sequential gene expression analysis also indicated the upregulation of T3-related genes during testicular regression. Furthermore, the effects of daily intraperitoneal administration of T3 on gene expression, apoptosis, and testicular mass resembled those of SL stimulation. Although contradictory effects of TH on seasonal breeding have been reported, we have revealed the role of TH in seasonal reproduction; in central tissues, TH is involved in seasonal testicular development and, in peripheral tissues, it is involved in seasonal testicular regression.

[P31] Do photoperiod and social cues modulate gonadotrophin-releasing hormone-I mRNA expression in male zebra finches?
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Zebra finches are opportunistic breeders that are hypothesized to maintain the hypothalamo-pituitary-gonadal axis in a tonic state of activation. Cues of a variety of sorts, including variation in photoperiod, are known to modulate reproductive activity in zebra finches. In this study, we investigated whether gonadotrophin-releasing hormone-I (GnrH-I) mRNA changes in male zebra finches maintained on different photoperiods and in the presence or absence of a female. There were 6 treatment groups: male birds, either housed singly or with a female that were maintained on one of three photoperiods: 8L:16D, 12L:12D, or 16L:8D. At day 56, brains were collected for later analysis via quantitative polymerase chain reaction (qPCR) for GnrH-I mRNA. Both daylength and social condition modulated the concentration of plasma testosterone (long day males had higher T, but males with females had lower T than those alone by day 56) though not testis volume. Despite this variation in peripheral endocrine measures, significant differences in GnrH-I mRNA were not detected. This suggests that finches, unlike some seasonally breeding temperate zone songbirds, do maintain a high reproductor potential in different stimulus conditions.
Brood patch development is one of the earliest morphological changes that occur during the preparation for incubation behavior. The body heat of hens is efficiently transmitted by the brood patch to eggs in their nests. Brood patch development is considered to be stimulated by increased levels of plasma sex steroid hormones and prolactin. However, we thought that brood patch development resembles molting because it involves feather loss. Therefore, in this study, we focused on the thyroid hormone triiodothyronine (T₃). We found that brood patch development began when T₃ secretion began to increase. Therefore, we planned a trial to inhibit brood patch development by suppressing T₃ secretion. Propylthiouracil (PTU) is a medicine used to decrease the amount of thyroid hormones produced by the thyroid gland. Hens were fed a diet containing 0.1% PTU from 1 week after the onset of laying eggs to the end of the experiment. We found that the plasma T₃ concentrations did not increase and the brood patch did not develop in the broody hens. Therefore, we concluded that T₃ is more important than sex steroid hormones and prolactin during the final stage of brood patch development.

Corticosterone induces fatty liver in laying hens by improving lipogenesis while impairing excretion

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The effect of Corticosterone on the laying performance and hepatic lipid metabolism were investigated. 26-wk-old laying hens were subjected to subcutaneously injection of corticosterone (2 mg/kg BW). The result showed that corticosterone treatment significantly decreased body weight and laying rate. The liver weight was significant increased by corticosterone treatment. The corticosterone chickens showed more vacuoles in hepatocytes. Blood concentrations of triglyceride and very-low density lipoprotein were decreased by corticosterone treatment at either feeding or fasting state. At fasting state, the gene expressions of acetyl coenzyme A carboxylase (ACC), fatty acid synthase (FAS), malic dehydrogenase (ME) were significantly upregulated by DEX treatment. The transcription levels of SCD1 and SREBP-1 were increased by DEX as well. Under feeding state, the mRNA level of FAS was upregulated while ACC and ME mRNA levels were not affected by CORT. In contrast, the gene expressions of Apo-100 and ApoVLDL-II were down-regulated by CORT regardless of feeding state. The present result indicated that the de novo lipogenesis was enhanced while the export of fatty acids to peripheral tissues was decreased by CORT treatment. The result suggests that the increased de novo hepatic lipogenesis and suppressed excretion of fatty acids result in enhanced hepatic lipid accumulation.

Smart birds stress less: Corticosterone levels are lower in species with bigger brains

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Behavioral plasticity is a key component of success in a changing environment. Behavioral plasticity and innovation have been found to be related to brain size in birds and mammals and reptiles and large brains are thought to have evolved to facilitate the information processing and the generation of adequate behavioral responses to environmental challenges. When the required cognitive apparatus is available (as it may be the case in large-brained species), and the organisms may be frequently challenged, a context-specific behavioral stress reaction (acclimation or stressor-avoidance) may be favored over an organism-wide physiological stress response. We tested this hypothesis by using an interspecific comparative approach. We have compiled the most extensive database of 122 avian species from various phylogenetical lineages containing published records of species-specific brain sizes, and circulating corticosterone levels from various life-history stages. Our study revealed a negative relationship between corticosterone concentrations and brain mass controlled for body size, which was consistent across life-history stages. This relationship was especially marked for peak levels, which can be interpreted as the magnitude of stress response within species, suggesting that big-brained birds can afford mitigating their neuro-endocrine stress response due to their cognitive capacities for coping with stressful situations.
[P36] Corticosterone and birdsong evolution: Comparative analysis of corticosterone levels and receptor expressions
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Bengalese finch (Lonchura striata var. domestica) has very complex song. The complex song is believed to have evolved by domestication because its wild ancestor white-backed munias' song was very simple. However, the mechanism of evolution is still obscure. Much evidence suggests that birdsongs are used as an indicator of male quality during mate selection. Because songbirds learn vocalizations during the juvenile stage, development of the song system are affected by developmental stress. Thus, the relaxation from environmental stress in wild conditions by domestication might evolve the complex song. We observed that stress hormone corticosterone were lower in domesticated finches than in wild finches. However, it remains unknown how stressful conditions affect song system. Here, to explore the relationship between corticosterone and song system, we analyzed detailed expression patterns of receptors of corticosterone glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in Bengalese finch brain by in situ hybridization method. We found thatGR and MR showed expression in song nuclei of juveniles and adults. Thus, these results suggest the possibility that song learning was regulated by corticosterone binding to corticoid receptors GR and MR. Song-nucleus-related expression of corticosteroid receptors might have had increasing growth condition-dependent influence on song traits.

[P37] Pre- and post-natal stress: Effects on the plasticity of stress physiology in a precocial bird
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Mammalian studies suggest that pre- and post-natal stress can induce differential effects on the Hypothalamic-Pituitary-Adrenal (HPA) axis. In mammals the physiological link between mother and offspring make it difficult to disentangle pre- and post-natal interactions. Here we used the Japanese quail to examine the effects of elevated corticosterone concentrations in the egg and/or in the endogenous circulation of hatchlings on (1) corticosterone and glucose stress responses in juvenile and adult birds and (2) oxidative stress biomarkers in different tissues during adulthood. In juveniles, post-natal treatment, regardless of pre-natal treatment, dampened HPA responsiveness in females, but not in males. Glucose responses were not affected in juveniles. In adulthood, birds previously stressed as embryos showed HPA hyper-responsiveness, regardless of sex. Importantly, this effect was not evident in birds that experienced either post-natal treatment or the combined treatments. Adult birds exposed to elevated yolk corticosterone showed opposite sex-specific basal glucose patterns compared with the other groups. Furthermore, analyses on oxidative stress revealed that early life corticosterone exposure induced a variation in the redox physiology, which was tissue-dependent. In conclusion, our results showed that early life stress can alter individual physiology in the short- and long-term depending upon the developmental stage.

[P38] Developmental stress: Reproductive consequences in the zebra finch
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The long-term effects of developmental stress on phenotype and performance are well-known. In comparison, the effects of developmental stress on fitness (e.g. reproductive success) remain largely unexplored. Developmental stress in known to decrease the quality of sexually selected traits (e.g. bird song) and, for this reason, is assumed to decrease reproductive success. However, animals exposed to developmental stress may compensate for poor quality sexually selected traits by pursuing alternative reproductive tactics such as increasing parental investment. Here, we explored the fitness consequences of developmental stress in male zebra finches (Taeniopygia guttata). Specifically, we investigated whether adult males exposed to stress during development sire fewer nestlings through extra-pair copulations, but may have greater nest success through greater investment in parental behavior. These data will allow us to empirically evaluate how developmental stress affects reproductive success and draw inferences about the role of developmental stress in shaping alternative reproductive tactics.
**[P39] Regulation of β-defensins expression by microbial components and proinflammatory cytokines in hen oviduct**

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The health of oviduct is essential for its normal functioning and production of safe eggs. Our goal was to examine innate immune function mediated by avian β-defensins (avBDs), antimicrobial peptides, which may play roles in host defense against pathogenic organisms. We identified expression of Toll-like receptors (TLRs) including TLR-4 and -21, which recognize lipopolysaccharide (LPS) and CpG ODN of microbes respectively, in the oviductal mucosa. The gene and protein expression of several types of avBDs were identified in the mucosal epithelium of oviduct. In the healthy laying hens, avBDs synthesized in the uterus were incorporated in the eggshell, probably to form a defense on the egg surface. The expression of avBDs was higher in older birds than younger, and declined in the regressed oviducts of non-laying hens. Inoculation of cultured vaginal cells with salmonella organisms increased avBD expression. Furthermore, the expression of IL-1β and IL-6 in the vaginal cells was upregulated by stimulation with LPS or CpG ODN, probably by interacting with TLR-4 and -21. Then, the expression of avBD-10 was increased by IL-1β, but not by IL-6. These results suggest that avBDs are synthesized in response to bacterial components, and cytokines may be partially involved in this process. The synthesized avBDs may play roles in local host defense against pathogens in oviduct.

**[P40] Effects of repeated stimulation by lipopolysaccharide on the antigen presenting cell-T cell associated immunity in hen vagina**

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The vagina of hen oviduct can be invaded by the microbes from cloaca. Antigen presenting cells (APCs) expressing major histocompatibility complex II (MHC-II) and T cells play important roles in host defense. The aim of this study was to determine whether multiple stimulation by antigen affects the antigen presentation-related factors and T cell subsets densities. White Leghorn hens were intravaginally injected 1 or 5 times in 10 days with saline (control) or lipopolysaccharide (LPS). The vagina of oviducts was collected 1 day after final injection. Frozen sections were prepared for double immunostaining for MHC-II and CD45, and immunostaining for CD4+, CD8+ or TCR αβ+ T cells. The vagina of hens was infiltrated with LPS or CpG ODN, probably by interacting with TLR-4 and -21. The expression of avBD-10 was increased by IL-1β, but not by IL-6. These results suggest that avBDs are synthesized in response to bacterial components, and cytokines may be partially involved in this process. The synthesized avBDs may play roles in local host defense against pathogens in oviduct.

**[P41] Gene transfer to chicks using lentiviral vectors administered via the embryonic chorionicallantoic membrane**

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The lack of affordable technologies for gene transfer in birds has inhibited the advancement of molecular studies in avian species. Recently, we have established a new approach for introducing genes into somatic tissues of chickens using the feline immunodeficiency virus (FIV) derived vector system. Recombinant FIV vectors harboring either the yellow fluorescent protein (YFP) or the recombinant α-melanocyte-stimulating hormone (α-MSH) genes, both driven by the cytomegalovirus (CMV) promoter, were injected into the chorionicallantoic membrane (CAM) of chick embryos on embryonic day 11 (E11). Transgene expression was detected in the induced chicks both one day and one month post hatch. Expression was detected by quantitative real-time PCR and for the FIV-YFP treated chicks, also by immunostaining and flow cytometry (FAX) analyses. Signals were detected primely in the liver and spleen tissues, and at a lower extent also in the kidney and brain. Using the Southern blotting technique we have demonstrated that the FIV vectors integrate into the chicken genome following infection, similarly to the reports in mammals. Since the liver is specialized in production and secretion of proteins, we suggest that it could provide an optimal target for long term studies of secreted hormones and peptides in chickens.

**[P42] Oviduct – the target tissue for growth hormone action in the domestic hen**

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The aim of the present study was to examine the expression and localisation of growth hormone receptors (GHR) as well as the in vivo effect of growth hormone (GH) on cell apoptosis in the chicken oviduct. Real-time PCR showed presence of GHR mRNA in all examined oviductal parts, i.e. the infundibulum, magnum, isthmus and shell gland with significantly lower expression in the magnum and correlated with GHR mRNA level. Administrations of GH to the chickens inhibited cell apoptosis examined by TUNEL and decreased caspase-1, -2 and -3 mRNA expressions in the oviductal parts. The results obtained suggest that chicken ovine secreted hormones and peptides in chickens.
Does oestradiol inhibit the collagen synthesis in the chicken liver?

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Hepatic stellate cells (HSCs) are primary collagen-producing cells in the liver. In the chicken liver, collagen content is larger in males than females. However, it is not known whether sex differences in collagen content are caused by gender-related sex hormone differences. In our previous study, we showed that the oestradiol levels were significantly higher in male chicken livers than in female chicken livers. The aim of this study was to determine whether oestradiol could affect the collagen synthesis of HSCs in the chicken liver. For this purpose, we treated liver tissue samples from male and female chickens with oestradiol and assessed collagen synthesis using a microarray analysis.

Egg formation occurs in the hen oviduct and steroid hormones are the key regulators in the mechanism of the egg forming process. Specifically, estrogen plays an important role in the development of the female reproductive system and production of egg white proteins. The estrogen treatment increases the expression of egg white proteins such as ovalbumin. The previous studies have shown that most of the estrogen-induced genes were greatly increased in juvenile oviduct by diethylstilbestrol (DES) treatment, which is a nonsteroidal synthetic estrogen. We examined novel transcript expression changes in the chick oviduct after an exposure to DES through microarray analysis. To confirm our microarray data, we performed reverse transcript PCR analysis and in situ hybridization for comparison of the expression pattern between ovalbumin and the novel transcripts. The results showed that novel transcripts, stimulated by estrogen treatment in chick oviduct, were detected specifically in the magnum of hen oviduct. Through further studies, we will investigate the biological functions of these transcripts and the mechanisms of their biological activities. The identified genes may facilitate the discovery of potential therapeutic peptides or proteins for the prevention and treatment of various diseases.
**[P47]** Costs and benefits of the within-clutch decrease in yolk androgen levels in zebra finches

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Yolk hormones are thought to be an important mechanism mediating adaptive maternal effects. Mothers can adjust competitive asymmetries between their offspring by differential allocation of hormones to their eggs. In many species, yolk androgen levels increase with the laying order, which is thought to benefit last-hatched offspring by increasing their begging behaviour and growth. In contrast, yolk androgen levels in zebra finches decrease with the position in the laying sequence, suggesting a double disadvantage for last-hatching offspring. This may ensure survival of at least the first-born offspring when there is not enough food, allowing last-hatching offspring to survive only if conditions are exceptionally good. To test this idea, we abolished within-clutch differences by experimentally elevating yolk hormone levels in eggs 2–6 to the level of egg 1, and assessed costs and benefits for junior offspring (eggs 2–6), senior offspring (egg 1), and their mothers. Contrary to our expectations, the treatment was detrimental for offspring from testosterone-treated eggs and had no indirect consequences for their older siblings. Also, mothers did not seem to suffer from rearing testosterone-treated clutches. This suggests that the natural pattern of within-clutch yolk testosterone allocation is beneficial for all family members also in zebra finches.

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**[P48]** Sexual maturation of testis in sex-linked dwarf chicken

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Growth hormone (GH) plays various functions such as controlling cell proliferation and development, nutrient metabolism, protein synthesis and reproduction as well as normal growth. GH actions are exerted after binding to GH receptor (GHR) localized in target tissues. The GH actions are well established in the pituitary gland and liver in mammals, although little is known about the role of GH in other tissues. Sex-linked dwarf (SLD) chicken lacking functional GHR is a good experimental model for study of GH actions. In the present study, we investigated effect of GH deficiency on testicular sexual maturation in the chicken. Testicular GH mRNA expression was high until 12 weeks old, but which was not detected in 16-weeks old to matured males. Opposite pattern was observed in the area of seminiferous tubule. Maturation of seminiferous tubule was delayed approximately 4-8 and in SLD chicken. Present results suggested that GH was directly or indirectly affected to sexual maturation of chicken. Especially, negative correlation with GH mRNA expression and area of testicular tubule was suggested that GH strongly effect on testicular development, before spermatogenesis.

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**[P49]** Gonadotropin-inhibitory hormone precursor mRNA is induced during depressed food intake in the heat-exposed chick brain

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The regulation of food intake in chickens represents a complex homeostatic mechanism involving multiple levels of control, but regulation during high ambient temperatures (HT) is poorly understood. In this study, we examined hypothalamic mRNA expression of gonadotropin-inhibitory hormone (GnIH) to understand the effect of HT on an orexigenic neuropeptide. We examined the effects of HT (35ºC ambient temperature for 1-, 24- or 48-h) on 14-d-old chicks. HT significantly increased rectal temperature and suppressed food intake, and also influenced plasma metabolites. The expression of GnIH precursor mRNA in the diencephalon was significantly increased in chicks at 24- and 48-h of HT when food intake was suppressed significantly, whilst no change was observed for GnIH precursor mRNA and food intake at 1-h of HT. Mass spectrometric analyses detected a dodecapeptide produced from the precursor polypeptide in the brain as an endogenous ligand. In situ hybridization and immunocytochemistry further revealed the cellular localization of chicken GnIH precursor mRNA and its peptide in the paraventricular nucleus (PVN) in the chick hypothalamus. These results indicate that not only a reduction in food intake but also the PVN-specific expression of GnIH, an orexigenic agent, may be induced by HT.

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**[P50]** Ontogenic profile of glucokinase and hexokinase mRNA expressions in embryonic chicken liver and muscle

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Blood glucose of the developing chicken embryo increases gradually, reaching at the end of the incubation to a level that is about twice higher than that of adult non-ruminant mammals. In an effort to understand biological implications and biochemical mechanisms underlying the high glucose level, we are conducting experiments to characterize gene expressions of enzymes responsible for glucose homeostasis. The present study was focused on hexokinase (HK; EC 2.7.1.1) and glucokinase (GK; EC 2.7.1.2, otherwise known as HK IV) that form the first step of glucose metabolism by the cells. The liver and skeletal muscle were collected from 11 to 21 day embryos (n=6) of layers and the mRNAs of HK I and II, two HKs known in the chicken, and GK were measured by semi-quantitative RT-PCR. In the liver, HK I mRNA gradually decreased during the experimental period, while GK gradually increased. In the skeletal muscle, HK I was almost stable, while GK decreased. HK II expression did not change considerably in the both organs. These results suggest a possibility that the well-established domination of GK to HK in the liver is gradually acquired during embryogenesis. Whether embryonic skeletal muscle expresses active GK protein should be studied further.
[P51] Heat stress and immunity in broiler breeders
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Most of today’s problems in broiler breeders are caused by combinations of factors such as management, stress, nutrition, immunosuppressant, and exposure to disease agents. Stress is an important cause of reduced performance and increased susceptibility to disease. Broiler breeders are subjected to frequent stress factors, and therefore it is important to have an effective management program to minimize their effects on the performance and health of the birds. Identifying and managing factors that cause stress in broiler breeders is a critical part of a successful breeding program. This paper reviews stress in poultry and examines specific causes of heat stress in broiler breeders. Several management practices and an overall strategy to prevent and reduce the effects of stress are discussed.

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Ileal lipid binding protein (FABP6) is a member of fatty acid binding protein family and has a major role in enterohepatic circulation of bile acids by mediating intracellular transport of bile acid across ilealocytes in mammals. However, FABP6 has not been characterized in birds. Thus, the objective of the present study was the cloning and determination of the tissue specific distribution of FABP6 transcripts and protein in chicken. Chicken FABP6 encodes a 128 amino acid with 66% amino acid identity to mouse FABP6. Consistent with a role in the enterohepatic circulation of bile acid, levels of FABP6 mRNA and protein increased along the small intestine with the highest levels expressed in the ileum. However, FABP6 was also expressed in the follicular hierarchy with highest levels being observed in the small yellow follicles (about 16 fold higher than in the duodenum). Levels of FABP6 were about 8 fold higher in the granulosa than in the thecal cell layer. In addition, significant concentrations of FABP6 were observed in the testes. In contrast to intestinal tissues, levels of ASBT in gonadal tissue did not correlate with FABP6. These data suggest that in addition to having a role in bile acid metabolism, FABP6 may have roles in intracellular trafficking in steroidogenic tissues.

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Neurotensin (NT) functions as a neurotransmitter and as a circulating hormone. In mammals, NT is involved in regulation of blood pressure, gastric and intestinal motility, gastric, pancreatic and biliary secretion, cell growth, and inflammatory response. However, in avian species, physiological function of NT is poorly understood. We have recently cloned cDNA for chicken neurotensin receptor 1 (NTR1) and characterized its primary structure and tissue distribution. In this study, we examined developmental changes in expression levels of chicken NT and NTR1 mRNAs in the chicken gastrointestinal tract and liver. Real-time PCR analysis of NT mRNA in the peripheral tissues of adult hen revealed preferential expression of the mRNA in intestinal tissues such as the duodenum, jejunum, ileum, and colon/rectum. The expression levels of NT mRNA in these intestinal tissues increased around hatching. The expression level of NTR1 mRNA in the liver markedly increased early post-hatch period when accumulated cholesteryl ester is rapidly metabolized into cholesterol and fatty acid. These results suggest that neurotensin produced in the intestinal tissues is involved in the regulation of the lipid metabolism in the liver at early post-hatch period.

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Dietary nutrient component, one of environments, effects on protein and energy metabolism. Little has been known about the effect of dietary amino acid content on plasma level of corticosterone (CORT) that is associated with protein and energy metabolism. The objective of this study was to examine the variation of plasma CORT level when chicks were fed a lysine-free diet. Firstly, we confirmed that there was no diurnal variation in plasma CORT level in chicks fed ad libitum a commercial diet. Then, plasma was gotten from chicks fed a lysine-free diet at one, 3, and 6 hours of feeding ad libitum the lysine-free diet. In another experiment we hourly prepared the plasma in chicks that were fed the lysine-free diet for 4 hours. Hourly food intake was simultaneously determined. Plasma CORT level was determined in RIA and ranged from 1 to 3 ng/mL at the beginning of each experiment. Plasma CORT level remained the beginning level at 3 or 4 hours after presenting the lysine-free diet, and subsequently increased by about 3-fold. Hourly food intake did not vary. It is unlikely that increased plasma CORT due to feeding the lysine-free diet caused food intake to decrease.
[P55] Effect of intracerebroventricular injection of orexigenic peptide on feeding behavior in dwarf chicks
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Dwarf chicks (S23MA line) have been developed from one of the White Leghorn strains (MA line). They have mutation in gene of growth hormone receptor on the Z chromosome. Since growth hormone cannot work properly in dwarf chicks, their body size is smaller than that of normal chicks (MA line). In addition, dwarf chicks show less food intake than the normal chicks. However, the cause of less food intake is still unknown. Then we hypothesized that the difference in feeding behavior may be due to the change in the function of orexigenic peptide in dwarf chicks. Therefore, we examined the effect of intracerebroventricular (ICV) injection of orexigenic peptides such as neuropeptide Y (NPY), somatostatin (SST), and β-endorphin (β-END), on food intake in dwarf chicks. ICV injection of NPY increased food intake of both strains, but the orexigenic effect in dwarf chicks was similar to normal chicks. On the other hand, SST increased food intake more in dwarf than in normal chicks. The decrease in food intake between the normal and dwarf chicks was more evident on β-END. The above results suggested that these peptides might be related to food intake in dwarf chicks.

Chickens have plasma glucose concentrations that are two times higher than that of mammals and develop resistance to insulin early in life. So far, little is known about the ontogeny of the glucose-insulin metabolism during late embryonic development. The aim is to explore this metabolism by the injection of insulin in the embryonic blood. Broiler embryos were injected on embryonic day (ED) 16 or 18 with 200 µl of insulin (1 mg/g embryo weight) dissolved in a saline solution. An injection of 200 µl saline was used as a sham group. Blood and tissues were taken every two hours after injection (until 23 hours thereafter) and plasma glucose concentrations were determined. Results showed that glucose concentrations have reached a minimum level 7 hours after injections on ED16 and 18 and the decrease was more pronounced on ED16. An increase to a basal level was more slowly on ED16, when compared with that of ED18. As the changes were more drastic on ED16, it seems they were more sensitive to insulin on ED16 than on ED18. Thus, embryos may develop a certain resistance to insulin already during late incubation.

[Vasoactive intestinal peptide (VIP)]

Vasoactive intestinal peptide (VIP) is expressed in peripheral and central nervous systems in vertebrates and has several physiological functions. Since intracerebroventricular (ICV) injection of rat VIP (VIP) inhibits feeding behavior in chicks, VIP is related to the feeding regulation in chickens. However, it is reported that rVIP is less potent in evoking cyclic AMP response in the hypothalamus of chicken than that of chicken VIP (cVIP), suggesting that cVIP should be used to clarify the effect of VIP on feeding in chicken. The purpose of the present study was to investigate whether cVIP affected feeding behavior of chick (Gallus gallus). ICV injection of cVIP decreased food intake of chicks. As expected, rVIP had less anorexigenic effect in chick than that of cVIP. We also found that ICV injection of cVIP stimulated corticosterone release and decreased the dienechophalic mRNA expression of corticotropin-releasing hormone (CRH). These results suggested that cVIP might be related to the activation of hypothalamus–pituitary–adrenal axis (HPA). This hypothesis was supported by the fact that the anorexigenic effect of cVIP was attenuated after co-injection of cocaine, a CRH receptor antagonist. In conclusion, the present study suggests that cVIP may have a role in the regulation of feeding behavior via HPA in chicks.
**[P59]** Antisense reduction of neuropeptide Y Y1 receptor modulates energy expenditure in chicks

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Neuropeptide Y (NPY) is one of the most potent neuropeptides known to enhance feeding in mammalian species and chickens. NPY appears to exert its actions via multiple receptor subtypes, and much interest has always been directed towards the postulated NPY feeding receptor. In mammals, conflicting data on the effect of NPY Y1 antisense oligodeoxynucleotides (Y1 antisense ODNs) on feed intake have been reported, describing either an increase or a decrease in feeding in antisense-treated animals. In the present studies Y1 antisense ODNs were used to investigate the functional importance of this receptor subtype in vivo in appetite and body temperature in chickens. Y1 antisense ODNs given intracerebroventricularly suppressed feed intake in ad libitum fed chicks (approximately -50% vs. control saline and scrambled sequence, respectively during 10-14 h post-administration). While rectal temperatures in Y1 antisense ODNs treated chicks were significantly above 0.5°C compared with control groups at 4, 6 and 8 h post-injection. However, leg temperatures were not different between groups during 24 h experimental period. These data suggest that the NPY Y1 receptor is implicated in feeding and thermoregulation in chick.

**[P60]** Brain insulin signaling controls feed passage in chicks

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Feeding regulation is essential for the maintenance of energy homeostasis, and has been counter-regulated with gastrointestinal function through vagus nervous activity in animals. To clarify the effect of insulin on feed passage in chicks, the chick emptying rate was investigated after intracerebroventricular treatment of insulin in the force-fed chick. The effects of central insulin on the levels of plasma monoamines and corticosterone were also examined. The amount of residual feed content of crop in insulin treated chicks was significantly higher, while that of gizzard was lower than those in control chicks (P<0.05). Regarding the estimated outflow from gizzard, it tended to be increased by insulin treatment (P<0.1). Both levels of noradrenaline and corticosterone in the treatment group were higher than those in the control group (P<0.05). These results suggest that brain insulin signal operates feed passage mediating monoaminergic and/or CRFergic pathways in chicks.

**[P61]** Gastrointestinal region-related change in motilin receptor mRNA expression and motilin response in the growing chicken

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Motilin regulates gastrointestinal motility in chickens as well as mammals. After hatching, chickens start to feed, so it would be interesting to compare motilin-induced responses at different days after hatching. Effect of motilin on isolated strips from different regions of the gastrointestinal tract was examined. Expression of motilin receptor (MOT-R) mRNA was also measured. Chicken motilin caused contraction of the crop, proventriculus, ileum and colon in region-dependent manner. The amplitude of maximum contraction was consistent with the expression levels of MOT-R. Atropine decreased the response to motilin in the proventriculus but not in the ileum, indicating the presence of muscle and neural MOT-R. Motilin-induced contraction in the proventriculus gradually decreased up to 100 days after hatching without changing EC50, but the motilin response in the ileum was almost the same from 1 to 100 days. The age-dependent decrease in the contraction was attenuated in atropine-treated proventriculus. MOT-R expression decreased depending on days after hatching but the change was more marked in the proventriculus than in the ileum. Motilin regulates gastrointestinal motility in region-specific manner. In addition, regulation of chicken gastric motility by motilin changed depending on the post-hatching day due to disappearance of neural MOT-R.

**[P62]** Effect of acute heat stress on gene expression of feed intake regulatory peptides in broiler chickens (Gallus gallus domesticus)

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Heat stress induced feed intake reduction results in a big problem in poultry production. One explanation is that the energy requirement declined significantly under high temperature, and so the poultry cut down the feed intake to avoid severe heat burden. However, the alterations of feed intake regulatory peptides during heat exposure remain unclear. Here we investigated the effect of acute heat stress (35°C, 6h, RH 65%) on the gene expression of appetite-related genes in broiler chickens (42 d old). Hypothalamic mRNA levels of neuropeptide Y (NPY), agouti-related peptide (AgRP), pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), corticotropin-releasing hormone (CRH), melanocortin receptor 4 (MCR-4), melanin-concentrating hormone (MCH), pre-orexin, cholecystokinin (CCK) and ghrelin did not significantly changed (P>0.05) in heat-exposed chickens. However, mRNA levels of ghrelin in the glandular stomach, duodenum and jejunum were increased (P<0.05) and the mRNA levels of CCK in the duodenum were decreased (P<0.05) in heat-exposed broilers. The present results indicated that acute heat stress showed little or no effect on gene expression of central appetite regulation peptides and the peripheral gastrointestinal peptides play key roles in feed intake regulation in broiler chickens under acute high temperature exposure.
[P63] Prenatal undernutrition of the chicken embryo leads to changes in plasma T3 and corticosterone levels
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In mammals, prenatal nutritional constraints are known to program offspring, resulting in an altered metabolic phenotype, characterized by increased risk of developing chronic diseases such as type II diabetes in later life. In mammals no distinction can be made between maternal and strictly nutritional effects. Chicken embryos however develop independent from the hen. Prenatal protein undernutrition was established in layer-type eggs by removal of 10% albumen at embryonic day 1 and replacing it with saline (Albumen-group), only a hole was drilled in the Sham-group and the Control-group received no treatment. At hatch, no differences in body weight could be observed. From day 3-21, chicks from the Albumen-group weighed significantly less than chicks from either the Control- or Sham-group. From day 13-21, chicks from the Albumen-group had a higher feed intake than chicks from the Control-group. The Sham-group had intermediate values. At day 7, the plasma T3 levels of the Albumen-group were significantly lower and the plasma corticosterone levels were significantly higher as compared to the Control-group. The Sham-group had intermediate levels for both hormones. These results already point to an effect of prenatal undernutrition on the thyroid and adrenal axis in post-hatch life and further investigations are currently being performed.

[P64] The proximate effects of food availability on the reproductive physiology of a Sonoran Desert songbird
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Birds use food availability to synchronize seasonal reproductive activity with local environmental conditions, but the mechanism(s) by which this cue influences the hypothalamo-pituitary-gonadal (HPG) axis remain(s) poorly understood. We examined the influence of food availability on the HPG axis of adult male Abert’s Towhees, Melozone aberti. In captivity, towhees were exposed to long days-ad libitum food consumption for two weeks, or (3) two weeks of food restriction followed by two weeks of ad libitum food, to stimulate reproductive development and randomly assigned to one of three groups: (1) ad libitum food consumption for four weeks, or (3) two weeks of food restriction followed by two weeks of ad libitum food. Two weeks of food restriction decreased body mass and cloacal protuberance (CP; an androgen-sensitive sexual characteristic) width. Reinstating ad libitum increased during the study in ad libitum birds but did not increase in food restricted or reinstated ad libitum towhees. However, at the end of the study the three groups had similar testis mass. Furthermore, food restriction decreased the area of brain gonadotropin-inhibitory hormone-immunoreactive cells. Food availability, therefore, influenced the HPG axis, but this influence varied at different points of the axis.

[P65] Does baseline corticosterone reflect energy costs? Seasonal changes in energy expenditure and baseline corticosterone in free-living red crossbills, Loxia curvirostra
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Glucocorticoids are involved in energy mobilization and stimulate catabolic processes (e.g., gluconeogenesis and lipolysis). Most models describing corticosterone (CORT) and the hypothalamic-pituitary-adrenal axis therefore predict that baseline levels of CORT are reflective of recent metabolic state. We tested this hypothesis in free-living, behaving red crossbills (Loxia curvirostra) using heart rate as a proxy for metabolic cost. Red crossbills offer a unique opportunity to compare energy costs of survival and reproduction across seasons, given their propensity to breed at high altitudes in the northern hemisphere in both winter and summer. Previous studies of free-living red crossbills found that baseline CORT does not vary across seasons, suggestive that either baseline CORT is not reflecting the higher energy costs of cold, northern winters or that energy costs are near equivalent across seasons. We took baseline CORT samples just prior to tracking individuals for 50 hours during summer, autumnal molt and winter. Average heart rate across 24 hours was higher in winter than summer, suggesting a higher metabolic cost in winter. With removal of a single outlier, our preliminary data suggest that CORT may (weak trend) reflect increasing energy demands. However, the outlier may be more revealing in that the much higher baseline CORT levels did not correspond with a high heart rate in this individual.

[P66] Mercury suppresses breeding readiness in an arctic seabird
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In the Arctic, mercury, a ubiquitous toxicant shows a dangerous trend in increasing. Because mercury can act as an endocrine disruptor, we tested whether high mercury concentration may suppress breeding readiness (to breed of not to breed) in an Arctic seabird via the disruption of lutetinizing hormone (LH) secretion, a crucial driver for breeding. To do so, we performed gonadotropin releasing hormone (GNRH)-challenges in a Svalbard population of pre-laying Black-legged Kittiwakes, a long-lived species known to show intermittent breeding. Mercury clearly affected breeding readiness, as non-breeders, both males and females, bear higher mercury levels than breeders, but did not affect egg-laying neither clutch size or breeding success. In male non-breeders, baseline LH levels decreased with increasing mercury concentration and GNRH-induced LH levels significantly increased with increasing mercury levels. Concerning female non-breeders, an opposite relationship was found with baseline LH levels increased with increasing mercury levels and similarly, GNRH-induced LH increased with increasing mercury levels. In this study we underlined for the first time in a free-living bird, a clear disruption of the pituitary, which is the brain region where LH is produced, by mercury pollution. Indeed GNRH-induced LH levels and baseline LH levels (in non-breeders only) were sharply related to mercury. As non-breeders mainly differ from breeders by hormonal levels, we suggest that other hormones like sex steroids may play an important role in the action of a toxicant and may prevent the adverse effects of mercury in breeding individuals.
Testosterone affects several traits of animals including physiology, morphology and behavior. During the breeding stage, testosterone is expected to mediate the trade-off between territorial and paternal behavior in males. The aim of this study was to investigate the relationship between testosterone and behavior in the male black redstart (*Phoenicurus ochruros*), a socially monogamous bird species that does not socially modulate testosterone. We measured baseline levels and GnRH-induced testosterone levels of males during two different substages: mating and parental care. We compared these hormone levels to territorial aggression during s simulated territorial intrusions and to feeding behavior during the parental phase. Our results show significant differences in baseline and GnRH-induced testosterone levels between mating and parental care, being higher during mating. There was, however, no relationship between baseline and GnRH-induced testosterone levels with territorial aggression or parental behavior of males. Hence, in black redstarts, testosterone levels vary during the breeding season, but the absence of a relationship between testosterone concentrations and agonistic and parental behavior suggests that testosterone may not modulate the trade-off between these behaviors.

**Testosterone and song behavior in an endemic Amazon songbird, *Ramphocelus carbo* (Thraupinae)**

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Throughout an individual’s life cycle physiological adjustments are made in response to environmental changes. At the same time different environmental cues can result in the activation or deactivation of seasonal behaviors. In oscine birds, many males show a vernal change in the song behavior that is used to defend territories and to attract mates. It is hypothesized that the increase in photoperiod stimulates gonadal testosterone secretion, which in turn increase singing activity. However most of our knowledge about song behavior comes from studies on temperate songbirds, and the role that hormones play in the regulation of seasonal behaviors in tropical species remains unclear. The Amazon Rainforest experiences little or no seasonal changes in temperature and photoperiod, but seasonal fluctuations in rainfall are common. We monitored plasma testosterone concentrations and singing activity of wild males Silver-beaked Tanager (*Ramphocelus carbo*) during the transition between the dry and rainy season in an equatorial population of the lowland Brazilian Amazon. At the meeting I will present testosterone levels in parallel with bioacoustics data and climatic registers. Because little is known about endemic Amazon oscine birds, this information has important implications for understanding the endocrine mechanisms and the environmental factors that synchronize reproductive behaviors in the Amazon ecosystem.

**Effects of food cues on reproductive development in two species of Cardueline finches**

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Birds may use a variety of environmental cues to time reproduction. It has been hypothesized that the extent to which species rely on non-photic cues for reproductive timing reflects differences in breeding schedules. Specifically, species that exhibit flexible or opportunistic breeding schedules are expected to be more sensitive to non-photic cues than are species with highly seasonal schedules. Here, we test this hypothesis by investigating the effects of changing food availability on reproductive development in two species of Cardueline finches: the flexibly breeding pine siskin (*Spinus pinus*) and the seasonally breeding house finch (*Carpodacus mexicanus*). Birds in this experiment experienced a naturally declining photoperiod while receiving ad libitum access to a maintenance pellet diet. Following winter solstice, birds were housed on a 12L:12D photoperiod and received maintenance diet only or maintenance diet plus ad libitum access to a seed mixture. In pine siskins, access to seeds had a positive effect on reproductive development. Pine siskins receiving seeds had significantly larger gonads and higher LH levels than did birds on the maintenance diet. In contrast, there was no significant effect of access to seeds on gonadal development in house finches. These results are consistent with the hypothesis that species with more opportunistic breeding schedules may be more sensitive to non-photic cues than are species with more seasonal schedules.
[P71] Are within-clutch patterns of maternal testosterone an adaptive maternal effect to modulate sibling competition? Evidence from a large comparative study

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Avian mothers deposit substantial quantities of testosterone into eggs that vary over the laying sequence in many species but in different patterns. Maternal testosterone exposure enhances competitive ability of nestlings; increasing or decreasing yolk testosterone over the laying sequence might be a way for mothers to modulate sibling competition. Hatching asynchrony produces competitive hierarchies in broods because synchronous early-hatching chicks grow substantially before last chicks (marginal chicks) hatch. Increasing testosterone in marginal eggs might provide a competitive boost that promotes their survival, whereas decreasing testosterone in marginal eggs might enhance the disadvantage of hatching late and promote adaptive brood reduction. To date the potential link between hatching asynchrony and yolk testosterone has generated a lot of interest, but has rarely been tested. Here we use a comparative analysis to investigate this question from an evolutionary point of view. We expect species with a small degree of competitive asymmetries within broods to aim for survival of the whole brood and therefore compensate marginal young with more testosterone whereas species with large asymmetries within broods anticipate brood reduction and allocate less testosterone to marginal eggs. Our analyses support this hypothesis, providing evidence for correlated evolution of maternal effects that influence sibling competition.

[P72] Life at differential pace: A comparison of annual itineraries in the non-migratory and migratory songbirds

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Birds exhibit annual life history stages in order to maximize their fitness in the periodic environment. The duration of such life history stages may however considerably vary among species as evolutionarily determined by the selection pressure. Therefore the prediction is that among summer breeders, reproductive life history stage is at faster pace in high latitude species than in low latitude species. We examined this by comparing the photoinduced seasonal cycle between high latitude migratory redheaded bunting (Emberiza bruniceps, a species that breeds at higher latitudes and overwinters in India) and low latitude (subtropical) non-migratory Indian weaverbird (Ploceus philippinus). Birds (n = 7 or 9) held under short days (8 h light: 16 h darkness) were subjected to weekly increments of 0.5 h of light became 13 h per day (13L:11D) in which birds were maintained for a further 32 weeks. In spite of both species being long day breeders, there was a clear difference in the timing and duration of testicular recrudescence and regression phases: buntings showed a faster induction of the gonadal cycles than weaverbirds. We interpret current results that avian life history stages have evolved as a result of close interactions of internal timing mechanisms with the environment they inhabit. *Equal contribution. Funded by DST-IRHPA Center for Excellence grant (IS/SO/LU-02/2005)
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