8th International Conference on Hormones Brain and Behavior

Official retirement and 65th birthday of Jacques Balthazart

Liège, Belgium
June 24-27, 2014

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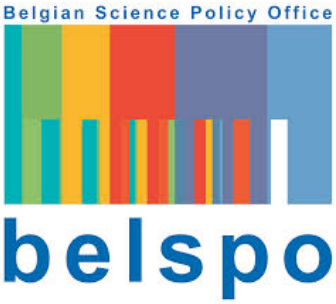

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The history of the International Conference on Hormones, Brain and behavior

Introduction

Jacques Balthazart will reach the age of 65 on June 29, 2014 and according to the Belgian rules will have to retire from all his official functions at the University of Liege, though he is not planning to end his scientific career at this point! On this occasion, we would like to celebrate him and organize one more time an International Conference on Hormones, Brain and Behavior (ICHBB) in the tradition that he had initiated in the mid-eighties. This conference met in Bielfeld Germany in 1982, in Liege Belgium in 1984 and 1989, in Tours France in 1993, in Torino Italy in 1996, in Madrid Spain in 2000 and again in Torino in 2009. We would like to organize it once more in Liege as a 3-day conference on June 24-27, 2014.

The conference will be focused on the themes that Jacques studied in his career including sexual differentiation of brain and behavior, role of brain steroid metabolism in particular aromatase in the control of sexual behavior, sexual motivation, seasonal changes and steroid-induced brain plasticity. The invited and plenary talks will thus be essentially concerned with these themes but other topics in behavioral endocrinology can be covered in the posters and contributed oral presentations.

The conference will include 6 symposia, including each 4 invited talks, and 3 plenary lectures (See program). In addition, a few shorter contributed talks will be included in the symposia and all participants will be able to present posters describing their recent work.

Charlotte A. Cornil (Liege B) Gregory F. Ball (Baltimore MD)

The ICHBB history: a personal view

The International Conference on Hormones, Brain and Behavior originates in my failure to find in Europe a conference that would adequately cover my field of research, Behavioral Endocrinology. I had attended in the seventies and early eighties a series of conferences focusing on Endocrinology (mainly the meeting of the European Society of Comparative Endocrinology) or on Animal Behavior (the Ethology conference and the annual meeting of the Association for the Study of Animal Behavior) but these conferences usually included only a very small number of talks on the effects of hormones on behavior. Interestingly, this was particularly the case for animal behavior meetings that were at the time strongly influenced by the emerging sociobiology and behavioral ecology and therefore devoted almost no interest to the study of the causation of behavior.

At the beginning of 1982, I was contacted by Raymond Gilles, Professor of Animal Physiology at the University of Liege, who had already organized a number of meetings on comparative aspects of animal physiology that had crystallized in the foundation of the European Society for Comparative Physiology and Biochemistry (ESCPB). He had planed to organize for ESCPB in collaboration with other scientists a series of symposia during the following summer but his colleagues were not really moving on and he feared that no meeting would be ready to take place that year. He thus offered me to help organize a meeting on the topics close and dear to my heart.

Having no experience in the organization of scientific conferences, I sent a letter of invitation to approximately 40 well-established specialists in the analysis of the endocrine control of behavior hoping that at least a few of them would answer positively and form a first nucleus for a conference program. To my great surprise, almost all of them answered positively and I had to juggle to fit all these talks within the three days of conference that had been planed originally. The conference was organized in September 1982 at the Zentrum für Interdisciplinäre Forschung (ZIF) a new conference facility created at the University of Bielefeld, Germany with the help of Ekki and Ragna Pröve who served as extremely efficient local organizers. This conference was attended by approximately 150 scientists from a variety of countries mostly in Europe and in North America and was a great success. Its proceedings were published by Springer (Balthazart et al., 1983) and this book was quite broadly distributed, being at the time one of the first published collection of reviews in behavioral endocrinology.

This initial success was a great incentive to continue on the same trajectory and two similar conferences therefore followed that were organized in Liège, Belgium in 1984 as part of the First International Congress of Comparative Physiology and Biochemistry and in Liège again in 1989 with proceedings being published respectively by Springer Verlag (Gilles and Balthazart, 1985) and Karger (Balthazart, 1990).

Two more conferences in this series were organized in Tours, France in 1993 with the late Jean Pierre Signoret and Claude Fabre Nys serving as local organizers and then in Torino, Italy in 1996 with Gian Carlo Panzica as a host. The proceedings of these two meetings were published as special issues of Psychoneuroendocrinology (Signoret et al., 1994) and of Brain Research Bulletin (Panzica and Balthazart, 1997) respectively.

By that time, a Society for Behavioral Neuroendocrinology (SBN) had emerged (in 1997) in the USA from the previous Conference on Reproductive behavior (colloquially known as "the Sex Conference"), a loosely organized meeting that had been organized annually since 1969 (Dewsbury, 2003). SBN had a clear international dimension. It was therefore decided that the next ICHBB would be organized as a joint meeting with the annual SBN conference. This would also be the last ICHBB that would become later fully incorporated into SBN activities and SBN would, in the future, run its annual meeting every four years outside the United Sates. The 6th ICHBB-SBN meeting was held in Madrid in 2000 (Antonio Guillamon, Luis-Miguel Garcia Segura and Santiago Segovia as local organizers) and assembled a larger number of scientists than ever. Its proceedings were published in Hormones and Behavior (McCarthy et al., 2001), the official journal of the SBN, another sure sign of the future merge of the meeting with SBN activities. I though this was the end of the story, but I was in for some surprise!

Gian Carlo Panzica while organizing the 5th ICHBB in Torino in 1996 was "infected by a nasty virus" compulsively pushing people into conference organization. He consequently decided to create, in collaboration with Roberto Melcangi (University of Milan) a cycle of conferences using essentially the same format as ICHBB that would be covering the specific field of steroid action in the nervous system. This meeting has now taken place on 7 occasions (2001, 2003, 2005, 2007, 2009, 2011 and 2013) and the next meeting is planned for 2015.

The 5th conference on steroid action in the nervous system that took place in February 2009 was associated with a symposium entitled 7th ICHBB! This one day meeting was organized in part for the 60th birthday of the two compulsive conference organizers concerned by this short history (JB and GCP) but also more seriously to celebrate the 50th anniversary of the publication of the seminal paper of Phoenix and collaborators (Phoenix et al., 1959) universally recognized as the founding paper for the research field analyzing the endocrine controls of sexual differentiation of brain and behavior.

This 8th edition of ICHBB organized in Liège will meet on the occasion of my 65th

birthday and official retirement from my official duties at the University of Liège. Talks will be presented during three days by a group of stellar scientists who will without a doubt provide exciting summaries of recent developments in the field of behavioral neuroendocrinology. This meeting will hopefully be scientifically and socially as rewarding as previous editions.

Jacques Balthazart
University of Liege
(June 2014)

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Gilles, R. and Balthazart, J. (Eds.), 1985. "Neurobiology, Current Comparative Approaches", Springer Verlag, Berlin.

McCarthy, M.M., Guillamon, A., Garcia-Segura, L.-M. and Segovia, S. (Guest Eds.), 2001. Hormones and Behavior, 40/2, pp. 85-366.

Panzica, G.C. and Balthazart, J. (Guest Eds.), 1997. Brain Research Bulletin, Vol. 44/4, pp. 319-557.

Phoenix, C. H., Goy, R. W., Gerall, A. A. and Young, W. C., 1959. Organizational action of prenatally administered testosterone propionate on the tissues mediating behavior in the female guinea pig. Endocrinology. 65, 369-382.

Signoret, J.-P., Fabre-Nys, C. and Balthazart, J. (Guest Eds.), 1994. Psychoneuroendocrinology, 19 (5-7), pp. 403-721.

Program and timetable of the 8th ICHBB in Liège (June 24-27, 2014)

Tuesday June 24

Chair: Charlotte Cornil

17.45 -18.00. Inaugural ceremony with a few welcome words by Rector Bernard Rentier

18.00-19.00. **Plenary keynote lecture**
Margareth M. McCarthy (Univ. Maryland, Baltimore MD, USA)
50 years on, sexual differentiation of the brain still surprises

Wednesday June 25

Symposium 1 : Sexual differentiation

Chairs: Anne Etgen & Barney Schlinger

09.00-09.30. **Geert De Vries** (Georgia State Univ., Atlanta GA, USA)
Sexual differentiation of the brain; a whole body perspective
09.30-10.00. **Elizabeth Adkins-Regan** (Cornell Univ., Ithaca NY, USA)
Avian sex differences: a Tinbergen-inspired approach
10.00-10.30. **Chuck Roselli** (OHSU, Portland OR, USA)
From barn to bench: using sheep to study sexual differentiation of the brain

10.30-11.00 . Coffee break

11.00-11.30. **Julie Bakker** (University of Liege, Belgium)
Sexual differentiation of the human brain : fMRI studies

11.30-12.30. Contributed talks

11.30-11.45. **Sakuma Y.** (University of Tokyo Health Sciences, Japan): Development and function of the sexually dimorphic nucleus of the preoptic area.
11.45-12.00. Raskin K., Marie-Luce C., Naulé L., Picot M., **Mhaouty-Kodja S.** (Sorbonne Universités, Paris, France): Revisting the role of androgen and estrogen receptors in the organization and activation of male behaviors by using conditional mutagenesis in mice
12.00-12.15. **Taziaux M. ,** Staphorsius M. , Swaab D.F., Bakker J. (University of Liège, Belgium): Sex difference in kisspeptin expression in the human infundibular nucleus: developmental and hormonal aspects.
12.15-12.30. **Kelly A.M.,** Goodson J.L. (Indiana University, Bloomington, IN): Sexually differentiated functions of hypothalamic oxytocin and vasopessin neurons.

12.30-14.00. Lunch with poster discussion

Symposium 2 : Sexual and social motivation

Chairs: Julie Bakker & Jeff Blaustein

14.00-14.30. **Lauren Riters** (Univ. Wisconsin, Madison WI, USA)
Pleasure seeking and birdsong: Neuroendocrine regulation of the motivation to communicate
14.30-15.00. **Jim Pfaus** (Concordia Univ., Montreal CANADA)
The pleasure principle revisited : How sexual pleasure links sexual arousal, desire, preference and performance

15.00-15 .30. **Rae Silver** (Columbia Univ, New York NY, USA)
Sex differences in hormonal remodeling of circadian plasticity

15.30.16.00. Coffee break

16.00-16h30. **Bob Meisel** (Univ Minnesota, Minneapolis MN, USA)
Why do animals have sex? A neurobiological perspective

16h30-18.00. Contributed talks

16.30-16.45. **Schneider J.E.** (Lehigh University, Bethlehem, PA): Our stolen figures: endocrine disruptors and obesity.
16.45-17.00. **Mong J.A.,** Cusmano D.M., and Viechweg S. (University of Maryland School of Medicine, Baltimore, MD): The median preoptic nucleus (MnPN): a key site for estradiol mediated changes in sleep.
17.00-17.15. Borrow A.P., **Cameron N.M.** (Binghamton University, Binghamton, NY): A natural model of gonadal steroid effects on emotional lability and risky behavior in females.
17.15-17.30. **Charlier T.D.,** Niessen N.A., Vesel C., Schmidt R, Wiemerslage L., Welch L.R., Balthazart J. (Ohio University, Athens, OH): Distinct roles of androgenic and estrogenic testosterone metabolites in gene regulation underlying male sexual behavior.
17.30-17.45. **Dalla C.,** Kokras N. (University of Athens, Greece): Antidepressant potential of subacute aromatase inhibition.
17.45-18.00. **Keller M.,** Chemineau P., Delgadillo J.A. (INRA/CNRS/University of Tours, Nouzilly, France): Male sexual behavior drives the reactivation of female gonadotrope activity in goats.

Thursday June 26

Symposium 3 : Aromatase and estrogen action

Chairs: Elizabeth Adkins-Regan & Chuck Roselli

09.00-09.30. **Per Södersten** (Karolinska Institutet, Huddinge SWEDEN)
Steinach versus Young: how the effects of estrogen in the male disappeared and how the male brain emerged
09.30-10.00. **Jon Levine** (Wisconsin Univ., Madison WI, USA)
Non-classical estrogen receptor alpha signaling and regulation of body weight
10.00-10.30. **Luke Remage-Healey** (UMass, Amherst MA, USA)
Rapid changes in brain estrogens and sensorimotor integration

10.30-11.00. Coffee break

11.00-11.30. **Jeff Blaustein** (UMass, Amherst MA, USA)
Peripubertal stressors influence behavioral response to estradiol later in life

11.30-12.30. **Plenary lecture**

Chair: Gregory Ball
Barney Schlinger (UCLA, Los Angeles CA, USA)
Androgens, courtship, and athleticism: lessons from an exuberant tropical bird

12.30-14.00. Lunch with poster discussion

Symposium 4 : Neuroprotection and endocrine disruptors

Chairs: Margareth M. McCarthy & Colin Saldanha

14.00-14.30. **Anne Etgen** (Albert Einstein, New York NY, USA)
Estradiol reduces death of hippocampal pyramidal neurons subjected to global ischemia: cellular mediators
14.30-15.00. **Luis Miguel Garcia Segura** (Cajal Institute, Madrid, SPAIN)
Neuroprotective actions of brain aromatase
15.00-15.30. **Michael Schumacher** (INSERM, Kremlin Bicêtre-Paris FRANCE)
Myelin regeneration with androgens

15.30.16.00. Coffee break

16.00-16.30. **Gian Carlo Panzica** (Torino Univ., Torino ITALY)
Sexually dimorphic effects of endocrine disruptors on brain and behavior

16.30-17.30. Contributed talks

16.30-16.45. **Grattan D.R.,** Cashen S., Larsen C.M., Kokay I.C., Brown R.S.E., Wyatt A., Le Tissier P.R. (University of Otago, Dunedin, New Zealand): Impaired postpartum maternal behaviour following conditional deletion of the prolactin receptor from GABAergic neurons.
16.45-17.00. **Wild J. M. (**University of Auckland, New Zealand): Involvement of the avian song system in reproductive behaviour.
17.00-17.15. **Groothuis T.G.G.,** Rettenbacher S., Hsu B-Y., Rie Henriksen R. (University of Groningen, the Netherlands): Context-dependent effects of maternal testosterone: Solving a paradox.
17.15-17.30. **von Engelhardt N.,** Langen E., Goerlich-Jansson,V. (University of Bielefeld, Germany): Transgenerational effects of the social environment transmitted through the avian egg.

17.30-20.00. Poster discussion and Belgian beers tasting

Friday June 27

Symposium 5 : Seasonality/Plasticity

Chairs: Elaine Hull & Luis Miguel Garcia-Segura

09.00-09.30. **Tyler Stevenson** (Aberdeen Univ., Aberdeen UK)
Reversible epigenetics and seasonal neuroendocrine plasticity
09.30-10.00. **Kiran Soma** (Univ. British Columbia, Vancouver CANADA)
Local steroid synthesis in the brain…and other organs
10.00-10.30. **Annemie Van der Linden** (Antwerp Univ., Antwerp BELGIUM)
Magnetic resonance imaging of brain plasticity in songbirds

10.30-11.00. Coffee break

11.00-11.30. **John Wingfield (**Univ. California, Davis CA, USA)
Changes in regulation of behavioral traits over the life course: diversity of mechanisms

11.30-12.30. Contributed talks

11.30-11.45. **Wade J.** (Michigan State University, East Lansing MI): Do estradiol and specific z-chromosome genes work in concert to masculinize the zebra finch song system?
11.45-12.00. **Steyaert S.,** Diddens J., Van der Linden A. , Vanden Berghe W., De Meyer T. (University of Ghent, Belgium): A genome-wide search for epigenetically regulated genes in zebra finch cell lines using methylcap-seq and RNA-seq.
12.00-12.15. **Alward B.A.,** Chan T.T., Rownd K., Weidenbenner H., Balthazart J., Ball G.F. (Johns Hopkins University, Baltimore, MD): Anatomical specificity in the action of testosterone in the regulation of song and underlying neuroplasticity in canaries.
12.15-12.30. **Caro S.P.,** Aldredge R.A., Schaper S.V., Dawson A., Sharp P.J., Visser M.E. (Netherlands Institute of Ecology , Wageningen, The Netherlands): Effect of an increase in ambient temperature on brain activity and endocrine concentrations in photostimulated female great tits.

12.30-14.00. Lunch with poster discussion

Symposium 6 : Brain plasticity

Chairs: Rae Silver & Gian Carlo Panzica

14.00-14.30. **Elaine Hull** (Florida State Univ., Tallahassee FL, USA)
Steroids in the MPOA: pathways to sexual sensitization and stress reduction
14.30-15.00. **Carolyn Pytte** (Queen College, CUNY, Flushing NY, USA)
Effects of song feedback on lateralized neurogenesis in the zebra finch
15.00-15.30. **Colin Saldanha** (American Univ., Washington DC, USA)
Estrogens where and when they're needed: compartment- and cell-specific aromatization in the songbird brain

15.30-16.00. Coffee break

16.00-16.30. **David Clayton** (Queen Mary Univ. London, London UK)
The deceptive simplicity of sex, steroids and genome responses

16.30-17.30. **Plenary lecture**

Chair: Jacques Balthazart
Gregory F. Ball (Johns Hopkins Univ., Baltimore MD, USA)
Steroid-induced adult neuroplasticity: What I learned from Jacques and the Birds

17.30-18.00. **Closing ceremony** including historical presentation by
Kathie Olsen (ScienceWorks, Washington DC, USA)
Reflections on the International Conferences on Hormones, Brain and Behavior : from Bielefeld to Liege

and concluding remarks by **Greg Ball** (Johns Hopkins Univ., Baltimore MD, USA)

18.00-19.00. Closing cocktail party

20.00-24.00. Final Banquet (Walking dinner in Colonster Castle)

Poster presentations (IN ALPHABETICAL ORDER)

Abdelkareem A.A., Ma W.Q., Ni Y.D., Zhou Q., Zhao R.Q. (Nanjing Agricultural University, Nanjing, China): Embryonic exposure to corticosterone modifies aggressive behavior through alterations of the hypothalamic pituitary adrenal axis and the serotonergic system in the chicken.

Ampatzis K., Dermon C.R. (University of Patras, Greece): Sexually dimorphic behavior is associated to cerebral metabolic activity and adrenoceptors in adult zebrafish brain.

Brus M., Bakker J. (University of Liège, Belgium): The impact of oestradiol on olfactory neurogenesis in the context of sexual behavior

Cornez, G., Ter Haar, S.M. , Cornil, C.A., Balthazart, J. (University of Liège): Sex differences in perineuronal nets and parvalbumin expression in the zebra finch *(taeniopygia guttata)* song system.

Cusmano D.M., Mong J.A. (University of Maryland, Baltimore, MD): The sleep promoting effect of dora-12 is sex dependent in rats.

de Bournonville C., Aourz N., van Eeckhaut A., Smolders I., Ball G.F., Balthazart J. , Cornil C.A. (University of Liège): The sexual behavior induced-inhibition of aromatase activity in the preoptic nucleus is mediated by glutamate release.

De Groof G., Balthazart J., Cornil C.A., Van der Linden A. (University of Antwerp, Belgium): Acute effect of aromatase inhibition on cognitive auditory processing in a seasonal songbird.

Desroziers E., Brock O., Baum M.J., Bakker J. (University of Liege, Belgium): Role of the progesterone receptor in the development of sexual behavior in female mice

Evrard H.C., Harada N., Balthazart J., Erskine M.S. (Max Planck Institute for BioCybernetics, Tübingen, Germany): Localization and Nociceptive Function of Aromatase in the Adult Rat Spinal Cord.

Feighery A., Josimovich J., Orr J., Blackshear, K., Saldanha C.J., Holloway K.S., Duncan K.A. (Vassar College, Poughkeepsie, NY): Interleukin 1 receptor signaling regulates anxiety behaviors in mice.

Franssen D.G., Parent A-S., Gérard A.L., Hennuy B.R., Bourguignon J-P. (University of Liège, Belgium): Delayed puberty, slowed down GnRH secretion and changed hypothalamic RNA expression after neonatal exposure to a very low environmentally relevant dose of bisphenol A.

García-Pupo L., Ramírez-Sánchez J., Zaldo Castro A., Ochoa E., Verdecia Y., Delgado-Hernández R., Vanden Berghe W., Nuñez-Figueredo Y. (Drugs Research and Development Center, Havana, Cuba): Neuroprotective effects of a synthetic sapogenin derivative in brain ischemia *in vivo* models.

Gennotte V., Balagizi D.A., Mélard C., Denoël M., Ylieff M., Cornil C., Rougeot C. (University of Liège, Tihange, Belgium): Influence of sexual genotype on agonistic behaviors and sex steroid levels of phenotypic males and females in the nile tilapia (*Oreochromis niloticus*).

Hamaide J., De Groof G., Van Audekerke J., Van Ruijssevelt L., Mai Z., Kara F., Cornil C. , Verhoye M., Van Der Linden A. (University of Antwerp, Wilrijk, Belgium): *In vivo* non-invasive structural imaging tools to investigate sex- and ontogeny related differences in the zebra finch brain.

Hellier V., Brock O., Prévot V., Boehm U., Bakker J. (University of Liege, Belgium): Functional characterization of the role of kisspeptin and GnRH receptor neurons in the neural circuit controlling the lordosis reflex.

Iyilikci O., Balthazart J., Ball G.F. (Johns Hopkins University, Baltimore, MD): Dopamine depletion in the medial preoptic nucleus impairs appetitive and consummatory sexual behaviors in male Japanese quail.

Jonckers E., Orije J., De Groof G., Verhoye M., Van der Linden A. (University of Antwerp, Belgium): Seasonal neuroplasticity of the auditory system of female starlings assessed with resting state functional MRI.

Koren L., Ciuti, S., Wynne-Edwards, K.E., Boyce, M.S., Musiani, M. (Bar-Ilan University, Ramat Gan, Israel): Hair testosterone reveals sex differences in wildlife behavioural strategies.

Krohmer R. W. (Saint Xavier University, Chicago, IL): Neuronal plasticity and courtship behavior in the male red-sided garter snake is dependent on estrogens aromatized from circulating testosterone during low temperature dormancy.

Kumar N., Gahr M., Groothuis T.G.G. (University of Groningen, The Netherlands): Hormone-mediated maternal effects: a potential role for the embryo.

Lacreuse A., LaClair M., Chang J. (University of Massachusetts, Amherst MA): Sex differences in gonadectomized marmosets performing an object reversal task.

Madison F.N., Alward B.A.,Ball G.F. (Johns Hopkins University, Baltimore, MD): Investigating Possible Intraspecific Variation in Testosterone-Induced Neuroplasticity by Comparing Two Canary Breeds.

Mommer B.C., Bell A.M. (University of Illinois at Urbana-Champaign, Urbana, Il) : maternal experience with predation risk influences genome-wide embryonic gene expression in threespined sticklebacks (G*asterosteus aculeatus)*

Naulé L., Marie-Luce C., Martini MA., Picot M., Albac C., Franceschini I., Keller M., Mhaouty-Kodja S. (Université Pierre et Marie Curie, Paris, France): Role of neural estrogen receptor beta (ERβ) in the control of behavioral and neuroendocrine responses in male and female mice.

Olvera-Hernández S., Fernández-Guasti A. (CINVESTAV, Mexico City, Mexico)**:** Change in sexual partner preference in male rats by prenatal letrozole administration.

Quintana L., Jalabert C., Zubizarreta, L., Pessina P., Texeira F., Meerhoff M., Silva, A. (IIBCE, Montevideo, Uruguay): The weakly electric fish *Gymnotus omarorum* as a novel model system for the study of neuroendocrine control of non-breeding territorial aggression.

Raymaekers S.R.M., Van Herck S.L.J. & Darras V.M. (KU Leuven, Belgium): Regulators of thyroid action in the songbird brain.

Rudzinskas SA., Mong, J.A. (University of Maryland, Baltimore, MD): Methamphetamine mediates increased female sexual motivation in response to relevant cues.

Ruploh T., Bischof H.J., von Engelhardt N. (University of Bielefeld, Germany): The zebra finch: An excellent model organism to study the physiological causes of experience-dependent variation in social behavior.

Santi S., Mélard C., Toguyeni A., Antoine N., Rougeot C. (University of Liège, Tihange, Belgium): Effect of high temperature on sex determination and sex differentiation process in african catfish, *Clarias gariepinus* .

Schneider J.E., Benton N., Kriegsfeld L. (Lehigh University, Bethlehem, PA): Gonadotropin inhibiting hormone and appetitive behavior.

Seredynski A.L., Ball G.F., Kelly M.J., Jacques Balthazart J., Cornil C.A. (University of Liège): Activation of ERβ by estrogens acutely modulates male sexual motivation.

Shevchouk O.T. , Cornil C.A. , Balthazart J. (University of Liège): Influence of social context on singing behaviour and song system plasticity.

Spencer K. A., Boogert, N. , Zimmer, C. (University of St Andrews, UK): Mum's the word: trans-generational transmission of phenotypes programmed by early-life stress.

Ter Haar, S.M. , Raymaekers, S. , Darras, V.M., Cornil, C.A˙, Balthazart, J. (University of Liège): The effect of thyroid hormones on zebra finch *(taeniopygia guttata)* brain plasticity during development.

Williams K.M., Mong J.A. (University of Maryland, Baltimore, MD 21201): Ovarian hormones modulate mapk in the nucleus accumbens but not the amygdala.

York J.E., Radford A.N., Groothuis T.G., Young A.J. (University of Exeter, Penryn, UK): Dawn song performance is unrelated to circulating testosterone in a subtropical, social songbird.

Abstracts of plenary, invited and contributed talks

(Listed in order of presentation)

50 YEARS ON, SEXUAL DIFFERENTIATION OF THE BRAIN STILL SURPRISES

Margaret M. McCarthy, PhD.

Department of Pharmacology, University of Maryland School of Medicine
Baltimore MD, 21230 USA

The developing brain is permanently and differently organized in males and females as a result of variance in hormonal exposure, with perinatal males producing high levels of testicular androgens that are aromatized to estrogens in neurons. Developing females experience far less hormone exposure and feminization is considered the default pathway in the process of sexual differentiation. Endpoints impacted by steroids in the developing brain include synaptic patterning, neurogenesis, glial genesis, differential cell death, migration and phenotypic differentiation. Multiple attempts to identify a neurotransmitter system subject to hormonal regulation and serving as the final common denominator of steroid-hormone induced masculinization of the brain have largely failed. We now understand this to be due to the origins of many sex differences in the brain being outside the realm of neurotransmission but instead involving inflammatory and immune mediators such as prostaglandins, microglia and mast cells, as well as another class of membrane derived signaling molecules, endocannabinoids, all of which are higher in males. Cell-to-cell communication via diffusible but short-lived signaling molecules expands the impact of steroids beyond those cells expressing steroid receptors and likely contributes to regional specificity. Once sex differences are established, they must also be maintained into adulthood to assure reproductive behavior and physiology are in register. Epigenetic modifications to the DNA imprint early life environment and experience onto the genome. Emerging evidence suggests the default female pattern involves epigenetic repression of the male genome which is emancipated by gonadal steroid inhibition of DNMT activity and subsequent demethylation of key genes, allowing for their expression. Understanding how male and female brains develop differently informs us as to sources of the well established gender bias in risk of developmental disorders which are more prevalent in males and increased in frequency and severity by early life injury or inflammation.

SEXUAL DIFFERENTIATION OF THE BRAIN; A WHOLE-BODY PERSPECTIVE

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The hundreds of sex differences found in the brain beg the question as to how they develop and what is their function. Factors that cause sex differences in the brain are sex chromosomal gene expression, gonadal hormones, and environmental interactions. Parsimony dictates that these factors act directly on the brain. However, these factors act on peripheral structures as well. Sex differences may therefore develop in part because brains reside in fundamentally different bodies. This has consequences for brain function as well. Brains may generate different output autonomously, but if they are wired up to different bodies, similar output will have different consequences. To generate similar behaviors, the nervous system may have to compensate by giving different commands. This interaction between body and brain has to be taken into account for a full understanding of the development as well as function of sex differences in the brain. These principles will be demonstrated by discussing the development and function of sex differences in vasopressin signaling in brain and body.

AVIAN SEX DIFFERENCES: A TINBERGEN-INSPIRED APPROACH

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In 1963 Tinbergen explained that a full understanding of behavior addresses four problems: ontogeny (development within individual lives), causation (in a narrow sense, meaning mechanisms), survival value (function, adaptation) and evolution (phylogeny, evolutionary history) [1]. Development and mechanisms are often referred to as proximate causes, and function and phylogeny as ultimate causes. Sex differences in behavior have inspired research addressing all four of Tinbergen's problems, and studies with birds continue to contribute importantly to these efforts. The programmatic and sustained research of the Balthazart lab with Japanese quail has provided much of what we now know about the mechanisms for avian behavioral and brain sex differences, especially the role of sex steroid hormones and their actions on the nervous system [2]. Research by multiple labs has established the zebra finch as the other key avian species for discovering neuroendocrine mechanisms of sexually differentiated behavior. Recent results from the Adkins-Regan lab with both species will be presented that, while focused primarily on mechanisms and development, also address the other two of Tinbergen's four categories. A developmental experiment with zebra finches has shed new light on the origins of the robust sex difference in pairing partner preference by showing that males reared by fathers only, instead of fathers and mothers, pair with other males instead of females. This suggests that cross-sex imprinting may be involved in the normal development of pairing partner preference [3]. The hypothesis is being tested that nonapeptides have organizational effects on affiliative behavior in zebra finches, including sex differences in those effects or effects on sexually differentiated behavior. With respect to ultimate causation, a function has now been firmly established for the foamy product of the androgen regulated sexually dimorphic foam gland of male Japanese quail. The foam increases a male's success in sperm competition, making it likely that the unusual gland arose through intrasexual selection [4]. The quail foam gland has opened up an opportunity to determine the genetic mechanisms involved in the evolution of a novel sex-specific reproductive structure [5]. Progress has also been made in understanding the phylogenetic distribution of the sex-determining systems that underlie sexual differentiation [6]. There is still much to be done, however, to integrate ultimate and proximate causes to better understand evolutionary changes in sex differences. Supported by NSF IOS-1146891.

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FROM BARN TO BENCH: USING SHEEP TO STUDY SEXUAL DIFFERENTIATION OF THE BRAIN

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The domestic ram is an ideal model to study the biological underpinnings of sexual preferences because a subpopulation of sheep, like some humans, exhibits naturally occurring durable and exclusive preferences for individuals of the same sex. Sheep also have a long period of gestation making them an excellent experimental model for the study of possible links between fetal neuroendocrine programming and adult sexual behavior. Sheep, like humans, possess a sexually dimorphic brain nucleus (SDN) that is larger in males that are attracted to females than in males or females that are attracted to males. The ovine SDN (oSDN) is a highly tractable neuroanatomical landmark that is localized in the medial preoptic area and characterized by abundant expression of the aromatase enzyme. The oSDN develops prior to birth and is enlarged (i.e., masculinized) by testosterone exposure during gestational days (GD) 60 to 90 (gestation length = 147 days). In addition to aromatase, the developing oSDN expresses messenger RNAs for the synthesis of androgen and estrogen receptors suggesting that both hormones could mediate developmental processes that lead to functional and structural changes important for male-typical differentiation. However, prenatal exposure of lamb fetuses to an aromatase inhibitor, ATD, failed to interfere with masculinization of the oSDN. Recent studies designed to test whether androgen receptor activation is responsible for brain masculinization found that exposure to the androgen antagonist flutamide reduced oSDN volume, but paradoxically so did the nonaromatizable androgen dihydrotestosterone (DHT). DHT also significantly decreased both pituitary gonadotropin and hypothalamic kisspeptin expression in males during the critical period. These results demonstrate that androgen receptors mediate much or all of the masculinization of the oSDN in males and suggest the effects of DHT on oSDN development are secondary to negative feedback on the fetal hypothalamus and pituitary which may be mediated, in part, through hypothalamic kisspeptin. Collectively, the results support the concept that the hypothalamus-pituitary-gonadal axis in the midgestation male fetus responds in the expected negative feedback manner to exogenous androgens and that tonic negative feedback by endogenous androgens is disrupted by flutamide. We conclude that, during the critical period of brain sexual differentiation, the fetal reproductive axis in long gestation species, such as sheep, is sufficiently developed to react to perturbations in serum androgen levels and defend against disruptions in brain masculinization. (Supported by NIH R01 OD011047).

THE SEXUAL DIFFERENTIATION OF THE HUMAN BRAIN: fMRI STUDIES

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The sexual differentiation of the human brain seems to be primarily driven by gonadal hormones during fetal development. However, direct genetic factors might also contribute to the sexual differentiation of the human brain since several behavioral and neuroimaging studies of Turner (XO) or Klinefelter (XXY) syndrome point to a role for X-chromosomal dosage in human brain differentiation. Since in both disorders there is an aberrant number of sex chromosomes, along with reduced gonadal hormone levels it is difficult to determine whether the results from these syndromes reflect genes on the X or Y chromosome, chromosomal dosage or sex hormone levels. Therefore, we compared 46, XY men and 46,XX women to 46,XY individuals with complete androgen insensitivity disorder (CAIS) on a mental rotation task using functional magnetic resonance imaging (fMRI) to study the separate effects of testosterone versus sex chromosomes on neural sex differences. Previously reported sex differences in neural activation during mental rotation were replicated in the control groups, with control men showing more activation in the inferior parietal lobe than control women. Individuals with CAIS showed a female-like neural activation pattern in the parietal lobe, indicating feminization of the brain in CAIS. This first neuroimaging study in individuals with CAIS thus provides evidence that sex differences in regional brain function during mental rotation are most likely not driven by genetic sex, but rather reflect gonadal hormone exposure.

The concept of gender identity is uniquely human. Hence we are left with the phenomenon of men and women suffering from Gender Dysphoria (GD) also known as transsexualism to study the origins of gender identity in humans. It has been hypothesized that atypical levels of sex steroids during a perinatal critical period of neuronal sexual differentiation may be involved in the development of GD. In order to test this hypothesis, we conducted several fMRI experiments in individuals with GD. Since GD is often diagnosed in childhood and puberty has been proposed to be an additional organizational period in brain differentiation, we included both prepubertal children and adolescents with GD in our studies. First, we measured brain activation upon exposure to androstadienone, a putative male chemo-signal which evokes sex differences in hypothalamic activation (women > men). We found that hypothalamic responses of both adolescent girls (female-to-male: FM) and boys (male-to-female: MF) were more similar to their experienced gender than their natal sex, which supports a sex-atypical brain differentiation. Second, we quantified regional gray matter (GM) volumes in both FM and MF adolescents, as well in control boys and girls. In control girls, larger GM volumes were observed in the bilateral superior medial frontal and left pre/postcentral cortex, while control boys had more volume in the bilateral superior-posterior cerebellum and hypothalamus. Within these regions of interest (ROIs) representing sexually dimorphic brain structures, GM volumes of both GD groups deviated from the volumetric characteristics of their natal sex towards those of individuals sharing their gender identity, again thus supporting a partial sex-atypical differentiation of the brain during early development in individuals with GD.

DEVELOPMENT AND FUNCTION OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA

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Transgenic (tg) rats and mice were exploited to study developmental process and function of the sexually dimorphic nucleus of the preoptic area (SDN-POA). In tg rats that express EGFP under the control of the ER alpha gene promoter 0/B, ER alpha immunoreactive (ir) neurons in the SDN-POA were fluorescent in both sexes, while those in the adjacent preoptic area were not. We termed the structure with the fluorescent neurons the 0/B SDN-POA, which overlapped with the SDN-POA visualized by Nissl staining or by calbindin D28k (cal) immunohistochemistry. The 0/B SDN-POA is larger in males than in females as the classic SDN-POA. Neonatal endocrine manipulations reversed the sexual phenotype [2]. Time-lapse movie of POA slices, which were prepared from the forebrain of 18-day embryos and incubated with or without estradiol, showed fluorescent cells to migrate to form cal-ir cell aggregate of different sizes over a period of 3 weeks, thus showing cell migration contributes to the establishment of the sex-typical SDN-POA. DNA microarray analysis, Western blotting, and immunohistochmistry showed estradiol drove PKC-d/Rac1/PAK1/LIMK1/cofilin pathway, culminating in actin remodeling to cause cell migration [5]. Excite toxin lesion of the preoptic area diminished behavioral capability to distinguish odor cues in alternate choice paradigm in female/male rats. The time spent sniffing stimulus animals diminished as well, which suggests decreased sexual motivation. We noted, however, no reversal in sexual preference in the POA-lesioned males [1]. In C57/BL6J and ddN strains of mouse, cal-ir visualized the SDN-POA, which is homologous in every respect to the rat structure in its sex difference and development [4]. Oxytocin-ligand male/female knockouts, backcrossed to C57/BL6J, lacked capability to distinguish odor cues emitted by the opposite sex. They had, however, the SDN-POA as in the wild type. As in the male rat nucleus accumbens [3], the SDN-POA may contain neurons responsible for the inhibition of male sexual behavior under certain contexts.

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REVISTING THE ROLE OF ANDROGEN AND ESTROGEN RECEPTORS IN THE ORGANIZATION AND ACTIVATION OF MALE BEHAVIORS BY USING CONDITIONAL MUTAGENESIS IN MICE

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Gonadal hormones play a key role in sexual differentiation of behaviors and neuroendocrine functions related to reproduction (mating, partner choice, aggression…). In males, testosterone acts during the perinatal/pubertal periods to irreversibly organize (masculinization et defeminization) the neural circuitry underlying the expression of male behaviors, and during adulthood to activate and maintain these responses. In the nervous system, gonadal testosterone can act directly through androgen receptors (AR) or be metabolized into estradiol, which activates estrogen receptors (ERs).
In order to precise the relative contribution of androgen and estrogen receptors in the organization and activation of male behaviors, without interfering with their peripheral functions, we generated mouse lines lacking *AR* or *ERs* in the nervous system. The behavioral characterization of males lacking neural *AR* showed that this receptor is involved in the expression of sexual and aggressive behaviors. The neuroanatomical analysis of sexually dimorphic neuronal populations revealed that this receptor is differentially implicated in the organization of brain and spinal nuclei involved in sexual and aggressive behaviors. Similar behavioral and neuroanatomical analyses were performed on mice lacking neural *ERb* and the obtained data will be presented.

SEX DIFFERENCE IN KISSPEPTIN EXPRESSION IN THE HUMAN INFUNDIBULAR NUCLEUS: DEVELOPMENTAL AND HORMONAL ASPECTS.

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The human brain is sexually differentiated during development under the influence of testosterone acting in the male foetus. Sex differences in the hypothalamus are thought to be at the basis of sex differences in the control of reproductive function, i.e. cyclical in women and tonic in men. Human genetic studies demonstrated that kisspeptin (KP) as well as neurokinin B (NKB) signalling are both potent regulators of GnRH secretion and are therefore thought to be essential for the onset of puberty and the maintenance of adult reproductive function. We recently demonstrated that NKB expression in the human infundibular nucleus (INF) is sexually dimorphic (women > men). We further demonstrated that this female-dominant sex difference appears to develop during puberty until adulthood and that it might be estrogen-sensitive. Interestingly, a female-typical NKB expression was observed in male-to-female (MTF) transsexuals suggesting that these individuals might have undergone an atypical sexual differentiation of the brain. At present, only a fragmented overview of KP expression throughout life is available in humans. Therefore, in the present study, we analyzed KP expression in the human INF to determine 1) when during development KP expression would potentially become sexually dimorphic, 2) whether this putative sex difference is reversed in MTF transsexuals and 3) whether menopause is accompanied by changes in KP expression. KP immunostaining was performed on post-mortem hypothalamus material of both sexes from the infant/prepubertal period into the elderly period and from adult MTF transsexuals. Quantitative analysis confirmed that the human KP system exhibits a female-dominant sexual dimorphism in the INF. During the first years after birth, both sexes displayed a moderate and equivalent number of KP-immunoreactive (KP-ir) cells. The adult features seemed to emerge progressively around puberty until adulthood when the female-dominant sex difference appears and this process continues into old age. In post-menopausal women, there was a significant increase in KP expression compared to pre-menopausal women. Finally, in MTF transsexuals, a female-typical KP expression was observed. Interestingly, a positive correlation was observed between the number of NKB-ir and KP-ir neurons in the INF suggesting that NKB and KP-expressing cells might be part of the same neuronal population, as has been observed in rodents and sheep ("KNDy neurons": a sub-population of neurons expressing KP, NKB and dynorphin in the arcuate nucleus). Overall, our results indicate that certain sex differences do not emerge until adulthood when activated by sex steroid hormones as well as that the sex-reversal observed in male-to-female transsexuals probably reflects, at least partially, an atypical brain sexual differentiation.

SEXUALLY DIFFERENTIATED FUNCTIONS OF HYPOTHALAMIC OXYTOCIN AND VASOPESSIN NEURONS

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Numerous pharmacological studies, such as those using site-specific infusions of agonists and antagonists, demonstrate that the vasopressin-oxytocin (VP-OT) nonapeptides modulate a diversity of social behaviors, including pair ponding and grouping. However, the contributions of specific VP-OT cell groups to such behaviors remains largely unknown. Using antisense oligonucleotides to knock down site-specific production of peptide synthesis, we have investigated the direct contribution of hypothalamic nonapeptide cell groups to behavior, and have demonstrated that the avian forms of VP and OT in the paraventricular nucleus of the hypothalamus (PVN) modulate behavior in sex- and phenotypic-specific ways. Antisense knockdown of VP production in the PVN demonstrates that this VP neuronal population promotes grouping in both sexes and modulates aggression in a sex-specific manner (i.e., suppresses aggression in males, promotes aggression in females). In addition, knockdown of PVN OT production profoundly impairs grouping and pair bonding only in females, but also produces deficits in intrapair affiliation in both sexes. Furthermore, we provide the first extensive description of complex phenotype structure in zebra finches and show that nonapeptide anatomy and phasic Fos responses of nonapeptide neurons are significantly predicted by personality, sex, and social context. We also show that personality is reflected in complex patterns of neuromodulation arising from multiple peptide cell groups. These findings provide novel insights into the mechanisms underlying sex-and phenotype-specific modulation of behavior, and should be broadly relevant, given that VP-OT systems are strongly conserved across vertebrates.

PLEASURE SEEKING AND BIRDSONG: NEUROENDOCRINE REGULATION OF THE MOTIVATION TO COMMUNICATE

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Songbirds are well known for singing at high rates within multiple distinct social contexts. This suggests that they are highly motivated to communicate and raises the possibility that the consequences of vocal production are rewarding (or alternatively that reward can facilitate vocal production). Much progress has been made in elucidating functions of song control nuclei in song production, learning, and sensorimotor processing; however until recently, little was known about the neural regulation of the motivation to communicate or the extent to which reward neural systems shape vocal production. Across vertebrates, dopamine and opioid neuropeptides underlie reward seeking and sensory pleasure associated with multiple behaviors. In songbirds, dopamine and opioids are found within brain regions implicated in motivation, reward, and singing behavior, including the medial preoptic nucleus (POM). Several lines of research indicate that dopamine and opioids in the POM play roles in birdsong that differ depending upon whether song is produced spontaneously in affiliative flocks (a type of song that can be considered socially-motivated) or used to attract mates (sexually-motivated song). Studies from my lab using qPCR, immunolabeling, autoradiography, HPLC, site-directed pharmacological manipulations, and behavioral tests of song and reward in male European starlings (*Sturnus vulgaris*) support the hypotheses that 1) distinct patterns of dopamine and opioid activity in the POM influence the motivation to produce socially- and sexually-motivated song, that 2) socially-motivated communication is facilitated and maintained by intrinsic reward induced by immediate release of opioids, and that 3) sexually-motivated communication is externally rewarded by opioids released upon successful mate attraction.

THE PLEASURE PRINCIPLE REVISITED: HOW SEXUAL PLEASURE LINKS SEXUAL AROUSAL, DESIRE, PREFERENCE, AND PERFORMANCE

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Although sexual behavior is orchestrated by hormonal and neurochemical actions in the brain, sexual experience allows animals to form instrumental and Pavlovian associations that predict sexual outcomes, thereby directing the strength of sexual responding. The behavioral and neurochemical mechanisms by which experience with sexual reward strengthens the development of sexual behavior and induces sexually-conditioned place and partner preferences will be discussed. In both male and female rats, early sexual experience with partners scented with a neutral or even noxious odor induces a preference for scented partners in subsequent choice tests [1]. Those preferences can also be facilitated by injections of oxytocin or dopamine D2 agonists paired with a male rat's first exposure to scented females, or injections of oxytocin, vasopressin, or dopamine agonists during a female rat's first rewarding sexual experiences with a male. Sexually naïve females that receive clitoral stimulation paired with a neutral odor form a sexual partner preference for a male bearing that odor during their first sexual encounter with a male [2], and despite being promiscuous, female rats display mate guarding behavior [3]. Conversely, conditioned place or partner preferences can be blocked by sexual frustration or by administration of the opioid receptor antagonist naloxone, suggesting that endogenous opioids are critical for sexual reward. Finally, a somatosensory cue (a rodent jacket) paired with sexual reward comes to elicit sexual arousal in male rats [4], such that paired rats with the jacket off show dramatic copulatory deficits. We propose that endogenous opioid activation forms the basis of sexual reward, which also sensitizes hypothalamic and mesolimbic dopamine systems in the presence of cues that predict sexual reward. Those systems act to focus attention on, and activate goal-directed behavior toward, reward-related stimuli. Thus, a critical period exists during an individual's early sexual experience that creates a "love map" or Gestalt of features, movements, feelings, and interpersonal interactions associated with sexual reward.

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SEX DIFFERENCES IN HORMONAL REMODELING OF CIRCADIAN PLASTICITY

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Sex differences in sleep-wake cycles currently receive tremendous attention attributable in part to differences in the "body clocks" of men and women. Men's average "circadian period" is longer than that of women (Duffy et al., 2011). The period of circadian rhythms is determined by a master clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Relevant in the present context is the fact that the localization of gonadal steroid receptors within the SCN regions differs in the sexes in most species studied (reviewed in Bailey and Silver, 2014).

The SCN has *two functionally and anatomically distinct regions. Cells of the core* lack detectable rhythmicity in clock gene expression and electrical activity, and they express FOS rapidly following a light pulse. Cells of the shell region are rhythmic in measures of clock gene expression and electrical activity, and their response to a light pulse is delayed. In the intact female, but not the male, estrogen receptor beta receptors are localized to the shell SCN region. In the intact male, but not the female, androgen receptors are localized to the core SCN region. The functional significance of these sex differences can be studied at the whole animal level, and at the level of individual cells and circuits. The focus here is on the male. Castration results in marked circadian alterations, including lengthened free-running period, decreased precision of daily activity onset, and elimination of early-evening activity bouts. Dihydrotestosterone (DHT) replacement restores these responses to pre-castration levels. Direct brain implants of testosterone in or near the SCN restore the normal free-running period of locomotor activity, and increase SCN AR expression indicating that the SCN is the site of action of androgen effects on the period of rhythmicity. DHT treatment of castrated animals restores SCN glial fibrillary acidic protein, postsynaptic density 95, and synaptophysin and reinstates normal molecular and behavioral responses to light. Thus androgens play a role in regulating SCN circuitry, with functional consequences for clock gene expression and behavioral responses to photic stimuli. Projections of AR-containing cells form a dense plexus in the core, with their fibers exiting the SCN dorsally. This indicates that directly retinorecipient cells of the core send efferents to targets in the adjacent hypothalamus, providing a mechanism whereby photic information from the external environment and endogenous signals from circulating hormone secretions are integrated within individual SCN cells and communicated to the brain.

Intuitively one might imagine that the 20,000 cells of the SCN are synchronized and produce a single coherent oscillation. Instead, precise timing of oscillation within individual neurons is dependent on their location within the nucleus; small groups of SCN neurons give rise to distinctive serially activated subregions over the day. Importantly, studies of mutant mice reveal independent cell and circuit contributions to rhythmicity. The link between rhythmic clock gene expression within individual cells, and serial activation of clusters of cells located in distinct parts of the SCN network are necessary for robust, high amplitude circadian oscillation. The results demarcate a hypothalamic neuroendocrine feedback loop in which the SCN regulates circadian rhythms in gonadal hormone secretion, and in turn, hormones act on their SCN receptors to modulate circadian oscillation of the SCN and its output to the brain.

WHY DO ANIMALS HAVE SEX? A NEUROBIOLOGICAL PERSPECTIVE

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"Why do animals have sex?" is a question that has been the focus of research in our lab for several decades. The conventional view for rodents and non-human mammals is that animals have sex to produce offspring. The answer for people may include the idea that sex is pleasurable. Our non-scientific view is that human teenagers do not have the cognitive capacity to causally link sex with pregnancy, so how can a rat (with presumably less cognitive abilities than a human teenager) make such a connection? Our guiding idea is that both human and non-human animals have sex because it is pleasurable. This view led us to study neurotransmission within the nucleus accumbens of female Syrian hamsters as a basis for understanding why animals have sex. Our research uncovered that sexual experience (not surprisingly) has rewarding consequences, which are paralleled by plasticity in dopamine release and postsynaptic alterations in biochemical processes and structural changes in medium spiny neurons in the core of the nucleus accumbens. These changes are mediated by signaling through dopamine D1 receptors, with structural changes in dendritic spines localized to neurons expressing dopamine D1 receptors. Further it appears that the stable transcription factor, deltaFosB, mediates these effects of sexual experience on the rewarding consequences of sexual experience and on copulatory interactions with male hamsters that would be predicted to increase reproductive success. Here viral overexpression of deltaFosB can augment the impact of subthreshold sexual experience, whereas viral overexpression of deltaJunD, a dominant negative protein which dimerizes with deltaFosB, prevents the behavioral consequences of sexual experience. The overall goal of this presentation is to honor the illustrious career of Professor Jacques Balthazart, whose research has been a continuous source of scientific inspiration.

OUR STOLEN FIGURES: ENDOCRINE DISRUPTORS AND OBESITY

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There is little doubt that reproductive processes are influenced by endocrine disruptors, natural and synthetic molecules that interfere with hormone action, often during fetal development. Furthermore, there is little doubt that endocrine disruptors interfere with sexual differentiation. Less well known is the fact that endocrine disruptors can alter energy balance (energy intake, storage and expenditure). Recent evidence indicates that endocrine disruptors affect many individual processes known to contribute to obesity, including the gut microbiome, adipocyte differentiation, energy metabolism, ingestive behavior, and the tendency to accumulate adipose tissue in response to certain diets. Understanding effects of endocrine disruptors on energy balance will be aided by attention to the processes involved in sexual differentiation. This is because many energy balancing traits are sexually dimorphic with the masculine phenotype most closely linked to metabolic diseases such as type II diabetes and heart disease. So far, it is clear that at least some endocrine disruptors have masculinizing effects via classical organizational effects on sexually dimorphic energy balancing traits during fetal development. In addition, we should expect endocrine disruptors to impact other defined mechanisms of sexual differentiation (e.g., sex chromosome action, aromatization, active feminization, and organizing actions at later periods of development, such as puberty). Investigators interested in effects of endocrine disruptors on peripheral metabolism often work in isolation from those interested in the effects of endocrine disruptors on ingestive behavior. In fact, changes in peripheral metabolism have organizational and activational effects on the neural circuitry that controls ingestive behavior. Together, all of these considerations demand a concerted multidisciplinary and integrative approach to the study of endocrine disruptors.

THE MEDIAN PREOPTIC NUCLEUS (MnPN): A KEY SITE FOR ESTRADIOL MEDIATED CHANGES IN SLEEP

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Sleep complaints such as insufficient sleep and insomnia are twice as prevalent in women than men. Symptoms of sleep disruption are often coincident with changes in the ovarian steroid profiles across a woman's lifespan. While gonadal steroids and gender have been implicated as risk factors for sleep disruptions and insomnia, the relationship between ovarian steroids and sleep is poorly understood. Understanding how steroids influence sleep is imperative if we are to gain a better understanding of how dysregulation of endocrine systems influence the risk for, and mechanisms, of sleep disorders in women.

In our recent work, we have employed a rodent model to examine the effects of ovarian steroids on sleep behavior and its underlying neurocircuitry. We have made significant inroads into how estradiol (E2) influences normal wake, non-rapid eye movement sleep (NREMS), and rapid eye movement sleep (REMS) under non-pathological conditions demonstrating that (1) when endogenous E2 is elevated, both NREMS and REMS are significantly reduced by up to 50% compared with phases of the estrous cycle where E2 is low; (2) ovariectomy (OVX) eliminates these fluctuations in sleep while exogenous E2 replacement reinstates the suppression of sleep and (3) E2-mediated suppression of sleep is correlated with an attenuation of neuronal activation in two main hypnogenic preoptic area nuclei, the ventrolateral preoptic area (VLPO) and median preoptic nucleus (MnPN). Furthermore, we recently found that estrogen receptor alpha expression was markedly greater in the MnPN versus the VLPO, suggesting that E2 may be acting directly in the MnPN to mediate the suppression of sleep.

To test whether the MnPN is a key site for estrogenic regulation of sleep, the estrogen receptor antagonist ICI 182,780 (ICI) was microinfused into the MnPN. Briefly, guide cannulae targeting the MnPN were implanted into adult OVX females. Following a within animal design, sleep/wake behavior, derived from electroencephalographic recordings, was assessed at baseline (systemic oil injection) and again following systemic E2 administration in animals receiving microinfusions into the MnPN of either ICI (50 ng in 500 nL; n=8) or vehicle (n=6; 500 nL 0.25% DMSO). The E2-mediated percent change from oil baseline for each vigilance state was calculated. The results demonstrated that ICI directly infused into the MnPN significantly attenuated the E2-mediated suppression of sleep (both NREMS and REMS) bringing total sleep to within 20% of baseline sleep ($U=7.00$, $p=0.029$). Additionally, ICI attenuated the E2-mediated reduction in the total number of REMS bouts compared to vehicle ($t_{12}=3.402$, $p=0.005$). Next, the ability of E2, directly infused into the MnPN of OVX animals, to recapitulate the systemic effects of E2 was tested. In the absence of systemic E2, wakefulness was significantly increased 6 hours after E2 infusion into the MnPN compared to controls ($t_{12}=2.204$, $p=0.048$). Taken together, these findings strongly implicate the MnPN as a key site for the estrogenic control of sleep in females and to our knowledge is the first demonstration of E2 acting directly on a sleep-associated nucleus. Thus, understanding the mechanisms underlying E2 modulation of sleep has the potential to shed new light on the roots of sleep disturbance in the female brain. Ultimately, the findings may serve to uncover novel drug targets for the treatment of sleep disorders in women.

A NATURAL MODEL OF GONADAL STEROID EFFECTS ON EMOTIONAL LABILITY AND RISKY BEHAVIOR IN FEMALES

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Parenting style influences anxiety and risk-taking behavior in children [1,2]. In the female rat, Low levels of licking/grooming (LG) received during the first week of life are correlated with increased anxiety and risk behavior relative to High LG offspring [3]. Anxious, depressive-like, risk-taking, and locomotor behaviors are all influenced by changing levels of progesterone [4,5]. These behaviors may be mediated by the activity of progesterone's metabolite, allopregnanolone. Given our previous finding that female offspring of Low LG mothers show a greater increase in progesterone at proestrus than High LG offspring [6], it is possible that only Low LG offspring show estrous cycle-dependent changes in emotional behaviors. Recent research has characterized two stable behavioral phenotypes in rats: Low Responders (LR) and High Responders (HR). Female HR rats engage in riskier behaviors, show increased exploration, and engage in lower levels of LG relative to LR rats [4,7]. Using a locomotor activity test, the elevated plus maze, and the forced swim test, we sought to evaluate both affective and exploratory behaviors during proestrus and metestrus in Low and High LG offspring. We hypothesized that Low LG females would resemble the HR rat, and show a difference in risk-taking behaviors between proestrus and metestrus as well as affective dysregulation, which we defined as abnormal responding to emotional events. We also believed that Low LG females would be more vulnerable to the effects of progesterone at proestrus. On the locomotor activity test, only Low LG females showed decreased activity at metestrus relative to proestrus. On the forced swim test, only Low LG offspring showed cycle-dependent differences in floating behavior, with increased floating at estrus relative to proestrus and at metestrus relative to estrus, and higher levels of plasma corticosterone at metestrus relative to estrus. High LG offspring showed a more stable affective profile, with no estrous cycle-related changes in behavior or corticosterone level. We additionally investigated whether estrous cycle-dependent differences in anxious and risk-taking behavior were driven by allopregnanolone levels by peripherally injecting placebo or finasteride, a 5 alpha-reductase inhibitor. On the elevated plus maze, High LG offspring displayed behavior consistent with typical female rat behavior [5], spending a greater percent of time in the open arms and showing a decreased number of entries into the closed arms during proestrus relative to metestrus. Contrary to these findings, Low LG offspring showed aberrant behavior on the elevated plus maze on many variables, including an increase in the percentage of time spent in the open arms during metestrus relative to proestrus. Interestingly, finasteride treatment successfully removed all estrous cycle-related differences in behavior. In summary, only Low LG female offspring exhibit estrous cycle-dependent differences in locomotor and forced swim test behavior. While it appears that allopregnanolone contributes to elevated plus maze behaviors for both LG phenotypes, Low and High LG animals show opposing behavioral profiles on this test. The deviation from normal elevated plus maze behavior exhibited by Low LG offspring may stem from an altered reactivity to a novel environment. Decreased locomotor activity at metestrus coupled with impaired risk assessment may explain why Low LG animals spent more time in the open arms during metestrus. Poor evaluation of risk is increased in HR females following progesterone withdrawal [4]. Risky behavior and emotional dysregulation may stem from dysfunctional prefrontal cortex-amygdala connectivity. Further research will investigate the effects of maternal care on connectivity of

these two brain areas in Low and High LG female offspring. These findings suggest a natural model of gonadal steroid effects on emotional lability and risky behavior in females.

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DISTINCT ROLES OF ANDROGENIC AND ESTROGENIC TESTOSTERONE METABOLITES IN GENE REGULATION UNDERLYING MALE SEXUAL BEHAVIOR

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Testosterone (T) controls a large array of physiological and behavioral processes, including male sexual behavior. T is able to directly bind and activate androgen receptors (ARs) but in numerous instances, T modulates physiology and behavior via its active metabolites. It can be reduced to the androgen 5α-dihydrotestosterone (DHT) that activates ARs or can be locally converted to 17β-estradiol (E2) that activates estrogen receptors (ERs). This metabolism of androgenic to estrogenic compounds is functionally important since it allows T to not only activate androgen receptors but also estrogen receptors and the related signaling pathways. These 2 testosterone metabolites modulate neuroplasticity in the medial preoptic nucleus (POM) in a large number of vertebrates, including the Japanese quail and by doing so, activate male sexual behavior. However, the complete array of transcriptional changes involved in this T-dependent neuroplasticity and the specific importance of ER and AR in regulating these changes are currently not known. The goals of the present study were to answer these questions using the specific receptor antagonists, flutamide (AR) and ICI182,780 (ER). We analyzed the effects of these systemic treatments on the appetitive and consummatory aspects of male sexual behavior in Japanese quail treated with a chronic implant of testosterone. We also quantified the consequences of blocking either steroid receptor on wide-scale gene transcription specifically in the POM to functionally link phenotypic plasticity and behavior. All animals were killed at the end of behavioral testing and the POM was microdissected out of the skull, RNA extracted and samples from the same group were pooled for microarray analysis, using the chicken GeneChip™ affymetrix. The normalization process was performed using RMA (R Bioconductor package affyPLM version 1.36.0). Behavioral observations confirmed the importance of ER and AR in the activation of both aspects of male sexual behavior. We identified 31 and 79 genes respectively up- and down-regulated by AR while 17 and 85 genes were respectively up- and down-regulated by ER. Surprisingly, both estrogen and androgen receptors seemed to affect the transcription of common genes: 3 transcripts were found to be up-regulated (ADAMTS3, ACE2, BACH1) while 8 genes were down-regulated (SLCL5A36, SYCE3, ABCC4, CCDC18, VPS41, NOD1, TARP and LOC100857201) by manipulations of both receptors. The functional analysis using DAVID highlighted 34 clusters regulated by androgen receptors, the main one linked to cation binding (22 transcripts), ATP binding (14 transcripts), transcription (8 transcripts), channel activity (7 transcripts), as well as neuron development (6 transcripts), and kinase activity (5 transcripts). The functional analysis of ER- regulated genes defined 28 clusters, including ATP binding (17 transcripts), protein transport (8 transcripts), kinase activity (6 transcripts) and transcription (6 transcripts). Additional analyses are required, as a different normalization process of the microarray data (MAS5) leads to a different output. Validation of these outputs is currently taking place using qPCR. Although much remains to be done, these data lay down a strong foundation for future studies and will help deciphering the cellular mechanisms and pathways underlying the activation of complex behaviors.

ANTIDEPRESSANT POTENTIAL OF SUBACUTE AROMATASE INHIBITION

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Aromatase inhibitors, such as letrozole, are widely-used for the treatment of hormone-responsive breast cancer in women. Recent findings have shown that sustained aromatase inhibition with letrozole for one week has no antidepressant effect in cycling female rats, but exerts a pro-depressive effect in ovariectomized females [1]. In the present study, we investigated whether subacute letrozole treatment has an effect in a test of antidepressant potential in cycling female rats. Adult cycling female Sprague-Dawley rats were subjected to the forced swim test (FST). Females were treated with either vehicle or the antidepressant fluoxetine for 28 days, in combination with subacute letrozole (3 injections in 24 hours) or sustained (7 days, 1 injection/day) letrozole treatment. Behavioral response in the FST was evaluated with the use of the software *Kinoscope*. Also, gonadal hormone levels were assayed following behavioral testing. Immobility duration in the FST was reduced following acute aromatase inhibition, indicating letrozole's antidepressant potential. Additionally, swimming duration was enhanced, suggesting letrozole's action on the serotonergic system [2]. Instead, aromatase inhibition for one week did not show an antidepressant response. Estrogen levels were undetected following letrozole treatment, as expected. Moreover, serum testosterone levels were elevated following acute letrozole treatment and this was associated with the decreased depressive behavior in the FST. On the other hand, the regression analysis revealed that progesterone levels explained the increased swimming behavior in the FST. These findings indicate that aromatase inhibitors have psychotropic attributes that depend on treatment duration [3]. Thus, it is of interest to investigate whether aromatase inhibitors can act as rapid antidepressants or as augmenting agents in the treatment of depression.

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MALE SEXUAL BEHAVIOR DRIVES THE REACTIVATION OF FEMALE GONADOTROPE ACTIVITY IN GOATS

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In ungulates, seasonal activity is regulated by photoperiodic variations, and females show a period of sexual activity followed by a period of sexual rest or anoestrus. However, this seasonal activity can be also influenced by social cues. Indeed, it is possible to reactivate the whole gonadotrope axis and to trigger ovulation during the anoestrus by exposing females to a male (the so called 'male''effect). Given that, during the anoestrus season, males are also under the inhibitory influence of the season, we have recently developed a model of sexually active males. Through the use of photoperiodic treatments (long days followed by a period of short days exposure during winter), these males exhibit, during the normal period of sexual rest, a high level of sexual activity assessed through the measure of testis volume, plasma testosterone level and sexual behavior. Our hypothesis was that such bucks could optimize the response of the females in comparison to males exposed to normal photoperiodic variations and therefore showing low sexual activity. Using this model, we demonstrated that male sexual behavior is a key factor for the successful induction of the male effect. Indeed, females exposed to sexually active males show a much higher level of ovulation than females exposed to sexually inactive males. In addition, we showed that the duration of interaction with the male could be highly reduced when females are exposed to these sexually active males, without reducing the female LH or ovulatory responses. Finally, we also demonstrated that olfactory cues, which are known to induce the reactivation of LH pulsatility, are inefficient to induce ovulation when presented in the absence of any male sexual activity, thus further highlighting the importance of male sexual behavior.

STEINACH VERSUS YOUNG: HOW THE EFFECTS OF ESTROGEN ON THE SEXUAL BEHAVIOR OF MALE RATS DISAPPEARED AND HOW THE MALE BRAIN EMERGED

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The hypothesis that male sexual behavior depends on estrogen, formed in the brain from testosterone in the circulation, was verified by Steinach in 1936 [1]. This discovery and his many other findings, including the bi-potential of the sexes, were broadly recognized and reviewed repeatedly by the Nobel Prize Committee, but Steinach´s research came to an end because of the political development in the 1930s, and his work was forgotten when Young launched sex differences as the focus of behavioral neuroendocrinology [2].

Steinach and his collaborators reported that injection of 5.8 μg estradiol benzoate (EB) or more induced lordosis in all adult castrated male rats and that sexually active male rats showed bisexual behavior if injected with 23.2 μg EB. They also reported that EB, but not androgens, increases cerebral blood flow, indicating an action of estrogen on the brain, that the threshold dose of testosterone for induction of ejaculation in castrated rats was reduced tenfold by addition of 8.3 μg EB, and they confirmed that male rats convert androgens into estrogen [1].

These effects of estrogen disappeared in 1959 because results, which were in line with Steinach´s results [3], were interpreted to show the opposite and because the synergistic effect of estrogen and androgen on sexual behavior was disregarded [2,4]. Instead, the male brain was introduced using the female guinea pig, treated with testosterone in utero, as a model, importantly a model without a penis [2]. However, these animals were not injected with testosterone and tested for mounting and while they mounted much more than animals with a female rather than a male brain, i.e., females, when they were injected with EB and progesterone, their behavior could not be related to their hypothetical male brain, because animals with a male brain, i.e., males, were not tested for comparison.

Lordosis, the penis, mounting, estrogen, and testosterone were mixed up when the male brain appeared. And the suggestion that the male brain is rather insensitive to estrogen was contradicted not only by the results used to demonstrate its existence [2,4] abut also by the results that Steinach had reported.

Because the effects of estrogen in the male vanished, the study of the role of gonadal hormone metabolism in sexual behavior was delayed and the field entered the blind alley of hormone specificity of a masculinized brain, a brain that has still not been found.

Young´s work remains useful in showing the strength of a conceptual framework in stimulating research [4]. It is also valuable in raising issues on how to review available literature in generating hypotheses and how to design experiments in testing these hypotheses.

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NON-CLASSICAL ESTROGEN RECEPTOR ALPHA (ERα) SIGNALING AND REGULATION OF BODY WEIGHT

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Ovarian estradiol is an important regulator of energy expenditure, food intake, and body weight. These actions are believed to be exerted in large part via activation of ERα in hypothalamic neurons, as mice lacking this receptor exhibit a metabolic syndrome characterized by obesity, reduced metabolic energy expenditure, reduced locomotor behavior, and dysregulation of insulin-glucose homeostasis. Our studies have focused on the ERα signaling mechanisms that mediate these effects. In classical ERα signaling, ERs bind directly to estrogen response elements in the promoters of target genes to regulate transcription. Non-classical signaling, by contrast, is mediated by ERα tethering via other transcription factors to DNA recognition sequences, or by activation of membrane-localized receptors coupled to cytoplasmic signaling cascades. We have assessed the role of non-classical signaling in energy homeostasis in non-classical ERα knock-in (NERKI) mice, in which classical signaling is abrogated, leaving isolated non-classical ERα signaling intact. Our studies have revealed that non-classical ERα signaling is sufficient to normalize energy homeostasis, locomotor activity, adiposity, and metabolic hormone secretions in otherwise complete ERα null mutants. In recent studies, we have additionally determined that the manifestation of a lean body weight phenotype in NERKI mice is dependent upon the presence of phytoestrogen-containing soy in the maternal diet during gestation and/or lactation. Our work thus supports the idea that non-classical signaling mechanisms mediate the major effects of estradiol on energy homeostasis, and that phytoestrogen exposure during early development may program the degree to which these signaling pathways are activated in adulthood.

RAPID CHANGES IN BRAIN ESTROGENS AND SENSORIMOTOR INTEGRATION

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It is now clear that the brain of males and females can produce its own local supply of estrogens. The signaling capacity of brain estrogen fluctuations is both rapid and spatially targeted, similar to classical neuromodulators. This talk will focus on how such brain-derived 'neuro-estrogens' can guide sensory perception and learning. Using the songbird model system, we pursue these questions with a combination of in vivo recording, neurochemical, and behavioral methods. I will discuss our recent work showing how brain estrogens (1) respond to sensory cues from the environment, (2) regulate how sensory stimuli are represented in auditory circuits, and (3) guide the consolidation of long-lasting memories. Building on the pioneering work of Jacques Balthazart and colleagues, this work reinforces the view that estrogens can act as intrinsic modulators of neural circuits and corresponding behaviors.

PERIPUBERTAL STRESSORS ALTER BEHAVIORAL RESPONSE TO ESTRADIOL LATER IN LIFE

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The peripubertal period is considered a second period of sexual differentiation of the brain, and during this time, an animal's response to stress undergoes development. We have discovered that particular stressors, but not others, during pubertal development of female mice result in enduring changes in behavioral responsiveness of the brain to estradiol and progesterone. We do not consider this an influence on sexual differentiation of the brain, *per se.* However, as with sexual differentiation, the pubertal stressors result in an enduring change in the brain's response to hormones. In the case of some behaviors, such as female sexual behavior, a pubertal stressor decreases behavioral response to ovarian hormones later in life; in other cases, as in particular cognitive tasks, it eliminates the effects of estradiol. In tests of depression-like behaviors, the pubertal stressor reverses the effects of estradiol. The ovaries are essential for the long-term effects of immune challenge; ovariectomy prior to immune challenge blocks the long-term effects on response to estradiol on female sexual behavior, even though it is without effect on the sickness behavior that results from the immune challenge. A pubertal stressor results in changes in the concentration of estrogen receptor-immunoreactive cells in key brain areas, suggesting a cellular mechanism for this remodeling of the brain's response to hormones. Microglia, the immune responsive cells within the brain, and which are known to play a role in development of the brain, display a more highly ramified phenotype during the peripubertal period. An immune challenge during the peripubertal period results in increased activation of microglia in some brain regions. Unlike both an inbred and outbred strain of mice, an immune challenge is ineffective in causing long-term changes in response to estradiol in rats, a species which typically has a very brief period of pubertal development. This may provide clues to the underlying cause of the sensitive period for the enduring effects of immune challenge on behavioral response to estradiol. A hypothesis will be discussed that predicts that particular adverse experiences in girls may cause enduring remodeling of the brain's response to estradiol and/or progesterone *via* activation of the immune system. This could then lead to altered response to any behavior influenced by estradiol in women.
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ANDROGENS, COURTSHIP, AND ATHLETICISM: LESSONS FROM AN EXUBERANT TROPICAL BIRD

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Pressures of sexual selection drive males of many species to evolve elaborate courtship displays to enhance reproductive success. Such displays can involve considerable visual and acoustic signaling that requires specialized functions and exquisite coordination of multiple neuromuscular systems. Presumably, testosterone serves as a global signal to both centrally motivate performance of courtship as well as optimizing function of peripheral motor systems. We have examined the likely multiple roles of testosterone in the activation of courtship behavior of the Golden-collared manakin (*Manacus vitellinus*) of Panamanian rainforests. Manakins are well known for their elaborate courtship displays, and *Manacus* displays are especially acrobatic and noisy resulting from their frequent powerful wingsnapping behaviors. Our work is motivated by several related hypotheses but I focus on two in this presentation that a) females choose males for the neuromuscular prowess (athleticism) and b) testosterone enhances physical capabilities by facilitating the function of neural and muscular systems. Consistent with hypothesis one, we find that individual males differ in their courtship performances and that females choose to mate with males that are most active and display courtship elements more quickly. Consistent with hypothesis two, we find that testosterone activates male courtship behavior and does so largely through activation of androgen receptor pathways. The actions of androgen are noticeably widespread. AR are expressed in unique patterns in the manakin brain, with significant levels in spinal motor and somatosensory neurons and in a variety of peripheral skeletal muscles. We find that activation of peripheral AR is especially important for optimal courtship performance. Gene expression studies of forelimb muscles reveal a considerable number of genes regulated by AR in both manakins and in zebra finches. Moreover, across a range of avian species, muscle AR expression levels strongly correlate with display complexity. Our work on manakins illustrates that androgens exert extensive control over diverse neuromuscular systems and may do so across a wide-range of avian species. By their actions, androgens likely promote optimal neuromuscular function enabling the concomitant evolution of complex vertebrate behavior. Supported by NSF IOS-0646459.

ESTRADIOL REDUCES DEATH OF HIPPOCAMPAL PYRAMIDAL NEURONS SUBJECTED TO GLOBAL ISCHEMIA: CELLULAR MEDIATORS.

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Transient global forebrain ischemia causes selective, delayed death of hippocampal CA1 pyramidal neurons, and estrogenic compounds including the endogenous ovarian hormone 17β-estradiol (E2) reduce neuronal loss in males and females (1). The neuroprotective efficacy of E2 after a prolonged period of hormone deprivation is controversial, and few studies examine this issue in aged animals treated with E2 after induction of ischemia. We investigated the neuroprotective effects of E2 administered immediately after global ischemia in aged female rats (15-18 months) 6 months after removal of the ovaries to mimic long-term hormone deprivation. We also used electrophysiological methods to test the hypothesis that CA1 synapses in the aging hippocampus remain responsive to E2 after prolonged hormone withdrawal. A single dose of 2.25 mg of E2 infused intraventricularly (icv) after reperfusion significantly increased cell survival in aged, long-term hormone-deprived females. Bath application of 1 nM E2 onto brain slices derived from non-ischemic aged females 6 months after ovariectomy significantly enhanced excitatory transmission at CA1 synapses, and high frequency stimulation induced normal long-term potentiation (LTP). The magnitude of LTP and of E2 enhancement of field excitatory postsynaptic potentials was indistinguishable from that recorded in slices from young female rats (2). We also assessed the possible role of signal transducer and activator of transcription-3 (STAT3) and its target genes as candidate cellular mediators of E2 neuroprotection in global ischemia in young rats. Injection of E2 icv immediately after ischemia promoted activation of STAT3 signaling, association of STAT3 with the promoters of target genes and STAT3-dependent mRNA and protein expression of pro-survival proteins in hippocampal CA1. In animals subjected to ischemia, E2 further enhanced activation and nuclear translocation of STAT3 and STAT3-dependent transcription of target genes. Importantly, STAT3 inhibitor peptide and STAT3 shRNA delivered directly into the CA1 of living animals abolished E2 neuroprotection. In addition, survivin, a member of the inhibitor-of-apoptosis family of proteins and known gene target of STAT3, is essential for E2 neuroprotection, as evidenced by the ability of shRNA to survivin to reverse neuroprotection (3). Thus, the aging hippocampus remains responsive to E2 administered either in vivo or in vitro even after prolonged periods of hormone withdrawal. Moreover, STAT3 and survivin are potentially important downstream mediators of E2 neuroprotection in an *in vivo* model of global ischemia.

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NEUROPROTECTIVE ACTIONS OF BRAIN AROMATASE

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Aromatase expression in the mammalian and avian brain is increased after different forms of brain injury, including excitotoxic and mechanical lesions [1,4,5,9,11], experimental stroke [2,10,11], chronic hypertension [7] and neuroinflammation [3]. The increased expression of aromatase after brain injury occurs mainly, but not exclusively [7], in reactive astrocytes [2,4,9].

The increased expression of aromatase in the injured brain probably represents an endogenous protective mechanism, since its pharmacological inhibition [1,5,11] or the central administration of aromatase antisense oligonucleotides [12] results in increased neurodegenration, a situation also observed in aromatase deficient animals [1,5,6]. The neurodegenerative effects of aromatase deficiency are counterbalanced by the administration of estradiol [1,6,8], indicating that the neuroprotective properties of aromatase lie in its ability to catalyze the formation of estradiol rather than in reducing estradiol precursor levels. By increasing local estradiol levels within the brain, aromatase modulates local inflammation, regulates the function of the neurovascular unit, promotes the release of growth factors and decreases neuronal death [1,4,5,8,9,11,12].

Several observations suggest that aromatase may have different neuroprotective effects in males and females. Therefore, it is possible that sex differences in brain aromatase activity or in brain vulnerability to aromatase deprivation may be involved in the sexually dimorphic expression of different brain pathologies, such as Parkinson's and Alzheimer's diseases.

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MYELIN REGENERATION WITH ANDROGENS

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Until recently, myelin was merely considered as an electrical insulator around axons. This is now changing with an increasing number of studies revealing that myelin sheaths are in fact highly dynamic structures. Myelin plasticity is thought to have a major impact on the functioning of neural circuits and to play an important role in cognition, brain aging and a wide range psychiatric disorders [1]. The concept of myelin plasticity also broadens the scope of investigations on the effects of steroid hormones on brain and behavior, so far mainly focused on neuronal activity and synaptic plasticity. Steroid hormones are indeed known to influence the formation of myelin during development and also its regeneration in adulthood. In contrast to the limited regenerative capacity of adult neurons, lost or damaged myelin of the central nervous system (CNS) can be replaced. This process, named "remyelination", requires the generation of new myelin forming oligodendrocytes from oligodendrocyte progenitor cells (OPC).

The role of steroid hormones in the regeneration and plasticity of myelin may be more important as previously thought. Thus, we have recently reported that testosterone stimulates the formation of new myelin sheaths in chronic demyelinated brain lesions resulting from cuprizone intoxication of male mice during 12 weeks. In this model, spontaneous myelin regeneration no longer takes place, and it thus mimics the failure of remyelination in chronic multiple sclerosis lesions. However, testosterone failed to stimulate the formation of new myelin in testicular feminized (Tfm) mice with a non-functional androgen receptor (AR). Importantly, testosterone did not stimulate the formation of new myelin sheaths after specific knockout of the AR in neurons and macroglial cells [2]. The potent synthetic testosterone analog 7alpha-methyl-19-nortestosterone (MENT), which has been developed for long-term male contraception and androgen replacement therapy in hypogonadal men and does not stimulate prostate growth, also efficiently promoted myelin repair. Thus, the neural brain AR is required for the remyelination effect of testosterone, without ruling out a role of testosterone aromatization into estradiol.

A key role of AR-dependent testosterone signaling in myelin regeneration was also demonstrated in two models of lysophosphatidylcholine (LPC)-induced acute and reversible demyelination. *In vitro*, after the demyelination of cerebellar slices in organotypic culture with LPC, testosterone stimulated the remyelination of axons, and its effect could be mimicked by 5 alpha-dihydrotestosterone, a metabolite which is not converted to estrogens, and blocked by the selective AR antagonist flutamide. Most import, after LPC-induced demyelination of the ventrolateral funiculus in the male mouse spinal cord, spontaneous remyelination was only observed in the presence of AR and functional testes or testosterone treatment. These results reveal an unexpected importance of AR signaling in the endogenous regeneration of myelin, and they qualify the AR as a promising drug target for promoting both remyelination and myelin plasticity.

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SEXUALLY DIMORPHIC EFFECTS OF ENDOCRINE DISRUPTORS ON BRAIN AND BEHAVIOR

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Several environmental substances (synthetic or natural) are able to impact endocrine function (endocrine disrupting chemicals, EDCs) and, therefore, they may have long-term consequences, especially if exposure occurs during embryonic development. Most of EDCs are agonists or antagonists of androgen or estrogen receptors, therefore they may interfere with brain and behavior sexual differentiation.
We present here data collected in our laboratory on two widely used animal models: the mouse and the Japanese quail. In the quail, we investigated the effect of several EDCs [diethylstilbestrol (DES), genistein or ethylene,1,1-dichloro-2,2-bis-p-chlorophenyl (DDE)] administered in eggs on the differentiation of male sexual behavior and of the parvocellular sexually dimorphic vasotocin system. In the mouse we investigated the effects of perinatal exposure to bisphenol A (BPA) or genistein on the sexual differentiation of NO producing system and of the kisspeptin system. We investigated also the organizational effects of these EDCs on sexual, social, and explorative behaviors.
Our data suggest that precocious exposure to EDCs through maternal administration (in mice) or in egg deposition (in quail) may permanently alter some sexually dimorphic circuits and influence in a gender-oriented way some behaviors. In particular, the timing of exposure to EDCs is a critical factor, such that the effects of a particular EDC will vary over the lifecycle of the animal as well as across species and phyla. Therefore, exposure to the estrogenic chemicals during embryonic development has consequences beyond impaired function of the reproductive axis. This makes it very challenging to evaluate the short and long-term effects of EDCs. These compounds are therefore a third player within the nervous system and the evolutionary implications of having them in the normal food supply for certain human populations (i.e. phytoestrogen derivatives from soy), as well as for wild and farm animals should stimulate a wide discussion about their beneficial or adverse role.

IMPAIRED POSTPARTUM MATERNAL BEHAVIOUR FOLLOWING CONDITIONAL DELETION OF THE PROLACTIN RECEPTOR FROM GABAERGIC NEURONS.

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The anterior pituitary hormone prolactin plays an important role in stimulating the onset of maternal behavior, acting through prolactin receptors (Prlr) in the medial preoptic nucleus, but the specific neurons involved are not known. Using dual-label in situ hybridization, we have recently observed that many of the Prlr in this region are localized to GABAergic neurons. Using mice expressing the fluorescent marker Tomato under control of the GABA-specific vesicular GABA transporter (vGat) promoter, we could identify prolactin-induced pSTAT5 in many GABAergic neurons in the medial preoptic area. To test the hypothesis that these prolactin-sensitive GABAergic neurons mediate prolactin action to promote maternal behavior, we generated mice lacking Prlr in GABA neurons using Cre-Lox technology, and examined post-partum maternal behaviour. Mice with LoxP sites flanking the Prlr gene (PRLRflox) were crossed with mice expressing Cre-recombinase under the control of the vGAT promotor (vGAT-Cre), or the neuron-specific calcium-calmodulin-dependent Kinase-2α (CamK-Cre) promoter. Cre-mediated recombination could be detected through activation of a green fluorescent protein (GFP) construct within the transgene. Female mice homozygous for the PRLRflox and expressing Cre, and control mice that lacked Cre, were monitored over several estrous cycles, and then placed with a wildtype male and allowed to mate. The male was removed when a post-coital mucus plug was present, and females observed throughout pregnancy and post partum. While CamK-Cre mice had abnormal cycles characterized by prolonged periods of diestrous, vGAT-Cre cycles were normal, and both groups were able to get pregnant and have an apparently normal pregnancy. The day of parturition was designated as postpartum day 1 (PPD1), and maternal behavior was tested on PPD2 using a pup-retrieval paradigm. Both lines of Cre-positive mice showed high levels of GFP in the medial preoptic nucleus (and other areas), indicating deletion of the prolactin receptor. vGAT-Cre mice showed significantly impaired maternal behaviour, compared to control mice, suggesting that GABAergic neurons mediated some of prolactin action to stimulate maternal behaviour. CamK-Cre mice, however, showed markedly worse maternal behavior, suggesting that additional non-GABAergic neurons are also involved in prolactin action on maternal behavior. Further characterization of the phenotype of these mice will be extremely informative about the mechanisms of prolactin action in the brain.

INVOLVEMENT OF THE AVIAN SONG SYSTEM IN REPRODUCTIVE BEHAVIOUR

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Since its discovery over 35 years ago, the song system of songbirds has become the prevalent model system for the neurobiological study of vocal learning and performance. A major function of song in males is to attract a female, and because singing is often correlated with reproduction, singing can be seen as a form of appetitive behaviour that may then be consummated by sexual union in which the male's sperm is transferred to the female by the apposition of the birds' cloacae. Here we ask whether the neural basis of consummatory as well as appetitive aspects of reproductive behaviour can be found in the song system itself. We show that the same medullary nucleus, retroambigualis (RAm), that projects upon spinal motoneurons innervating expiratory muscles – which provide the pressure head for vocalization - and upon vocal motoneurons for respiratory-vocal coordination, also projects upon cloacal motoneurons. Furthermore, RAm neurons projecting to sacral spinal levels housing cloacal motoneurons receive projections from the same premotor nucleus of the forebrain song system, robustus arcopallialis (RA), that projects upon vocal motoneurons and expiratory premotor neurons in RAm. Thus, by indicating a disynaptic relationship between RA and motoneurons innervating the reproductive organ, these results potentially extend the role of the song system to include consummatory as well as appetitive aspects of reproductive behaviour.

Cloacal contact during mating is facilitated in female songbirds by the copulation solicitation display (CSD), the suite of postural responses signalling estrogen-dependent sexual receptivity resembling lordosis in mammals. In female canaries the elicitation and intensity of the CSD is under the control of highly specific, possibly innate, acoustic components of male song, called sexy syllables. Moreover, although partial lesions of one of the main song system inputs to RA, namely HVC, causes female canaries to present the CSD indiscriminately, their CSDs remain most intensely evoked by canary sexy syllables. This would seem to indicate that the pathway originating in HVC and descending via RA to RAm contributes in some way to the control of the CSD; in particular, it may provide a *raison d'etre* for these projections in species in which the female sings very little, such as canaries. Although males do not normally present the CSD, cloacal positioning and associated movements are presumably as important as those in females for successful insemination and may, therefore, also be controlled in part by the same pathways as those in females. In sum, a neural pathway such as here described, originating in the song control nucleus HVC and projecting upon RAm, via RA, could mediate continuity between appetitive and consummatory aspects of reproductive behaviour.

How this continuity might be achieved will be discussed.

CONTEXT-DEPENDENT EFFECTS OF MATERNAL TESTOSTERONE: SOLVING A PARADOX

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In many animal species mothers differentially bestow their eggs with hormones. The increasing pattern of yolk testosterone (T) concentrations over the avian laying sequence is interpreted as mitigating effects of hatching asynchrony by boosting the later hatching chicks. However, why would avian mother first produce hatching asynchrony, classically regarded as an adaptation, and then compensate its effect by maternal T? We hypothesized that maternal T is only beneficial for the chick under good food conditions, when mothers aim to raise the full brood, but detrimental under poor food conditions when brood culling is needed and in the chick benefits do not outweigh the costs of elevated exposure to testosterone. We studied this in the rock pigeon, in which first eggs contain much lower T concentrations than last and second eggs. Since hormone deposition was not affected by the food condition, food condition should interact with maternal hormone deposition to optimize reproductive success.. To test this, we created clutches of two first eggs, one injected with T to the level of the second egg (T chicks), and one injected with vehicle (C chicks). Pairs were then housed under either good or poor food conditions. Only in the good condition T chicks grew faster than C chicks. Only in the poor condition, T chicks had a much higher early mortality than C chicks. These results indicate that higher levels of yolk T functions in concert with hatching asynchrony to optimize the brood size in a changing environment. This not only solves the above paradox, it also explains contradictory results of *in ovo* T injections in the literature.
The results also point to a general problem in the study of hormone mediated maternal effects. In most studies, the postnatal condition in which the prenatal manipulations are evaluated consists of the standard laboratorial context and / or optimal rearing conditions, that may provide either a match or a mismatch with the condition in which the mother may have programmed her offspring for. Such studies may tell only half of the story. A mismatch often occurs when studying the effect of maternal stress on offspring development under standard conditions. We studied this in quail showing clear match-mismatch effects, in which offspring from stressed mother may do even better under later stressful conditions that offspring from non-stressed mothers.

TRANSGENERATIONAL EFFECTS OF THE SOCIAL ENVIRONMENT TRANSMITTED THROUGH THE AVIAN EGG

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The social environment during breeding can influence egg quality and thereby offspring quality with potential consequences over multiple generations. Such transgenerational effects can enable individuals to adjust to conditions anticipated by earlier generations, but may also constrain adaptive responses if environmental conditions change. Even though the adaptive significance of such transgenerational effects is clearly acknowledged, hardly any studies have tried to understand their consequences and underlying mechanisms over multiple generations. We studied effects of the social environment during breeding (pairs versus groups) on morphology, behaviour and physiology in Japanese quail (*Coturnix japonica*) over multiple generations. Precocial birds are ideal to disentangle egg-mediated transgenerational effects from other maternal effects since eggs can be artificially incubated and chicks can forage independently immediately after hatching. Housing female quail in pairs or groups during egg laying affected offspring growth and behavioural response to standardized challenges, even before offspring experienced different social environments themselves. Social conditions affected maternal circulating testosterone levels, but not average yolk testosterone, and there was no evidence for differences in offspring stress physiology (baseline & challenge levels of corticosterone). Maternal and grand-maternal social conditions as well their interactions affect grand-offspring growth and/or behaviour, suggesting that prenatal social conditions are indeed transmitted over more than one generation and depend upon both anticipated and actually experienced social context. Finally, using data from the fourth generation we demonstrate whether there are true transgenerational effects on offspring developing from germ cells that were never directly exposed to the egg environment shaped by the starting generation.

REVERSIBLE EPIGENETICS AND SEASONAL NEUROENDOCRINE PLASTICITY.

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Epigenetic modification such as DNA methylation and histone acetylation, are important molecular events that act to regulate gene transcription. The general assumption is that epigenetic events are permanent. Seasonal rhythms are associated with extensive genomic plasticity in a diverse range of tissues and cellular phenotypes. Using a seasonally breeding species, this presentation will demonstrate light and hormone dependent regulation of DNA methylation in the adult hypothalamus. In Siberian hamsters, a simple change in day length leads to marked variation in the levels of DNA methyltransferase 1 and 3b expression in the hamster hypothalamus. One genomic region in particular, Type 3 deiodinase (DIO3) is one target of the light induced change sin DNMT1/3b expression with greater levels of *dio3* promoter methylation in hamsters when housed in long days compared to short days. Studies that assessed the sufficiency of melatonin revealed timed injections were able to reduce DNMT1/3b expression and DIO3 promoter methylation. The development of refractoriness in hamsters was associated with a complete reversal in dnmt1/3b enzyme expression and dio3 promoter methylation. Data obtained from male European starlings brains that support light induced, reversible changes in the expression of enzymes involved in demethylation. Altogether, the data will indicate the DNA methylation in the adult brain is dynamic and reversible.

LOCAL STEROID SYNTHESIS IN THE BRAIN…AND OTHER ORGANS

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Traditionally, the regulation of blood-borne steroid hormone concentrations has been viewed as a multi-organ process involving the hypothalamic-pituitary-gonadal (HPG) axis for sex steroids and the hypothalamic-pituitary-adrenal (HPA) axis for glucocorticoids. However, active steroids can also be synthesized locally in target tissues, either from circulating inactive precursors or *de novo* from cholesterol. For such reasons, it is now clear that steroid profiles in blood and various organs can differ. I will present several examples, from studies of the brain and also other organs, such as the prostate gland, thymus, and bone marrow. The insights gained about steroid synthesis in the brain can inform studies of steroid synthesis in other organs and *vice versa*. The balance between systemic and local steroid signaling is dynamic and can shift from systemic to local signaling during particular contexts or ages. I propose that the shift to local synthesis and regulation of steroids within target tissues represents a "Balkanization" of the endocrine system, whereby individual tissues and organs become capable of autonomously synthesizing and regulating local steroid signals, perhaps independently of the HPG and HPA axes.

MAGNETIC RESONANCE IMAGING OF BRAIN PLASTICITY IN SONGBIRDS

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Songbirds represent an outstanding model for studying both critical period and adult neuroplasticity, vocal communication, brain steroid hormone action and lateralization of brain function. For several decennia the model has been the focus of many behavioral and molecular studies aiming at unraveling its specific and exclusive features. The recent unraveling of the zebra finch genome has fostered many discoveries and will allow future translation of data from this exciting model system to humans. For a long time this model has been deprived from in vivo neuroimaging investigations which on the contrary were used already decennia ago in rodent models and to a large extend in parallel with the unraveling of the mouse genome and the subsequent development of a variety of transgenic mice models for studying basic mechanisms in health and disease. The bio imaging lab has initiated in vivo neuroimaging in songbirds and developed over the years a songbird customized in vivo micro Magnetic Resonance Imaging (MRI) toolbox that proved to enable detection and quantification of structural and functional plasticity in songbirds (1).

The most important asset of in vivo neuroimaging remains the capacity of repeated measurements permitting to follow individuals over time with repeated assessment of brain structure and function while observing the functional outcome in the animal's behavior. Voxel based image analysis of repeated measurements of the same individual bird led to assumption free discoveries of brain regions involved in seasonal plasticity and this has headed new discoveries of seasonal structural changes in brain regions beyond the highly investigated song control system, more specifically the social behavioral network, the visual and the auditory system (2). With the intrinsic capacity of imaging to speed up temporal assessment of structural brain changes we are currently moving in the direction of linking temporal patterns of brain changes with behavioral changes and investigate the causality of sex hormones in this interaction. We are also monitoring structural and functional changes in the zebra finch brain during ontogeny, disentangling when and where in the brain tutor song discrimination, recognition and learning evolves. Other neuroplasticity studies required rather higher spatial resolution using specific in vivo MR imaging tools allowing to unravel functional changes in the smallest sensory system representation in the songbird brain; the olfactory system. This way we revealed seasonal changes in olfactory discrimination capacity for relevant odor cues in starling (3). Another advantage of repeated measurements and voxel based analysis is to study brain activity upon differential successive stimuli in the same bird enabling to spot brain regions with specific discrimination capacity by subtracting stimulus specific activation maps. This led to discoveries of lateralization of birds own song - and heterospecific song recognition in the zebra finch brain (4) and to assessment of fast hormone induced neuromodulation of auditory discrimination of specific socially relevant vocalisations in starling.

The same toolbox inevitably leads us to a new research era characterized by using 'in vivo high resolution imaging' for discriminating -in live birds- the key time points and target regions in the brain for hormone induced neuroplasticity and use this information as escort to target succeeding ex-vivo molecular investigations up to the (epi)genetic level. This type of image guided unravelling of molecular mechanisms of neuroplasticity was made possible through a newly started (Belgian) funded large research network (IAP - PLASTOSCINE).

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CHANGES IN REGULATION OF BEHAVIORAL TRAITS OVER THE LIFE COURSE: DIVERSITY OF MECHANISMS.

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Planet Earth is changing rapidly in so many ways and the responses of organisms to those changes include shifts in the predictable life cycle (e.g. seasons) as well as increasing frequency, intensity and duration of unpredictable events (e.g. weather events, human disturbance and other perturbations of the environment). Perception-transduction-response is the first reaction chain of an organism to any environmental change - physical, social and internal. Neuroendocrine and endocrine systems respond to environmental social and internal systems to adjust the course and timing of events of the life cycle as well as allow individuals to cope with a capricious world. Following perception of environmental change and transduction of this information, neuroendocrine systems classically regulate central responses as well as trigger cascades of endocrine responses down stream. There is a three-component regulation system of hormone secretion, transport and target cell responses that is well conserved across vertebrates. However, which components are regulated to control morphological, physiological and behavioral responses are very diverse and can occur at any level from neuroendocrine control to the target cell. We need to understand them more thoroughly if we are to also appreciate the many ways in which different species, and individuals can adjust to environmental change – or not. Birds are excellent models to explore these diverse mechanisms because we know so much about their morphological, physiological and behavioral changes over the life cycle from field as well as laboratory investigations. Focus here will be on the role of a relatively newly discovered peptide called gonadotropin-inhibitory peptide synthesized in the paraventricular nucleus of the hypothalamus. There is growing evidence that this peptide can regulate functions of several neuroendocrine- endocrine cascades to adjust behavioral and physiological responses to environmental change. These include reproductive behavior, territorial aggression and adrenocortical responses to stress.

DO ESTRADIOL AND SPECIFIC Z-CHROMOSOME GENES WORK IN CONCERT TO MASCULINIZE THE ZEBRA FINCH SONG SYSTEM?

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Sex differences in brain and behavior are widespread among vertebrates. A substantial body of data exists on endocrine mechanisms, but we have less information about other factors critical to sexual differentiation of neural structure and function. The zebra finch song system is highly sexually dimorphic. Only males learn to produce these stereotyped vocalizations, and the brain regions regulating the development and adult production of the behavior are substantially enhanced in males compared to females. They contain more and larger cells, and in some cases the projections between regions are more robust in males. It has long been known that sexual differentiation occurs in the weeks following hatching and that the differences in brain and behavior become stable by adulthood. The mechanisms regulating their development have remained a bit of a puzzle, however.

Administering estradiol (E2) to post-hatching females has potent, but incomplete, masculinizing effects on structure and function of the song system, which suggests a role for this steroid among other factors in masculinization. However, consistent evidence for sex differences in hormone levels, neural estrogen synthesis and estrogen receptors has been difficult to come by. Attempts to inhibit masculinization by treating juvenile males with aromatase inhibitors and estrogen receptor blockers have been largely unsuccessful, with a few exceptions. These include data suggesting that the projection from HVC to RA is facilitated by E2 produced in the male brain [1], and that expression of brain derived neurotrophic factor (BDNF [2]) and androgen receptor [3] are masculinized by endogenous E2.

We have become interested in the role that Z-chromosomes play in masculinization (males = ZZ; female = ZW), as birds have limited dosage compensation and we and others have detected specific Z-genes that exhibit increased expression in the song system of developing males compared to females. We are testing the hypothesis that E2 exposure and expression of particular Z-chromosome genes work in concert to masculinize structure and function of the song system. Current work is focused on TrkB (the high affinity receptor for BDNF) and tubulin specific chaperone protein A (TBCA), which is important for microtubule formation.

Experiments include tests of whether: (1) E2 modulates Z-gene expression in specific song control regions; (2) the proteins have direct masculinizing effects; (3) they alter responsiveness to E2; and (4) additive effects exist between E2 and TBCA or the TrkB/BDNF system.

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A GENOME-WIDE SEARCH FOR EPIGENETICALLY REGULATED GENES IN ZEBRA FINCH CELL LINES USING METHYLCAP-SEQ AND RNA-SEQ

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DNA methylation is an epigenetic mark tightly linked with gene expression regulation and when located in the gene's promoter region, it generally leads to transcriptional silencing. DNA methylation has traditionally been regarded as a highly stable epigenetic mark in postmitotic cells, defining their cellular identity. However, it recently became clear that postnatal brains appear to show stimulus-induced de novo CpG methylation or active DNA demethylation associated with brain-specific genes related to neuronal plasticity [1,2,3]. Due to striking homologies between the brains of birds and mammals, songbirds have become a widely used model system for neuroscientists. One songbird in particular, the zebra finch (*Taeniopygia guttata*), has been the focus of much research because of its rapid maturation and tendency to sing and breed in captivity [4]. Therefore, the zebra finch represents an attractive model for investigating DNA methylation reconfiguration during brain development and to assess the impact of hormonal treatments on the genome-wide DNA methylation profile in brain regions of interest.

In order to assess a first 'map of the zebra finch methylome', two recently cultured zebra finch cell lines, G266 and ZFTMA [5], were analyzed with MethylCap-seq experiments, including DMSO- (= control) and AZA-treated samples, the latter resulting in a netto demethylation effect. By analyzing the DMSO samples a total of 719,917 possibly methylated regions, i.e. Methylation Peaks, were identified. Next, a quantitative comparison was made between the DMSO- and AZA-treated samples making use of the EdgeR package [6]. Normalization was based on the reads that mapped between Methylation Peaks. At a False Discovery Rate (FDR) cut-off of 10%, 30,700 Methylation Peaks showed significantly less methylation after AZA-treatment, and significant enrichment for these regions was found in exons and promoters. To assess the influence of (variable) methylation on gene expression, also RNA-seq experiments were performed. After normalization (Poisson-seq goodness-of-fit statistic [7]) and EdgeR analysis (FDR of 10%), 172 and 3,457 genes were significantly down- and upregulated after AZA-treatment, respectively. As we were particularly interested in demethylation that results in upregulated expression, we determined the AZA-induced upregulated genes that were featured by significant AZA-dependent demethylation in the promoter region, i.e. genes under putative DNA methylation control. Comparison of the RNA-seq and MethylCap-seq results showed that 357 of the 3,457 upregulated genes were featured by at least one Methylation Peak exhibiting significant AZA-dependent demethylation in their promoter region.
Although the used cell lines were not derived from neuronal tissue, a pathway analysis of these 357 genes identified various interesting targets related to neuronal plasticity and neuronal or psychological disease. For a subset of neuroplasticity related genes, we validated the expression- and methylation changes following AZA treatment using respectively quantitative RT-PCR and CpG pyrosequencing on bisulfite treated samples. To our knowledge, this study provides the first genome-wide DNA methylation map of the zebra

finch genome as well as list of genes that are putatively regulated by DNA methylation in the promoter region.

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ANATOMICAL SPECIFICITY IN THE ACTION OF TESTOSTERONE IN THE REGULATION OF SONG AND UNDERLYING NEUROPLASTICITY IN CANARIES

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Steroid hormones coordinate multiple behaviors into a functional response, as exemplified by reproduction or stress. In the case of the regulation of behavior both motivational and performance aspects of behavior as well cognitive components are often activated by the same hormone. How steroids coordinate these distinct aspects of an integrated behavioral response is not well understood. Songbirds like canaries are especially amenable to investigating this phenomenon. Birdsong is a species-typical, stereotypic set of generally long, learned, complex vocalizations produced in a reproductive context. Castration drastically reduces song output and treatment with T rescues this decline. A discrete network of interconnected brain nuclei orchestrates song learning and production. Areas such as HVC (acronym is proper name) and the robust nucleus of the arcopallium (RA) regulate the production of song while areas such as Area X and the lateral magnocellular nucleus of the nidopallium (lMAN) are involved in the auditory feedback needed for song learning. These forebrain nuclei can undergo remarkable plasticity (as exemplified by changes in nucleus volume) in response to seasonal changes in testosterone. There is also evidence that other factors such as singing activity via the activity-dependent release of neurotrophins can contribute to the occurrence of seasonal neuroplasticity independently of T. Importantly, throughout the songbird brain there are multiple sites of steroid action. For instance, androgen receptors are expressed in HVC, RA, lMAN and throughout the hypothalamus and midbrain and estrogen receptors are expressed in HVC as well as in the hypothalamus. Moreover, androgen receptors are also expressed in the syrinx, the birds' vocal production organ. Here, we investigated the sites of hormone action in relation to the activation of different aspects of song to illustrate how steroid hormones integrate different traits into a functional response. First, we implanted T into the POM of castrated male canaries (Border strain) and assessed song rate and acoustic features as well as stereotypy, a measure of song quality. We demonstrate that T in the POM was sufficient to induce song rate to levels similar to those in birds with globally circulating T. On the other hand, T in the POM was unable to induce song loudness and stereotypy to a degree exhibited by birds with global T. Castrated birds treated with T peripherally had larger volumes of song nuclei as compared to castrates with blank implants but strikingly canaries receiving T in the POM had song control nuclei volumes as large as birds receiving global T, suggesting activity-induced neuroplasticity. We next implanted T in the HVC of canaries that also had T implanted in the POM. Preliminary evidence suggests that T in the HVC enhances the loudness and the bandwidth stereotypy of songs to levels of birds exposed to globally circulating T. Finally, we implanted canaries peripherally with bicalutamide (BICAL), a potent androgen receptor blocker that does not cross the blood-brain barrier to investigate selectively the role played by T on the syrinx. Birds implanted with BICAL sang songs that were indistinguishable from normal birds with respect to their rate, the loudness, and bandwidth stereotypy. While we are still in the process of completing our analysis, these results suggest that testosterone acts in a non-redundant manner throughout the brain and periphery to coordinate different song traits into a functional response and regulate the underlying neuroplasticity.

EFFECT OF AN INCREASE IN AMBIENT TEMPERATURE ON BRAIN ACTIVITY AND ENDOCRINE CONCENTRATIONS IN PHOTOSTIMULATED FEMALE GREAT TITS

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The annual timing of events like reproduction or hibernation in mammals and birds is strongly influenced by ambient temperature. Correlational studies on the impact of ambient temperature on timing have recently been supported by experiments demonstrating a direct, causal effect: e.g., birds fed *ad libitum* in captivity breed earlier when exposed to increasing temperatures. We are, however, still lacking an understanding about the mechanistic (physiological) pathways that integrate temperature information to fine-tune timing decisions in endotherms. Using 31 climate-controlled aviaries, we tested whether an increase in ambient temperature influences the brain activity and the endocrine concentrations of female great tits that were in a reproductive-like state (i.e. photostimulated). Females were sacrificed on the 3rd or 5th day after the temperature increase. We examined plasma concentrations of prolactin (PRL) and luteinizing hormone (LH), as well as hypothalamic contents of gonadotropin-releasing (GnRH) and -inhibitory (GnIH) hormones, and immediate-early gene Zenk. Preliminary data suggest that plasma PRL, LH, and the number of GnRH neurons were not affected by the temperature increase. The amount of Zenk staining might have been affected but in the opposite direction than expected: Zenk tended to decrease in birds that experienced a temperature increase 5 days earlier, compared to control birds that did not experience any temperature change. Preliminary conclusions suggest that temperature variation, which influences timing of breeding in great tits, might not affect the highest levels of the hypothalamo-pituitary-gonadal axis. Knowledge of the physiological mechanisms involved in temperature perception is, however, crucial for a better understanding on how timing of life-cycle stages in endotherms will be affected by increasing temperatures due to climate change.

STEROIDS IN THE MPOA: PATHWAYS TO SEXUAL SENSITIZATION AND STRESS REDUCTION

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The medial preoptic area (MPOA) is critical for male sexual behavior in all vertebrate species that have been tested. Dopamine is released in the MPOA as soon as a gonadally intact male rat detects the presence of an estrous female. Dopamine agonists, microinjected into the MPOA, facilitate copulation, and dopamine antagonists inhibit the behavior. Male rats that were castrated two weeks before a mating test do not show the extracellular dopamine increase and do not copulate; however, most males castrated only one week before the test do show the dopamine increase and do copulate. Basal extracellular dopamine is also decreased in two-week castrates; however, tissue levels of dopamine are actually higher in castrates, suggesting that release, but not synthesis, is inhibited in castrates. Nitric oxide in the MPOA promotes dopamine release, and neuronal nitric oxide synthase (nNOS), which produces nitric oxide (NO), is greatly reduced in castrates. Many, but not all, nNOS immunoreactive neurons in the MPOA of intact males are co-localized with either androgen receptors or estrogen receptor alpha. In addition, a nNOS antagonist impairs both copulation and the ability of non-copulatory exposures to an estrous female to facilitate mating on the first mating test. My current graduate student, Chris Robison, has shown that carbon monoxide (CO) produced in the MPOA also promotes copulation in male rats. Heme oxygenase 2 (HO-2), which produces CO, is increased in the MPOA of male rats after multiple mating experiences, and a CO releasing molecule, microinjected into the MPOA, facilitates copulation. On the other hand, an HO-2 antagonist does not inhibit copulation, as an nNOA antagonist does, but it does inhibit the ability of noncopulatory exposures to a female to enhance mating on a later mating test. The HO-2 antagonist also increases anxiety-like behavior in an elevated plus-maze and in an open field apparatus. Both NO and CO activate guanylyl cyclase and have some similar, and some dissimilar effects on behavior. Jenna McHenry, my recent graduate student, showed that repeated plus acute mating experiences decrease anxiety-like behavior in several behavioral tests and also decreases restraint stress-induced c-Fos activation in corticotropin releasing hormone (CRH) immunoreactive neurons in the paraventricular nucleus (PVN). Activation of androgen receptors in the MPOA contributes to that stress reduction, and repeated mating increases the number of androgen receptors in the MPOA. At least some androgen receptors are in GABAergic neurons that project from the MPOA to the PVN. Therefore, one mechanism for mating-induced stress reduction is activation of androgen receptors in the MPOA, at least some of which are in GABAergic neurons that project to the PVN.

EFFECTS OF SONG FEEDBACK ON LATERALIZED NEUROGENESIS IN THE ZEBRA FINCH

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New neurons continue to be added to specific regions of the vertebrate brain throughout life. Although many of these regions exhibit lateral differences in structure and function, differences in new neuron incorporation between the hemispheres have not been described. The songbird provides a model system for assessing the possible contribution of new neurons to hemispheric specialization because songbird brain areas that serve song processing and production are functionally lateralized and also receive a continuous influx of new neurons in adulthood. Song is produced by the syrinx, which is bilaterally innervated by the tracheosyringeal (ts) motor nerve -- the output of the ipsilateral descending song motor pathway. Here we asked whether altering song-related sensory feedback by unilaterally sectioning the ts nerve in adult male zebra finches would impact new neuron survival in the song system regions HVC and Area X, and in the caudo-medial nidopallium (NCM), an auditory area that is involved in discrimination of and memory for the complex vocalizations of individual conspecifics. Specifically, we were interested in whether altering singing unilaterally would impact new neuron survival unilaterally. After unilateral nerve section, birds still sang, but acoustic structure was changed. We labeled new neurons using the mitotic marker BrdU 1 week prior to unilateral ts nerve section and sacrificed birds 3 weeks later. New neurons were identified using immunohistochemistry to label BrdU and the neuron-specific protein, Hu. We found that altered song feedback via either left or right ts nerve section decreased new neuron survival in HVC and Area X equally in both hemispheres. In contrast, altered song feedback decreased or increased new neuron survival in NCM depending on side of nerve cut. Moreover, in untreated controls, we found more new neurons in left NCM than right NCM. We then assessed learning and memory correlates of new neuron asymmetry in unmanipulated birds by comparing behavioral and electrophysiological measures to numbers of new neurons in NCM. We found that the degree of left-side asymmetry in new neurons in NCM was correlated with the quality of song learning, and that asymmetry in new neuron numbers predicted the strength of neuronal memories for recently heard conspecific songs. Together this work suggests that new neuron lifespan in HVC and Area X is bilaterally influenced by the accuracy of song feedback whereas new neurons in left and right NCM are differentially influenced by song feedback. In addition, asymmetrical addition of new neurons to NCM may contribute to, or reflect, a division of function in NCM between the hemispheres that underlies the learning and processing of complex vocal signals.

ESTROGENS WHERE AND WHEN THEY'RE NEEDED: COMPARTMENT- AND CELL-SPECIFIC AROMATIZATION IN THE SONGBIRD BRAIN.

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Estrogens like 17β-estradiol (E) are established modulators of vertebrate brain and behavior, exerting powerful influences throughout the lifespan. Given the considerable diversity of E-dependent behaviors and mechanisms, how E is provided to discrete targets with spatial and temporal precision is of considerable interest. In the songbird, the telencephalon is a source of abundant E due to the constitutive expression of aromatase in neurons, often in brain areas with documented E-sensitivity. However, other E-sensitive loci, such as the hippocampus (HP) and HVC have few to no aromatase-expressing neuronal profiles. Since these areas do contain aromatase activity, we hypothesized that this enzyme may be present in an ultrastructural component distinct from the soma. Using immunoelectron microscopy with an antibody specific to passerine aromatase, we detected presynaptic boutons and post-synaptic dendrites containing aromatase in the zebra finch HP and HVC, areas where somal aromatase was low to undetectable. Thus, E-sensitive synapses may be provided with E with extreme precision via presynaptic and post-synaptic aromatization, perhaps to the exclusion of adjacent structures. This synaptocrine E-provision lacks the range of endocrine signaling, but greatly increases the precision of E-delivery compared to paracrine and autocrine pathways. To test the behavioral consequences of synaptocrine E, we targeted the zebra finch HP, a superficial telencephalic region rich in synaptic, but very low in somal aromatase expression. Local application of the aromatase inhibitor ATD, reduced local E in the HP, but not adjacent brain areas, and impaired learning and performance on a spatial memory task. Importantly, the compromise of memory function following ATD-treatment was similar to that achieved following a lesion of HP circuits. Thus, HP aromatization, likely at the synapse is a critical modulator of HP-dependent function. Additional work towards understanding the precise role of constitutive synaptic aromatase on synaptic physiology is underway. We have also found that aromatase can be induced in non-neuronal, astroglia; cells that do not express aromatase in the unperturbed homeotherm brain. Specifically, mechanical damage induces aromatase expression in vimentin-positive reactive astrocytes and radial glia. This induction is considerable, as mechanical injury almost doubles immunoreactive aromatase and almost quadruples local E around the injury. Glial aromatase is neuroprotective since aromatase inhibition and E-replacement exacerbates and mitigates injury-induced apoptosis respectively. This influence completely obscures the wave of degeneration characteristic of the injured mammalian brain. We now focus on the interaction between injury-dependent aromatase and the innate immune system in an attempt to understand the mechanism of glial aromatase induction. In adult of both sexes, mechanical injury first induces cytokine expression in microglia followed by the aforementioned induction of astrocytic aromatase. Females demonstrate a quicker response to brain injury with more injury-dependent reactive microglia and astrocytes sooner after brain damage relative to males. Studies aimed at understanding the interactions between microglial cytokines and astrocytic aromatase are ongoing. Taken together, these studies reveal the remarkable specificity of E-provision in the vertebrate brain, evidenced by the compartment- and cell-specific expression of aromatase in the songbird. This specificity may underlie the considerable spatial and temporal precision of E-provision, and its influence on brain structure and function.

THE DECEPTIVE SIMPLICITY OF SEX, STEROIDS AND GENOME RESPONSES

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The avian song control system provides one of the classic examples of the impact of steroids on neural development, plasticity and sexual differentiation. In this presentation I review some of the efforts over the past 25 years to interpret or analyze the role of steroids in the song system through the lens of genome biology. Following on the development of DNA cloning technology in the 1980s, early studies identified a few genes that showed transcriptional responses in the song system to steroid manipulation, e.g., androgen receptor itself [6] , and synelfin, now known as alpha-synuclein [2; 3]. Further work to establish a controlled culture system for studying song system development lead to direct evidence that steroids are at once necessary and sufficient for masculinizing the system, and must also be synthesized de novo in or around the song nuclei [4]. By 2001, the first draft of the human genome sequence had been released and the era of high-throughput genomics was in full swing. The Songbird Neurogenomics (SoNG) Initiative was launched to capitalize on this progress, by generating a core microarray platform and applying it to a broad array of research questions through a collaborative community-focused program [7]; steroids and sex differences were central to several of the SoNG Initiative projects [5; 8-10]. A general conclusion emerging from these studies is that steroids trigger complex genomic responses with differing structures and timecourses in different brain regions [1]. This challenges any simple model of steroid action in the nervous system, and will prompt future efforts to develop systems-biology approaches to understand the logic of steroid effects on neuroplasticity at the genomic level.

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STEROID-INDUCED ADULT NEUROPLASTICITY: WHAT I LEARNED FROM JACQUES AND THE BIRDS

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One of the major goals of the field of Behavioral Neuroendocrinology is to establish where and how hormones act in the brain to facilitate the activation of behavior. In collaboration with Jacques Balthazart I have pursued this goal over the past 25 year by focusing on steroid hormone effects in two neural systems present in avian species: the medial preoptic area of Japanese quail and songbirds in relation to the regulation of appetitive and consummatory aspects of male-typical sexual behaviors as well as the forebrain circuit that controls the learning and production of complex vocalizations in oscine songbirds. Behavioral studies had indicated that estrogenic and androgenic metabolites of testosterone were involved in behavioral regulation. Anatomical studies of the localization of androgen receptors, estrogen receptors and the metabolizing enzyme aromatase were helpful in identifying candidate areas where the hormones might be acting. However, certain areas such as the preoptic medial nucleus (POM) of the preoptic region as well as the song control nucleus HVC expressed AR and ER, exhibited male-biased sex differences in volume, and were sites of marked steroid-induced neuroplasticity in adulthood. This plasticity could be revealed by a variety of measures including just the assessment of nucleus volume in Nissl-stained material. Stereotaxic and lesion studies could link the action of steroids in these nuclei to activation of particular behaviors. Several themes emerged from this experimental approach to the study of hormone-brain-behavior interrelationships. First, steroid hormone effects on brain and behavior were much more rapid than expected based on traditional models of genomic action. Second, sites of plasticity and causal action in some cases corresponded to the anatomical site of steroid receptors and aromatase but this was not always the case. This observation along with the observation about the fast action of steroids led to a realization that steroids were acting more like neuromodulators in a paracrine fashion or akin to the volume transmission characteristic of biogenic amine action. Third, it became apparent that steroid action could vary markedly as a function of its site of action and that an understanding of the functional topography of a brain area and its hodology was essential if one were to understand the action of steroids on behavior in an integrative manner. Recent work has assessed the fast and slow actions of steroids in specific anatomical regions in relation to a characterization of the function of each brain area in the context of a neural circuit that regulates many aspects of a behavioral system such as reproduction. These studies have been conducted in quail and songbirds in both gonadectomy/testosterone replacement design as well as in the context of seasonal modulation. These studies will facilitate the development of more general rules about how steroids regulate behavioral systems.

REFLECTIONS ON THE INTERNATIONAL CONFERENCES ON HORMONES, BRAIN AND BEHAVIOR: FROM BIELEFELD TO LIEGE

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This presentation will address the history of the International Conferences on Hormones, Brain and Behavior and its impacts on advancing the field of Behavioral Neuroendocrinology. Starting with the first meeting held in Bielefeld, Germany in 1982 to the 2014 Conference, efforts were made to bring an international focus to the field and foster interactions among participants and their students. Interweaving stories and photos with data, a focus will be on the leadership role of Prof. Jacques Balthazart, PhD, on the occasion of his 65th birthday.

Abstracts of poster presentations

(In alphabetical order based on first author last name)

EMBRYONIC EXPOSURE TO CORTICOSTERONE MODIFIES AGGRESSIVE BEHAVIOR THROUGH ALTERATIONS OF THE HYPOTHALAMIC PITUITARY ADRENAL AXIS AND THE SEROTONERGIC SYSTEM IN THE CHICKEN

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Exposure to excess glucocorticoids (GCs) during embryonic development influences offspring phenotypes and behaviors and induces epigenetic modifications of the genes in the hypothalamic-pituitary-adrenal (HPA) axis and in the serotonergic system in mammals. Whether prenatal corticosterone (CORT) exposure causes similar effects in avian species is less clear. In this study, we injected low (0.2 μg) and high (1 μg) doses of CORT into developing embryos on day 11 of incubation (E11) and tested the changes in aggressive behavior and hypothalamic gene expression on posthatch chickens of different ages. *In ovo* administration of high dose CORT significantly suppressed the growth rate from 3 weeks of age and increased the frequency of aggressive behaviors, and the dosage was associated with elevated plasma CORT concentrations and significantly downregulated hypothalamic expression of arginine vasotocin (AVT) and corticotropin-releasing hormone (CRH). The hypothalamic content of the GR protein was significantly decreased in the high dose group ($p<0.05$), whereas no changes were observed for GR mRNA. High dose CORT exposure significantly increased platelet serotonin (5-HT) uptake, decreased whole blood 5-HT concentration ($p<0.05$), downregulated hypothalamic tryptophan hydroxylase 1 (TPH1) mRNA and upregulated 5-HT receptor 1A (5-HTR1A) and monoamine oxidase A (MAO-A) mRNA, but not monoamine oxidase B (MAO-B). High dose CORT also significantly increased DNA methylation of the hypothalamic GR and CRH gene promoters ($p<0.05$). Our findings suggest that embryonic exposure to CORT programs aggressive behavior in the chicken through alterations of the HPA axis and the serotonergic system, which may involve modifications in DNA methylation.

SEXUALLY DIMORPHIC BEHAVIOR IS ASSOCIATED TO CEREBRAL METABOLIC ACTIVITY AND ADRENOCEPTORS IN ADULT ZEBRAFISH BRAIN.

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In most vertebrates, differences in behavior and physiology between males and females are reflected in sexual dimorphisms in brain structure and function. Neurotransmitter systems such as, noradrenergic are known to control sexually dimorphic behaviors in avian [1,2] and mammalian [3] species. Here we questioned whether sexual dimorphisms are conserved in zebrafish (*Danio rerio*) brain. For this we studied alpha$_2$- and beta-adrenoceptors (AR) in adult male and female zebrafish brain, by means of *in vitro* quantitative autoradiography. Zebrafish brain adrenoceptors [4,5] sharing similar characteristics to those of avian and mammalian brain differ in densities between sexes in pallium (Dl), preoptic (PPa and PPp), hypothalamic (Hd) nuclei, in addition to the central gray (GC), and cerebellar areas (CCe gr, LCa gr). Female ventral telencephalic areas, corresponding to the septal formation, exhibited higher adrenoceptor numbers than males. In agreement to sex differences in alpha-adrenoceptor densities reported in the rat hippocampus [3], the zebrafish homologues pallial area, the lateral zone of dorsal telencephalon (Dl), involved in spatial learning in teleost fish [6], included higher alpha$_2$-adrenoceptor densities in females than males. In support, further examination of exploratory spatial behaviors in zebrafish, showed a clear and well-defined sex-related behavior pattern. To relay this sex-specific swimming behavior with cerebral activity, we subject the animals to *in vivo* [^{14}C]-deoxyglucose metabolic mapping, evaluating the regional brain glucose metabolism. Interestingly, in most areas studied, high densities of adrenoceptors coincided with high metabolic activity, with females exhibiting higher glucose uptake rates than males. Taken together, our results suggest that sex differences in adrenoceptors and metabolic activity are conserved in zebrafish limbic areas and possibly play an important role in regulating gonadotropin release, sexual differentiation, reproductive behavior, as well as spatial learning in a sex-specific way. Partly supported by EU, FP7 grant COPEWELL

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THE IMPACT OF OESTRADIOL ON OLFACTORY NEUROGENESIS IN THE CONTEXT OF SEXUAL BEHAVIOR

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Many mammalian species use olfaction as main sensory modality for the expression of behavior including sexual behavior. Two different sensory pathways are involved in the processing of olfactory cues in the brain. The main olfactory system is mostly implicated in the processing of environmental odors, whereas the accessory olfactory system processes odors associated with sexual behavior. Interestingly, both the main and the accessory olfactory bulbs (MOB and AOB) continue to receive new olfactory neurons during adulthood. These neurons are born in the subventricular zone (SVZ) and migrate along the rostral migratory stream to the olfactory bulb. Recent studies have demonstrated a role for olfactory neurogenesis in sexual behavior in female mice. Sexual behavior is mediated by different hormones such as estradiol, which appears to play a key role in mate preferences. Furthermore, estradiol can modulate the survival of newborn neurons. Our working hypothesis is thus that estradiol might play a role in learning odors of potential sexual partners by modulating olfactory neurogenesis. To address this question, we used aromatase knockout (ArKO) mice which are unable to produce estradiol across their life span. In these mice, both males and females showed reduced sexual behavior associated with deficits in olfactory investigation of odors of conspecifics. Using bromodeoxyuridine, a marker of cell division, in combination with endogenous markers of neuronal maturation (DCX, NeuN), and cell activation (Zif268), we wanted to characterize the population of newborn olfactory neurons which might be involved in learning the odor of a potential sexual partner. Thus, female and male WT and ArKO mice were exposed to male or female bedding, respectively, each day during seven days. In males, both WT and ArKO, cell proliferation was reduced in the SVZ following exposure to female bedding. By contrast, the number of neuroblasts (DCX+ cells) was increased in the MOB and AOB in WT, but not in ArKO males. In females, no effect of exposure to male bedding was observed on cell proliferation or on the proportion of neuroblasts in either WT or ArKO. By contrast, the proportion of newborn olfactory neurons was reduced in female WT mice in response to male bedding but only in the AOB, with no effect observed in ArKO mice, probably due to their estradiol deficiency. We are currently evaluating the functional integration of newborn olfactory neurons in the context of sexual behavior under the control of estradiol by measuring the population of newborn neurons activated by exposure to odors of a sexual partner (BrdU+/Zif268+ cells) in WT and ArKO mice.

SEX DIFFERENCES IN PERINEURONAL NETS AND PARVALBUMIN EXPRESSION IN THE ZEBRA FINCH *(TAENIOPYGIA GUTTATA)* SONG SYSTEM.

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Songbirds including zebra finches (*Taeniopygia guttata*) have been widely used as a model for studying vocal learning and the associated neural plasticity. Males learn to produce song and develop a set of brain nuclei, called the song system, during a sensitive phase for song learning in their ontogeny. Females do not sing but early exposure to conspecific song permanently affects their adult auditory preferences suggesting the existence of a possible sensitive phase in females as well. In parallel, the female song system nuclei are absent or decrease in size during development and probably also demonstrate experience-dependent plasticity. Recently, two neural markers for critical periods in brain plasticity were suggested to be related to song learning in males: parvalbumin (PV) expression would be associated with the onset of experience-dependent plasticity whereas perineuronal nets (PNN, chondroitin sulfate proteoglycans surrounding neurons) would limit potential plasticity at the end of sensitive phases [1,2]. In the present study we investigated whether adult male and female zebra finch brains show different levels of PV and PNN expression. In two song system nuclei of the caudal song control motor pathway, the Robust Nucleus of the Arcopallium (RA) and HVC (formerly High Vocal Center but now used as a proper name), the percentage of PV-expressing cells surrounded by PNN was significantly higher in males than in females. Furthermore, in HVC and in the Lateral Magnocellular Nucleus (LMAN), the percentage of cells surrounded by PNN that were expressing PV was higher in males than in females. The Area X of males was compared in females to the corresponding area of medial striatum. A larger proportion of the total number of cells as identified by DAPI (diamidino-2-phenylindole) staining was surrounded by PNN in the male Area X than in the corresponding area of females but the co-localization of PV and PNN was not significantly different between sexes in this area. These data largely confirm earlier findings showing elevated PNN-PV co-localization in adult males compared to juvenile males [1] and to females [2] in the posterior motor pathway (RA and HVC). In contrast to the present study however, Meyer et al. (2014) found no sex differences related to PV and PNN in area X and LMAN, two nuclei of the anterior forebrain pathway (AFP). They reported elevated levels of PNN expression in AFP nuclei of both males and females and suggested that these structures limit social learning and critical periods in both sexes. The pattern of changes observed here is slightly different but the origin of these differences remains to be investigated. However, the sex differences observed here clearly indicate that PV, PNN and their co-localization is sexually differentiated in an anatomically specific manner. These anatomical sex differences suggest the existence of sex-specific patterns of plasticity in the song control system that should be investigated by a combination of anatomical and behavioral experiments.

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THE SLEEP PROMOTING EFFECT OF DORA-12 IS SEX DEPENDENT IN RATS

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Sleep disruptions are more commonly reported in women and typically coincide with periods of hormonal fluctuation like during the menstrual cycle, pregnancy, or menopause. We have previously shown using a rat model that estradiol (E2) suppresses sleep in females and that sex differences in sleep are due to activational effects of E2 on sexually differentiated circuitry. Masculinization of the brain renders sleep behavior in males and masculinized females insensitive to the suppressive effects of E2. We have also identified the median preoptic nucleus (MnPN) as a key site of E2 action. The MnPN is a sleep-promoting region that sends inhibitory projections to arousal nuclei like the lateral hypothalamus (LH). E2 reduces the activation of sleep-associated MnPN neuron and antagonism of estrogen receptors within the MnPN attenuates the E2-mediated suppression of sleep. Orexinergic neurons in the LH are regulated by MnPN activity and orexin is a key neuropeptide involved in arousal. Preliminary data from our lab indicate that E2 increases activation of orexinergic neurons. Others have shown that prepro-orexin mRNA expression is sexually dimorphic in the hypothalamus, such that females have higher levels compared to males. Circulating levels of gonadal steroids also modulate expression of orexin and its receptors. Therefore, we hypothesize that orexin is the mediator of E2's suppressive effects on sleep. To test this, we obtained a dual orexin receptor antagonist (DORA-12, gift from Merck), which has been shown to promote sleep in male rats. If orexin mediates E2's suppression of sleep, then we predict that antagonism of orexin receptors will attenuate E2's effect. Additionally, due to the sex difference in prepro-orexin mRNA and steroidal modulation of receptor expression, we predict that sleep promotion by DORA-12 will be sex and E2 dependent. Gonadectomized female and intact male rats were fitted with EEG/EMG transmitters (DSI) and randomly assigned to DORA-12 or vehicle (VEH) groups. DORA-12 (30mg/kg) or VEH was given orally prior to lights out (ZT12) each day. Females received an oil injection and then two doses (5µg and 10µg) of E2 benzoate 24hrs apart at ZT21, while males received only oil injections. 12hr dark periods designated "oil baseline" and "E2" were scored for wake and sleep behaviors. In females, DORA-12 significantly reduced wake and promoted sleep, but it did not block the suppression of total sleep by E2. The percent change in each vigilance state induced by E2 is not significantly different between VEH and DORA-12 treated females. DORA-12's sleep-promoting effect in females is greater than in males. In males, DORA-12 significantly suppressed wake 3-4hr post-administration. Wake suppression in females was steady across the 12hr dark phase following DORA-12 administration. These findings suggest that the efficacy of sleep-promoting drugs may differ amongst men and women. Sex differences in sleep circuitry and/or drug metabolism may contribute to increased sleep promotion in females. We are currently exploring this hypothesis by testing the metabolism of DORA-12 in males and oil/E2 treated females.

THE SEXUAL BEHAVIOR INDUCED-INHIBITION OF AROMATASE ACTIVITY IN THE PREOPTIC NUCLEUS IS MEDIATED BY GLUTAMATE RELEASE

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Besides their long-lasting effects mediated by a modulation of gene transcription, brain-derived estrogens can rapidly regulate (within minutes) reproductive behaviors. *In vitro*, the activity of aromatase (AA), the enzyme responsible for the conversion of androgens into estrogens, is also regulated on a similar short time-scale, via phosphorylation of the enzyme resulting from changes in neuronal activity or glutamate release. Acute changes in AA have been shown *ex vivo* following exposure to various environmental stimuli. For example, AA in the medial preoptic nucleus (POM) rapidly increases after exposure to an acute stress and decreases after 5 minutes in the presence of a female. The simple view of a female is sufficient to induce these effects suggesting that rapid changes in AA are linked to changes in sexual motivation rather than to sexual performance. The mechanism underlying these regulations is not completely understood but more and more evidence seems to point to glutamate as a candidate mediating these effects. Indeed, exposing quail explants maintained *in vitro* to glutamatergic agonists such as kainate leads to a significant inhibition of AA within 5 minutes. NBQX, a glutamatergic antagonist blocked this kainate effect. We thus hypothesized that the sexual behavior induced-inhibition of AA in the POM could be mediated by a release of glutamate in this region.
We therefore performed two studies to determine (1) whether glutamate is involved in rapid changes in AA and (2) whether copulation is able to induce a release of glutamate in the POM. In the first experiment, male quail received a unilateral injection of kainate in the POM. The left and right preoptic areas were collected 20 min later and assayed separately for AA by the tritiated water technique. As shown previously in preoptic explants maintained *in vitro*, AA was down regulated in the kainate-injected hemisphere as compared to the non-injected side. In the second experiment, extracellular glutamate concentration was measured by *in vivo* microdialysis with a probe implanted in the POM of sexually mature males. Dialysate was collected every 3 minutes over three periods of 15 min when the male was (a) alone, (b) allowed to freely copulate with a female and (c) alone again. A transient rise in extracellular glutamate concentration was observed specifically and immediately after the expression of cloacal contact movements, when semen is transferred to the female. Glutamate returned to a basal level after the female was removed. Together, these results indicate that the acute regulation of aromatase activity by glutamate identified *in vitro* is potentially responsible for the acute regulation of the enzyme observed *in vivo* following copulation. As rapid changes in brain estrogen synthesis and its actions are apparently related to the control of sexual motivation rather than sexual performance, follow-up experiments should now determine whether the release of glutamate in the POM occurs following visual exposure to a female only (change in sexual motivation) or whether the performance of the copulatory sequence is required.

ACUTE EFFECT OF AROMATASE INHIBITION ON COGNITIVE AUDITORY PROCESSING IN A SEASONAL SONGBIRD

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Estrogens affect multiple reproductive traits, such as reproductive behaviors, but also non-reproductive traits, such as cognition. Although usually associated with long-term changes in physiological state [1], it has recently been demonstrated that the synthesis of brain estrogens is regulated within minutes by neuronal activity [e.g. 2,3]. Also recent studies demonstrate that the rapid (behavioral) effects of E2 are modulated by the photoperiod in mice [4] and by seasons in songbirds [5]. Recently, we detected a seasonal change in auditory processing of male starlings using functional Magnetic Resonance Imaging (fMRI) [6]. Here, we examined using fMRI whether acute inhibition of E2 synthesis alters auditory processing in the highly social European starling (*Sturnus vulgaris*). Subjects were exposed to synthetic pure tones and to two types of male starling song: species-specific (used in species and population recognition) and individual-specific songs (used in individual recognition) [6]. With fMRI, we measured the neural responses to these behaviorally relevant songs in medetomidine-anesthetized adult male starlings (N=9). All males were implanted with testosterone (T) for three weeks when photosensitive (kept in 8L:16D for 6 months) and then measured using fMRI in December. They were then switched to a natural photoperiod and measured again 10 weeks later in early March when photoperiod had reached 11 hours of light per day. Each fMRI experiment consisted of two sessions: a control session directly followed by a session in which Vorozole (an inhibitor of aromatase, the enzyme that synthetizes estrogens) was injected intraperitoneally (30 mg/kg) 10 min before the recording session. In December, direct comparison of the neural activation elicited by the songs between the control and Vorozole session identified no significant difference. In March, the same comparison revealed a clear statistically significant difference in the left NCM/Field L complex (P=0.008) but not in the right NCM/Field L complex. The differential response to E2 synthesis inhibition between the two periods could indicate either a differential aromatase expression/activity [7] (although this expression is mostly correlated with an increase in plasma T [8,9]) or a change in sensitivity to E2 that would be associated with the increasing photoperiod possibly resulting in a limited photostimulation. However, unlike most previous studies we clamped T to stable concentrations during the entire experiment so that the observed changes must be attributed to other factors than T. Lateralization of the effects of Vorozole on auditory responses in starlings is fascinating. A similar finding was reported in zebra finches in which a direct aromatase inhibition in the left but not right NCM decreased the behavioral preference for bird's own song [10]. Further analysis of responses to different stimuli will be needed to determine whether neuroestrogens modulate song processing in the left NCM in general or specifically modulate responses to certain stimuli.

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ROLE OF THE PROGESTERONE RECEPTOR IN THE DEVELOPMENT OF SEXUAL BEHAVIOR IN FEMALE MICE

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The classical theory of brain sexual differentiation holds that testosterone secreted during perinatal development and aromatized into estradiol (E2) masculinizes brain circuits controlling sexual behavior in male rodents. Recently, we showed that E2 is also implicated in the organization of female sexual behavior during a pre-pubertal period. We also showed that progesterone (P) receptor (PR) expression, known to be up-regulated by E2, increased over the pre-pubertal period in WT but not in aromatase knock-out (ArKO) mice, which are deprived of estrogen throughout life. This raised the question of whether any feminizing effects of E2 are mediated by the prepubertal induction of PR in the female brain. To investigate the role of PR in the development of sexual behavior in female mice, WT mice were treated with either a specific PR antagonist (ZK 137316, 6mg/kg; Bayer Schering Pharma, Berlin, Germany) or sesame oil vehicle between postnatal days P15 and P25. In our previous preliminary study, adult females treated with ZK during pre-pubertal development showed significantly less lordosis behavior than control females when given ovarian hormones. By contrast, olfactory mate preference and mounting behavior were not affected. Moreover, no differences were observed in PR expression between ZK and oil-treated females indicating no long term effects of ZK on PR expression. Further investigation by immunohistochemistry to determine the effects of our treatment on the neural circuitry underlying sexual behavior suggest that ZK-treated females showed an increase in kisspeptin activation compared to OIL-treated females. In conclusion, our preliminary results suggest that the progesterone receptor may play a role prepubertally in the development of neural circuits that control the later expression of lordosis behavior. In a new experiment, we treated female and their male counterparts with ZK at 6mg/kg or vehicle (sesame oil) during two different critical periods of brain sexual differentiation: the neonatal (P0, P5 and P10) and prepubertal period (P15, P20 and P25). We are currently investigating whether we can replicate our earlier findings observed in females as well as whether blocking PR signaling over the neonatal period affects reproductive behavior and function in male mice.

LOCALIZATION AND NOCICEPTIVE FUNCTION OF AROMATASE IN THE ADULT RAT SPINAL CORD

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We examined the distribution and nociceptive effect of the estrogen-synthesizing enzyme aromatase in the adult rat spinal cord. Aromatase-immunoreactive fibers and varicosities (but not perikarya) occurred throughout the entire rostrocaudal extent of laminae I, IIo and X, the lateral spinal nucleus and the intermediolateral cell column in both males and females. Western blots revealed ~60 and ~40 kDa bands of proteins likely corresponding to the classical and truncated forms of rat brain aromatase, respectively. Reverse transcription and polymerase chain reaction amplified specific aromatase mRNA sequences. Intrathecal injections of testosterone or 17β-estradiol in castrated male rats significantly reduced the hindpaw withdrawal latency within 5 minutes in a Hargreaves radiant heat test. The effect of testosterone was blocked using a concurrent intrathecal injection of the aromatase inhibitor vorozole. The present results are consistent with the results obtained in quail in prior studies (Evrard, 2006) with the exception that, in quail, both fibers and perikarya were labeled with the immunohistochemistry and only the classical ~60 kDa band of proteins was labeled in the Western blot. Taken together, the present results suggest that the overall neuroanatomical distribution of aromatase is highly conserved across amniotes but may have undergone species-specific adaptation with spatially (and temporally) refined "synaptocrine signaling" in the rat. This conservation imbues spinal aromatase with a possibly crucial role in nociception and more broadly in interoception and its corresponding autonomic and behavioral efferent outflow both in the context and independent of reproduction.

INTERLEUKIN 1 RECEPTOR SIGNALING REGULATES ANXIETY BEHAVIORS IN MICE

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Activation of the immune response influences a range of behavioral phenotypes. Proinflammatory cytokines, the mediators of this immune response, orchestrate inflammatory responses to infection and injury both in the periphery and the CNS. Interleukin 1 (IL-1β), a key player in both the physiological and behavioral responses to inflammation, mediates a specific behavior complex referred to as sickness behavior. Sickness behavior is characterized by reduced locomotor activity, sleep disorders, and diminished social interactions. IL-1β, has also been implicated in emotional disorders such as depression and anxiety. In the present study, we investigated the role of the IL-1 signaling pathway in anxiety-like behaviors. Adult male mice (IL-1 receptor knockouts (IL-1R KO) and wild type C57BL/6) were tested for measures of both sickness and anti-thigmotaxis (anxiety) behaviors two hours following an injection of LPS or vehicle. Measures of anxiety suggest that IL-1R KO mice differ in their thigmotaxis response prior to treatment. This effect is further exacerbated following LPS treatment and immune system activation. These data suggest that IL-1 signaling is associated with anxiety-like behaviors, and may provide another site for treatment of depression and anxiety disorders.

DELAYED PUBERTY, SLOWED DOWN GnRH SECRETION AND CHANGED HYPOTHALAMIC RNA EXPRESSION AFTER NEONATAL EXPOSURE TO A VERY LOW ENVIRONMENTALLY RELEVANT DOSE OF BISPHENOL A

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We recently reported that neonatal exposure of female rats to 1 or 10 µg/kg.day of diethylstilbestrol could respectively cause late or early puberty and consistent changes in maturation of pulsatile GnRH secretion [1]. Endocrine disrupting effects of low bisphenol A (BPA) doses in the µg range are a matter of controversy. We studied the effects of neonatal exposure to a very low dose of 25 ng/kg.day in comparison with 5mg/kg.day. Newborn female rats were exposed to vehicle (corn oil) or BPA injected subcutaneously from postnatal day 1 (PND 1) to 5 or from PND 1 to 15. The rats were followed for vaginal opening (VO) and estrous cyclicity. The GnRH interpulse interval that was known to decrease between PND 10 and 25 was studied *ex vivo* using hypothalamic explants obtained at PND 15, 20 or 25. Gene expression in the retrochiasmatic hypothalamus was assessed by whole exome RNA-sequencing on PND 20 (3 samples per condition). After neonatal exposure to 25 ng/kg.day of BPA for 15 days, the age at VO was delayed (35.3 ± 0.7 days vs 33.5 ± 0.5 days in controls) while advancement (32.1 ± 0.6 days) was observed using 5 mg/kg.day. The difference in pubertal timing between the two doses was significant. The late VO after exposure to 25 ng/kg.day of BPA was preceded by a significant increase in GnRH interpulse interval (52.5 ± 0.8 min vs 44.6 ± 0.7 min in controls) at PND 20. By contrast, early VO after exposure to 5 mg/kg/d was preceded by a significant decrease in GnRH interpulse interval (40.3 ± 0.1 min vs 42.8 ± 0.4 min). Similar dose-related changes in GnRH secretion were observed after BPA exposure from PND 1 to 5. At PND 20, after exposure from PND 1 to 15, RNA expression of 10 genes showed significant opposing changes in the high vs low BPA dose groups. Fourteen genes displayed an expression that was only affected by 25 ng/kg of BPA. The dose of 5 mg/kg resulted in modified expression level of 472 genes versus controls. A significant difference in level of RNA expression was observed for 1407 genes when comparing the two BPA dose conditions. In conclusion, neonatal exposure to a very low dose of BPA was followed by a delay in pubertal timing with consistent changes in pulsatile GnRH secretion. Changed hypothalamic RNA expression confirmed the effects of the two BPA doses with opposing changes of similar genes in relation to BPA dose and alteration of distinct genes by each of the two doses.

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NEUROPROTECTIVE EFFECTS OF A SYNTHETIC SAPOGENIN DERIVATIVE IN BRAIN ISCHEMIA *IN VIVO* MODELS

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Cerebrovascular diseases remain as the third cause of death in Cuba, where ischemic stroke shows one of the highest incidences among the elder population [1]. The ischemic ictus occurs when the blood flow is permanent or transiently interrupted in a major brain artery or one of its branches. This occlusion triggers a sequence of molecular and cellular events, described as the ischemic cascade [2]. The mechanisms involved in neuronal cell death, after ATP loss and anoxic depolarization, are related with excitotoxicity, disturbances in calcium homeostasis, oxidative stress, mitochondrial and DNA damage, inflammation and programmed cell death [2]. Also other brain cell types, such as glial cells and vascular endothelial cells, are involved in the pathobiology of ischemia and all interplay as the so called neurovascular unit [3, 4]. Besides microglia and astrocytes, peripheral immune cells also plays a role in tissue damage, inflammatory responses after the ischemic ictus, neuroprotection and even tissue repair [5]. Despite the intensive research about molecular mechanisms related to stroke, and the numerous of tested therapeutic candidates, there is a lack of neuroprotective clinically approved therapies [3]. The behavioural and neuroprotective effects of gonadal hormones are widely described in several neurological diseases [6]. However the clinical use of endogenous estrogens has been questioned, due to a higher risk of breast cancer incidence and thromboembolism in women subjected to hormone replacement therapy [7]. Therefore design and synthesis of new non-feminizing steroid analogues, is still an intensive preclinical research topic. In the present work, we evaluate a newly synthesized sapogenin (S15) in a transitory middle cerebral artery occlusion rat model. The treatment with the derivative S15, 1h before the occlusion, significantly decreased the infarct volume, in a dose-dependent manner. However the lesion volume did not significantly change when the S15 treatment was applied 1h after the occlusion, according to the TTC histology results. On the other hand, daily doses of S15 (4mg/kg) significantly decreased the asymmetry in forelimb use of ischemic rats, subjected to thermocoagulation of the blood in the pial blood vessels of the motor and sensorimotor cortices. These results show that S15 analogue is neuroprotective in transient, as well in permanent, ischemia in vivo models. The versatile pharmacological activity of S15 is probably related to early neuroprotective mechanisms as well as late events, perhaps related to neuroregeneration.

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INFLUENCE OF SEXUAL GENOTYPE ON AGONISTIC BEHAVIORS AND SEX STEROID LEVELS OF PHENOTYPIC MALES AND FEMALES IN THE NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Mechanisms of sex determination and differentiation are extremely labile in fish, as demonstrated by the numerous sex reversal experiments performed on teleosts. In Nile tilapia, sex reversal processes using exogenous sex steroids allow to produce individuals with atypical sexual genotypes that constitute major tools to investigate the mechanisms of sex determination and differentiation, from gonad differentiation to sexual differentiation of brain and behavior.

The aim of this study was to assess the influence of sexual genotype and the role of circulating sex steroids on the expression of agonistic behaviors in Nile tilapia breeders.

Observations were carried out on fights staged between one male (M) and one female (F) acclimatized in 250-L aquaria at 27°C. Agonistic behaviors were recorded (for 25 min) in five crosses (6 repetitions with different pairs): MXY×FXX (control), MXY×FXY, MXY×FYY, MXX×FXX and MYY×FXX. Quantified behaviors were: fin rising, throat swelling, chasing, lateral attack, frontal display, tail beating, mouth fighting and biting. For steroid assay, blood was sampled on 10 individuals of each phenotype/genotype combination. Testosterone (T), 17beta-estradiol (E2) and 11-ketotestosterone (11KT) were measured by radioimmunoassay.

Expression of aggressive behaviors was significantly higher in couples with a XY or YY female than in MXY×FXX. The mean durations (in % of total time) of threatening behavior expression were respectively 41 ± 3 and 8 ± 1 %; and the mean frequencies of attacking behaviors were 110 ± 7 and 25 ± 5 n h^{-1}. Expression level of agonistic behaviors in MXY staged with FXY or FYY seems to be adjusted to the aggressiveness level of females. Aggressiveness level was low and similar in MXY×FXX, MXX×FXX and MYY×FXX crosses. When comparing males together in these 3 crosses, only MXX showed a slightly but significantly higher expression of aggressive behaviors.

Compared to normal MXY, MXX had significantly higher levels of circulating 11KT (16.0 ± 4.1 and 26.5 ± 4.2 ng mL^{-1} respectively), that could be related to their higher aggressiveness. However, no similar difference was reported between females. E2 concentrations were similar between males (mean: 4.0 ± 0.4 ng mL^{-1}) and increased in females with the presence of Y chromosome(s) (FXX: 6.5 ± 1.4, FXY: 9.5 ± 1.7 and FYY: 14.1 ± 2.2 ng mL^{-1}). These results raised the question of an involvement of E2 in the control of agonistic behaviors in females. No influence of the genotype was observed on T levels.

Our results suggest that the presence of a Y chromosome increases aggressiveness in females. However, since the same relationship between aggressiveness and the Y chromosome is not observed in males, in which the level of aggressiveness is paradoxically higher in XX, we can hypothesize that the differences in aggressiveness are not directly dependent on the genotype but on the sex reversal procedures which young fry were exposed to during their sexual differentiation. These hormonal treatments could have permanently modified the development of the brain and consequently influenced the behavior of adults independently to their genotype. The role of endogenous steroids in agonistic behaviors needs further clarification.

IN VIVO NON-INVASIVE STRUCTURAL IMAGING TOOLS TO INVESTIGATE SEX- AND ONTOGENY RELATED DIFFERENCES IN THE ZEBRA FINCH BRAIN

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Introduction - So far structural investigation of the zebra finch (ZF; *Taeniopygia guttata*) brain was mainly performed *ex vivo* using histology (e.g. [1, 2]). This methodology imposes several disadvantages such as the impossibility to perform repeated measures. Here we present data obtained with two *in vivo* magnetic resonance imaging techniques, i.e. Diffusion Tensor Imaging (DTI) and 3D high resolution anatomical imaging (3D). Both studies pick up structural differences resp. between male and female adult ZFs and within the same ZFs over time.

Materials and Methods - Adult (n=11 males and n=11 females) and juvenile (n=10 males, preliminary data acquired at 20 and 65 days post hatching (dph)) ZFs were imaged on a 7 Tesla MRI system (PharmaScan, Bruker BioSpin, Germany). The birds were anaesthetized with isoflurane after which diffusion weighted SE-EPI images and a 3D anatomical scan (3D) were acquired. A Region-of-Interest and a Voxel-based analysis was performed on the Fractional Anisotropy (FA) maps calculated from the DTI data (two sample t-test; voxel-based outcome was FWE corrected; $p<0.05$ is considered significant). All data was processed in SPM8 supplemented with the Diffusion II toolbox (http://www.fil.ion.ucl.ac.uk/spm/ext/).

Results - The ROI-based analysis in adult birds (male versus female) found significant differences in FA near Area X and NCM, pointing to significant structural differences in these regions. It appears that in males the specific region encapsulating Area X displays a differential FA in males as compared to females ($p=0.019$ right; $p=0.006$ left hemisphere). NCM displays higher FA values in females compared to males. The voxel-based outcome confirms this and informs about the precise location of the peak differences i.e. mid-NCM extending to HVC and RA ($p<0.001$ both right and left hemisphere). Histology will be performed to validate the *in vivo* findings. Follow-up of structural brain development in juvenile males (20 versus 65 dph) illustrates that the *tractus occipitomesencephalicus* comes out as strongest difference between 20 and 65 dph in the voxel-based analysis ($p<0.001$ bilaterally). The 3Ds show that the *commissura anterior* is not yet visible at 20dph where it is clearly visible at 65 dph in juvenile males.

Discussion - The optimized DTI protocol is sensitive enough to pick up known but also detect novel sexual dimorphic regions in the adult ZF brain. Voxel-based analysis allows to find differences in the ZF brain without the need for an *a priori* hypothesis about the brain regions involved. The implementation of DTI in ZF opens up exciting new avenues of research where the entire brain can be followed upon ageing, social, environmental and hormonal modulations etc. and where the structural outcome can be correlated to behavioural findings from the same animal.

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FUNCTIONAL CHARACTERIZATION OF THE ROLE OF KISSPEPTIN AND GNRH RECEPTOR NEURONS IN THE NEURAL CIRCUIT CONTROLLING THE LORDOSIS REFLEX

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In rodents, female reproductive behavior involves the display of lordosis which is characterized by immobility on the part of the female along with an arching of the back and hindleg extension that elevates the rump and head. Remarkably, lordosis can be facilitated by GnRH indicating that GnRH neurons may thus have other functions in addition to gonadotrope control. However, elucidating the functional role of GnRH in the central nervous system has been hampered because of the difficulty in identifying GnRH signaling targets in brain tissue. It has been discovered that GnRH neurons are controlled by a set of neurons expressing the peptide kisspeptin. We recently showed that ablation of kisspeptin neurons by systemic administration of diphtheria toxin to a transgenic line of mice expressing diphtheria toxin receptor off the kisspeptin promoter (KissIC/R26-iDTR) but not those expressing the kisspeptin receptor, GPR54, greatly decreased lordosis suggesting that kisspeptin neurons are part of the neural circuit controlling the lordosis reflex. The finding that ablation of GPR54-expressing neurons did not affect lordosis gives rise to two different hypotheses: 1) kisspeptin acts on GnRH neurons through a different receptor other than GPR54 leading to the release of GnRH peptide within the central nervous system to act on downstream neurons expressing GnRHR; 2) kisspeptin neurons express another neuropeptide or neurotransmitter that will affect directly the lordosis circuit. My main research objective is thus to unravel the role of kisspeptin and GnRHR neurons in the lordosis reflex.

DOPAMINE DEPLETION IN THE MEDIAL PREOPTIC NUCLEUS IMPAIRS APPETITIVE AND CONSUMMATORY SEXUAL BEHAVIORS IN MALE JAPANESE QUAIL

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There is ample evidence indicating that dopamine plays a critical role in the regulation of male sexual behavior. In particular, studies have demonstrated that dopamine release in the medial preoptic nucleus (POM) facilitates the onset of sexual behaviors in male quail and rats [1, 2]. To further elucidate the role of dopamine in POM for different aspects of sexual behavior and to investigate the source of these dopaminergic inputs, we stereotaxically injected a catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), into the POM of male Japanese quail. To enhance the dopamine-selectivity of the lesions, an intraperitoneal injection of desipramine was administered thirty minutes prior to the delivery of 6-OHDA. Both appetitive and consummatory aspects of sexual behavior were monitored 1hr prior to surgery and 5hr, 24hr, 1week, and 2weeks after the surgery. Animals exposed to 6-OHDA exhibited a rapid impairment in both aspects of sexual behavior and this impairment persisted 5hr and 24hr after surgery; however, there was complete recovery of these behaviors 1 week after surgery. We also examined the distribution of tyrosine hydroxylase immunoreactive (TH-ir) fibers in POM and TH-ir incerto-hypothalamic dopamine cells. Our initial results demonstrated a decrease in TH-ir fibers within the POM, indicating an impairment of dopaminergic inputs to POM. Overall, this study demonstrates that dopamine release in POM is necessary for appetitive and consummatory sexual behavior in male quail. Currently, we are investigating possible causes of recovery of sexual behaviors.

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SEASONAL NEUROPLASTICITY OF THE AUDITORY SYSTEM OF FEMALE STARLINGS ASSESSED WITH RESTING STATE FUNCTIONAL MRI

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Introduction: European starlings sing both during and outside of the breeding season but the primary function of male song shifts depending upon the season [1]. During the breeding season (BS) the male starling song becomes highly sexually motivated and is used for e.g. mate attraction. The fact that female starlings select mates based on song characteristics such as bout length [2] could indicate an increased relevance for auditory discrimination in BS compared to non-breeding season (NBS). In this study we investigated if this increased discrimination is reflected in an increased local functional connectivity (FC) within higher level structures of the auditory system (NCM-Field L complex), which are responsible for song perception and discrimination. Furthermore, we investigated whether seasonal variation also changes the inter-hemispheric FC between left and right NCM-Field L complex.
Materials and methods: Female European starlings (*Sturnus vulgaris*) (N=11) were studied with resting state fMRI (rsfMRI) both in BS (March-April) and in NBS (October). During scanning the birds were anesthetized with a mixture of medetomidine (Domitor, Pfizer, 1mg/ml) and ketamine (Anesketine, Eurovet, 100mg/ml). The rsfMRI data were acquired on a 9.4T Biospec scanner (Bruker, Ettlingen, Germany) using single shot GE-EPI (TE/TR = 16/2000ms, 13 axial slices, slice thickness 0.8mm, 150 repetitions). To estimate FC, Independent Component Analysis was performed using a pre-set of 25 components, applied in GIFT. Resulting components or clusters were matched to anatomical meaningful regions using a homemade MRI starling atlas.
Results: Two components, one in each hemisphere, could be identified co-localized with the major auditory areas (NCM-Field L complex) and were selected for further analysis. Overall, these components were present in both seasons. The cluster size of both left as right component was more spread out laterally in BS compared to NBS, indicating that the local FC increased significantly ($p=0.002$ (left), $p<0.0001$ (right)). Moreover, comparison of the cross-correlation values, indicating the inter-hemispheric FC between left and right NCM-Field L complex, showed a significant lower correlation in NBS (0.19+/-0.02) compared BS (0.46+/-0.02) ($p=0.003$).
Discussion and conclusion: In line with the current study, increased spontaneous and induced activity of Field L in BS has been reported using electrophysiological measurements [3]. This finding could be explained by a seasonal improvement of local inter-communication between neurons, which seems to be reflected as an increased local FC or cluster size. In addition we observed a stronger inter-hemispheric FC in BS compared to NBS, suggesting an increased relevance of interconnectivity between these homotopic regions during BS. By using rsfMRI, a seasonal variation in neuronal communication within the auditory system, both locally and inter-hemispheric, has been observed and quantified. This opens the possibility to study seasonal neuroplasticity in other brain networks in a non-invasive manner with rsfMRI.

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HAIR TESTOSTERONE REVEALS SEX DIFFERENCES IN WILDLIFE BEHAVIOURAL STRATEGIES

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Sex differences in vertebrate behaviour, as well as in peripheral sex steroids have been well documented. Less established are relationships between behaviours that are not in a reproductive context and sex steroids. Testosterone is an androgen, whose levels are predominantly measured in males, although it is also synthesized and circulates in females. Testosterone is related to movement on multiple levels and scales. In this study, we heli-trapped, measured, hair sampled, and marked with GPS collars 168 wild male and female elk during five consecutive winters, in the Rocky Mountains, Canada. We than followed their movement, and used generalized estimating equations to relate their habitat and movement parameters with testosterone levels that we measured in their hair. We found that testosterone levels in both sexes were related to their movement strategy (resident, migratory, or dispersing), however, other movement parameters showed opposing tendencies between sexes. For example, males that spent more time in higher altitudes during the summer had higher testosterone levels. High altitude areas, during the summer, contain high-quality non-renewing food sources that trigger aggressive interactions with competing males. Since male elk tend to migrate in search of food, they are more susceptible to agonistic social interactions, and dangers. For females that are caring for young, immobilizing testosterone to ingest energy may not be a viable option. Males that spent more time in open areas during the summer had higher testosterone levels. Spending time in open areas to feed may involve risk-taking behaviour, whose relationship with testosterone is well established in males. Females, on the other hand, showed opposite trends in the above parameters, and dissimilar to males, females that moved faster in open areas during the summer had higher testosterone levels. Moving faster in open areas likely means spending less time in potentially dangerous situations. This may imply a decoupling of testosterone from risk-taking behaviour in females, which may be adaptive to maternal behaviour. Our study non-invasively collected information on elk movement behaviour and accumulated steroid levels for a large social mammal in its natural habitat. The significant interactions that we detected between sex and movement parameters may reflect the choices that individual elk made based on their personal strategies, which take into account sexually unique trade-offs related to reproduction, growth, and survival.

NEURONAL PLASTICITY AND COURTSHIP BEHAVIOR IN THE MALE RED-SIDED GARTER SNAKE IS DEPENDENT ON ESTROGENS AROMATIZED FROM CIRCULATING TESTOSTERONE DURING LOW TEMPERATURE DORMANCY

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Numerous studies have reported variations in regional morphology in the brains of seasonally breeding vertebrates. This neural plasticity has been shown to be in response to changes in sex steroid hormone levels and, in many cases, occur within pathways essential for the control of courtship behavior and mating. The red-sided garter snake (*Thamnophis sirtalis parietalis*) (RSGS) is one of a few species where courtship and mating is initiated when the gonads are fully regressed. And, although androgen levels are elevated at the beginning of the breeding season [4], the only cue found to initiate courtship behavior in the RSGS is a prolonged period of low temperature dormancy (LTD) [1]. Interestingly, pathways regulating reproductive behavior in male RSGS contain sex steroid concentrating neurons that respond to both testosterone (T) and estrogen (E) [2]. Subsequently, the presence of the aromatase enzyme (ARO) throughout the forebrain of male RSGS [3] suggests E may play an important role in the initiation of reproductive behaviors. In many seasonally breeding species, modifications in dendritic spine density appear to be an active process within neural regions that control reproductive behaviors. The current study examined seasonal and hormonal influences on the density of dendritic spines in the RSGS brain. Our data found dendritic spines within pathways controlling reproductive behaviors to be denser in spring, actively courting animals compared to fall, non-courting individuals. Animals maintained under conditions of LTD exhibited increased spine density that increased in association with time maintained in LTD. Although animals that received either T or estradiol (E2) exhibited greater dendritic spine density than controls, animals treated with E2 had a significantly greater density than either T-treated or control animals. In a separate study, the role of LTD and E on the initiation of spring courtship behavior and mating was investigated. Prior to being placed into a 16-week LTD, adult male RSGS received one of three treatments: 1) an empty silastic tube, 2) a silastic tube containing the anti-aromatase, 1,4,6-androstatriene-3,17-dione (ATD), or 3) a silastic tube containing ATD and a tube containing E2. Upon emergence, animals were tested daily with attractive females. Control animals exhibited normal courtship behavior whereas animals implanted with only ATD failed to exhibit courtship behavior. However, animals receiving both ATD and E exhibited normal courtship behavior. These results add to the increasing amount of evidence suggesting that testosterone may play a critical, although indirect, role in the regulation of reproductive activity in the male RSGS.

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HORMONE-MEDIATED MATERNAL EFFECTS: A POTENTIAL ROLE FOR THE EMBRYO

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In vertebrates, the exposure of the developing embryo to maternal steroid hormones can have long-lasting organizing effects on the development of embryonic gonads and brain; as well as on post-natal morphology, physiology and behaviour. Such hormone-mediated maternal effects have been postulated as a potential tool for mothers to adjust offspring's development to prevailing environmental conditions, and thus to enhance offspring's adaptive phenotypic plasticity, enabling evolution of local adaptations. Most studies in this field have been performed with bird species where maternal steroids deposited in the egg yolk show clear and systematic variation among species, females of the same species, nests of the same mother, and eggs of the same nest. However, the mechanisms underlying hormone-mediated maternal effects are largely unknown.

Recent studies found that androgen concentrations in the yolk decline strongly from the follicle stage to the day of oviposition, and decline further in the course of embryogenesis. Moreover, in the incubated eggs of birds and turtles it has been found that the maternal gonadal hormones are converted during embryogenesis to other hormones, such as conjugated hormones, probably to facilitate uptake by the embryo. The latter are probably biologically inactive, suggesting that the embryo converts them back to active components. This opens the possibility that the embryo is not a slave of the mother's signal but possibly is able to modulate this signal, perhaps depending on contextual cues. That may also depend on its own sex, as the relation between maternal gonadal steroids and embryonic sexual differentiation is as yet far from clear. There is also the possibility that the mother herself deposits enzymes for hormonal conversion in the yolk.

We will present our recent findings on these topics based on determination of a wide array of hormones and their metabolites in mature pre-ovulatory follicles; as well as in egg yolk, albumen and embryonic tissues upto two-third of embryogenesis, using rock pigeon. Rock pigeon females lay two eggs per clutch and the embryo of the second egg is exposed to nearly three times higher maternal testosterone, making it suitable to study context-dependent embryonic regulation.

SEX DIFFERENCES IN GONADECTOMIZED MARMOSETS PERFORMING AN OBJECT REVERSAL TASK

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The common marmoset (*Callithrix jacchus*) has been proposed as a new model of human aging and may be particularly useful in studying the effects of sex steroids on cognitive aging. In this study, the effect of hormonal treatment was examined in adult gonadectomized (GDX) female (n=11, mean age = 3.7 years old) and male (n=10, mean age = 5.5 years old) marmosets performing an object reversal task with 3 reversals. Females were implanted with 17β-estradiol capsules (E2, n = 6) or empty capsules (controls, n = 5); males were given weekly injections of either testosterone cypionate (T, n = 5) or vehicle (controls, n = 5). After controlling for age differences, no effect of sex or treatment was found for the number of trials to reach criterion (sex: $F(1,16) = 0.23$, ns; treatment: $F(1,16) = .79$, ns) or the number of errors to reach criterion (Sex: $F(1,16) = 0$, ns; treatment ($F(1,16) = 1.4$, ns). However, an effect of sex ($F(1, 16) = 6.97$, $p < .02$) was evident in response latencies, with males being significantly slower (M = 7.2 s $\pm$ 0.68 SEM) than females (M = 4.47 s $\pm$ 0.65 SEM), independent of hormonal status. In addition, males, but not females, had longer response latencies (M = 7.67 s $\pm$ 0.65 SEM) on incorrect trials relative to correct trials (M = 6.72 s $\pm$ 0.74 SEM; outcome X sex: $F(1,16) = 6.98$, $p < .02$), suggesting that they made errors due to distraction or that they were particularly sensitive to the uncertainty of their choices. T levels were not associated with response latencies in males, but were negatively correlated with performance (trials and errors to criterion) in the third reversal. In contrast, E2 levels in females were associated with shorter response latencies and worse performance in the second reversal. We conclude that GDX male and female marmosets achieve similar levels of performance on reversal learning, despite slower responding in males. Interestingly, hormone manipulations had only subtle effects on learning performance: elevated E2 in females impaired performance by increasing speed-accuracy trade-offs, while elevated T in males impaired performance by a mechanism independent of speed. Further studies in gonadally intact monkeys are necessary to examine the relationships between endogenous sex hormones and cognitive performance in each sex.

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INVESTIGATING POSSIBLE INTRASPECIFIC VARIATION IN TESTOSTERONE-INDUCED NEUROPLASTICITY BY COMPARING TWO CANARY BREEDS

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In our previous work we found that male canaries of the American Singer strain did not exhibit photoperiodic responses characteristic of wild-type canaries or of other strains such as the Border canary. American singers do not regress their gonads when exposed to long days and exhibit photorefractoriness. We also found that castrated males responded to high exogenous testosterone (T) in a variable manner. For example, various doses of T did not consistently induce an increase in the volume of the song nucleus HVC. These findings suggest that domestication of the American Singer canary, via human selection perhaps to lock-in a certain level of song quality and quantity, has led to a decrease in neuroplasticity associated with changes in photoperiod and hormonal action. In this study, we investigate possible strain differences in response to T (high) on HVC volume in adult male American Singer and Border canaries exposed to identical environmental conditions. Fifteen male American Singer (AM) and fifteen male Border canaries (BDR) were housed an 8L:16D (light:dark) light cycle for at least eight weeks, rendering them photosensitive and were placed into groups of intact male (IC), castrated male (CX), and castrated male implanted subcutaneously with one Silastic implant (12mm) filled with crystalline T (CX+T). Immediately after implantation, birds were individually housed in sound attenuated chambers on the 8L:16D light cycle for three weeks. After three weeks of T treatment, brains were collected from male AM and BDR canaries, sectioned, and HVC volumes were measured based on Nissl stained sections. Song behavior was recorded for three weeks. Preliminary data suggest testosterone stimulates castrated American singers to sing sooner (1 day post-T treatment) than castrated borders (3 days post-T treatment). Interestingly though, CX AM treated with T sang as much as their intact counterparts throughout the experiment while CX BDR treated with T sang more than their intact counterparts from day 9 to day 17 and were indistinguishable from both groups of AM that were exposed to T. Castrated BDR treated with T sang songs with lower entropy (noise or variance) than intact BDR as well as both groups of AM exposed to T. Importantly, CX AM treated with T sang songs with entropy that was indistinguishable from their intact counterparts. Lastly, all groups showed a linear increase in the energy (measure of loudness or amplitude) of their songs. These preliminary results suggest testosterone differentially stimulates song features in these two strains of canaries and may be due to differences in the neural substrate upon which testosterone acts to stimulate song. Consistent with this hypothesis, our preliminary analyses suggest that HVC volume increased more robustly in response to T in BDR than in AM.

MATERNAL EXPERIENCE WITH PREDATION RISK INFLUENCES GENOME-WIDE EMBRYONIC GENE EXPRESSION IN THREESPINED STICKLEBACKS (*GASTEROSTEUS ACULEATUS)*

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There is growing evidence for nongenetic effects of maternal experience on offspring. For example, previous studies have shown that female threespined stickleback fish (*Gasterosteus aculeatus*) exposed to predation risk produce offspring with altered behavior, metabolism and stress physiology. Here, we investigate the effect of maternal exposure to predation risk on the embryonic transcriptome in sticklebacks. Using RNA-sequencing we compared genome-wide transcription in three day post-fertilization embryos of predator-exposed and control mothers. There were hundreds of differentially expressed transcripts between embryos of predator-exposed mothers and embryos of control mothers including several non-coding RNAs. Gene Ontology analysis revealed biological pathways involved in metabolism, epigenetic inheritance, and neural proliferation and differentiation that differed between treatments. Interestingly, predation risk is associated with an accelerated life history in many vertebrates, and several of the genes and biological pathways that were identified in this study suggest that maternal exposure to predation risk accelerates the timing of embryonic development. Consistent with this hypothesis, embryos of predator-exposed mothers were larger than embryos of control mothers. These findings point to some of the molecular mechanisms that might underlie maternal effects.

ROLE OF NEURAL ESTROGEN RECEPTOR BETA (ERβ) IN THE CONTROL OF BEHAVIORAL AND NEUROENDOCRINE RESPONSES IN MALE AND FEMALE MICE

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Sexual reproduction in vertebrates is based on anatomical and behavioral dimorphisms, which allow sexual attraction and mating between male and female partners. In male rodents, gonadal testosterone exerts organizational effects during the perinatal period to masculinize and defeminize the nervous system and activational roles in adulthood, reinforcing typical male behaviors and neuroendocrine functions. Testosterone can signal directly through the androgen receptor (AR) or be converted into estradiol (E2), which then stimulates the estrogens receptors ERα and ERβ. In female rodents, the ovaries are inactive during the perinatal period and start synthesinzing E2 postnatally. Brain feminization is taught to occur independently of hormones, but recent studies suggest that prepubertal E2 may enhance female features of sexual behavior. Ubiquitous models of gene invalidation have been extensively studied in order to delineate a role of ERα and ERβ in the regulation of male and female behaviors. Given the importance of estradiol in both peripheral and central regulation of reproductive functions, the use of conditional genetic models where invalidation of each receptor is restricted to the nervous system could allow to learn more about the neural effects and targets of E2. The present study aims to determine the role of neural ERβ in the regulation of sexual behavior in male and female mice. For this purpose, we generated and characterized conditional mutant mice selectively lacking ERβ in the nervous system by using Cre-loxP technology. Results obtained by combining behavioral, neuroanatomical and hormonal approaches will be presented.

CHANGE IN SEXUAL PARTNER PREFERENCE IN MALE RATS BY PRENATAL LETROZOLE ADMINISTRATION

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Sexual partner preference is the individual election for a sexual partner for one or the other sex, when given the choice. In males during development, testosterone conversion to estradiol, by the enzyme aromatase is necessary for the expression of the typical female preference and masculine sexual behavior in adulthood. Several experiments have demonstrated that the inhibition of this conversion results in permanent changes in these behaviors [1,2].

The prenatally administration of 0.56 μg/kg of letrozole, from gestational day 10 to delivery altered the partner preference and sexual behavior of the male offspring tested when adults. This dose produced a subpopulation of around 30% of males that showed preference for the sexually active male, whereas the rest 70% and the prenatally vehicle-treated males had a female partner preference. Lower letrozole doses (0.10 or 0.31 μg/kg) had no further effects on male-sexual preference, while higher doses (1, 3.1 and 5.6 μg/kg) prevented parturition. When the males with sexual preference for other males were tested for masculine sexual behavior in circular arenas in the presence of a receptive female no differences were found in the percentage of animals that displayed mounts, intromissions or ejaculations. Only the males prenatally treated with letrozole (0.56 μg/kg) that showed preference for the female showed a reduction in the percentage of subjects that achieved ejaculations. Regarding female sexual behavior we found that the males prenatally treated with vehicle did not display lordosis or proceptive behaviors when faced to a sexually male. However, 24% of the males prenatally treated with letrozole (0.56 μg/kg) with a female preference displayed lordosis and proceptive behaviors, and this percentage increased to 56% in the group of males with a same sex preference. Interestingly, no changes in the serum levels of sex steroid hormones and gonadotrophins were found between the groups. These results show that the aromatize inhibition during this critical period of development impairs the complete defeminization of the partner preference and the lordosis behavior, but does not participate in establishment of masculine sexual behavior.

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THE WEAKLY ELECTRIC FISH GYMNOTUS OMARORUM AS A NOVEL MODEL SYSTEM FOR THE STUDY OF NEUROENDOCRINE CONTROL OF NON-BREEDING TERRITORIAL AGGRESSION

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Non breeding territorial aggression in vertebrates is uncommon and its underlying mechanisms are under ongoing research in birds and mammals. The weakly electric fish *Gymnotus omarorum* is a monomorphic, solitary, seasonal breeder, which aggressively defends its territory all year round. During the non-breeding season, *G. omarorum* exhibits non-sex biased territorial aggression, which makes it an interesting model system to study the neuro-endocrine mechanisms of territoriality. We postulate non-breeding aggression in this species is independent of plasmatic levels of steroid hormones. To test this hypothesis we will take two complementary approaches, one in the field, in a natural population of *G. omarorum*, and in the lab analyzing dyadic male-male contests.
Agonistic behavior is a key factor in territory establishment. Our first approach is to understand territory value, relating environmental parameters and individual spacing to morphometric and physiological traits in a natural population of *G. omarorum* during the non-breeding season. We carried out a diurnal electrical census of 7 homogeneous sites in the littoral area of Laguna del Sauce, Uruguay; and measured ecological variables, fish length, weight, sex, blood samples and electrophysiological features in 60 individuals. Our results show that territory size correlated with fish size and that oxygen saturation had a positive relationship with the territory size, suggesting the importance of this parameter as indicator of territory value. We expect to find no correlation between territory value and circulating hormones levels of E2, T and 11KT.
In order to test if aggression is independent of hormone levels, we first characterized behavioral and physiological features of non-breeding territorial aggression in intermale dyadic contests (n=6) tested in a plain arena of equally sized compartments with 5-20% weight asymmetry between contenders. As *G. omarorum* is a sexually monomorphic species, gonadal sex was identified by surgical inspection 45 days before agonistic experiments.We validated this approach by demonstrating that surgery did not affect predicted dominance relationships according to weight asymmetry, or the locomotor and electric patterns of male-male aggression in comparison to intact individuals. Plasma hormone measurements of 11KT in dominant and subordinate fish, and experiments with gonadectomized males are being performed.
The advantages of this model system and of the complementary field/lab approach will allow us to gain insight into the neuroendocrine mechanisms of non breeding aggression.
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REGULATORS OF THYROID ACTION IN THE SONGBIRD BRAIN

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The songbird song control system is an excellent model to study neural development and plasticity. This collection of brain nuclei is responsible for song production and learning and displays a large sexual dimorphism. In zebra finches (*Taeniopygia guttata*), the song nuclei undergo drastic changes during song ontogeny, while in starlings (*Sturnus vulgaris*), they additionally follow a seasonal cycle. Both processes display a degree of brain plasticity unseen in many other animals. A major factor influencing neural plasticity in all vertebrates are thyroid hormones (THs). They mainly exert their function by binding to nuclear receptors which influence gene transcription. Because the requirements for TH availability and action are time- and tissue-specific, tight regulation of local TH levels and availability of receptors is crucial. To study the possible effects of THs on songbird brain plasticity at the cellular level, we partially cloned and sequenced several regulator genes from both zebra finch and starling. These regulators include the iodothyronine deiodinases (*DIO1*, *DIO2* & *DIO3*), four TH transporters (*OATP1C1*, *MCT8*, *MCT10* & *LAT1*) and the two TH receptors (*THRA* & *THRB*). Amino acid alignment of these partial zebra finch and starling sequences showed a 95-100% match. Comparison of these songbird sequences with other model organisms showed various degrees of similarity. Both partial TH receptors were a perfect match (100%) with corresponding chicken sequences and had a 94-97% similarity with corresponding mouse sequences. In case of the transporters there was a high similarity with chicken (87-99%) but a much lower similarity with mouse (61-92%). Finally, while the partial *DIO2* and *DIO3* amino acid sequences were highly similar to chicken (~97%), *DIO1* only had a 82% similarity. Furthermore, similarity with mouse sequences was much lower for *DIO1* (~57%) and *DIO3* (65%) than for *DIO2* (86%). The active site of all three deiodinases was however completely conserved in the different models. These results indicate a general high degree of function conservation for these regulators of TH action. More detailed studies involving *in situ* hybridisation on brain sections in specific stages of song learning/production will show whether THs influence song learning and seasonal song variability, and will help to elucidate their role in the underlying mechanism of neural plasticity.

METHAMPHETAMINE MEDIATES INCREASED FEMALE SEXUAL MOTIVATION IN RESPONSE TO RELEVANT CUES

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Methamphetamine (MA) is a psychomotor stimulant associated with increases in sex drive and sexual activity in both men and women. This increased sex drive results in 'high risk' sex behaviors, such as unprotected intercourse and numerous sexual partners. In women, these behaviors pose significant health concerns such as unplanned pregnancies and STD transmission, as well as an enormous societal economic burden. In an attempt to understand the cellular mechanisms underlying MA effects on sexual motivation in females, our lab has developed a rodent model where female rats treated with MA show significant increases in proceptive behaviors in the presence of a sexually active male. Proceptive behaviors are considered to be a relevant gage of the female's motivation for sexual activity, as they represent solicitation attempts for the male's attention. Although we have previously demonstrated that MA does not significantly alter overall locomotor behavior at the time of sex behavior testing, there remains a possibility that the increased proceptive behavior is a consequence of heightened motor responsivity stemming from generalized arousal in the brain. Therefore, we sought to understand whether the observed increases in female sexual motivation following MA exposure were specific to the sexual stimulus (i.e. sexual cues) present. We predicted that if MA increases only goal-directed sexual motivation, MA-increased proceptivity would not occur in the presence of an inappropriate sexual stimulus, such as a castrate (CX) male. Castration reduces sex behavior and alters the pheromonal profile of the male; as such, female rats show little proceptive behavior toward CX males. Here, ovariectomized female rats were treated with estradiol and progesterone (Horm; n=8), or MA + Horm (n=10) according to our established paradigm. Four hours following the last injections, rats were exposed to a CX male for 25min, removed for 2hrs, and then exposed for another 25min to a dihydrotestosterone-treated-CX (DHT-CX) male. DHT restores the normal state of accessory sex glands and with it a number of sensory and behavioral cues, without fully restoring male sex behavior. Sexual, social, and exploratory behaviors were then analyzed with a repeated measures 2-way ANOVA, which revealed a significant interaction between stimulus male and MA-treatment for overall proceptive behavior [$F_{(1,16)}$=6.041, p=0.0258]. MA-treated females exhibited significant increases in proceptive behaviors toward DHT-CX males (Bonferroni t= 3.896, p<0.01) but *not* toward CX-only males. These results support the prediction that MA-induced increases in proceptive behavior are specific to sexually-relevant cues. Furthermore, these results suggest that certain male sensory cues are sufficient to induce increased female sexual motivation in MA-treated rats. We are currently testing whether one potential cue is pheromones, as our previous work has demonstrated that the medial amygdala posterior dorsal region (MePD), which receives projections from the olfactory bulb and the accessory olfactory system, is necessary for MA-induced female sexual motivation. Additionally, we are also testing potential cellular mechanisms underlying MA-induced female sexual motivation. Since oxytocin and its cognate receptor mediate social reinforcement, we predicted that one mechanism by which MA may increase sexual motivation is via increases in oxytocin receptor (OXTR) within the MePD. Surprisingly, our preliminary data show that MA significantly downregulates the OXTR in the MePD, suggesting that MA may inhibit mate preference and increase promiscuous sex behavior.

Thus, future experiments will directly investigate whether MA-induced changes in oxytocin signaling affect sexual motivation and promiscuity.

THE ZEBRA FINCH: AN EXCELLENT MODEL ORGANISM TO STUDY THE PHYSIOLOGICAL CAUSES OF EXPERIENCE-DEPENDENT VARIATION IN SOCIAL BEHAVIOR

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Interactions with conspecifics constitute an essential part of most animals' daily life. Interestingly, individuals within one species often differ consistently in the way they behave during social interactions. Such individual differences may be due to genetic effects, but can especially be explained by variation in social experience during specific ontogenetic phases. The neuroendocrine mechanisms underlying such experience-dependent individual variation in social behavior have, however, not been investigated in much detail so far. Here, we propose the zebra finch (*Taeniopygia guttata*) as an excellent model organism to study this important topic. We demonstrate that an experimental manipulation of the social environment during adolescence (pair vs. group conditions) causes predictable and stable individual differences in adult male zebra finches social behavior, with pair-reared males exhibiting higher courtship rates and higher levels of aggressiveness during mate competition than group-reared males. Since the zebra finch has already been used frequently to study the neuroendocrine control of social behavior, there is already a strong background to develop clear hypotheses and predictions concerning the physiological causes of experience-dependent individual variation in social behavior. Some of our recent ideas will be discussed here.

EFFECT OF HIGH TEMPERATURE ON SEX DETERMINATION AND SEX DIFFERENTIATION PROCESS IN AFRICAN CATFISH, *CLARIAS GARIEPINUS*

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Gonochoristic fish generally display two sex determination systems: a genetic sex determination (GSD) and environmental sex determination (ESD). In species with ESD, environmental factors only drive the direction of sex differentiation. In these species, temperature seems to be the main factor influencing the sex-ratio. Several studies were carried out on thermosensitivity of several species, but none of these focused on the African catfish *Clarias gariepinus*. The aim of this study was to confirm the existence of temperature sex determinism (TSD) in African catfish and determine its thermosensitive period. Eggs and larvae from 17 full-sib families were obtained by artificial reproduction. A three days high temperature treatment (36°C) was applied every three days at different moment of ontogenesis from fertilization until D29 post-hatching. Before and after each treatment, whole individuals were reared in 50l aquarium in a recirculating water system at 28°C. Sex-ratio was analysed using the acetocarmine squash method at 70 days post-hatching (dhp). Parallely, histological observations of the developing gonads (hematoxylin-eosin coloration) were performed by sampling ten individuals at 10, 15, 20, 25, 35, 45, 55 and 70 dph. Our results demonstrate for the first time that *C. gariepinus* display a TSD process. The more thermosensitive period ranged from D6 to D8 post-hatching. High temperature treatment applied during this period induced from 25 to 100% of masculinization rate. We also observed high variability of this thermosensitivity between the families. The histological observations showed that African catfish gonads were still undifferentiated until 20 dph; the gonadal differentiation clearly appeared at 45 dph. Thus, the critical period of thermosensitivity (6 to 8 dph) occurred long time before the histological differentiation of the gonad (45 dph). These results suggest that the high temperature do not act directly on the differentiating gonad but on other organ implicated into the sex differentiation (brain).

GONADOTROPIN INHIBITING HORMONE AND APPETITIVE BEHAVIOR

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Gonadotropin-inhibiting hormone (GnIH) was originally identified in birds, and was subsequently discovered in other vertebrates, including Syrian hamsters (*Mesocricetus auratus*). This peptide was formerly known in mammals as RFamide-related peptide-3. As the name GnIH implies, treatment with the peptide decreases gonadotropin synthesis and/or secretion, and in female hamsters, its action on gonadotropins occurs upstream of gonadotropin releasing hormone. More recently, we have shown that activation of GnIH cells in the hamster dorsomedial nucleus of the hypothalamus (DMH) is more closely associated with appetitive behaviors than with consummatory behaviors or with function of the hypothalamic-pituitary-gonadal (HPG) system. Appetitive behaviors are interesting because they provide a window into motivation that is independent of the ability to perform ingestive or copulatory behavior. For example, food hoarding, but not food intake, is significantly positively correlated with cellular activation in GnIH cells, and vaginal scent marking, but not lordosis duration, is significantly negatively correlated with cellular activation in GnIH cells. Furthermore, in females fed *ad libitum*, appetitive sexual behaviors are high over the entire estrous cycle, whereas by contrast, in females that are mildly food restricted, high levels of appetitive sex behavior are limited to the periovulatory period. Mildly food-restricted females spend nonestrous days hoarding food, whereas *ad libitum*-fed females neglected these duties in favor of courtship every day of the estrous cycle. Similarly, food restriction increased activation of GnIH cells on nonestrous days, but failed to do so on the day of estrous. This pattern of GnIH activation was the same as the pattern of appetitive ingestive behavior (food hoarding) and the exact inverse of appetitive sex behavior (vaginal scent marking and the time spent with the male vs. food). These levels of food restriction had no significant effect on consummatory behavior (food intake and lordosis duration) and no significant effect on circulating levels of estradiol or progesterone on any day of the estrous cycle. In addition, intracerebroventricular (ICV) treatment with GnIH increases food hoarding in female Syrian hamsters. Others have shown that ICV GnIH treatment inhibits appetitive sex behaviors. Together, these results are consistent with the idea that metabolic signals influence sexual and ingestive motivation via GnIH cells in the DMH. These mechanisms appear to orchestrate the appetites for food and sex to optimize reproductive success in environments where energy availability fluctuates or is unpredictable.

ACTIVATION OF ERβ BY ESTROGENS ACUTELY MODULATES MALE SEXUAL MOTIVATION

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Estradiol (E2) modulates cell functions within minutes through non-genomic actions involving membrane-associated receptors (mERs). These cellular effects can result in the modulation of behavioral processes including sexual behavior. We previously showed that blocking the action of estrogens or their synthesis decreases sexual motivation within minutes without affecting sexual performance in male Japanese quail. However, the specific receptors and mechanisms by which estrogens mediate these behavioral responses are unknown. Several putative mERs have been proposed: classical ERs (ERα and ERβ), GPR30, ER-X and Gq-mER. We tested here whether acute intracerebroventricular injections of agonists and antagonists specific to these mERs modulate sexual motivation in male quail. As previously shown, acute aromatase inhibition significantly inhibited sexual motivation. DPN, an ERβ-specific agonist prevented this effect. In contrast, drugs targeting ERα (PPT and MPP), GPR30 (G1 and G15), ER-X (17α-estradiol) and the Gq-mER (STX) did not restore (agonists) nor inhibit (antagonists) sexual motivation. It has been suggested that metabotropic glutamate receptors (mGluRs) are involved in the membrane actions of E2. We tested whether intracerebroventricular injections of mGluR1a, mGluR5 and mGluR2/3 antagonists would acutely alter sexual motivation. MPEP, a mGluR5 antagonist and LY341495, a mGluR2/3 antagonist, did not produce any effect. In contrast, LY367385, a mGluR1a antagonist, significantly inhibited sexual motivation suggesting that mGluR1a could be involved. We then investigated whether the acute action of E2 on this response depends on mGluR1a. The restoration of sexual motivation by E2 following its acute inhibition by the aromatase inhibitor vorozole was completely blocked by the coadministration of LY367385. The same results were obtained when E2 was replaced by DPN, the specific ERβ agonist. Altogether, these results indicate that brain-derived estrogens, acting via ERβ, rapidly modulate male sexual motivation potentially through the transactivation of mGluR1a.

INFLUENCE OF SOCIAL CONTEXT ON SINGING BEHAVIOUR AND SONG SYSTEM PLASTICITY

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As seasonal songbirds, canaries undergo neural and behavioural plasticity of the song system across seasons, an effect largely mediated by the rise in steroid hormone levels in response to increasing daylengths. However, other factors in the birds' environment also change across seasons and some of these have been shown to have effects on both singing behaviour and the song control system at least partially independently of steroid hormones. One such factor is social context. Boseret and colleagues (2006) [1] showed that male canaries have a larger HVC if housed with a female (M-F) than if housed with another male (M-M), despite the latter group displaying more singing behaviour. We investigated the mechanism of this increase in HVC volume by comparing these two social conditions to isolated male canaries either with (M-mir) or without a mirror (M-alone), as well as female canaries alone. All males were castrated and received subcutaneous testosterone implants that produce blood concentrations similar to physiological levels. Females received estradiol implants to increase their receptivity. The social condition manipulation continued for 21 days during which singing behaviour was recorded. To investigate the modulation of HVC neurogenesis by social treatments, we injected the subjects with two thymidine analogs at different time points to evaluate the number of newborn neurons surviving at 21 days (BrdU injected at the start of the social treatment) and 10 days post-injection (EdU injected 11 days later). Blood samples were collected one day before the start of the social treatment, 4 days later and during sacrifice and will be analysed for corticosterone, as well as testosterone.

Males housed alone without a mirror sang more than all other groups, which did not differ from each other. There was a trend for long songs (>5 seconds and complex structure) to be more frequent in the M-M as compared to M-F group. Analysing male-male dyads as a unit rather than two individuals did not change these results. Half of the male-male dyads developed a hierarchy without additional intervention whereas the other males only showed aggression when competing over temporarily available eggfood. Dominance did not correlate with amount of singing. There was a trend for higher HVC volumes in dominant compared to subordinate males. HVC volume was higher in the M-M compared to M-F condition. There was also a trend for higher HVC volumes in the M-alone compared to M-F group. In females, HVC volume was smaller than in males, however, social context (with a male or alone) had no effect on HVC size. In males Area X volume showed the same pattern as HVC, with a smaller volume in the M-F compared to M-M and M alone conditions, but only the comparison to M-alone was significant. The number of BrdU- and EdU-positive cells in the HVC of birds in the different social conditions is currently being quantified. The discrepancy between the present results and those of Boseret et al [1] could be explained by differences in the daylength birds were exposed to, in the strain of canaries used or by the number of manipulations (injections and blood sampling) that could have stressed birds in the current study. These interpretations will be tested via corticosterone assays and additional experiments.

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MUM'S THE WORD: TRANS-GENERATIONAL TRANSMISSION OF PHENOTYPES PROGRAMMED BY EARLY-LIFE STRESS

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The traditional view of developmental stress is one of constraint. However, an alternative hypothesis recognises the potential for adaptive developmental programming of behaviour, which enhances fitness if developmental environments match those experienced later in life [1, 2]. One intriguing possibility is that these effects are transmitted to the next generation, increasing offspring fitness. We present experimental data on the neuroendocrine and behavioural effects of early-life stress within and across generations of Japanese quail. F1 birds that experienced pre-natal stress exhibited attenuated corticosterone responses to acute stress in adulthood [3]. This attenuated stress response was mediated by a higher intracellular glucocorticoid receptor expression in the hippocampus. These birds were also more willing to begin exploring a novel environment. Post-natal stress did not affect the stress response in later life, but did facilitate higher levels of food acquisition in the novel environment. F1 individuals that experienced both pre- and post-natal stress exhibited the highest levels of exploration and found more food in a novel environment. Each F1 female then bred twice, under both *ad libitum* and unpredictable food availability, to create an F2 population. F2 quail from pre-natally stressed mothers showed the same heightened explorative behaviour and neuroendocrine changes as their mothers; the same was true for the offspring of mothers exposed to both early-life stressors. These results show that phenotypes programmed by early-life stress, particularly at the pre-natal stages, can be transmitted to offspring. Interestingly, the mother's developmental experience seems to be the major factor affecting offspring behaviour, regardless of the environmental cues experienced by the offspring during development. This trans-generational transmission of a mother's stress copying phenotype may serve to enhance their offspring's ability to cope with stressful environments.

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THE EFFECT OF THYROID HORMONES ON ZEBRA FINCH *(TAENIOPYGIA GUTTATA)* BRAIN PLASTICITY DURING DEVELOPMENT.

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Thyroid hormones are essential for the development of the nervous system. In seasonal songbirds, thyroid hormones have been shown to be involved in the annual changes in neural plasticity as well. Furthermore, thyroid hormones play a role in the control of the duration of the sensitive phase for filial imprinting. The male zebra finch *(Taeniopygia guttata)* learns his song during a sensitive phase associated with pronounced neural plasticity in nuclei of the song control system. Together these data suggest that thyroid hormones may be implicated in the control of neural plasticity in the song system during the sensitive phase for song learning. In the present study we injected juvenile zebra finches every other day with either a low (0.2 µg T4 + 0.04 µg T3) or a high (2 µg T4 + 0.4 µg T3) dose of thyroid hormones or vehicle during development to increase levels of thyroid hormone. In addition, to decrease the concentrations of active thyroid hormones we injected another group of birds with iopanoic acid (IOP; 500 µg every other day), a compound that blocks deiodinase activity and therefore the conversion from T4 to T3 (the active form of the hormone). All treatments were performed between 20 and 50 days post hatch (dph). At 35 dph, all birds received bromodeoxyuridine (BrdU) injections to study cell proliferation and survival. Birds were sacrificed at 50 dph and brains were perfused with paraformaldehyde so that they could be analyzed by histological and immunohistochemical techniques for total volume of the song system nuclei and number of neurons in these nuclei. In addition, body weight, gonad weight and size were measured. An effect of age, but no effect of treatment, was found on body weight and no significant effect of treatment on gonad size or weight was found. Preliminary results show a possible tendency for larger volume of RA in birds treated with the low dose of thyroid hormones. This trend however needs to be confirmed with a larger sample size (work in progress).

OVARIAN HORMONES MODULATE MAPK IN THE NUCLEUS ACCUMBENS BUT NOT THE AMYGDALA

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Methamphetamine (Meth) is a psychostimulant drug with a high potential for addiction. While Meth use and effects are similar between sexes; among Meth abusers, women report an increase in sex drive and risky sex behavior that leads to higher rates of sexually transmitted diseases and unplanned pregnancy. In a rodent model, Meth administration increases proceptive behavior in female rats in the presence of ovarian hormones. Previous studies have shown that this behavioral response is dependent upon the presence of progesterone and activation of dopamine type 1 receptors (D1Rs) in the posterio-dorsal medial amygdala. It is still unclear whether these pathways (dopaminergic GPCRs & nuclear steroid receptors) function independently of each other or if they interact at a central nexus to mediate the increases in proceptive behavior. The MAPK pathway presents itself as a viable nexus by which the dopaminergic and progesterone pathways may converge. The D1R activation leads to increases in PKA which is able to phosphorylate ERK. Ligand-activated progesterone receptor is also capable of interacting with Src-kinase which is an upstream kinase of ERK phosphorylation. In addition ERK has been shown to contain binding sites that are able to interact in a ligand-dependent manner with activated progesterone receptor. In this way, progesterone can indirectly lead to increases in phosphor-ERK transcriptional activity which may contribute to the changes we see in proceptive behavior. I hypothesize that MAPK/ERK is the interaction site of these two receptors; specifically that both Meth, via the dopamine type-1 receptor, and ligand-activated progesterone receptor increase phosphorylation of ERK over either treatment alone, and that this effect is region specific. To determine if the actions of Meth and hormones converge at ERK, ERK activation in the form of phospho-ERK (pERK) was measured by immunohistochemistry within the brain after Meth and hormone treatments. Female OVX Sprague Dawley rats (n=6/group) were given subcutaneous oil vehicle or ovarian hormones over three days, during which they received intraperitoneal Meth or saline vehicle. An hour after the last injections, animals were sacrificed and brains processed for pERK immunohistochemistry. All brain sections were analyzed using stereologic software by an investigator blind to the treatment groups. The current study shows that while pERK does not appear to significantly change with Meth or hormone treatment in the amygdala sub-nuclei, hormones do appear to ameliorate Meth-induced increases in pERK within the nucleus accumbens. While this suggests that ERK is not the convergence site of Meth and hormone action in the amygdala, it provides insight to how stages of the menstrual cycle may influence Meth's addictive properties in women.

DAWN SONG PERFORMANCE IS UNRELATED TO CIRCULATING TESTOSTERONE IN A SUBTROPICAL, SOCIAL SONGBIRD

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Circulating testosterone (T) plays a key role in the development and maintenance of masculine traits and has been proposed to enforce the honesty of male sexual signals. While numerous studies provide experimental support for the role of T in promoting the production of birdsong, surprisingly few directly examine whether natural variation in song is related to natural variation in T. Here, we investigate the relationship between song and T in the cooperatively breeding white-browed sparrow weaver (*Plocepasser mahali*), a sedentary passerine of sub-Saharan Africa. Social groups consist of a dominant pair, who monopolise reproduction, and up to 10 subordinates of either sex [1]. Males sing duets and choruses year-round, and also produce solo song from a separate repertoire sung at dawn only during the breeding season [2]. Previous studies of a Zimbabwean population of our focal subspecies (*P.m.mahali*) found that subordinate males do not produce dawn solo song and that circulating T differs with dominance status [2]. Our findings reveal that while subordinate males in this South African population do produce dawn solo song, they demonstrate marked dominance-related differences in song production. Despite seasonal variation in both dawn solo song and T in both classes, there was no dominance-related difference in T. In addition, T levels were on average appreciably higher in our population than in a more northerly subspecies (*P.m.pectoralis)* [3], suggesting a latitudinal gradient in T may exist. Finally, we found no correlation between the T of dominant males and their dawn song duration, song rate or the proportion of time spent singing. Together these findings suggest that T does not play a major role in the modulation of dawn solo song output in this species. More widely, these results highlight the need to continue investigating alternative mechanisms utilized in the expression of male display behaviours, and they promote further questioning of a T-mediated mechanism for enforcing signal honesty.

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2. Voigt, C., Leitner, S. & Gahr, M. 2007 Socially induced brain differentiation in a cooperatively breeding songbird. *Proc. Biol. Sci.* 274, 2645–2651.
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