Abstract #2:

In-vitro effects of BPA, BP2 and BP3 on cell proliferation in mature GnRH neurons

Riaño Gómez JM[1], Sorianello EM[1], Lux-Lantos VAR[1] and Fernandez MO[1].
1-Instituto de Biología y Medicina Experimental-CONICET, Email: juanrianog@gmail.com

Previously we showed that the in-vitro exposure to BPA, BP2 and BP3, endocrine disruptors, (ED, $1x10^{-7}$ and $1x10^{-9}$ M, 24 hs) increased cell proliferation in an immature GnRH cell line, GN11 cells (Susan Wray, USA). Kisspeptin also has a proliferative effect on these cells. The aim of this study is to evaluate the effects of the in-vitro exposure of the aforementioned compounds on cell proliferation in GT1-7 cells (mature GnRH neurons, Pamela Mellon, UCSD, USA).
Cell proliferation was evaluated using a Non-Radioactive Cell Proliferation Assay, MTS (Promega, WI, USA), after BPA, E_2, BP2 and BP3 exposure ($1x10^{-7}$ and $1x10^{-9}$ M, 12 or 24 hs). Participation of the nuclear estrogen receptors was evaluated using the estrogen receptor antagonist ICI 182780, ($1x10^{-6}$ M). Effects of Kisspeptin (Kiss, $1x10^{-9}$ M) on cell proliferation was also evaluated. Results were recorded as Abs490/Abs490(Control), presented as Mean±SE and analyzed by Repeated measures ANOVA with a Fisher posttest (Statistica, StatSoft, OK, USA).
Neither BPA nor BP2 modified cell proliferation (ANOVA ns, n=5). BP3 on the other hand increased cell proliferation compared to control values both after 12 and 24 h exposure [12h-exposure: Control=1±0.08, BP3-9=1.27±0.12, BP3-7=1.39±0.13, BP2-9=1,04±0,05, $BP2^{-7}$=1,01±0,06, $BP3^{-7}$ different from Control $p<0.05$, n=5. 24h-exposure: Control=1±0.03; $BP2^{-7}$=0.92±0.12; $BP2^{-9}$=0.94±0.11; $BP3^{-7}$=1.29±0.13; $BP3^{-9}$=1.29± 0.09; Repeated measures ANOVA $p<0.05$, $BP3^{-7}$ and $BP3^{-9}$ different from Control $p<0.05$, n=5], at 24-hour exposure both $1x10^{-7}$ and $1x10^{-9}$ M increased proliferation, whereas at 12-hour exposure only $1x10^{-7}$ increased proliferation. The estrogen antagonist ICI 182780 only blocked the effects of $BP3^{-9}$ after 24 h exposure (Repeated measures ANOVA $p<0,05$, n=5). Exposure to Kiss itself ($1x10^{-9}$ M) did not increase proliferation relative to control values, but co-treatment with BP2 increased cell proliferation at 24 h exposure (Repeated measures ANOVA $p<0,05$, n=5).
The results obtained show that exposure to ED have different effects on mature and immature GnRH neurons. This reinforces the notion that effects of the exposure to ED depend on the developmental period, duration and level of exposure, among other factors. (Supported by CONICET, ANPCYT, International Society for Neurochemistry, Fund. Williams, Fund. R. Barón).

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #4:

Sex-biased dependence of puberty and fertility on microRNA biogenesis in Kiss1 neurons

Juan Roa Rivas[1], Miguel Ruiz-Cruz[1], Francisco Ruiz-Pino[1], Rocio Onieva[1], María Jesús Vázquez[1], María Jesús Sánchez-Tapia[1], José Manuel Ruiz-Rodriguez[1], Alexia Barroso[1], Violeta Heras[1], Inmaculada Velasco[1], Cecilia Perdices-Lopez[1], Marisol Avendaño[1], Vincent Prevot[2], Matti Poutanen[3], Leonor Pinilla[1], Francisco Gaytan[1], Manuel Tena-Sempere[1,3]

[1]Instituto Maimónides de Investigación Biomédica de Córdoba; Department of Cell Biology, Physiology and Immunology, University of Córdoba; Hospital Universitario Reina Sofia (IMIBIC/ HURS); and CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, 14004, Cordoba, Spain; [2]INSERM, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Jean-Pierre Aubert Research Centre, 59045, Lille, France; [3]Institute of Biomedicine & Turku Center for Disease Modeling, University of Turku, 20520, Turku, Finland

Introduction/Aim:
Kiss1 neurons, which produce kisspeptins, are an essential component of the GnRH pulse generator, with indispensable roles in the control of puberty and fertility. However, the molecular mechanisms driving the activity of theses neurons remain unfolded.

Methods/Results:
Here, we report that mice with congenital ablation of the miRNA-synthesizing enzyme, Dicer, in Kiss1-expressing cells (named KiDKO) display hypogonadotropic hypogonadism (HH) of postpubertal-onset in both sexes. However, analyses at peripubertal and young adult ages documented that failure to complete puberty and early-onset infertility occurs selectively in females. Hormonal and pharmacological analyses evidenced that central, rather than peripheral, alterations are primarily responsible for the reproductive phenotype caused by ablation of Dicer in Kiss1 cells. Interestingly, Dicer elimination affected differentially ARC and AVPV Kiss1 populations during pubertal transition. Thus, while the number of ARC Kiss1 neurons was largely preserved during the infantile-pubertal transition in KiDKO mice, *Kiss1* expression and kisspeptin protein levels were reduced at the time of puberty, with a more obvious drop at the protein level. These changes seem to be associated with increased expression of Kiss1 promoter repressors, Mkrn3, Cbx7 and Eap1. In contrast, the AVPV Kiss1 population was fully preserved in KiDKO animals. Yet, steroid-induced LH surge, which is seemingly associated to AVPV Kiss1 population, was absent in KiDKO females.

Conclusions:
Our data unveil that miRNA biosynthesis in Kiss1 neurons is indispensable for pubertal completion and fertility in females, but dispensable for initial neuronal survival and early stages of postnatal sexual maturation in both sexes. This role seems to be conducted via fine-tune regulation of Kiss1 repressors by miRNAs during pubertal maturation, acting differentially on AVPV vs. ARC Kiss1 populations.

Grant Support: This work was supported by grants BFU2017-83934-P (Agencia Estatal de Investigación) and grants PI16/01243 and PI19/00257 (Instituto de Salud Carlos III, Ministerio de Sanidad, Spain; co-funded with EU funds from FEDER Program)

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #6:

LH cells in tilapia pituitary comprise a gap-junction coupled functional syncytium

Yaron Cohen[1,2], Berta Levavi-Sivan[1], Michael Gutnick[2]

[1]Department of Animal Sciences, Hebrew University of Jerusalem, Rehovot, Israel; [2]The Robert H. Smith Faculty of Agriculture, Food, and Environment, Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel

In the fish pituitary, LH and FSH are released from distinct cells. The LH cells, which exhibit many excitability characteristics in common with neurons and generate action potentials, are anatomically arrayed in dense clusters. Using electrophysiological and imaging techniques in slices from the pituitary of transgenic tilapia, we now show that these clusters comprise electrically coupled networks which respond to GnRH by generating sustained, synchronous electrical and calcium oscillations. In intracellular recordings, direct application of a GnRH did not produce any consistant immediate effect, but did lead within 1 to 10 minutes to slow (0.45 ± 0.2 Hz, N=20) oscillations in membrane potential and intracellular calcium concentration. In double intracellular recordings, nearby cells were strongly electrotonically coupled. The coupling coefficient was frequency dependent, such that slow potentials passed from cell to cell easily while action potentials were significantly attenuated. Thus, the frequency of GnRH-induced oscillation is optimal for synchronizing the extended network of LH cells. Application of a gap-junction blocker terminated the oscillations. This, along with previous evidence from our laboratory that LH cells are dye-coupled, indicates that the LH cells are connected via gap-junctions. Cell-specific RNAseq shows that these cells express several connexins. We show that LH release avoked by GnRH application decreased in the presence of a gap-junction blocker, suggests that the sychronous oscillation is directly related to hormone release. Interestingly, the oscillation can persist for hours after washout of GnRH. This leads us to conclude that the network of coupled LH cells acts as a functional syncytium that, while triggered by GnRH, itself plays an active role in regulation of the HPG axis.

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #8:

Serotonin excites preoptic area kisspeptin neurons via serotonin type 2 receptor activation in female mice

Carrie Buo, Anna Anello, Jordan Dakin, Robin Bearss, **Richard Piet**

Kent State University, Kent, Ohio, United States

Introduction/Aim:
Ovulation is controlled by brain circuits that regulate the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus and luteinizing hormone (LH) from the anterior pituitary. Kisspeptin (Kiss1) neurons, which project to GnRH neurons, have emerged as key regulators of GnRH neuron activity and LH secretion. Kiss1 neurons located in the rostral periventricular area of the third ventricle ($RP3V^{Kiss1}$) of the hypothalamus are involved in generating the preovulatory surge in LH secretion that triggers ovulation. In addition to Kiss1, other neurotransmitters and neuropeptides likely regulate the surge. Previous studies have indicated that serotonin (5-HT) contributes to generating the LH surge in female rodents with effects mediated by serotonin type 2 receptors (5-HT_2). Here, we hypothesized that the impact of 5-HT on the LH surge results from 5-HT exciting Kiss1 neurons via these 5-HT_2 receptors.

Methods/Results:
We used brain slices from mice that express Cre recombinase (Cre) in Kiss1 cells and a Cre-dependent, genetically encoded fluorescent calcium indicator GCaMP6f, and live-cell epifluorescence imaging to determine changes in intracellular calcium concentrations ($[Ca^{2+}]_i$) in multiple $RP3V^{Kiss1}$ neurons simultaneously, as an index of their activity. In female mice, 10 μM 5-HT increased $[Ca^{2+}]_i$ (mean normalized change in fluorescence 4.12±0.35%) in 85% of $RP3V^{Kiss1}$ neurons (n=397 cells in 32 slices from 24 mice). This effect was smaller in male mice (-0.25±0.10%; $p<0.0001$, unpaired *t* test; n=73 cells in 4 slices from 4 mice) where 18% of $RP3V^{Kiss1}$ neurons ($p<0.0001$, Fisher's exact test) showed $[Ca^{2+}]_i$ rises. We tested the effect of preventing action potential firing in the slice using tetrodotoxin (TTX, 0.5 μM) to determine if the effect of 5-HT on $RP3V^{Kiss1}$ neuron activity is direct. 5HT-induced rises in $RP3V^{Kiss1}$ neuron $[Ca^{2+}]_i$ in the presence of TTX (2.27±0.36%) were comparable to those evoked without TTX (2.59±0.35%; $p=0.34$, paired *t* test; n=68 cells in 7 slices from 4 mice). We next tested the involvement of 5-HT_2 receptors using ritanserin (5 mM), a 5-HT_2 receptor antagonist. Ritanserin significantly reduced the effect of 5-HT on cell activity when applied before and during 5-HT application (8.10±0.97% vs 0.04±0.06%; n=90 cells in 5 slices from 5 mice; $p<0.0001$, paired *t* test).

Conclusion:
Together these data indicate that 5-HT directly stimulates the activity of female $RP3V^{Kiss1}$ neurons through 5-HT_2 receptors. Our data suggest a potential cellular mechanism through which 5-HT might regulate the preovulatory surge in female rodents.

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #10:

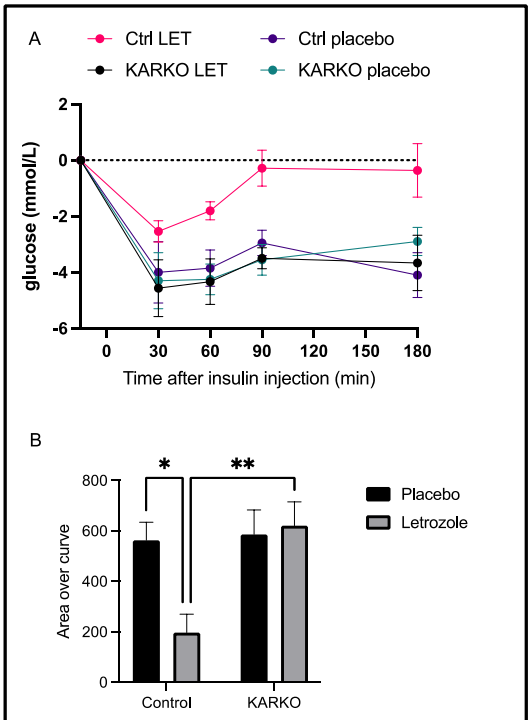
Androgen receptors deletion from kisspeptin neurons can prevent PCOS features in a letrozole mouse model

Caroline DeCourt, Megan Inglis, Greg Anderson

University of Otago, Dunedin, New Zealand

Introduction/Aim:
PCOS is the most common form of anovulatory infertility, affecting 1 in 10 women worldwide. It is characterized by ovulatory dysfunction, polycystic ovaries and hyperandrogenism. PCOS appears to be due to an increase of GnRH activity and LH release, and elevated androgen production. This increase of androgen levels is also linked with an impaired steroid hormone feedback into the brain. Infertility is often associated with a metabolic phenotype, including weight gain and insulin resistance. Kisspeptin is a master regulator of GnRH/LH secretion. Kisspeptin neurons express androgen receptors, but their role in PCOS is unclear. Therefore, we generated mice with a specific deletion of androgen receptors in kisspeptin neurons, named KARKO, using the cre-lox technology, to decipher its role.

Methods/Results:
To induce a PCOS-like phenotype in mice we used an aromatase inhibitor, letrozole (LET). This model is known to induce both ovulatory dysfunction and metabolic syndrome.
Control mice and KARKO mice received a 90 day release subcutaneous implant of LET or placebo from D26 of age. Testosterone levels were higher at D50 in mice receiving the LET treatment compared to the ones that received the placebo.
Control mice and KARKO mice that received placebo showed regular cycles (10/10), while most of the control mice that received LET were in constant diestrus (6/8). Interestingly, most of the KARKO mice that received LET showed regular cycles (8/11). LET mice were significantly heavier from D50 compared to placebo mice, but no differences were observed within the genotypes.
An insulin tolerance test was performed at D60, to assess the ability of insulin to decrease glucose levels. Control mice that received LET showed a faster recovery from hypoglycemia (figure A) and a significantly smaller decrease of the amount of glucose secreted after insulin injection (figure B), compared to control mice that received placebo, indicating an insulin resistance. Interestingly, KARKO mice that received LET showed a similar profile (figure A and B), to both placebo groups.



Assessment of insulin resistance:
Insulin (0.25U/kg) was injected after 3 hours of fasting. (A) Adjusted mean change of glucose from the baseline (B) Area over the curve. Values are mean ± SEM. (* $p<0.05$; $p<0.01$, data were analyzed by two-way ANOVA with Tukey *post hoc* analysis).

Conclusions:
All together these data suggest that direct actions of androgen receptors in kisspeptin cells causes the development of PCOS-like reproductive and metabolic phenotypes in mice.

Abstract #12:

KNDy neurons maintain gonadotropin pulses and folliculogenesis as the GnRH pulse generator

Mayuko Nagae[1], Yoshihisa Uenoyama[1], Hitomi Tsuchida[1], Masumi Hirabayashi[2], Naoko Inoue[1], Hiroko Tsukamura[1]

[1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi, Japan; [2]Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, Okazaki, Aichi, Japan

Introduction/Aim:
The gonadotropin-releasing hormone (GnRH) pulse, which governs tonic gonadotropin release and consequent folliculogenesis/spermatogenesis, is fundamentally important for mammalian reproduction. Circumstantial evidence suggests that KNDy neurons, located in the hypothalamic arcuate nucleus (ARC), expressing kisspeptin (encoded by the *Kiss1* gene), neurokinin B (encoded by the *Tac3* gene), and dynorphin A (encoded by the *Pdyn* gene) serve as a GnRH pulse generator in mammals including ruminants and rodents. However, no direct evidence was available. The present study aims to investigate whether the ARC KNDy neurons serve as a GnRH pulse generator and, if so, how many percent of KNDy neurons are required to maintain folliculogenesis and GnRH/gonadotropin pulses.

Methods/Results:
Global *Kiss1* knockout (KO) female rats were transfected with *Kiss1* cDNA utilizing adeno-associated virus (AAV) vectors carrying CAG-promoter–driven *Kiss1* (AAV-*Kiss1*) into the ARC to rescue KNDy neurons. Rescuing more than 20% KNDy neurons by transfecting *Kiss1* cDNA inside the ARC *Tac3*-expressing neurons, but not outside of the neurons, recovered luteinizing hormone (LH) pulses, an indicator of GnRH pulses. Furthermore, the rescue of >20% KNDy neurons recovered follicular development up to the Graafian follicles. In addition, newly generated *Kiss1*-floxed female rats were transfected with *Cre* cDNA utilizing AAV vectors carrying CAG-promoter–driven *Cre* (AAV-*Cre*) into the ARC to delete *Kiss1* gene in the ARC KNDy neurons. Few or moderate number of *Kiss1*-expressing cells were found throughout the ARC of AAV-*Cre*-treated *Kiss1*-floxed female rats. Highly (>90%) ARC *Kiss1* KO rats showed either a lack or severe suppression of LH pulses.

Conclusions:
These results provide direct evidence that KNDy neurons are the GnRH pulse generator, and at least 20% of KNDy neurons are sufficient to maintain folliculogenesis via generating GnRH/gonadotropin pulses.

Abstract #14:

Identification of Novel Reproductive Neuropeptide, Phoenixin (PNX) in Nile tilapia (Oreochromis niloticus) Brain

BoonLee Chiam, Tomoko Soga, Mageswary Sivalingam, Faizul Jaafar

Jeffrey Cheah School of Medicine and Health Sciences, Monash University, Malaysia

Introduction/Aim:
Phoenixin (PNX) was discovered *in-silico* and involves in reproduction, stress regulation and neurohypophysis. PNX is involved in the reproductive system by potentiating the neurones of kisspeptin and gonadotropin-releasing hormone in the hypothalamus. While in stress, PNX is a regulator for arginine vasopressin (AVP); a neuropeptide that stimulates the secretion of adrenocorticotropic hormone (ACTH), a stress regulator. Hypothetically, increased expression of PNX prevents or counteracts the overshooting reaction to stress. However, the role of PNX in social stress, a pathophysiology in the development of psychological problems due to strain that is formed from one's relationships with their social environment, remains unclear. To understand further the role of PNX in stress regulation, this study aims to identify and localise the PNX using Nile Tilapia (*O. niloticus*), a model with distinct social hierarchy of dominant and subordinate relationship.

Methods/Results:
To isolate the *pnx* gene in the *O. niloticus*, set of primer were designed from the sequence retrieved from NCBI (XM_003449339.5). The primer was able to amplify the *pnx* gene sequence from RNA extracted from the whole brain of *O. niloticus*. The brain of *O. niloticus* was divided into three parts; i) frontal, ii) mid, iii) dorsal. Real time RT-PCR was performed to elucidate the expression of *pnx* gene in the three different brain regions. The *pnx* gene showed no significant difference in the expression pattern between the three different brain regions.

Conclusions:
Our study is the first to investigate the expression of *pnx* gene in three different brain regions of social stress animal model, *O. niloticus*. The presence of PNX in the mid brain suggest its role in mediating reproduction and stress regulation. Nonetheless, the expression in multiple brain regions shows it have a pleiotropic effect apart from reproduction and stress regulation

Abstract #16:

Different estrogenic regulation of arcuate and preoptic kisspeptin neuron transcriptomes in ovariectomized mice

Miklós Sárvári[1], Balázs Göcz[1], Szabolcs Takács[1], Katalin Skrapits[1], Éva Rumpler[1], Norbert Solymosi[2], Szilárd Póliska[3], William Colledge[4], Erik Hrabovszky[1]

[1]Institute of Experimental Medicine, Budapest, Hungary; [2]University of Veterinary Medicine, Budapest, Hungary; [3]Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [4]University of Cambridge, Cambridge, UK

Introduction/Aim:
Kisspeptin neurons residing in the arcuate nucleus (KP_{ARC}) and the anteroventral periventricular nucleus (KP_{AVPV}) mediate negative and positive estrogen feedback, respectively, on GnRH neurons. Estrogenic regulation of the two kisspeptin neuron populations holds the key to understand the central regulation of reproduction in females. Here, we aimed to reveal the differences in estrogenic regulation of the KP_{ARC} and KP_{AVPV} neuron transcriptomes.

Method/Results:
Transgenic mice were ovariectomized and supplemented with 17β-estradiol (E2) or vehicle. Fluorescently tagged KP_{ARC} and KP_{AVPV} neurons collected by laser-capture microdissection were subjected to Illumina-based RNA sequencing. Subsequent data analysis with stringent criteria identified 1592 and 223 E2 regulated protein coding genes in KP_{ARC} and KP_{AVPV}, respectively. Regulated genes were classified into functional categories such as neuropeptides, receptors, transporters and transcription factors, among others. Comparative analysis of KP neuron transcriptomes revealed 1486 genes which showed estrogenic regulation only in KP_{ARC}, and 127 genes which showed estrogenic regulation only in KP_{AVPV} neurons. 62 and 34 genes displayed analogous and inverse estrogenic regulation, respectively. Strikingly, besides Kiss1 some neurotransmitter receptor subunits, granins, processing enzymes and dense-core vesicle associated genes showed inverse estrogenic regulation.

Conclusion:
These results provide evidence that E2 differentially regulates gene expression in the two distinct KP neuron populations and shed new light on the molecular mechanism of estrogen feedback.

Abstract #18:

A versatile LCM/RNA-seq method for differential expression analysis of fluorescently-tagged cholinergic neuron populations

Éva Rumpler[1], Balázs Göcz[1,2], Miklós Sárvári[1], Katalin Skrapits[1], Szabolcs Takács[1], Norbert Solymosi[3], Szilárd Póliska[4], Erik Hrabovszky[1]

[1]Institute of Experimental Medicine, Budapest, Hungary; [2]János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary; [3]Centre of Bioinformatics, University of Veterinary Medicine, Budapest, Hungary; [4]Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Introduction/Aim: Recent advancements in single-cell transcriptomics provided powerful tools to identify neuronal cell types based on a cluster of phenotype-specific transcripts, at the expense of a compromised sequencing depth, lack of information about rare transcripts and limited power to reveal differential gene expression. In this paper we report an alternative bulk sequencing protocol for deep transcriptome profiling of a few hundred fluorescently-tagged neurons isolated and pooled with laser-capture microdissection (LCM) from the brain of transgenic mice.

Methods/Results: Formaldehyde-fixation of ZsGreen marker, sampling of ~300-400 neurons with LCM and optimized RNA isolation, library preparation and RNA-sequencing methods were key to successfully characterize fluorescently-tagged cell populations with the expression of over 10.000 different transcripts (cut-off: cpm>5).

Conclusions: This versatile method is highly sensitive and compatible with differential gene expression analysis, shown by thousands of transcripts expressed differentially (FDR<0.05) between the functionally distinct cholinergic neuron populations of the dorsal caudate-putamen and the medial septum.

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #20:

Glutamatergic signalling in the Ventral Premammillary Nucleus mediates leptin action on the reproductive axis

Cristina Saenz de Miera, Nicole Bellefontaine, Carol Elias

University of Michigan, Ann Arbor, MI, United States

Introduction/Aim:
Leptin is an essential regulator of reproduction, but the neural pathways involved are not entirely known. The Ventral Premammillary nucleus (PMv) of the hypothalamus is glutamatergic, rich in leptin receptor (LepR) expressing cells and essential for the metabolic control of reproduction. However, conditional deletion of LepR in vesicular glutamate transporter 2 (vGlut2) expressing neurons results in virtually no reproductive deficits. In this study, we aimed to disclose the role of glutamatergic signaling from leptin responsive PMv neurons on puberty and fertility.

Methods/Results:
We injected the stimulatory form of Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) on the PMv of LepR-Cre female mice to assess if stimulation of PMv neurons is sufficient to induce LH release in adult female mice. We sequentially collected blood for 1h following a clozapine-N-oxide or Clozapine injection to activate the DREADDs. 80% of animals correctly targeted to the PMv showed an increase in LH release, and maximum LH level was correlated to the number of cFos immunoreactive neurons in the PMv. Also, females with deletion of *Vglut2* in LepR neurons ($LepR^{\Delta VGlut2}$) showed delayed age at first estrus and disrupted estrous cycles. Finally, the LepR-null mice ($LepR^{loxTB}$), which carry a reactivable deletion of *Lepr,* were crossed with a VGlut2-floxed line. In these $LepR^{loxTB}$;$Vglut2^{floxed}$ mice, we stereotaxically injected an adenoassociated virus expressing Cre recombinase (AAV-Cre) in the PMv to selectively rescue *Lepr* expression while deleting *Vglut2*. Control $LepR^{loxTB}$ mice with PMv LepR rescue showed vaginal opening, follicle maturation and became pregnant, while experimental $LepR^{loxTB}$;$Vglut2^{floxed}$ mice did not reach puberty or show gonadal maturation.

Conclusions:
In contrast to previous studies, our results indicate that glutamatergic signalling from leptin responsive PMv neurons regulates the reproductive axis and is required for leptin action on sexual maturation. Future studies are needed to determine the downstream pathways.

Abstract #22:

Sexually dimorphic modulation of arcuate kisspeptin neurons activity in response to fasting

Renata Frazao & **Naira Mansano**

Universidade de Sao Paulo, Sao Paulo, Brazil

Kisspeptin neurons are part of an intricate brain circuit that receives neuronal inputs related to nutritional status from at least three neuronal populations, including NPY/AgRP and POMC neurons of the arcuate nucleus of the hypothalamus (ARH) and cells in the ventral premammillary nucleus. In addition to its well-known action on the HPG axis, hypothalamic kisspeptin neurons also modulate the activity of specific neurons of the paraventricular and the dorsomedial nuclei of the hypothalamus, regions involved in the regulation of appetite and energy expenditure. However, it is unknown if changes in energy status influence the HPG axis through the modulation of kisspeptin neurons activity.

To characterize spontaneous currents of kisspeptin neurons in mice fed a regular chow or fasted for 24 hr, we performed whole-cell voltage-clamp recordings. The hypothalamic slices were obtained from adult female (diestrus-stage) or male Kiss1/hrGFP mice. We recorded the spontaneous inhibitory postsynaptic currents (sIPSC) and spontaneous excitatory postsynaptic currents (sEPSC) in cells located at the anteroventral periventricular and the rostral periventricular nuclei (AVPV/PeN^{Kisspeptin}) or in the ARH (ARH^{kisspeptin}). The slices were maintained in artificial cerebral spinal fluid (ACSF) containing amino acid receptor antagonists to record sIPSC.

We observed no effect on sEPSC frequency or amplitude recorded from AVPV/PeN^{Kisspeptin} or ARH^{kisspeptin} neurons in fasted female mice compared to control animals (7-8 animals/group; P> 0.05). Fasting induced no effect on sIPSC frequency or amplitude recorded from AVPV/PeN^{Kisspeptin} in female mice (5 animals/group; P> 0.05). Interestingly, ARH^{Kisspeptin} neurons exhibited a significant reduction of sIPSC frequency and amplitude (0.7 ± 0.1 Hz, 46.9 ± 3.3 pA, n= 22 cells) compared to control animals (1.3 ± 0.2 Hz, n= 17 cells; P= 0.006; 61.5 ± 4.3 pA, n=17 cells, P = 0.009; 5 animals/group). To determine whether gender could contribute to the observed effect, we next recorded ARH^{Kisspeptin} neurons from male mice. Surprisingly, we observed no change in sIPSC frequency and amplitude by recording ARH^{Kisspeptin} cells in fasted males (0.9 ± 0.1 Hz; 43.5 ± 3.3 pA, n= 21 cells) compared to ad libitum fed male mice (0.8 ± 0.1 Hz, P = 0.2; 49.2 ± 3.2 pA, P= 0.2; n= 17 cells, 5 animals/group).

In conclusion, fasting led to a decrease in the inhibitory transmission to ARH^{Kisspeptin} neurons, an effect observed only in female mice. We postulate that lower GABAergic transmission to ARH^{Kisspeptin} neurons may contribute to the suppression of the LH surge when energy status is not favourable for reproduction.

Abstract #24:

Kisspeptin-10 and GnRH have similarly potency as stimulators of the reproductive axis in African lions (*Panthera leo*) in contrast to in humans

Robert P Millar[1,3], Claire Newton[3], Caitlin McIntyre[2], Deyana Ivanova[2], Xiao Feng Li[2] Kevin T O'Byrne[2] and Mike Ludwig[1]

[1]Centre for Discovery Brain Sciences, The University of Edinburgh, Edinburgh, UK; [2]Department of Women and Children's Health, School of Life Course Sciences, King's College London, London, UK; [3]Department of Immunology, Faculty of Health Sciences, Centre for Neuroendocrinology, University of Pretoria, Pretoria, South Africa.

Introduction/Aim:
Understanding the hypothalamic factors regulating reproduction is key to maximising reproductive success of breeding programmes, management and conservation of threatened species, including African lions. To provide insight into the physiology of the hypothalamic-pituitary reproductive axis in lions, we studied the luteinising hormone (LH) rand steroid hormone responses to GnRH and its upstream regulator, kisspeptin.

Methods/Results:
Ten male lions (Ukutula Conservation Centre, South Africa) were used: six young (13.3±1.7 months of age, 56.2±4.3kg, testes 1.68±0.2cm) and four adult (40.2±1.4 months of age, 174±6kg, testes 3.84±0.6cm). Lions were immobilised with a combination of Medetomidine and Ketamine (2-2.5mg plus 60-80mg for young, 9.5mg plus 280mg for adults). After moving to a surgical theatre, they were maintained under light surgical anaesthesia throughout the experimental procedure by receiving supplementation as needed. An intravenous catheter was placed in a medial saphenous vein and 22 blood samples collected at 10-min intervals. Kisspeptin (KP-10, 1µg/kg) was given i.v. after sample 7 and GnRH (1µg/kg) after sample 16. On completion of the experiment the lions were returned to the management camp and anaesthesia reversed. Ethics and permission for experimentation were obtained from the University of Pretoria animal ethics committee. A veterinarian administered drugs and took care of the welfare of the animals. LH was measured by ELISA as described previously (1).
Figure 1 shows the LH response to KP-10 and GnRH. Basal LH levels were similarly low between the two groups; due to anaesthetic thus providing a low baseline over which to determine LH responses to GnRH and Kisspeptin and prevent spontaneous LH pulses. The LH responses to KP and GnRH was higher in adult lions compared to young lions ($p<0.05$, Fig 1). The response to KP was slightly less than to GnRH. This contrasts with our studies in humans in which responses to GnRH are 5-10 fold higher than to KP-10. Intriguingly young male lions show an immediate response (i.e., do not require priming) indicating that the gonadotrophs are already in an active state prior to puberty. Mass spectrometry analysis demonstrated that there were significant increases in 17OH progesterone and testosterone in adult males but no change in young males.

Conclusions:
Provocative tests of LH stimulation with kisspeptin and GnRH provide a tool to determine the reproductive capacity of male lions and have the potential to be sensitive tests to determine stress, nutrition and infection impacts on lion's reproductive health.

Abstract #24 (cont)

Figure:

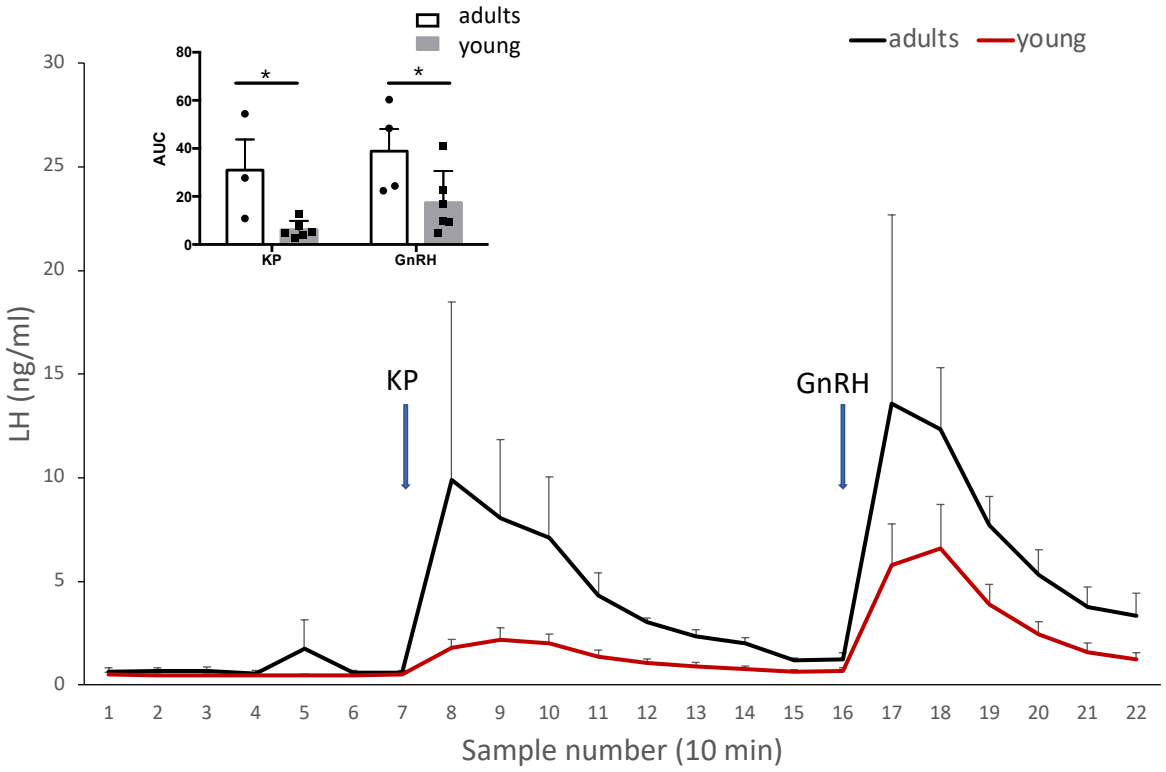


Figure 1: LH release profile in response to Kisspeptin-10 (KP) and GnRH in young and adult lions. AUC shown in inset. Mean±SEM, *p<0.05.

Ref:

1) Ivanova D. et al. Endocrinology 2021.

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #26:

Bradykinin B2 receptor expression in kisspeptin cells is required for mice energy homeostasis

Henrique R. Vieira[1]; Naira da S. Mansano[1]; José Donato Júnior[2]; Frederick Wasinski[2]; Renata Frazão[1]

[1]Department of Anatomy, Institute of Biomedical Sciences - University of São Paulo, São Paulo, Brazil; [2]Department of Physiology and Biophysics Institute of Biomedical Sciences - University of São Paulo, São Paulo, Brazil.

Introduction/Aim:
It is known that bradykinin (BK) inhibits the luteinizing hormone (LH) surge, an effect that depends on the BK 2 receptor (B2R). The BK effects on LH secretion were investigated before the advent of kisspeptin in the reproductive field. A recent study showed that kisspeptin neurons at the rostral hypothalamus express the gene coding for the Bk receptor (*Bdkrb*). However, whether BK acts on kisspeptin cells to modulate the HPG axis or the metabolism remains unknown. To determine if B2R expression in kisspeptin cells is required for kisspeptin-related functions, we induced the selective deletion of the B2R from kisspeptin cells.

Methods/Results:
The selective deletion of the B2R from kisspeptin cells was induced by breeding mice carrying the loxP-flanked *Bdkrb2* allele *($B2R^{flox/flox}$*) with Kiss1-Cre mice. Heterozygous offspring were crossed with homozygous *$B2R^{flox/flox}$* mice, which then generated mice homozygous for *$B2R^{flox/flox}$* alleles, expressing *Kiss1*-dependent deletion (Kiss1-B2RKO), whereas animals that did not express Cre were used as controls. Kiss1-B2RKO and control animals were born in the same litters. We collected a punch of the **medial preoptic area (POA)** to validate the animal model, measuring the *Bdkrb2* and *Kiss1* gene expression. We evaluated mice sexual maturation and body weight gain through development. In addition, metabolism features, such as bioimpedance, and respiratory rates, were also assessed in adulthood.

The Kiss1-B2RKO mice showed reduced *Bdkrb2* expression (*p= 0.0401*) compared to control animals. The *Kiss1* mRNA levels were similar between groups (*p= 0.2810*). Female and male Kiss1-B2RKO mice exhibited lower body weight gain during development (♀ *p= 0.0013*; ♂ *p= 0.0001*) compared to the control animals. Female Kiss1-B2RKO mice exhibited late vaginal opening (*p= 0.0084*) compared to the control animals. In contrast, Kiss1-B2RKO and control male animals exhibited similar timing for puberty onset (*p> 0,05*). Adult female Kiss1-B2KO mice exhibited reduced lean mass compared to control animals (*p= 0.0074*), an effect not observed in male mice (*p> 0,05*). Surprisingly, female and male Kiss1-B2RKO animals exhibited increased VO2 consumption (♀ and ♂ *p< 0.0001*) and VCO2 release (♀ and ♂ *p< 0.0001*) compared to the control group. The respiratory exchange rates were increased in females Kiss1-B2RKO during the night (*p= 0.0044*).

Conclusions:
Our results indicated that the pathway mediated by the B2R in kisspeptin cells may be involved in the control of energy homeostasis and probably in the sexual maturation of female mice. Whether the sexual maturation of female mice depends on body weight needs to be further verified.

Abstract #28:
Effects of pulsatile infusion of kisspeptin on puberty in the male monkey: A case report

Nell A. Bekiares[1], Erica M. Gelman[1], Brady D. Rose[1], Casey B. Fitz[1], Saverio V. Capuano[1], Stephanie B. Seminara[2], Joseph R. Kurian[1], and **Ei Terasawa**[1,3]

[1]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI 53715; [2]Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, and [2]Department of Pediatrics, University of Wisconsin, Madison, WI 53792, USA

Introduction/Aim:
The kisspeptin neuron in the hypothalamus is critical for puberty onset, as mutations of the *KISS-1* gene result in absence or abnormal timing of puberty onset (Seminara *et al*., *N Engl J Med*, 349:1614, 2003; de Roux *et al*., *PNAS*, 100:10972, 2003). A series of studies in this lab further indicate that in both male and female rhesus monkeys pubertal increases in kisspeptin release are accompanied with the pubertal increase in GnRH release (Guerriero *et al*., *Endocrinology*, 153:825, 2012; Garcia *et al*., *Endocrinology,* 159:3048, 2018). However, the question of whether pulsatile kisspeptin infusion into prepubertal monkeys leads to precocious puberty, similar to that shown with pulsatile infusions of GnRH (Wildt *et al*., *Science*, 207:1371, 1980), is unknown. In the present study, we examined the effect of pulsatile infusions of kisspeptin-10 (KP10), a kisspeptin agonist, on puberty in a male monkey at 16.9 months of age. For control, an age and bodyweight matched male received saline infusions.

Methods/Results:
Prior to the experiment the males were well adapted to the monkey jackets. Hourly bolus infusion of KP10 or saline was delivered subcutaneously using a portable pump, which was housed in the monkey jacket. Both KP10 and saline were replenished weekly and doses of KP10 (2.5-10 µg/h) were modified at 5-7 wk intervals. The KP10 infusion was terminated 30 wks after (24 months of age). Changes in the testicular volume, body weight, and circulating LH and testosterone levels were assessed weekly. The results indicate that 1) KP10 infusion of 2.5 µg/h resulted in a clear nocturnal LH increase by 5 wks, followed by nocturnal increase in testosterone and testicular volume by 7 wks; 2) KP10 infusion of 5 µg/h also increased the testicular volume initially, but subsequently both LH and testosterone levels returned to the pre-infusion level; 3) KP10 at 10 µg/h was not effective in increasing any parameter, perhaps due to KP receptor down-regulation; and 4) complete termination of KP10 regressed the testicular volume. These changes were not seen in the control, and it remained at the prepubertal stage. Importantly, after the initiation of the treatment, the KP10 male's body weight increased ~5% higher than the control, lasting throughout the study.

Conclusions:
Therefore, in non-human primate males KP10 infusion leads to puberty onset, although it appears not to accelerate the pubertal progress. Further technical refinements are needed. (Supported by R37HD043341)

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #30:

Role of the Melanocortin 3 Receptor in Sexual Maturation

DT Porter[1,2], L Guo[3], RD Cone[1,2]

[1]Life Sciences Institute, University of Michigan; [2]Molecular and Integrative Physiology, University of Michigan; [3]Undergraduate Program in Neuroscience, University of Michigan.

Energy metabolism and reproduction are mediated by neural systems within the hypothalamus containing many reciprocal connections. One such system is the central melanocortin system that has been shown to be at the heart of the control of energy homeostasis. In particular, the melanocortin 3 receptor plays a critical role in the communication between nutritional and reproductive states — MC3R knockout (Mc3r -/-) mice display defective regulation of reproductive development as indicated by delayed onset of puberty. Male Mc3r -/- mice had a 2-day delayed puberty compared to their wildtype counterparts. Female mice similarly had delayed puberty, delayed first estrus, and dysregulation of estrus cyclicity, and spent more time in proestrus-estrus phase cycle. Most striking is the observation that MC3RKO mice are resistant to fasting-induced suppression of the HPG axis. Importantly, the role of the MC3R in reproductive development has been demonstrated in both heterozygous and homozygous loss of MC3R function in humans, with one homozygous patient not reaching puberty until 20 years of age. To further elucidate the role of MC3R in modulating both development of the reproductive axis, and its regulation by nutritional state, our lab has created the first MC3R floxed mouse to delete MC3R in adult animals as well as specific neuronal populations known to be involved in reproduction. We found that when we selectively delete MC3R in kisspeptin neurons, there was no effect on time to puberty in males or females, and no effect on metabolic measurements including body weight, fat mass, and lean mass in males or females. This suggests that there could be an afferent MC3R neuron upstream of kisspeptin that relays nutritional information to modulate reproductive maturation. We recently reported that MC3R neurons are expressed in all AgRP neurons, AgRP has been reported to facilitate the onset of puberty, and directly stimulating AgRP fibers inhibit Kiss1 neurons in the arcuate nucleus. Using targeted deletion of MC3R, we are currently studying if or whether AgRP neurons are the neuronal mediators of pubertal activation that are dysregulated by the loss of MC3R. We are also currently testing the role of MC3R in kisspeptin cells in transmitting information on nutritional state to the HPG axis. Overall, these data expand upon the developing understanding of a role for the MC3R in modulation of energy homeostasis by reproductive state.

Abstract #32:

The modulatory potentials of kisspeptin/neurokinin B/dynorphin neurons and RFamide-related peptide-3 on Gonadotropin-releasing hormone

Babatunde Ibitoye, Olugbemi T. Olaniyan, Imole Ayobami Yemitan, Olutayo Margaret Alese, Francisca Omolara Ibitoye, Ayobami Dare, Olaleke Bashir Fasasi

Department of Anatomy, Ekiti State University, Ado Ekiti, Nigeria

Introduction:
We sought to critically analyze how gonadotropin-releasing hormone (GnRH), which is the key hypothalamic neurohormone that modulates the reproductive function of invertebrates is regulated in a complex hypothalamic circuit; that interacts with the reproductive and non-reproductive hormones in form of a negative feedback loop. Emotional status, environmental and local brain factors such as oxidative stress, inflammation, hormones, and paracrine cell communication can influence the output of GnRH. Understanding of this circuit is to be applied to solve reproductive diseases through drug design.

Materials and methods:
A focused literature search was conducted to include studies published in Cochrane, Pubmed, google scholar and Web of Science databases between the years 2005 and 2022.

Conclusion:
There is a need for a better understanding of the circuit regulatory mechanism for pharmacological manipulation of reproduction and treatment of reproductive diseases. Hence, this review elaborated on the cytoarchitectural arrangement of neurons involved in the regulation of reproductive function and their mechanism which involves the pulsatile release of gonadotropin-releasing hormone by gonadotropin hormone-releasing neurons in the hypothalamus. There is a consideration of the possibility of pharmacological interventions in solving the mirage of gynecological and andrological pathologies resulting from malfunctioning of this circuitry.

Abstract #34:

GABAergic Signalling in the Medial Amygdala Mediates Psychological Stress-induced Suppression of the GnRH Pulse Generator

Caitlin McIntyre [1], Xiaofeng Li [1], Ross de Burgh [1], Deyana Ivanova [1], Juin Wang [1], Gefen Lass [1], Shen Xi [1,2], Kevin T O'Byrne [1]

[1]Department of Women and Children's Health, Faculty of Life Sciences and Medicine, King's College London, Guy's Campus, SE1 1UL, UK; [2]Department of Assisted Reproduction, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, People's Republic of China.

Introduction/aim:
Psychological stress is linked to reproductive dysfunction by suppressing the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator and pulsatile luteinising hormone (LH) secretion. The posterodorsal subnucleus of the medial amygdala (MePD) is a key upstream regulator of GnRH pulse generator activity. The MePD is primarily a GABAergic nucleus with a strong GABAergic projection to hypothalamic reproductive centres, however, their functional significance has not been determined. We hypothesis that MePD GABAergic signalling is a crucial mediator of psychological stress-induced suppression of pulsatile LH secretion.

Method/Results:
We used a chemogenetic approach to selectively silence MePD GABA neurones during psychological stress to determine the effect on pulsatile LH secretion. Female ovariectomised Vgat-cre mice (n=7) were virally infected to selectively express inhibitory hM4DGi-DREADDs in MePD GABA neurones. Mice were exposed to either predator odour (PO) or restraint stress and given an intraperitoneal injection of either saline or the DREADD activator clozapine-N-oxide (CNO, 5mg/kg). Statistical significance was determined using a one-way ANOVA and post-hoc-Tukey test. Data are presented as mean ± SEM and $p<0.05$ was considered significant. PO exposure significantly increased LH inter-pulse interval (IPI) in the saline group (prestress: 19.05±1.16 min vs. PO: 29.28 ± 3.16 min, $p<0.05$, n=7), this effect was blocked by DREADD-mediated inhibition of MePD GABA neurones (prestress:20.59±1.12 min vs. PO:21.31±1.76 min, $p>0.05$, n=7). Restraint stress dramatically increased LH IPI in the saline group (prestress:17.78 ± 1.02 min vs. restraint:45.71±5.50 min, $p<0.05$, n=7) and DREADD-mediated inhibition of MePD GABA neurones blocked this effect (prestress:18.57±1.97 min vs. restraint:27.26±4.02 min,$p>0.05$, n=7). Direct projections from the MePD to the GnRH pulse generator in the hypothalamic arcuate nucleus (ARC) have been identified, however, their phenotype is unknown. In this study, we optogenetically stimulated potential MePD GABAergic projection neurone terminals in the ARC and determined the effect on LH IPI. MePD GABA neurones in female Vgat-cre ovariectomised mice (n=6) were virally infected to express channelrhodopsin 2 and MePD GABAergic terminals in the ARC were selectively stimulated by blue light via a chronically implanted fibre-optic cannula. Sustained optogenetic stimulation at 10 and 20Hz of MePD GABAergic terminals in the ARC dose-dependently suppressed pulsatile LH secretion (LH IPI; control:23.34±2.11 min vs. 10Hz:38.08±3.61 min; 20Hz:56.67±3.33 min, $p<0.05$, n=6).

Conclusions:
These findings confirm a functionally significant MePD GABAergic projection to the ARC and highlight the importance of GABA signalling in the MePD in mediating stress-induced suppression of reproductive function. Collectively, these findings provide insight into the neural circuitry underpinning stress-induced reproductive function.

Abstract #36:

Administration of NKB into the arcuate nucleus accelerates the GnRH pulse generator activity in goats

Satoshi Ohkura[1], Takashi Yamamura[2], Sho Nakamura[1], Yoshihiro Wakabayashi[2]

[1]Nagoya University, Nagoya, Japan; [2]National Agriculture and Food Research Organization, Tsukuba, Japan

Introduction/Aim:
Kisspeptin neurons in the arcuate nucleus (ARC), which co-express neurokinin B (NKB) and dynorphin A, are referred to as KNDy neurons. These neurons are candidates for the intrinsic source of the gonadotropin-releasing hormone (GnRH) pulse generator. The central and peripheral administration of NKB or its receptor (NK3R) agonist evokes GnRH pulse generator activity and the subsequent pulsatile GnRH/luteinizing hormone (LH) secretion. However, the mechanism responsible for neural activation of the GnRH pulse generator is unclear. The present study aims to test the hypothesis that NKB acts on the KNDy neurons directory and that the signal is transmitted bilaterally to a population of KNDy neurons in the ARC using the electrophysiological and histochemical technique in goats.

Methods/Results:
Bilateral electrodes aimed at a cluster of KNDy neurons were inserted into the ARC of ovariectomized goats. We observed the GnRH pulse generator activity, represented by characteristic increases in the multiple unit activity (MUA volleys) in the ARC. The unilateral administration of NKB or vehicle in the close vicinity of KNDy neurons under simultaneous MUA recording from both sides revealed that only NKB evoked MUA volley(s) immediately after administration. The timing of the MUA volley(s) evoked on the ipsilateral side was synchronized to that on the contralateral side. The double-labeled *in situ* hybridization for *KISS1* and *TACR3*, which encode kisspeptin and NK3R, respectively, revealed that most KNDy neurons co-expressed *TACR3* (96.2 ± 0.2%). Tract tracing histochemistry using biotinylated dextran amine (BDA), an anterograde tracer, indicated that axons projecting from NKB neurons in the ARC were directly apposed to other NKB neuronal cells located bilaterally in the ARC, indicating that KNDy neurons are bilaterally interconnected in the ARC via NKB-containing fibers.

Conclusions:
These results suggest that NKB administered locally into the ARC directly stimulates KNDy neurons, following which the stimulatory signal is immediately transmitted to the entire population of KNDy neurons on both sides of the ARC via connection with their fibers. This mechanism might play a critical role in synchronizing bursting activity among KNDy neurons, thereby generating neural signals that govern pulsatile GnRH secretion.

Day 2 Poster Abstracts (even #s): Saturday, August 6, 2022

Abstract #38:

Effect of chronic shift on the daily reproductive rhythms and fertility of female mice

Marine Simonneaux[1], Mathilda Kretz[1,2], Thibault Bahougne[1,2], Paul Klosen[1], Nathalie Jeandidier[2], Valérie Simonneaux[1]

[1]CNRS, UPR 3212 - Institute of Cellular and Integrative Neuroscience, University of Strasbourg, Strasbourg, France
[2]Department of Endocrinology, Diabetes and Nutrition, Strasbourg University Hospitals, Strasbourg, France

Introduction/Aim:
In female mammals, the timing of the preovulatory LH surge depends on the combination of the positive estrogen feedback and a circadian signal which synchronizes the LH surge with the transition between the resting and active period at the end of the follicular phase, when arousal is maximal. Since the correct timing of the LH surge is critical for optimal fertility, we are investigating the consequences a chronodisruptive environment could have on the female mammals' gonadotropic axis. It is a relevant issue since an increasing number of women are working in non-standard work schedules in our modern 24h/7d society, and shift work is associated with reproductive deficits.

Methods/Results:
Adult female mice were either kept in regular light/dark schedules or exposed to a chronic (three weeks) alternation of a 10-hour phase advance for three days and a 10-hour phase delay for four days (a model of shift work conditions). Daily LH secretion and daily activity of the anteroventral periventricular nucleus kisspeptin neurons, as well as fertility parameters, were then compared between both groups of mice. The chronodisruptive protocol abolishes the preovulatory LH surge and the activation of kisspeptin neurons typically observed at the light/dark transition of the day of proestrus. Furthermore, when female mice exposed to chronic shift are mated with a control male, their fertility is significantly decreased. The results show that chronic exposure to shifted light/dark schedules disrupts the daily activation of kisspeptin neurons which can explain the altered LH secretion and the declining fertility. We are currently investigating the effect of this experimental model of shift work results in changes on the activity of the vasopressin-containing neurons located in the suprachiasmatic nucleus known to transmit the daily information to the kisspeptin neurons.

Conclusions:
Chronic exposure to disrupted light/dark cycles desynchronizes the hypothalamic-pituitary-ovarian axis and leads to fertility troubles in female mice. In future experiments, we will investigate whether peripheral clocks within the gonadotropic axis are also altered by chronic shift. Altogether, these experiments will provide a better understanding of circadian disruption's potential on the daily reproductive rhythms of female mammals.