**Oral Presentation Schedule and Abstracts for Session 1: Friday, August 5, 2-3PM**

**2:00-2:10PM**

**Abstract #1: Characterization of voltage-gated calcium currents in hypothalamic arcuate *Kiss1* neurons in mice**

**Speaker: Jian Qiu**

Department of Chemical Physiology and Biochemistry, Oregon Health and Science University, Portland, Oregon 97239

**2:15-2:25PM**

**Abstract #2: The evolutionary conserved miRNA-137/325 regulates female puberty via coordinated repression of hypothalamic kisspeptin and neurokinin-B**

**Speaker: Cecilia Perdices-Lopez**

[1]Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC); [2]University of Cordoba; and [3]Spanish Network of Research on Obesity and Nutrition (CIBEROBN), Cordoba, Spain.

**2:30-2:40PM**

**Abstract #3: Amygdala Urocortin3 efferents to the hypothalamic paraventricular nucleus control corticosterone and LH secretion in mice**

**Speaker: Deyana Ivanova**

Department of Women and Children's Health, Faculty of Life Science and Medicine, King's College London, London, UK

**2:45-2:55PM**

**Abstract #4: *Kiss1* and *Kiss1R* have sexually dimorphic expression in the periaqueductal gray**

**Speaker: Karen J. Tonsfeldt**

Obstetrics, Gynecology and Reproductive Sciences, University of California San Diego

**Oral Presentation Abstract #1**

## Characterization of voltage-gated calcium currents in hypothalamic arcuate *Kiss1* neurons in mice

**Jian Qiu**[1], Martha A. Bosch[1], Oline K. Rønnekleiv[1,2] and Martin J. Kelly[1,2]

[1]Department of Chemical Physiology and Biochemistry, Oregon Health and Science University, Portland, Oregon 97239 and [2]Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Oregon 97006

### Introduction/Aim:

Pulsatile secretion of GnRH, and consequently pulsatile release of LH, is required for normal reproductive function, and this activity is controlled by kisspeptin neurons in the hypothalamic arcuate nucleus (*Kiss1*$^{ARH}$). The activation of *Kiss1*$^{ARH}$ neurons releases neurokinin B that depolarizes and recruits other *Kiss1*$^{ARH}$ neurons resulting in synchronous firing through activation of TRPC5 channels. Intracellular $Ca^{2+}$ not only potentiates TRPC5 channel activity, but also facilitates the release of neurotransmitters. However, the contribution of voltage-gated calcium channels to $Ca^{2+}$ homeostasis in *Kiss1*$^{ARH}$ neurons has not been elucidated. Therefore, we conducted molecular and cellular physiological analysis of these channels in *Kiss1*$^{ARH}$ neurons.

### Methods/Results:

We used whole-cell recording and selective pharmacological blockers to measure low-voltage activated (*i.e.,* T-type) and high-voltage activated (HVA) calcium currents in *Kiss1*$^{ARH}$ neurons from ovariectomized (OVX) *Kiss1*$^{Cre}$ female mice. In addition to the prominent T-type calcium current (Qiu *et al., eLife* 2018), Kiss1$^{ARH}$ neurons expressed the full complement of HVA channels: the R-type channels conducted most of the HVA calcium current at 30%, followed by the N-type (22%), the L-type (20%) and the P/Q-type (7%) channels. Using single cell real-time qPCR, we quantified the effects of 17beta-estradiol (E2) on calcium channel mRNA expression in *Kiss1*$^{ARH}$ neurons (10-cell-pools, n = 5 animals/group). Compared to vehicle-treated OVX females, E2 treatment increased the mRNA expression of all the channels: T (2.7-fold), L (1.8-fold), R (1.7-fold), P/Q (1.7-fold) and N (1.3-fold), all of which translated into a 1.8-fold increase in the peak HVA $Ca^{2+}$ current density. The mean $V_{1/2}$ values for channel activation were not significantly different for cells from oil-treated ($V_{1/2}$ = −32.3 ± 2.1 mV, n = 13) versus from E2-treated animals ($V_{1/2}$ = −33.6 ± 2.5 mV, n = 11). Also, $V_{1/2}$ values for steady-state channel inactivation were similar for both groups ($V_{1/2}$ = −48.9 ± 4.8 mV, n = 6 for control versus $V_{1/2}$ = −44.1 ± 1.9 mV, n = 5 for E2-treated group).

### Conclusions:

*Kiss1*$^{ARH}$ neurons express L-, N-, P/Q-, R-type calcium channels, all of which are regulated by E2 similar to T-type calcium channels. Since the kinetics of the HVA calcium current did not change with E2 treatment, increased expression of the mRNA is functionally translated into more HVA current. Therefore, these findings provide a foundation for examining the role of calcium channels in the synchronous activity of *Kiss1*$^{ARH}$ neurons and subsequently neuropeptide and glutamate co-neurotransmission. [Supported by National Institute of Health grant DK068098-14]

**Oral Presentation Abstract #2**

## The evolutionary conserved miRNA-137/325 regulates female puberty via coordinated repression of hypothalamic kisspeptin and neurokinin-B

**Cecilia Perdices-Lopez**[1,2,3], Yolanda Guerrero[1,2], Marco A. Calzado[1,2], Veronica Sobrino[1,2], Maria Jesús Vazquez[1,2,3], Maria Soledad Avendaño[1,2]*, Manuel Tena-Sempere[1,2,3]*

[1]Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC); [2]University of Cordoba; and [3]Spanish Network of Research on Obesity and Nutrition (CIBEROBN), Cordoba, Spain.

*Equal senior authors

**Introduction/Aim:**

Changes in the age of puberty onset have been reported recently, mostly in girls. Yet, the basis for this phenomenon, with potential durable consequences for later health, remains unfolded. Kisspeptin (encoded by *Kiss1*) and Neurokinin B (NKB; encoded by *Tac2* in rodents and *TAC3* in humans), co-expressed by KNDy neurons, are potent puberty-activating signals. However, the mechanisms for the precise regulation of kisspeptin and NKB during the pubertal transition are largely unknown. Our recent data suggest that microRNAs (miRNAs) are putative regulators of Kiss1/KNDy neurons. Yet, the physiological role of specific miRNAs operating on Kiss1 and/or NKB, as mechanism for pubertal control, has not been explored to date.

**Methods/Results:**

Four different algorithms were applied to seek for putative regulatory miRNAs with consensus seed regions in the 3'-UTR of *Kiss1* and *TAC3/Tac2* genes. Selection of miRNA candidates was based on the following criteria: (i) to be identified in at least two different databases; (ii) to show evolutionary conserved seed regions in rat, mouse and human species; and (iii) to have previous evidence for hypothalamic expression in rodents. Using these criteria, the miR-137-3p/miR-325-3p tandem was selected, as putative modulator of the 3'-UTRs of both *Kiss1* and *TAC3/Tac2* genes. The predicted repressive actions of miR-137-3p and miR-325-3p on *KISS1* and *TAC3* 3'-UTRs were documented in vitro using luciferase assays. Hypothalamic expression of miR-137-3p and miR-325-3p gradually increased during postnatal maturation in female rats. Yet, in models of delayed puberty, due to postnatal undernutrition or manipulation of sex steroid milieu during the critical neonatal period, the elevation of hypothalamic miR-137-3p and miR-325-3p levels was markedly enhanced. Repeated central injections of miRNA-mimics for miR-137-3p and miR-325-3p to immature female rats decreased hypothalamic levels of kisspeptin and NKB, and delayed puberty onset, with deferred vaginal opening and first estrus, as well as reduced ovarian weight and ovulatory rates. Conversely, selective blockade of the repressive interaction between miR-137-3p and miR-325-3p specifically at the *Kiss1* and *Tac2* 3'-UTRs in vivo, using central infusion of tailored Target Site Blockers (TSBs) to immature female rats, elevated the hypothalamic content of kisspeptin and NKB, and advanced puberty onset, with earlier vaginal opening and first estrus, along with increased ovarian weight and ovulatory rates.

**Conclusions:**

Our results demonstrate that the evolutionary conserved miR-137-3p/miR-325-3p tandem has a major role in the physiological control of the timing of female puberty, by a joint repressive action on kisspeptin and NKB at the hypothalamus.

**Oral Presentation Abstract #3**

## Amygdala Urocortin3 efferents to the hypothalamic paraventricular nucleus control corticosterone and LH secretion in mice

**Deyana Ivanova**, Xiao-Feng Li, Caitlin McIntyre, HongBei Xu and Kevin T O'Byrne

Department of Women and Children's Health, Faculty of Life Science and Medicine, King's College London, London, UK

### Introduction/Aim:

Psychosocial stress profoundly interferes with reproductive function and suppresses pulsatile luteinizing hormone (LH) secretion. The posterodorsal subnucleus of the medial amygdala (MePD) is an upstream modulator of the hypothalamus pituitary gonadal (HPG) axis and the hypothalamus pituitary adrenal (HPA) axis. Stress alters neuronal activity within the MePD, increasing the expression of Urocortin3 (Ucn3) while enhancing the inhibitory output from the MePD to key hypothalamic reproductive centres. Designer receptor exclusively activated by designer drugs (DREADDs) inhibition of MePD Ucn3 neurons prevents psychosocial stress-induced suppression of LH pulses in female mice. Moreover, MePD Ucn3 neurons have been shown to project directly to the hypothalamic paraventricular nucleus (PVN). We investigate whether MePD Ucn3 signalling is involved in modulating the stress induced rise in corticosterone (CORT) release and whether MePD Ucn3 projection terminals in the PVN control CORT secretion and LH pulsatility.

### Methods/Results:

Ucn3-cre-tdTomato female ovariectomised (OVX) mice were bilaterally injected with inhibitory DREADDs virus AAV-hM4D targeting the MePD. The mice received an I.P injection of clozapine-N-oxide (CNO; 5 mg $kg^{-1}$) and we monitored the effect on CORT secretion during 1 h of predator odor, 2,4,5-Trimethylthiazole (TMT), exposure. A separate group of Ucn3-cre-tdTomato OVX mice with oestradiol replacement were unilaterally injected with AAV-ChR2 in the MePD and implanted with optic fibre targeting the PVN. We optically stimulated MePD Ucn3 neurons with blue light at 20 Hz, 15 mW and monitored the effect on CORT secretion and LH pulses. We found that DREADDs inhibition of MePD Ucn3 neurons blocked predator-odour induced rise in CORT secretion compared to controls (DREADD group at 60 min: 166.52 ± 14.55 vs. control virus group 356.06 ± 59.60 ng/ml; mean ± SEM; n=6-9). Optogenetic stimulation of MePD Ucn3 neuron terminals in the PVN elevated CORT secretion compared to controls (AAV-ChR2 group: 265.14 ± 27.20 vs. control virus group 69.13 ± 13.84 ng/ml; mean ± SEM; n=3-4). Optical activation of MePD Ucn3 projection terminals in the PVN reduced LH pulsatility compared to controls (LH pulse interval for AAV-ChR2 group: 32.50 ± 5.30 vs. pre-stimulation control period: 15.17 ± 0.59 vs. control virus group 17.67 ± 1.67 min; mean ± SEM; n=3-4).

### Conclusions:

We reveal a functional role for MePD Ucn3 neurons and efferents to the PVN in modulating the activity of the HPA and HPG axis, thus the MePD may act as a central hub integrating anxiogenic cues with the stress and reproductive axis.

**Oral Presentation Abstract #4**

## *Kiss1* and *Kiss1R* have sexually dimorphic expression in the periaqueductal gray

**Karen J. Tonsfeldt**[1], Jacqueline Hernandez[1], Andy V. Babwah[2], Pamela L. Mellon[1]

[1]Obstetrics, Gynecology and Reproductive Sciences, University of California San Diego;
[2]Department of Pediatrics, Robert Wood Johnson Medical School, Rutgers University.

### Introduction/Aim:

Women are disproportionately affected by chronic pain, reporting more pain from the same conditions as males and having more chronic pain conditions. Chronic pain is believed to arise from sensitization of the descending modulation pathway, in which neurons in the ventrolateral periaqueductal gray (vlPAG) project to the rostral ventromedial medulla, which then projects to the dorsal horn of the spinal cord to regulate the sensation of noxious stimuli (nociception). Kisspeptin is a neuropeptide that has been well-studied in the hypothalamus for its role in reproduction; it has also recently been described to have be pro-nociceptive. Importantly, kisspeptin expression is sexually dimorphic in the brain, and may contribute to sexual dimorphism in chronic pain. In this study, we sought to characterize sex steroid and chronic pain regulation of kisspeptin and its receptor, KISS1R, in the vlPAG.

### Method/Results:

We first examined *Kiss1* and *Kiss1r* expression in whole PAG punches. We studied gonadectomized males with or without testosterone replacement (GDX, GDX+T) and ovariectomized females with or without estradiol replacement (OVX, OVX+E). We found that *Kiss1* expression was significantly higher in females than males in both groups [$F(1,18) = 15.33$, $p = 0.0010$], and that there was no significant effect of sex steroid treatment on either males or females [$F(1,18) = 0.2719$, $p = 0.6084$]. Similarly, we found that *Kiss1r* mrNA was significantly higher in females than in males [$F(1,20) = 7.691$, $p = 0.0117$], and the levels were not affected by sex steroids [$F(1,20) = 7.459e\text{-}005$, $p = 0.9932$]. We also performed a preliminary study in which mice were treated with Complete Freund's Adjuvant (CFA) for 5 days; CFA is used as a model of persistent arthritic pain. In a small cohort of males and females, we found that CFA treatment significantly upregulated *Kiss1* expression in the PAG [$F(1, 17) = 4.668$, $p = 0.0453$]. Finally, using a novel 2XHA-KISS1R reporter mouse, we identified a population of KISS1R-containing GABAergic neurons in the vlPAG.

### Conclusions:

In this study, we have identified increased *Kiss1* and *Kiss1r* expression in the PAG in females compared to males, and that *Kiss1* expression in these regions is not sex-steroid dependent as it is in other regions. We have also identified a GABAergic vlPAG neuron population that expresses 2XHA-KISS1R. Future studies will focus on the action of kisspeptin on vlPAG neurons, and plasticity during persistent inflammation.