Oral Presentation Schedule and Abstracts for Session 3:

Saturday, August 6, 2:00-2:50PM

2:05-2:15PM

Abstract #11: Kisspeptin signaling in astrocytes centrally modulates the reproductive axis

Speaker: Encarnacion Torres

Instituto Maimonides de Investigacion Biomedica de Cordoba (IMIBIC), Cordoba, Spain; Department of Cell Biology, Physiology and Immunology, University of Cordoba, Cordoba, Spain; Hospital Universitario Reina Sofia, Cordoba, Spain

2:20-2:30PM

Abstract #12: The KiNG of reproduction: Kisspeptin/nNOS interaction shaping hypothalamic GnRH release

Speaker: Virginia Delli

Univ. Lille, Inserm, CHU Lille, UMR-S1172 - LilNCog - Lille Neuroscience & Cognition, F-59000 Lille, France; FHU, 1000 Days for Health, F-59000 Lille, France

2:35-2:45PM

Abstract #13: Role of KNDy neurons and arcuate Kiss1R neurons in the LH surge of female sheep

Speaker: Max Griesgraber

West Virginia University, Morgantown, WV

Oral Presentation Abstract #11

Kisspeptin signaling in astrocytes centrally modulates the reproductive axis

Encarnacion Torres[1,2,3], Giuliana Pellegrino[4], Inmaculada Velasco[1,2,3], Antonio Carlos Fuentes-Fayos[1,2,3], Melisa Granados-Rodriguez[1,2,3], Shel Hwa Yeo[5], Stephen Manchisi[5], Maria Jesus Sanchez-Tapia[1,2,3], Juan Roa[1,2,3,6], Jesus Argente[6,7,8], Raul M Luque[1,2,6], Vincent Prevot[4], Julie Chowen[6,7], Ariane Sharif[4], William H College[5], Manuel Tena-Sempere[1,2,3,6,9], Antonio Romero-Ruiz[1,2,3]

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Introduction/Aim:

Reproduction is safeguarded by multiple, often cooperative regulatory networks. Kisspeptin signaling, via KISS1R, plays a fundamental role in reproductive control, primarily by regulation of hypothalamic GnRH neurons. We disclose herein a pathway for direct kisspeptin actions in astrocytes that contributes to central reproductive modulation.

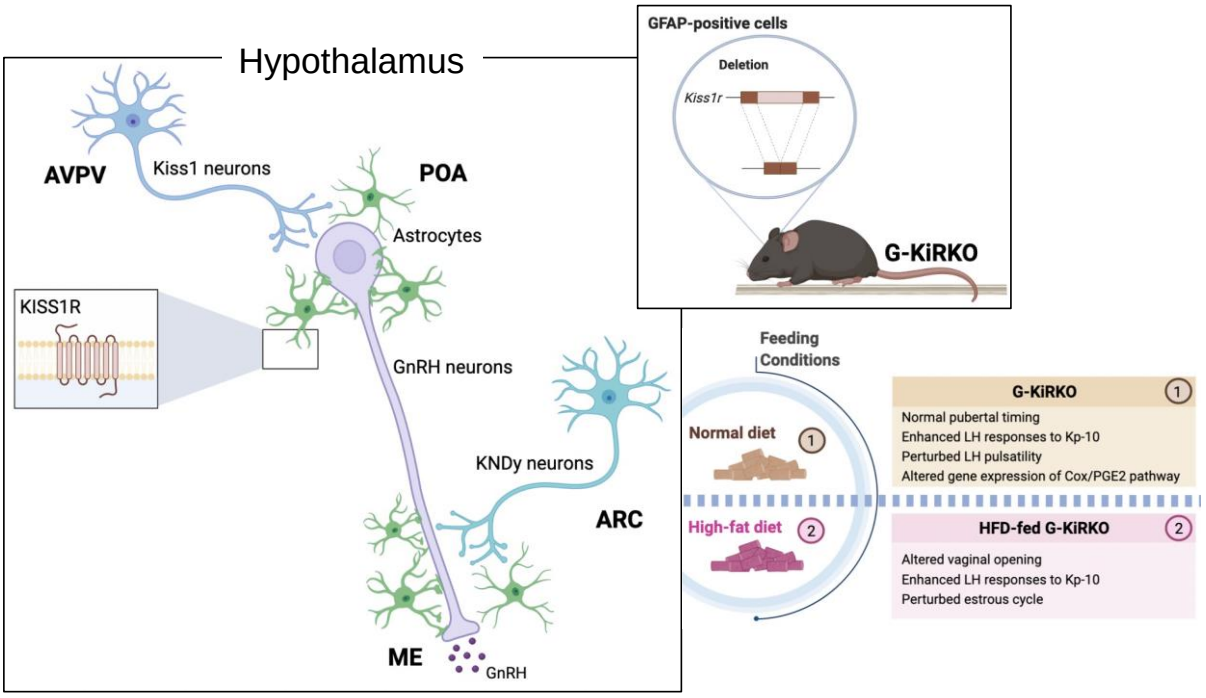
Methods/Results:

Protein-protein-interaction and ontology analyses of hypothalamic proteomic profiles after kisspeptin stimulation revealed that glial/astrocyte markers are regulated by kisspeptin in mice. This glial-kisspeptin pathway was validated by the demonstrated expression of Kiss1r in astrocyte cultures from rats and mice, where kisspeptin was able to activate canonical intracellular signaling-pathways. Cellular co-expression of Kiss1r with the astrocyte marker, GFAP, occurred in different brain regions, with higher percentage in Kiss1- and GnRH-enriched areas, while GFAP-immunoreactivity was altered in mouse models of disrupted kisspeptin signaling. Conditional ablation of Kiss1r in GFAP positive cells, in the G-KiRKO mouse, altered gene expression of key factors in PGE2 synthesis in astrocytes, and perturbed LH responses to kisspeptin and LH pulsatility, as surrogate marker of GnRH secretion. In addition, G-KiRKO mice displayed changes in reproductive responses to metabolic stress induced by high-fat diet, affecting female pubertal onset and estrous cyclicity.

Conclusions:

Our data unveil a non-neuronal pathway for kisspeptin actions in astrocytes, which cooperates in fine tuning the reproductive axis and its responses to metabolic stress.

Oral Presentation Abstract #11 (continued)



Graphical summary for **Kisspeptin signaling in astrocytes centrally modulates the reproductive axis.**

Oral Presentation Abstract #12

The KiNG of reproduction: Kisspeptin/nNOS interaction shaping hypothalamic GnRH release

Virginia Delli[1,2], Tori Lhomme[1,2], Vincent Prévot[1,2], Konstantina Chachlaki[1,2,3]

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Introduction:

GnRH is the master regulator of the HPG axis, and therefore of fertility and reproduction. The mechanisms regulating the release pattern of GnRH by the hypothalamus - including both pulses and surges - remain under debate. Kisspeptin is the most well-known stimulator of GnRH, while nitric oxide (produced by nNOS neurons), has been associating with the negative regulation of GnRH secretion, facilitating thus GnRH response to repeated stimulatory inputs. Kisspeptin and nNOS may form a neuronal microcircuit responsible for rhythmically shaping GnRH release pattern all across the estrous cycle.

Methods:

RNAscope is used to identify the expression pattern of *Kiss1*, Kiss1 receptor (*Kiss1R*) and *Nos1* mRNAs in female mouse hypothalamus. A chemogenetic approach is applied to selectively manipulate kisspeptin and nNOS afferences *in vivo*. The concentration of NO being released is measured *in vivo* and *ex vivo* using a newly designed cGMP biosensor. Electrophysiological approaches are applied to a $Nos1^{KO}$::$GnRH^{gfp}$ mouse model in the presence or absence of kisspeptin, in order to decipher the dynamics of the KiNG network.

Results:

nNOS and kisspeptin are distinct neuronal populations, found to anatomically associate and interact with GnRH neurons in both the mediobasal hypothalamus (MBH) and the preoptic area (POA). *GPR54* is found expressed by nNOS neurons and kisspeptin-induced nNOS activation is observed in both MBH and POA. Kisspeptin is able to stimulate the production of NO in a dose dependent fashion, this relying on nNOS functioning.

Conclusions:

Kisspeptin and nNOS interplay leads to the generation of GnRH pulses and surges, crucial for the proper development and function of the reproductive axis. We postulate that kisspeptin and nNOS create a dynamic system in which kisspeptin provides the “ON” signal, promoting GnRH release, while NO mediates the “OFF” signal, acting as a tonic brake on GnRH secretion.

Oral Presentation Abstract #13

Role of KNDy neurons and arcuate Kiss1R neurons in the LH surge of female sheep

Max Griesgraber[1], Eliana Aerts[1], Elizabeth Bowdridge[1], Kayla Onslow[2], Steven Hardy[1], Lique Coolen[2], Stanley Hileman[1], Michael Lehman[2], Robert Goodman[1]

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Introduction/Aim:

KNDy neurons containing kisspeptin and neurokinin B (NKB) in the arcuate nucleus (ARC) play an important role in tonic luteinizing hormone (LH) secretion. In sheep, kisspeptin also plays a role in the LH surge as a Kiss1r antagonist decreases surge amplitude by 50%. However, the particular role of KNDy neurons remains unclear. This study tested the role of KNDy neurons and determined if kisspeptin actions within the ARC are also important for the LH surge.

Methods/Results:

The ARC of adult female sheep was bilaterally targeted with injections of an NKB-saporin conjugate (NK3-SAP, n = 8), a kisspeptin-saporin conjugate (Kiss-SAP, n = 10), or a blank-saporin conjugate (Blank-SAP, n = 7) as a control. NKB-SAP would be expected to lesion NK3R-containing (i.e. KNDy) neurons, while Kiss-SAP would be expected to lesion Kiss1r-containing, but not KNDy, neurons. Ewes were ovariectomized and a silastic estradiol (E2) implant inserted subcutaneously on the day of neurosurgery. Two luteal phases were then simulated with progesterone-containing CIDRs, immediately followed by E2 treatment via subcutaneous implants to induce an LH surge. LH was monitored in blood samples taken every 2 to 4 hours for 46 hours. Six out of eight NKB-SAP treated ewes displayed an LH surge with a significantly reduced maximum amplitude (49.5 ± 11.7 ng/mL) when compared to that of blank-SAP-treated ewes (156.7 ± 20.2 ng/mL), an effect similar to that previously seen with a Kiss1r antagonist in ewes. RNAscope is being performed to quantify ARC NK3R and kisspeptin expression in these animals, but ewes displaying reduced surge amplitudes have so far exhibited markedly reduced NK3R and KNDy cell numbers. Nine of ten Kiss-SAP-treated ewes either did not display a surge or exhibited a surge with greatly decreased amplitude, with the mean maximum LH level at the time of the expected surge being 16.6 ± 5.3 ng/mL. All Kiss-SAP animals examined so far have significantly reduced ARC Kiss1r cell numbers, except for the single ewe that displayed a normal surge wherein there was no reduction in Kiss1r cell numbers due to a misplaced injection. Importantly, there was no change in gonadotropin-releasing hormone cell numbers or fiber density in Kiss-SAP treated ewes.

Conclusions:

Based on these data, we propose that in ewes, ARC KNDy and/or other NK3R neurons are needed for an LH surge of normal amplitude, while ARC Kiss1r-containing neurons are essential for a functional LH surge.