Monday April 19

Session Co-Chairs: Sasha Kauffman and Alexander Comninos

5:40 pm Edouard Mills (Imperial College London)

5:50 pm Juan Roa Rivas (University of Cordoba)

6:00 pm Su Young Han (University of Cambridge)

6:10 pm Stephania Guzman (Rutgers University)

6:20 pm Elizabeth McCarthy (Harvard Med. Sch./Brigham & Women's Hospital)

6:30 pm Rick McCosh (University of California, San Diego)

6:40 pm Richard Piet (Kent State University)

6:50 pm Mengjie Wang (University of Toledo)

(all times EDT [New York time])

KISSPEPTIN ADMINISTRATION MODULATES GAMMA-AMINOBUTYRIC ACID LEVELS IN THE HUMAN BRAIN

Edouard G. Mills[1], Alexander N. Comninos[1,2], Lisa Yang[1], James O'Callaghan[3], Matthew B. Wall[3], Lysia Demetriou[4], Victoria C. Wing[1], Layla Thurston[1], Bryn M. Owen[1], Ali Abbara[1], Eugenii A. Rabiner[2], Waljit S. Dhillo[1,2].

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Gamma-aminobutyric acid (GABA) is a key inhibitory neurotransmitter that has been implicated in reproductive signalling, as well as in common mood and behavioural disorders. Pre-clinical animal models demonstrate that kisspeptin and GABA closely interact in several brain regions, however, there is currently no data in this regard in humans.

We therefore sought to explore whether an *in vivo* change in neurotransmitter levels following kisspeptin administration could be demonstrated in humans, and specifically whether a change in central GABA levels could be detected. To do this we selected the anterior cingulate cortex (ACC) as our region of interest, based on its established involvement in kisspeptin signalling and its extensive prior human investigation using proton magnetic resonance spectroscopy (MRS).

We performed a randomised, double-blinded, placebo-controlled, cross-over study in 19 healthy men (age 26.1±1.2y). Firstly, we observed that kisspeptin administration increased luteinising hormone levels, as expected, confirming bioactivity of this dose of kisspeptin (intravenous 1nmol/kg/h, $p<0.0001$). Secondly, by employing MRS, we observed that kisspeptin administration decreased total endogenous GABA levels in the ACC compared to vehicle. This effect of kisspeptin on GABA levels endured when corrected both against water (GABA/H2O:14.1%,$t(18)=2.17$,$p=0.043$) and creatine (GABA/Cr:15.7%,$t(18)=2.44$,$p=0.025$).

This is the first data identifying a change in a central neurotransmitter following kisspeptin administration in humans and is in keeping with animal data demonstrating interactions between kisspeptin and GABA. Furthermore, our work may help explain previous human behavioural findings observed with kisspeptin administration, given the extensive role of GABA limbic signalling in mood and emotions. In this regard, a similar magnitude of GABA change to that we observed in our study, has been previously reported in psychological studies with functional significance, including in modulating impulsivity. Finally, these data also provide important information in humans for the escalating development of kisspeptin-based therapies for common reproductive disorders of body and mind.

SEXUALLY DIMORPHIC DEPENDENCE OF PUBERTY AND FERTILITY ON MICRORNA BIOGENESIS IN KISS1 NEURONS

Juan Roa[1], Miguel Ruiz-Cruz[1], Francisco Ruiz-Pino[1], Rocio Onieva[1], Maria J. Vazquez[1], Maria J. Sanchez-Tapia[1], Jose M. 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]

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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. 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 and *Kiss1* expression were largely preserved during the infantile-pubertal transition in KiDKO mice, kisspeptin protein levels were dramatically reduced already in infantile animals. In contrast, the AVPV Kiss1 population was fully preserved in KiDKO animals. Nonetheless, despite the massive suppression of kisspeptin content in the ARC, during the pubertal transition, the developmental increase in kisspeptin immunoreactivity was also detected in KiDKO mice, even if at lower magnitude than in controls. All in all, 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. The disparate impact of Dicer ablation between sexes and Kiss1-neuronal populations surfaces intrinsic differences in the roles of Kiss1 miRNA-machinery in the fine control of male and female reproduction.

VISUALIZATION OF ARCUATE KISSPEPTIN NEURON SYNCHRONIZATION WITH SINGLE-CELL RESOLUTION IN FREELY-BEHAVING MICE

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The episodic pattern of gonadotrophin-releasing hormone (GnRH) secretion drives luteinizing hormone (LH) pulses which are critical for maintaining normal reproduction. Using GCaMP fiber photometry, it has recently been shown that kisspeptin neurons in the arcuate nucleus (ARN) are the GnRH pulse generator exhibiting synchronized episodes of activity preceding LH pulses in a perfectly correlated manner. However, the cellular mechanism underlying such synchronization is not well-understood.

Using an *in vivo* GCaMP microendoscope approach, we have examined the activity of ARN kisspeptin neurons at single-cell resolution in freely-behaving gonadectomized (GDX) male mice. *Kiss1-Cre* mice were given stereotaxic injections of AAV9.CAG.Flex.GCaMP6s.WPRE.SV40 into the ARN followed by implantation of a 500 µm-diameter Gradient-Index lens (Grintech) placed 250 µm above the middle-caudal ARN. Following 5-8 weeks of habituation, an open-source UCLA miniscope was mounted on the head of the mouse, allowing for 10-40 min recordings in freely-behaving mice at 10Hz sampling.

Recordings imaged 36±13 GCaMP-expressing kisspeptin neurons simultaneously (n=4 mice). Individual kisspeptin neurons were found to exhibit repeated, abrupt increases in GCaMP fluorescence that were highly synchronized amongst ~ 80% of the population. The duration of each activation varied from 30 to 130 s between cells with the mean half maximum of the rising and falling phase being 8±3 s and 34±5 s, respectively. Although synchronized in the timeframe of seconds, the initiation of enhanced activity was not found to occur at exactly the same time amongst recorded kisspeptin neurons. Additionally, not every kisspeptin neuron contributed to every burst of activity when series of synchronization events were recorded. These data are highly consistent with our prior *in vivo* population-GCaMP imaging and demonstrate a remarkable degree of homogeneity amongst the ARN kisspeptin population indicating that the great majority of cells operate together as the GnRH pulse generator.

TARGETING HEPATIC KISSPEPTIN RECEPTOR AMELIORATES NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease worldwide. The incidence of NAFLD correlates with the rise in obesity, Type 2 diabetes, and metabolic syndrome. A key feature of NAFLD is excessive hepatic fat accumulation or steatosis (ie NAFL), due to dysregulated hepatic fat metabolism which can progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis and eventually to hepatocellular carcinoma (HCC). Currently, there are no approved pharmacotherapies to treat this disease. The G-protein coupled kisspeptin receptor (KISS1R) and its ligand kisspeptin are expressed in the liver, but their function is unknown. This study provides the first evidence that hepatic KISS1R signaling provides a protective role in the liver against steatosis. Using high fat diet (HFD) fed insulin resistant mice, we demonstrated that a deletion of hepatic *Kiss1r* worsened hepatic steatosis and increased markers for inflammation and fibrosis, in addition to enhancing glucose intolerance and insulin resistance phenotype. In contrast, administration of a KISS1R agonist protected against steatosis, improved insulin resistance and decreased markers for inflammation and fibrosis. Mechanistically, we found that hepatic KISS1R signaling inhibits lipogenesis via activation of the master energy regulator, AMPK, in addition to increasing fatty acid oxidation. In NAFLD patients and in HFD-fed mice, hepatic KISS1/KISS1R expression and plasma kisspeptin levels were elevated, suggesting a compensatory mechanism to reduce triglyceride synthesis. Overall, this study establishes KISS1R as a novel target for preventive treatment against the development of NAFLD.

INHIBITING KISS1 NEURONS WITH KAPPA OPIOID RECEPTOR AGONISTS TO TREAT POLYCYCTIC OVARY SYNDROME (PCOS) AND VASOMOTOR SYMPTOMS

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Recent evidence suggests that vasomotor symptoms (VMS) or hot flashes in the postmenopausal reproductive state and polycystic ovarian syndrome (PCOS) in the premenopausal reproductive state emanate from the hyperactivity of Kiss1 neurons in the hypothalamic infundibular/arcuate nucleus (KNDy neurons). Here, we demonstrate in two murine models simulating menopause and PCOS that a peripherally-restricted kappa receptor agonist (PRKA) inhibits hyperactive KNDy neurons and impedes their down-stream effects. First, chronic administration of a PRKA to ovariectomized (OVX) mice with experimentally-induced hyperactivity of KNDy neurons reduces the animals' elevated body temperature, mean plasma luteinizing hormone (LH) level, and LH pulse amplitude. Second, chronic administration of a PRKA to a murine model of PCOS, having elevated plasma testosterone levels and irregular ovarian cycles, suppresses circulating levels of LH and testosterone and restores normal ovarian cyclicity and *Kiss1* expression in the arcuate nucleus. Thus, the inhibition of Kiss1 neuronal activity by activation of peripheral kappa receptors shows promise as a novel therapeutic approach to treat both VMS and PCOS in humans.

ACTIVATION OF THE A2 POPULATION OF NOREPINEPHRINE NEURONS SUPPRESSES LH PULSES AND INCREASES CORTICOSTERONE SECRETION IN FEMALE MICE

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Stress causes robust neuroendocrine changes, including suppression of luteinizing hormone (LH) pulses and elevated corticosterone (CORT) secretion. Although the neural pathways involved in conveying stress signals to the hypothalamic centers controlling LH and CORT secretion are not well defined, A2 neurons (norepinephrine neurons in the nucleus of the solitary tract [NTS] of the brainstem) are implicated in both neural pathways. We used chemogenetics to test the hypothesis that activation of the A2 neuronal population is sufficient to suppress LH pulses and elevate CORT concentrations in ovariectomized female mice. Dopamine beta-hydroxylase (DBH) Cre positive and negative mice received bilateral NTS injections of either a Cre-dependent stimulatory Designer Receptor Exclusively Activated by Designer Drugs (DREADD) virus (AAV1-DIO-hM3Dq-mCherry) or a control virus (AAV1-DIO-mCherry; n=4-11/group). Frequent blood samples for LH and CORT were collected before and after CNO or saline in a crossover design in which each animal received both treatments in a random order separated by two weeks. All control and vehicle-treated animals showed clear and robust LH pulses (mean: 6.0 ± 0.2ng/mL, pulses/60 min: 3.4 ± 1.5) and low basal CORT (<200ng/mL) throughout the sampling period. In contrast, CNO caused a robust (>50%) suppression in mean LH and pulse frequency ($p<0.05$) in DBH-Cre positive mice with hM3D virus. In addition, circulating CORT was low prior to CNO (146±33ng/mL), but was significantly augmented (>260% of pre-injection values, $p<0.05$) by 30 min after CNO and remained elevated for the duration of the 3hr post injection period. These data demonstrating suppression of LH and enhancement of CORT secretion support the broad hypothesis that A2 neurons are critical for modulating hypothalamic-pituitary-gonadal and -adrenal axes during stress and raise the possibility that A2 neurons influence arcuate kisspeptin cell and paraventricular nucleus corticotropin releasing hormone neuron activity (analysis is of cell activation is ongoing).

SUPRACHIASMATIC NUCLEUS VASOPRESSIN NEURONS CONTROL OF PREOPTIC KISSPEPTIN NEURON ELECTRICAL ACTIVITY ACROSS THE ESTROUS CYCLE

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The preovulatory gonadotropin surge in female rodents begins prior to the daily onset of activity, ensuring that ovulation and sex behavior coincide. Timing of the surge relies on axonal projections from the suprachiasmatic nucleus (SCN), the locus of the central circadian clock, to circuits that control gonadotropin secretion. This includes projections from SCN arginine vasopressin (SCN^{AVP}) neurons to kisspeptin (Kiss1) neurons in the rostral periventricular area of the third ventricle ($RP3V^{Kiss1}$), responsible for generating the preovulatory surge in gonadotropin secretion. Despite prior knowledge that exogenous AVP evokes surge-like gonadotropin secretion *in vivo* and stimulates $RP3V^{Kiss1}$ neuron electrical activity *in vitro*, the function of the $SCN^{AVP} \rightarrow RP3V^{Kiss1}$ circuit *in situ* and its regulation during the estrous cycle remain to be elucidated. To investigate the impact of SCN^{AVP} neuron projections on $RP3V^{Kiss1}$ action potential firing, we targeted channelrhodopsin (ChR2) expression to the SCN of female mice expressing Cre recombinase in AVP cells and the green fluorescent protein (GFP) in Kiss1 neurons. Patch-clamp recordings of GFP-expressing Kiss1 neurons in brain slices containing the RP3V were subsequently undertaken. In slices from proestrous mice, sustained stimulation of ChR2-expressing SCN^{AVP} projection fibers in the RP3V resulted in a significant increase in Kiss1 neuron action potential firing, which likely resulted from AVP release acting at $RP3V^{Kiss1}$ neuron AVP V1 receptors. This effect was less prominent in diestrus and absent in estrus, following ovulation. Remarkably, in estrus, activation of SCN^{AVP} projections resulted in an inhibition of $RP3V^{Kiss1}$ neuron firing, an effect which was rarely seen in other cycle stages and was dependent on activation of $GABA_A$ and $GABA_B$ receptors. Together, these observations reveal functional plasticity in the output of SCN^{AVP} neurons, driving opposing effects on $RP3V^{Kiss1}$ neuron activity across the ovulatory cycle. This might contribute to gating activation of the preovulatory surge to the appropriate estrous cycle stage.

NOVEL AND COOPERATIVE ROLES OF IGF-1 RECEPTORS AND INSULIN RECEPTORS IN KISSPEPTIN NEURONS IN REPRODUCTION AND METABOLISM

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The neuropeptide kisspeptin, encoded by the *Kiss1* gene, is critical for puberty and fertility. It is also increasingly being recognized as a major determinant of metabolic functions, but the factors that regulate *Kiss1* neurons need to be clarified. Our lab has previously found that mice with deletions of insulin receptors (IRs) in *Kiss1* neurons (IR^{Kiss1} mice) experienced delayed puberty but normal adult fertility. To test whether insulin like growth factor 1 receptors (IGF1Rs) in *Kiss1* neurons affect reproductive and metabolic functions, we generated transgenic mice lacking IGF1Rs exclusively in *Kiss1* neurons ($IGF1R^{Kiss1}$ mice). Because IGF1R and IR signaling induce overlapping activation of the phosphoinositide 3-kinase (PI3K)-Akt pathway, we also generated mice with simultaneous deletions of IGF1Rs and IRs in *Kiss1* neurons ($IGF1R/IR^{Kiss1}$ mice). We found that $IGF1R^{Kiss1}$ mice and $IGF1R/IR^{Kiss1}$ mice experienced delayed puberty and adult reproductive deficits in both sexes. However, no differences were seen between $IGF1R^{Kiss1}$ mice and $IGF1R/IR^{Kiss1}$ mice, suggesting IGF1R in *Kiss1* neurons plays a major role in the regulation of puberty and fertility. Female $IGF1R^{Kiss1}$ mice exhibited a more “metabolically healthy” phenotype with decreased body weight and food intake but increased energy expenditure and physical activity, which was associated with increased brown adipose tissue (BAT) thermogenesis. Interestingly, this phenotype was normalized in female $IGF1R/IR^{Kiss1}$ mice. $IGF1R/IR^{Kiss1}$ mice had an increased fat mass, glucose intolerance, and insulin insensitivity in both sexes, suggesting IGF1Rs and IRs in *Kiss1* neurons cooperatively regulate body composition and glucose homeostasis. Our study demonstrates the unique and cooperative roles of IGF1Rs and IRs in *Kiss1* neurons in the regulation of reproduction and metabolism.