

## Neuroendocrinology and Pituitary

### ADVANCES IN NEUROENDOCRINOLOGY

#### **Connect-seq to Superimpose Molecular on Anatomical Neural Circuit Maps**

*Naresh Hanchate, PhD<sup>1</sup>, Eun Jeong Lee, PhD<sup>1</sup>, Andria Ellis, B.S.<sup>2</sup>, Kunio Kondoh, PhD<sup>3</sup>, Donghui Kuang, PhD<sup>1</sup>, Ryan Basom, MS<sup>1</sup>, Cole Trapnell, PhD<sup>2</sup>, Linda Buck, PhD<sup>1</sup>.*

<sup>1</sup>FRED HUTCHINSON CANCER RES CENTER, Seattle, WA, USA, <sup>2</sup>University of Washington, Seattle, WA, USA, <sup>3</sup>National Institute for Physiological Sciences, Okazaki, Japan.

#### SUN-LB55

Animals exhibit instinctive behavioral and physiological responses to a variety of stressors to overcome danger and restore homeostasis. The physiological response to stress is governed by hypothalamic corticotropin-releasing hormone (CRH) neurons which regulate the hypothalamic-pituitary-adrenal axis to control blood levels of stress hormones. At present, the neural circuits and signaling mechanisms through which different stress signals are transmitted to CRH neurons are poorly understood. Here, we devised a new method, termed “Connect-Seq,” which couples single-cell transcriptomics and retrograde viral tracing to define the molecular identities of individual neurons in neural circuits. As a proof of concept, using Connect-Seq, we profiled single-cell transcriptomes of 124 brain neurons upstream of CRH neurons and identified subpopulations that are likely to communicate stress-related signals to CRH neurons. Analyses of single-cell transcriptomes for ‘fast-acting’ neurotransmitters revealed subsets of upstream neurons that expressed markers of inhibitory GABAergic neurons or excitatory glutamatergic neurons. Further analyses showed a number of other neuromodulators/neurotransmitters in upstream neurons, including acetylcholine, dopamine, histamine, and 43 different neuropeptides, each expressed in individual neurons or subsets of neurons. These findings reveal extreme molecular heterogeneity among upstream neurons and suggest the upstream neurons use diverse neurochemical messengers to transmit signals to CRH neurons. Many neurons coexpressed different neurotransmitters/neuromodulators, suggesting the co-release of neurochemical messengers. Dual labeling of brain sections verified expression of specific neuromodulators in virus-infected neurons upstream of CRH neurons in selected brain areas. Our results indicate that Connect-Seq can be applied to genetically dissect neural circuits and uncover molecular identities of neurons upstream of specific neuronal types of known function. Molecular markers identified in those neurons lay a foundation for the application of cell-specific genetic tools to investigate the functions and physiological significance of diverse neuronal subsets within complex neural circuits.

## Neuroendocrinology and Pituitary

### ADVANCES IN NEUROENDOCRINOLOGY

#### **Assessing the Requirement of Arcuate Nucleus Kisspeptin Neurons in Puberty Onset and Adult LH Secretion**

*Eulalia A. Coutinho, PhD, Lourdes A. Esparza, MS, Shreyana Bolleddu, HSD, Alexander S. Kauffman, PhD.*  
University of California San Diego, La Jolla, CA, USA.

#### SUN-LB57

Puberty is a critical developmental period marking the transition to adulthood and attainment of reproductive capability. A hallmark of puberty is increased pulsatile secretion of pituitary luteinizing hormone (LH) which is itself driven by increased gonadotropin-releasing hormone (GnRH) from the forebrain. The mechanisms governing GnRH neuron activation at puberty still remain unclear, but likely include enhanced stimulation from upstream reproductive neural circuits, including kisspeptin neurons. Kisspeptin is a potent stimulator of GnRH and is required for proper puberty onset. However, the specific brain site(s) from where kisspeptin signaling arises to trigger puberty remain unclarified. Kisspeptin is expressed in two primary nuclei in the hypothalamus, the arcuate nucleus (ARC) and anteroventral periventricular (AVPV) region. Studies suggest that, in adulthood, ARC *Kiss1* neurons are involved in driving pulsatile secretion of GnRH (and hence, LH) in both sexes whereas AVPV *Kiss1* neurons participate in the preovulatory GnRH/LH surge in females. However, the specific role of either kisspeptin neuron population in puberty onset still remains unknown. We previously showed that both kisspeptin populations show increased *Kiss1* gene expression across the pubertal period, yet whether just one or both (or neither) population is needed for puberty to occur has not been determined. Here, we sought to tease out the role—if any—of ARC and AVPV *Kiss1* neurons in the pubertal onset process. Since ARC *Kiss1* neurons are abundant in both sexes and drive pulsatile GnRH secretion in adulthood, we hypothesized that ARC *Kiss1* neurons are necessary for normal puberty onset and, conversely, that AVPV *Kiss1* neurons are not sufficient on their own to induce normal puberty. To test this hypothesis, we used a Cre-specific diphtheria toxin approach to ablate just ARC *Kiss1* neurons in juvenile mice (~2 weeks old) while leaving AVPV *Kiss1* neurons intact. Preliminary data thus far indicates that site specific ablation of just ARC *Kiss1* neurons during the juvenile period significantly delays puberty onset in both sexes, as measured by vaginal opening, first estrous, and preputial separation. In addition, selective ARC *Kiss1* neuron ablation in juvenile life diminishes pulsatile LH secretion levels measured in adulthood, but does not alter LH surge generation in adult females. These preliminary findings empirically demonstrate that, in mice, ARC *Kiss1* neurons are required for proper activation of the reproductive axis during puberty but not the LH surge in adulthood, and AVPV *Kiss1* neurons are not sufficient to trigger normal pubertal onset.

## Bone and Mineral Metabolism

### BONE DISEASE FROM BENCH TO BEDSIDE

#### **A Comparison Between Weight Loss Associated Bone Loss From Lifestyle Measures Versus Bariatric Surgery**

*Betty La, M.D., Francesca Vigevano, MS, Prathyusha V. Chitrapu, MD, Anisha Gupta, MD, Vimlin Auetumrongsawat, BS, Dennis Tan Villareal, MD, Reina Condevillamar Armamento-Villareal, MD.*  
Baylor College of Medicine, Houston, TX, USA.

#### SUN-LB69

**Background** Despite improvement in many metabolic outcomes, bone loss remains a concern with weight loss