Abstract #1:

β-Nerve Growth Factor acts upstream of GnRH neurons to trigger ovulation in mice.

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Timed induction of ovulation requires a cascade of events in which GnRH and Kisspeptin (Kp) neurons play a key role. In mammals, ovulation is either spontaneous or induced by mating. In species with induced ovulations, β-Nerve Growth Factor (βNGF) is acknowledged as an important stimulatory factor whereas this role is attributed to Kp in species with spontaneous ovulations. However, recent studies suggested that the βNGF could also contribute in spontaneous ovulatory species.

We tested whether βNGF induced ovulation in mice, a spontaneous ovulatory species, and investigated if GnRH and Kp systems are involved in its effect.

Fifty mice (21 days) received 5IU of PMSG and, 48 hours later, one of the following treatments: 0.9% NaCl, 5 IU of hCG, or βNGF (0.1µg, 1 µg, or 10 µg/mouse). All three βNGF doses induced ovulation ($p < 0.001$) with an efficacy similar to that of hCG. Ovulation rates were 80% for hCG or βNGF (0.1 µg and 1µg), 100% for βNGF (10 µg), and 10% for NaCl.

To determine if βNGF action on ovulation required GnRH receptor activation, we tested responses in mice pre-treated with Cetrorelix, a GnRH receptor antagonist. Prepubertal mice (128) were allocated to five treatment groups: βNGF (1 µg), Cetrorelix (50 ng) + βNGF (1 µg), GnRH (50 ng), Cetrorelix (50 ng) + GnRH (50 ng), or 0.9% NaCl. Both βNGF and GnRH triggered ovulations and this effect was blocked by Cetrorelix (ovulation rate: Cetrorelix + βNGF vs βNGF, $p<0.05$). These results suggest that the GnRH is implicated in the βNGF-induced ovulation pass through.

We then tested responses in Kiss1 and Kiss1r null mice at two ages: adult (KO Kiss1: 23; KO Kiss1r: 16) and prepubertal (KO Kiss:5 ; KO Kiss1r: 9) mice. Each type of mice was allocated into two groups of treatment: βNGF (0.2 µg) or hCG (5UI). In all groups treated with βNGF, the rate of ovulation and the number of *corpora lutea* per ovary was significantly? lower than in groups treated with hCG. These results suggest that βNGF requires the action of Kp to trigger ovulation.

Using double immunohistochemical procedures on adult OVX+E2 female mice, we found both βNGF receptors, $p75^{NTR}$ and TrkA, localized in the organum vasculosum of the lamina terminalis (OVLT). $p75^{NTR}$ was also expressed in the arcuate nucleus (ARC) and Median Eminence (ME). $p75^{NTR}$ was found in neurons and tanycytes, but was not expressed by GnRH or Kp immunoreactive neurons suggesting the involvement of interneurons.

In conclusion, βNGF induces ovulation in the mouse and this probably involves the release of GnRH and Kp. However, because none of these neurons expresses $p75^{NTR}$, the transduction of βNGF information is relayed either by interneurons or by glial cells. Among them, tanycytes appear as interesting candidates.

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Keywords: βNGF, $p75^{NTR}$, ovulation, GnRH, Kisspeptin.

Abstract #3:

Lack of GABABR in *Kiss1* cells alters metabolism that worsens with age in male mice.

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Introduction/Aim:
Kisspeptin and GABA are expressed in various peripheral organs/tissues critical to metabolic control (liver/pancreas/adipose). Moreover, kisspeptin neurons coexpress GABAB receptors (GABABR) and GABA controls the expression and secretion of kisspeptin. However, our understanding of how these two factors interact to regulate metabolism is still incomplete. We have developed a unique mouse lacking GABABR exclusively from kisspeptin cells/neurons (Kiss1-GABAB1KO) to evaluate the impact on metabolism/reproduction. 3-month-old Kiss1-GABAB1KO females showed increased body weight (BW), non-fasted glycemia (NFG), insulin secretion and HOMA-beta-cell index. 3-month-old Kiss1-GABAB1KO males showed normal BW and NFG, higher fasted glycemia (FG), serum insulin and HOMA-IR index, altered response to glucose overload and lower insulin sensitivity compared to control males. Here determined whether these metabolic alterations persisted or worsened with age.

Methods/Results:
We evaluated metabolic parameters in 9-months-old Kiss1-$GABA_{B1}$KO and control mice.
Interestingly, Kiss1-GABAB1KO males had higher BW and increased in total white adipose tissue (WAT) and also in WAT/BW. Although NFG and FG were similar between genotypes, Kiss1-GABAB1KO males showed increased fasted serum insulin and pancreatic insulin content. Furthermore, HOMA-beta-cell and HOMA-IR indexes were increased in Kiss1-GABAB1KO males. We also evaluated serum cholesterol and triglycerides but they were similar between genotypes. We did not find differences between genotypes either in serum kisspeptin levels or hepatic kisspeptin content. However, kisspeptin levels were decreased by 35% in the pancreas of the Kiss1-GABAB1KO males, and this decrease could be leading to the increased pancreas insulin observed at this age.
In contrast, 9-months-old Kiss1-GABAB1KO females showed similar BW, NFG, FG, fasted insulin levels, HOMA indexes, serum cholesterol and triglycerides levels compared to controls. We need to further investigate kisspeptin levels in the main metabolic tissues in females to determine one possible cause of this striking phenotype reversion with age.

Conclusions:
In sum, lack of GABABR specifically in *Kiss1* cells has a clear impact on BW, WAT, glucose homeostasis, pancreatic kisspeptin, insulin levels and insulin resistance in male mice, reinforcing the proposed kisspeptin involvement in metabolic regulation. Not only the peripheral response to insulin worsened, in line with the increased BW, but also pancreas function was affected by aging in males. These metabolic alterations may be due to altered autocrine/paracrine regulation of the pancreatic islet, which will be further studied. Our results highlight the impact of GABA through GABABR in the regulation of the peripheral pancreas kisspeptin system in contrast to the liver kisspeptin system that was not affected, suggesting tissue specific regulation.

Abstract #5:

Nitric Oxide deficiency linking a defective minipuberty to the appearance of comorbidities: new therapeutic possibilities

Konstantina Chachlaki [1,2,3,4,5], Andrea Messina [4,5], Virginia Delli [1,2], Valerie Leysen [1,2], Csilla Maurnyi [6] Georgios Papadakis [4,5], Sonal Shruti [1,2] , Maria Kapanidou [7], Katalin Skrapits [6], Xu Cheng [4,5], Rademaker Jesse [4,5], S Rasika [1,2], Richard Quinton [8], Marek Nedziella [9], Dagmar L'Allemand [10], Duarte Pignatelli [11], Mirjam Dirlewander [12], Mariarosaria Lang-Muritano [13], Patrick Kempf [14], Sophie Catteau-Jonard [1,2,15], Philippe Ciofi [16], Nicolas J. Niederländer [4,5], Manuel Tena-Sempere [17,18,19], Laurent Storme [2,20], Paul Avan [21], Erik Hrabovszky [6], John Garthwaite [22], Federico Santoni [4,5], Paolo Giacobini [1, 2], Nelly Pitteloud [4,5 ©*], Vincent Prevot [1, 2 ©*]

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Introduction:
Prematurity is associated with alterations in the maturation of the Hypothalamic-Pituitary-Gonadal (HPG) axis, and specifically with its transient activation during infancy, i.e. minipuberty. Minipuberty, and the resulting surge in gonadotropin levels (luteinizing and follicle stimulating hormones; LH, FSH) influences neuronal network maturation, growth, blood pressure, body composition, and lipid and glucose metabolism. Indeed, hyperandrogenism or altered follicular development, both occurring because of aberrant FSH levels at minipuberty, contribute to the risk of developing many noncommunicable diseases. The neuromodulator nitric oxide (NO) has long

been recognized as a key player in the central hormonal regulation of ovulation. However very little is known about its role in the minipubertal activation of the HPG axis.

Methods:
Extensive hormonal characterization of minipuberty, reproductive, metabolic and cognitive assessment was carried out in both a newly developed mouse model of premature birth (preterm mice), as well as a *Nos1*-deficient mouse model. *In vivo* pharmacological manipulation of minipubertal NO levels was used to identify the timely regulation of minipuberty and its association with cognitive and non-cognitive comorbidities. Therapeutic options targeting the restoration of minipuberty have been pre-clinically validated.

Results:
Nos1 deficiency results in dose-dependent defects not only in sexual maturation but also olfaction, hearing and cognition. *In vivo* pharmacological manipulation of NO levels (blockade or replenishment) revealed a critical time window during which Nos1 activity shaped minipuberty and sexual maturation, but also reproductive and behavioral phenotypes in adulthood. Prematurely born mice phenocopied the effects of Nos1-deficiency, revealing the crucial role of minipubertal NO in the maturation of the neuroendocrine brain and the physiological outcomes in adulthood.

Conclusions:
Minipuberty is altered in premature infants, and the non-communicable neurological and physiological deficits associated with premature birth, including intellectual disability, constitute a major public health issue. Effective early-life treatments that can limit or reverse these comorbidities are lacking, and the interests of this growing population of individuals with lifelong handicap are largely neglected. Our work is the first to associate the maturation of the neuroendocrine axis with brain development (including the development of higher brain functions), identifying NO's key role in the underlying mechanism. By identifying a targetable cause for the deficits linked to premature birth (and altered minipuberty) our work aims to creating novel therapeutic avenues. This ground-breaking work forms the basis of a large-scale European study, "MiniNO", financed by the European Research Council in 2020 (No847941), and including medical and research teams from 6 European countries.

Abstract #7:

Reproductive Hormone Secretion is Increased by Intranasal Kisspeptin Administration in Healthy Volunteers and Hypogonadal Patients

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Introduction/Aim:
Kisspeptin is a critical activator of hypothalamic gonadotrophin releasing hormone (GnRH) neurons and has significant potential to treat common reproductive disorders. To date, kisspeptin has solely been administered to humans via the intravenous or subcutaneous routes. However, intranasal administration could offer a novel non-invasive delivery route. We therefore sought to determine the effects of intranasal kisspeptin on reproductive hormone release in humans for the first time.

Methods/Results:
Randomised, double-blinded, placebo-controlled, cross-over study in 12 healthy men (mean±SEM age 28.3±1.7 years; BMI 24.5±0.7 kg/m^2). After monitored self-administration of intranasal kisspeptin-54 (3.2, 6.4, 12.8 and 25.6 nmol/kg) or 0.9% saline, serum reproductive hormone levels were measured every 15 minutes for four hours. Subsequently, four women (mean age 29.8±3.7 years; BMI 21.2±1.1 kg/m^2) with hypothalamic amenorrhoea (HA) attended for the same protocol comparing intranasal kisspeptin-54 (12.8 nmol/kg) and 0.9% saline. Mean±SEM was presented. Time profiles of hormone levels were compared using two-way ANOVA, and multiple means using one-way ANOVA.
In healthy men, intranasal kisspeptin dose-dependently increased mean luteinising hormone (LH) levels at doses between 3.2-12.8 nmol/kg (p=0.008 and <0.0001 for 6.4 and 12.8 nmol/kg vs saline, respectively), with the maximal rises occurring 30-45 minutes post-administration. The maximal LH change from baseline was significantly elevated following all kisspeptin doses vs saline (*saline:* 1.54±0.30 IU/L; *3.2 nmol/kg:* 2.46±0.30 IU/L [p=0.01]; *6.4 nmol/kg:* 3.08±0.48 IU/L [p=0.04]; *12.8 nmol/kg:* 4.45±0.59 IU/L [p=0.002]; *25.6 nmol/kg:* 4.07±0.66 IU/L [p=0.003]). Follicle stimulating hormone (FSH) levels followed a similar trajectory to LH. Kisspeptin at 12.8 nmol/kg increased serum testosterone from 120 minutes onwards (p=0.02), with a maximal change from baseline of 4.9±0.7 nmol/L (p=0.03).
In women with HA, intranasal kisspeptin increased mean LH (p=0.002 vs saline), with the peak levels occurring 30-45 minutes post-administration. The maximal LH change from baseline was 4.06±0.89 IU/L, compared with 0.20±0.38 IU/L for saline (p=0.03). Intranasal kisspeptin increased mean FSH (p=0.01 vs saline). No significant changes in downstream serum oestradiol or progesterone were observed during the acute four-hour study.

Conclusions:

We report the first investigation of the effects of intranasal kisspeptin delivery on reproductive hormone release. Our results demonstrate that intranasal kisspeptin robustly and dose-dependently stimulates reproductive hormone release in healthy men and in a patient-group of women with hypogonadism. Given the ongoing development of kisspeptin therapeutics, intranasal

kisspeptin offers a novel, safe, effective and non-invasive route of administration for the management of reproductive disorders that would be preferred by patients and clinicians alike.

Abstract #9:

Kisspeptin Improves Sexual Brain Processing in Women with Hypoactive Sexual Desire Disorder

Layla Thurston[1], Tia Hunjan[1], Natalie Ertl[1,2], Matt B Wall[1,2], Edouard Mills[1], Sofiya Suladze[1], Bijal Patel[1], Emma Alexander[1], Beatrice Muzi[1], Eugenii A Rabiner[2], Paul Bech[1], David Goldmeier[3], Ali Abbara[1], Alexander N Comninos[1,3], Waljit S Dhillo[1,3]

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Introduction/Aim:
Sexual desire is a key component of the sexual response model. Absence or deficiency of sexual desire can lead to marked distress or interpersonal difficulty, termed 'hypoactive sexual desire disorder' (HSDD). HSDD is the most common female sexual health complaint worldwide, affecting up to 10% of women. Despite its detrimental impact on psychological well-being and quality of life, treatment options are currently limited. The hormone kisspeptin is a key endogenous activator of the hypothalamic-pituitary-gonadal axis, with emerging roles in sexual and emotional behaviour, and thus could serve as a novel treatment option in women with HSDD.

Methods/Results:
We performed a randomized, double-blind, two-way crossover, placebo-controlled study in 32 premenopausal women with HSDD. We used psychometric, functional neuroimaging, and hormonal analyses to investigate the effects of kisspeptin administration on brain activity, in response to erotic stimuli (erotic videos) and facial attraction (images of faces of varying attractiveness).

Kisspeptin administration resulted in an increase in self-reported ratings of feeling 'sexy', compared to placebo, measured using the Sexual Arousal and Desire Inventory (t[31]=2.27, P=0.03). On functional MRI, kisspeptin administration deactivated the left inferior frontal gyrus and activated the postcentral and supramarginal gyrus in response to erotic videos (Z=2.3, P<0.05). Kisspeptin administration deactivated the secondary somatosensory cortex (Z=2.3, P<0.05) and enhanced activation in the posterior cingulate cortex on viewing male faces, which correlated with a reduction in self-reported sexual aversion (r=0.476, P=0.005). Kisspeptin resulted in a mean increase in LH of 2.75 iU/L (F(1, 62) = 6.084, P=0.02) and FSH of 0.37 iU/L (F(1, 62) = 4.030, P=0.05) across the 75-minute duration of the study as expected, with no effect observed on downstream circulating oestradiol, progesterone or testosterone levels.

Conclusions:
Our results demonstrate that kisspeptin administration to women with HSDD increases their self-reported ratings of feeling 'sexy'. Our brain activity changes provide mechanistic insight for this, with deactivation of the left inferior frontal gyrus, likely serving to reduce internal monologue and response inhibition. Furthermore, kisspeptin's deactivation of the secondary somatosensory cortex can reduce a woman's focus on herself, her body image, and related negative thoughts, thus augmenting her judgement of male facial attractiveness. Finally, kisspeptin's actions in the posterior cingulate cortex can serve to increase feelings of romantic love and reward processing, thereby reducing sexual aversion and increasing sexual desire. These behavioural and mechanistic findings in women with HSDD lay the foundations for clinical applications for kisspeptin in psychosexual disorders.

Abstract #11:

Histological analysis of visualized Kiss1 neurons using newly generated Kiss1-Cre rats

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Introduction/Aim:
Hypothalamic kisspeptin neurons play a key role in regulating mammalian reproduction by stimulating gonadotropin-releasing hormone (GnRH) secretion. Kisspeptin neurons in the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) have been suggested to be involved in the GnRH pulse and surge generation, respectively, in female rodents. On the other hand, in male rodents, few kisspeptin neurons were found in the AVPV. This suggests that *Kiss1* (kisspeptin gene) expression would be irreversibly suppressed in AVPV kisspeptin neurons, or AVPV kisspeptin neurons may be disappeared because of the cell death in male rodents. Here we newly generated *Kiss1-Cre* rats, and the present study aims to evaluate the *Kiss1-Cre* rats. Further, the *Kiss1*-dependent tdTomato-expressing reporter rats were used to examine the sex difference in *Kiss1* neurons in the AVPV and ARC in the several developmental periods.

Methods/Results:
Newly generated *Kiss1-Cre* rats were bred with *Cre*-activated tdTomato reporter rats and the resultant offspring were subjected to the histological analysis for the distribution of tdTomato-expressing cells at several developmental periods as well as adulthood. tdTomato signals were colocalized in most of the *Kiss1*-positive cells in the AVPV of adult ovariectomized (OVX) rats treated with estrogen and in the ARC of OVX rats without estrogen treatment. *Kiss1* signals, but not tdTomato signals, were suppressed in the ARC and increased in the AVPV by estrogen in OVX rats. At 1 day of age, tdTomato-positive cells were found in the ARC of both sexes and AVPV of males, while no tdTomato-positive cells were found in the AVPV in females. At 3 weeks and other later ages, tdTomato-positive cells were found in the ARC and AVPV of both sexes.

Conclusions:
These results suggest that *Kiss1* neurons were successfully visualized by tdTomato fluorescence using the *Kiss1-Cre* rats and that tdTomato expression was not affected by estrogen milieu. Further, the current results suggest that *Kiss1* expression in the female AVPV is started in peripubertal period, whereas in the male AVPV, *Kiss1* is expressed in perinatal period and the cells exist even after the sexual maturation.

Abstract #13:

Involvement of central dynorphin and β-endorphin signaling in glucoprivic LH pulse suppression and gluconeogenesis/feeding

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Introduction/Aim:
It has been well-known that reproductive function in mammals is suppressed during malnutrition. The suppression is considered to be mainly due to the suppression of pulsatile gonadotropin-releasing hormone (GnRH)/gonadotropin secretion. Accumulating evidence suggests that kisspeptin neurons in the hypothalamic arcuate nucleus (ARC) play a critical role in controlling reproductive functions via regulation of pulsatile GnRH/gonadotropin secretion. The present study aims to examine if hypothalamic dynorphin-κ opioid receptor (KOR) and β-endorphin-μ opioid receptor (MOR) signaling mediate the suppression of GnRH/gonadotropin secretion during malnutrition.

Methods/Results:
Adult female rats peripherally administered with 2-deoxy-D-glucose (2DG), an inhibitor of glucose utilization, were used as a malnutrition model. A KOR or MOR selective antagonist was injected into the 3rd cerebroventricle immediately before intravenous (iv) 2DG administration. The central injection of KOR or MOR selective antagonist blocked the 2DG-induced suppression of luteinizing hormone (LH) pulses in female rats. Double *in situ* hybridization for dynorphin gene (*Pdyn*) and *fos*, a marker gene of neuronal activation, in the hypothalamus of female rats injected with iv 2DG or xylose (control) revealed that the number of *Pdyn*-positive cells co-expressing *fos* was significantly higher in the paraventricular nucleus (PVN) of the 2DG-injected rats compared to the xylose-injected controls. On the other hand, there was no significant difference in the number of the *Pdyn* and *fos* co-expressing cells in the ARC between the groups. Double *in situ* hybridization for the kisspeptin gene *(Kiss1*) and KOR gene (*Oprk1*) revealed that around 60% of ARC *Kiss1*-expressing cells co-expressed *Oprk1*. Moreover, double *in situ* hybridization revealed that ARC *Kiss1*-expressing cells and preoptic GnRH gene (*Gnrh1*)-expressing cells co-expressed little MOR gene (*Oprm1*), while around 10% of ARC glutamatergic marker gene (*Slc17a6*)-expressing cells co-expressed *Oprm1*. In addition, the central injection of the MOR selective antagonist blocked the 2DG-induced increase in blood glucose level and food intake in female rats, while the KOR selective antagonist failed to block the 2DG-induced increase in blood glucose level and food intake.

Conclusions:
These results suggest that hypothalamic dynorphin-KOR and β-endorphin-MOR signaling, at least in part, mediate the glucoprivic suppression of pulsatile LH secretion, and that β-endorphin-MOR signaling, but not dynorphin-KOR signaling, is also involved in gluconeogenesis and increases in food intake during malnutrition in female rats.

Day 1 Poster Abstracts (odd #s): Friday, August 5, 2022

Abstract #15:

Transcriptome profiling of kisspeptin cells uncovers estrogen feedback mechanisms

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Introduction/Aim:
Kisspeptin neurons in the mediobasal hypothalamus (MBH) are critical targets of ovarian estrogen feedback regulating mammalian fertility. To reveal molecular mechanisms underlying this signaling, we thoroughly characterized the estrogen-regulated transcriptome of kisspeptin cells from ovariectomized transgenic mice substituted with 17β-estradiol or vehicle.

Methods/Results:
MBH kisspeptin neurons were harvested using laser-capture-microdissection, pooled and subjected to RNA-Sequencing. 17β-estradiol significantly (p.adj.<0.05) upregulated 1190 and downregulated 1139 transcripts, including transcription factors, neuropeptides, ribosomal and mitochondrial proteins, ion channels, transporters, receptors and regulatory RNAs. Reduced expression of the excitatory serotonin receptor-4 transcript (Htr4) diminished kisspeptin neuron responsiveness to serotonergic stimulation. Many estrogen-regulated transcripts have been implicated in puberty/fertility disorders. Patients (N=337) with congenital hypogonadotropic hypogonadism (CHH) showed enrichment of rare variants in known and new putative CHH-candidate genes (LRP1B, CACNA1G, FNDC3A…).

Conclusions:
Comprehensive characterization of the estrogen-dependent kisspeptin neuron transcriptome sheds light on the molecular mechanisms of ovary-brain communication and informs genetic research on human fertility disorders.

Abstract #17:

What role does minipuberty play in the organization of hypothalamic circuits underlying female reproductive function?

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Introduction/Aim:
Sexual differentiation of the brain is governed by sex steroid hormones, especially testosterone and its metabolite estradiol. During the perinatal period, estradiol masculinizes and defeminizes male neural circuits underlying the regulation of the hypothalamic-pituitary-gonadal (HPG) axis and the expression of reproductive behaviors. In females, the ovaries are inactive during the fetal period and the brain is protected from the masculinizing action of perinatal estradiol by the action of α-fetoprotein. Mouse ovaries start releasing estradiol from postnatal day (PND7), rising between PND12 and PND17 resulting from a transient gonadotropin surge (Francois et al., 2017). This period, referred to as minipuberty, appears to play a critical role in the feminization of female reproductive functions, but its exact role and mechanisms of action remain unclear. This study aims to investigate the role of minipuberty in organizing the neural structures involved in hypothalamic estradiol negative and positive feedbacks and expression of female sexual behavior.

Methods/Results:
We used a pharmacological model, called GonadoSTOP, in which female mice were injected daily with a GnRH-R antagonist (Ganirelix, 10µg/mouse/day), or saline for control mice, from PND10 to PND16, to suppress gonadotropin surges (Francois et al., 2017). In adulthood, immunohistochemistry analyses showed no significant difference of the number of GnRH neurons in the rostral preoptic area ($p > 0.05$), nor in the mean density of GnRH-immunoreactivity (ir) in the median eminence ($p > 0.05$) between intact control- and Ganirelix-treated females at the diestrus phase. No effect was observed either in ovariectomized females supplemented with estradiol and progesterone mimicking the estradiol positive feedback of the proestrus phase ($p > 0.05$). Moreover, there was no difference in the mean density of kisspeptin-ir in the arcuate nucleus between control and Ganirelix-treated females in both conditions ($p > 0.05$). Interestingly, while no difference in the number of kisspeptin-ir cells in the rostral periventricular area of the third ventricle (RP3V) was observed between intact control and Ganirelix-treated mice, a significant decrease was observed in the Ganirelix-treated group supplemented with estradiol and progesterone ($p = 0.0384$).

Conclusions:
These results suggest that estradiol released during minipuberty participates in organizing the estradiol positive feedback on RP3V kisspeptin neurons. Studies of the impact of postnatal treatment of ganirelix on the expression of lordosis behavior are underway. It will allow us to decipher the contribution of the minipuberty period in the feminization of the HPG axis and on the neural circuits underlying the expression of lordosis behavior.

Abstract #19:

Deep transcriptome profiling of peptidergic neurons from formalin-fixed postmortem human hypothalamic

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Introduction/Aim:
RNA sequencing (RNA-Seq) is a powerful approach providing insight into the transcriptome of cells, however the characterization of the gene expression profile of specifically identified cell populations in human remains challenging. Here we present a novel “immuno-LCM/RNA-Seq” method which enables deep transcriptome profiling of immunohistochemically identified peptidergic neurons from postmortem human brains.

Methods/Results:
The protocol is based on optimized immersion-fixation of brain slabs with buffered paraformaldehyde. RNA-protecting additives allow storage of floated sections in cryoprotective solution for months/years. Agent 1 and Agent 2 are shown to preserve RNA integrity during immunohistochemical procedures. Laser-capture-microdissection and pooling of ~300 immunostained neurons provide high-quality RNA and reliable RNA-Seq library for Illumina sequencing. Using this method we could study the gene expression profile of the agouti-related protein and proopiomelanocortin cells in the infundibular nucleus. Furthermore, the newly-developed “immuno-LCM/RNA-seq” protocol now allows us to characterize the transcriptome of other neuroendocrine cell types in the human hypothalamus, including kisspeptin neurons.

Conclusions:
This highly versatile “immuno-LCM/RNA-Seq” method will open the way to transcriptome profiling of various cell populations from the postmortem human brain or other tissues, and has vast potential in promoting our understanding of biological mechanisms and human diseases.

Abstract #21:

Developmental profile of murine kisspeptin neuron transcriptome sheds new light onto molecular mechanism of puberty

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Introduction/Aim:
Puberty in mammals is a complex process of sexual development which leads to complete gonadal maturation and the attainment of full reproductive capacity. Kisspeptin (KP) encoded by the Kiss1 gene is a potent stimulator of pubertal development. KP neurons of the arcuate nucleus (ARC) mediate the negative feedback of gonadal steroid hormones. They are also responsible for the pulsatile activity of gonadotropin-releasing hormone (GnRH) neurons which is a hallmark of puberty onset. ARC KP neurons express high levels of Tac2 and Tac3r encoding neurokinin B and its receptor, NK3R, respectively. Mutations of Kiss1, Tac2 or Tacr3 cause pubertal failure in mice and humans, indicating that KP neurons of the ARC play a critical role in pubertal development. We hypothesized that the pubertal awakening of the GnRH pulse generator is preceded by profound regulatory events in these neurons. To shed light on the molecular events accompanying puberty, we determined the developmental changes in the gene expression profile of ARC KP neurons from early infantile period to adulthood.

Methods/Results:
KP neurons were harvested from female transgenic mice using laser-capture-microdissection and subjected to deep RNA-Sequencing. Samples were obtained on postnatal days (PN) 8, 15, 22 and on the days of vaginal opening (VO), first estrus (FE), and the proestrus (P) and metestrus (M) stages of adulthood. Pair-wise comparison of the gene expression profiles of the 7 groups identified 3589 significant differences at FDR<0.05, with the highest numbers of changes between PN8 and P (643 upregulation and 673 downregulation) and between PN22 and P (734 upregulation and 509 downregulation). 253 upregulated and 127 downregulated transcripts were detected between the adult M and P stages. Genes showing significant developmental changes belonged to various functional categories, including neuropeptides, receptors and transcriptional factors. Many of them were associated with the regulation of peptidergic and amino acidergic neurotransmission (Pdyn, Sv2c, Nr4a2, Nr5a2, Chgb, Scg2, Pgr, Prlr, Oprk1, Nhlh2, Slc17a6, Bdnf). Kiss1 known to promote puberty, showed maximal expression at P8, followed by an unexpected decrease by VO and FE. Tacr3 increased robustly between PN22 and VO and was downregulated in adult animals between M and P, in accordance with their negative regulation by E2.

Conclusions:
Identification of developmental changes in the transcriptome of the pulse generator KP neurons increases our understanding of the mechanisms that trigger the onset of episodic GnRH release at puberty.

Abstract #23:

Role of the A2 population of norepinephrine neurons in suppression of estrous cycles by undernutrition

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Introduction/Aim:
Chronic undernutrition suppresses reproduction, at least in part, via inhibition of gonadotropin secretion. We recently demonstrated that chronic undernutrition disrupts ovarian cycles in mice resulting in prolonged diestrus. Although the precise neural mechanism(s) for this effect is unknown, one possibility is that nutrient status is detected in the brainstem and conveyed to the hypothalamus via the A2 population of norepinephrine ($A2^{NE}$) neurons. $A2^{NE}$ neurons located in and around the nucleus of the solitary tract (NTS) of the brainstem are marked by the expression of the enzyme dopamine beta hydroxylase (DBH). To test the hypothesis that $A2^{NE}$ neurons are important for suppression of ovarian cyclicity during chronic undernutrition, we conducted 3 experiments to determine if (1) $A2^{NE}$ cells are activated during chronic undernutrition, (2) activation of $A2^{NE}$ cells is sufficient to suppress ovarian cyclicity, and (3) silencing of $A2^{NE}$ cells reverses the inhibitory effect of chronic undernutrition on ovarian cyclicity.

Methods/Results:
Following a 2-wk baseline period, female C57/BL6 mice were randomly assigned to receive unlimited feed (fed control) or receive 70% of their daily baseline feed (feed restricted) for 5 d and neural tissue was collected and processed for immunohistochemistry. Although, the number of DBH neurons and the percentage of DBH neurons in the NTS that contained cFos (a marker of neuronal activation) were not altered by feed restriction, the intensity of DBH immunoreactivity (i.e. relative fluorescent units) was significantly greater in feed restricted animals, revealing an upregulation of norepinephrine synthesis. Next, DBH-Cre positive and DBH-Cre negative mice received virus containing Cre-dependent stimulatory designer receptors exclusively activated by designer drugs (DREADDs) into the NTS. Ovarian cyclicity was assessed daily for 10 d before and after administration of clozapine N-oxide (CNO, DREADD receptor agonist) in drinking water. DBH-Cre negative animals continued to cycle normally. In contrast, DBH-Cre positive mice exhibited rapid disruption of cyclicity, resulting in prolonged diestrus. Finally, DBH-Cre positive mice received virus containing inhibitory DREADDs or an mCherry control, and then were administered CNO in drinking water during feed restriction. In preliminary analysis, mice that received the control virus had rapid suppression of cycles during feed restrictions, whereas mice given the inhibitory DREADD virus displayed a partial reversal in cyclicity. Ongoing analysis of additional animals will permit quantification of this reversal.

Conclusions:
Together, these data support the hypothesis that $A2^{NE}$ neurons are important for suppression of ovarian cyclicity during feed restriction.

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Abstract #25:

Fasting reduces *Kiss1* and *Th* mRNA expression in the ARH and increases circulating FSH levels

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Introduction/Aim:
The arcuate nucleus of the hypothalamus (ARH) is a critical integrative site for the control of metabolism and reproduction. Kisspeptin neurons are closely associated with neuropeptide Y and agouti-related peptide (NPY/AgRP), and proopiomelanocortin (POMC) neurons. However, how the molecular and cellular mechanisms that mediate energy homeostasis are closely connected to reproduction is not fully understood.

Methods/Results:
To determine whether fasting was sufficient to disrupt the estrous cycle and suppress the proestrus-stage, female mice were isolated on the second day of diestrus and fasted for 24hr or fed *ad libitum* (control animals). We evaluated the effects of fasting on body weight, lean and fat mass, and the estrous cycle length. Additionally, a group of mice was used to determine the fasting effects on gonadotropins secretion and mRNA expression in the medial preoptic area (POA) or ARH punches. Fasted mice showed a significant decrease of body weight (n= 17/group; $P<0.0001$), lean mass ($P<0.0001$) and fat mass (n= 7/group; $P=0.003$), as well as, increased the estrous cycle length (n= 9-13/group; $P=0.03$) compared to control animals. Most of the fasted (65%) and control mice (70%) exhibited cornified cells in the vaginal smear. However, control mice that exhibited cornified cells at the vaginal smear showed increased uterine weight ($P=0.01$) and increased luteinizing hormone (LH) levels (P=0.02) compared to fasted mice, suggesting that control animals were at proestrus-stage. Fasted or control mice that exhibited leucocytes at the vaginal smear showed no changes in uterine weight ($P= 0.3$) or LH levels ($P= 0.8$). Fasted mice exhibited a significant increase in FSH levels compared to control animals ($P=0.02$), without apparent correlation with vaginal cytology, uterine weight, or LH levels. As expected, fasting induced a significant reduction in *Pomc* mRNA levels (P 0.02), while increased *Npy* ($P=0.0004$) and *Agrp* mRNA expression (P<0.0001) in ARH punches compared to the control group (n= 6-7/group). In addition, we observed a significant reduction of *Kiss1* (P= 0.005) and *Th* (P= 0.001) mRNA expression in the ARH punches of fasted animals, despite no significant differences in *Kiss1* and *Th* expression in the POA (P>0.05) compared to control animals.

Conclusions:
Fasting is sufficient to suppress the HPG axis of female mice, by modulating *Kiss1* and *Th* expression in the ARH, preventing LH surge and increasing FSH levels. Our results suggest a pathway in which acute changes in energy metabolism modulate the HPG axis by acting on the ARH.

Abstract #27:

Plasma kisspeptin and its neurons are reduced in perimenopausal animal models following acute restraint stress

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Introduction/Aim:
Perimenopause is a transitory period during which many women experience stressful life changing events, coupled with the stress of daily living. Corticosteroids synthesis are increased in animal models of perimenopause and interestingly stress-induced elevation in glucocorticoids has been shown to affect hypothalamic neurons that secrete kisspeptin (KISS1). Thus, this study aimed to assess plasma kisspeptin levels and quantify its hypothalamic neurons in perimenopausal animal models following acute restraint stress.

Methods/Results:
Female 28-day old rats were divided into 2 groups; Control (injected, Corn oil 2.5 µl/g BW); VCD (injected, 4-vinylcyclohexene diepoxide 160 mg/kg BW diluted in Corn oil) both for 15 days; and Aged group allowed to age naturally till 180 days. Sixty (60) days after VCD/corn oil administration, and 180 days in Aged group, rats were further divided into 3 sub-groups: CON+KISS group injected as CON group plus kisspeptin dissolved in saline+gelofusine and administered through intraperitoneal (IP) route for additional 30 days; VCD+KISS group: injected as VCD group plus kisspeptin dissolved in saline+gelofusine and administered through IP route for additional 30 days; Aged+KISS group: as Aged group plus kisspeptin dissolved in saline+gelofusine and administered through IP route for additional 30 days. At 130 days in Control and VCD groups, and 210 days in Aged group on diestrus morning, animals were subjected to acute restraint stress for 30 minutes, followed by 30 minutes recovery period. Blood samples were drawn at rest and during recovery period from tail vein, diluted in PBST solution, frozen at -70ºC. Animals were perfused, brains processed for kisspeptin neurons in the anteroventral periventricular nucleus (AVPV) of the hypothalamus

Plasma kisspeptin levels at rest were significantly reduced ($p<0.05$) in VCD group compared to Control and Aged groups. During recovery, it was significantly lower ($p<0.05$) in VCD and Aged groups compared to Control group. Likewise hypothalamic kisspeptin neurons were significantly reduced ($p<0.05$) in VCD and Aged groups compared to Control group. However, exogenous kisspeptin administration did not significantly ameliorate this low plasma kisspeptin level or its neurons in these animal models of perimenopause.

Conclusions:
It could be inferred from this preliminary study that kisspeptin neurons might play a significant role in these animal models of perimenopause based on our recently published result of a decreased β-endorphin inhibition of LC neurons in VCD perimenopausal animal model.

Abstract #29:

Investigating puberty-related neuronal plasticity in kisspeptin neurons of the arcuate nucleus in female mice

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Introduction/Aim:
Puberty is a transition period from infertility to fertility and is governed by specific neuronal circuits in the hypothalamus that control the hypothalamic-pituitary-gonadal (HPG) axis. Kisspeptin neurons located in the arcuate (ARC) and the anteroventral periventricular (AVPV) regions of the hypothalamus control the activity of the HPG axis. We hypothesised that arcuate kisspeptin (*Kiss1*^{ARC}) neurons from female mice undergo neuronal plasticity from an immature to a mature phenotype as they transition through puberty. We have investigated their molecular and electrophysiological properties before puberty (22 to 34 days old with no vaginal opening) and after puberty (6-9 weeks old with a stable estrous cycle).

Methods/Results:
We used three measures of neuronal plasticity: gene expression of specific synaptic receptors; excitatory synaptic activity; and action potential firing. We prepared brain slices from female mice expressing TdTomato in *Kiss1*^{ARC} neurons for cell isolation and quantitative reverse transcription PCR (qRT-PCR) for gene expression or for whole cell patch clamp recordings of identified *Kiss1*^{ARC} neurons (spontaneous synaptic activity and evoked action potential firing). Expression of the following genes was assessed: *Kiss1, Gria1, Grin2b, Grm5, Kcnh2, Kcnd2, Kcna2, Hcn2 and Hcn3.* Expression of *Kiss1* in *Kiss1*^{ARC} neurons was significantly lower after puberty ($P<0.05$). *Grin2b* expression could not be reliably quantitated by Ct analysis in the qRT-PCR so was analysed by product formation using agarose gel electrophoresis. *Grin2b* was detected in 60% of pre-puberty and 11% of post-puberty mice but this did not reach significance ($P=0.09$). None of the other genes showed any significant differences. Action potentials evoked in response to depolarising current injections under physiological conditions in *Kiss1*^{ARC} neurons from female mice after puberty (n = 31) showed a significantly greater maximum number of action potentials ($P=0.016$) than *Kiss1*^{ARC} neurons from female mice before puberty (n = 43). There was no significant difference in the frequency or amplitude of spontaneous excitatory postsynaptic currents in *Kiss1*^{ARC} neurons before and after puberty.

Conclusions:
These data suggest that specific forms of neuronal plasticity occur in *Kiss1*^{ARC} neurons either before or during puberty in female mice.

Abstract #31:

The relationship of kisspeptin with depression in premature ovarian insufficiency

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Introduction:
Premature ovarian insufficiency (POI) is the cessation of ovarian function before the age of 40.Diagnostic criteria of POI: amenorrhea or oligomenorrhea for at least four months and elevated follicle-stimulating hormone (FSH) levels > 25 IU/L. The etiopathogenesis of the disease in most cases remains unclear.
Being the main cause of infertility and a decrease in the quality of life of women, the POI is one of the most difficult problems of female reproduction.
The COVID-19 virus itself, as well as quarantine measures taken to reduce its spread, have seriously affected the lives of the world's population, which has led to loneliness, social isolation, financial stress, as well as anxiety and fear of infection with the virus and uncertainty about the future. It is known that periods of stress and psychological stress can affect women's reproductive health. Under stress, the level of kisspeptin decreases, which is the main regulator of the hypothalamus-pituitary-ovary axis.
Aim of study was to determine the level of serum kisspeptin in 2 groups: women with POI and postmenopausal women; identification the severity of depression.

Methods/Results:
40 women with POI and 20 postmenopausal women were examined (comparison group). Serum kisspeptin (HUMAN KISS1) was tested using Human Elisa Kit assay in the laboratory of the Republican Centre of Endocrinology. Statistical analysis was performed using Minitab 14.
Women with POI had a history of moderate to severe COVID-19 and menstrual cycle disorders is associated with stress during the coronavirus pandemic. In women with POI (age 29 ± 2), the level of kisspeptin was significantly lower (280.55 ± 11.54 pg/ml, $p < 0.005$) than in postmenopausal women aged 52 ± 1 (420.25 ± 25.82 pg/ml, $p < 0.03$). The kisspeptin plasma levels are correlated negatively with severity of stress. The level of kisspeptin depended on the severity of COVID-19 and the severity of stress. A Beck depression questionnaire was conducted in both groups. According to the results, women with POI (30.7 ± 1.1 points, $p < 0.005$) have moderate to severe depression, compared to postmenopausal women, the degree of depression was low (14.2 ± 1.3 points, $p < 0.06$). Decreased levels of kisspeptin negatively correlated with levels of depression.

Conclusions:
Reduced levels of kisspeptin due to stress may be the cause of the development of premature ovarian insufficiency. It showed that patients with POI were more likely to have deficiency of kisspeptin and are more prone to depression.

Abstract #33:

Pattern of gonadotropin secretion along the estrous cycle of C57BL/6 female mice

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Introduction/Aim:
The pattern of gonadotropin secretion along the estrous cycle was elegantly described in rats. Although we observe many aspects in common between rats and females, some neuroendocrine differences may exist between these species. In addition, less information exists about the pattern of gonadotropin secretion in gonad-intact mice, particularly regarding the follicle stimulating hormone (FSH).

Method/Results:
We performed experiments using different approaches to determine circulating LH and FSH levels, especially at the moment expected for the LH surge. **Additionally,** we investigated the activation of the central components of the HPG axis during the LH surge. Serial blood collections from the tail-tip of gonad-intact C57BL/6 mice at the first day of cornification (transition from diestrus to estrus) showed that luteinizing hormone (LH) and FSH surge were not consistently detected since only one out eight females (12%) increased LH levels during the transition from the light to the dark phase. In contrast, a high percentage of mice (10 out 12 animals; 83%) exhibited LH and FSH surge at the transition from diestrus to estrus when a single serum sample was collected from the right atrium ($P < 0.0001$ **compared to** diestrus group for both, LH and FSH). All mice that exhibited LH and FSH surge at the transition from the light to the dark phase in the first day of cornification showed increased c-Fos expression in gonadotropin-releasing hormone-expressing (MPO^{GnRH}) neurons (83.4% of co-localization, $P < 0.0001$) and in kisspeptin-expressing neurons of the anteroventral periventricular nucleus ($AVPV^{Kisspeptin}$; 42.3% of co-localization, $P < 0.001$), both **compared to** diestrus group. Noteworthy, out the two animals that were perfused in the first day of cornification but did not have LH surge, one of them showed a small, but evident percentage of MPO^{GnRH} and $AVPV^{Kisspeptin}$ neurons presenting c-Fos (12.5% and 7.7%, respectively). Finally, 96 serial blood samples were hourly collected in eight regular cycling C57BL/6 females to describe the pattern of LH and FSH secretion along the estrous cycle. Curiously, only small elevations in LH and FSH levels were detected at the time expected for the LH surge.

Conclusions: Unlike rats, in some situations, gonad-intact mice may present a subliminal activation of the HPG at the time expected for the LH surge. These findings improve our understanding regarding the pattern of gonadotropin secretion and the activation of central components of the hypothalamic-pituitary-gonadal axis along the estrous cycle of C57BL/6 female mice.

Abstract #35:
Neuroendocrine role of Bisphenol A in controlling hypothalamic pituitary gonadal axis in adult male monkey

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Introduction/Aim:
Bisphenol A (BPA) has been implicated as an endocrine disruptor involved in impairment of reproductive function. BPA exposure during development caused impediment of reproductive axis in rodents. However, in case of higher primates, the data regarding effects of BPA on hypothalamic neuroendocrine activity is scarce. Investigations regarding effect of chronic peripheral BPA exposure on neuroendocrine testicular axis of non-human higher primates will be helpful to translate its negative consequences in humans. Therefore, the effect of chronic administration of BPA on neuroendocrine perturbations by quantifying the variation in gene expression of *GnRH1, KISS1* and *KISS1R*; and immunoexpression of GnRH and KISS1 in medio basal hypothalamus (MBH) with downstream peripheral parameters/consequences were evaluated.

Methods/Results:
Four healthy adult male rhesus monkeys were divided into two groups: BPA treated group (n=2) was exposed to BPA (1mg/kg) twice a day through subcutaneous route and control group (n=2) was injected with vehicle in similar manner. Hemi-hypothalamus was used to observe relative gene expression in both groups while other half of the hypothalamus was processed for fluorescent immunocytochemical detection of GnRH and kisspeptin-like (ir) cell bodies and fibers with specific primary antibodies.
The mean body weight, testicular, epididymis and prostate gland weight along with testicular volume showed no significant difference ($p>0.05$) between BPA treated and control group. Testicular histology showed nonsignificant change in morphometric parameters (seminiferous tubule diameter, epithelial height and lumen diameter), however, some impairment in morphology of epididymis and prostate gland was observed after BPA treatment. Moreover, daily sperm production rate was significantly ($P<0.0005$) decreased in BPA treated group. Non- significant decrease ($P>0.05$) in mean number of GnRH and kisspeptin positive cell bodies and fibers was observed in MBH of BPA treated group. Correspondingly, significant reduction in relative expression of *GnRH1* ($P<0.005$) and *KISS1* ($P<0.0005$) mRNA while increased ($P<0.05$) relative expression in *KISS1R* was observed in BPA treated group.

Conclusions:
Current findings suggest that the chronic dose BPA exposure influences *GnRH1, KISS1* and *KISS1R* mRNA expression levels in non-human primate hypothalamus without affecting histological and morphological parameters of testes. Immuncytochemical data revealed the decrease in number of GnRH and kisspeptin-ir cell bodies and fibers in MBH suggesting the possible effect of BPA in suppressing GnRH and kisspeptin neuronal activity. Further studies, however, are warranted to understand cellular and molecular mechanisms by which BPA disrupts GnRH and kisspeptin pathways in higher primates.

Abstract #37:

Effects of Manipulation of Hypothalamic Arcuate Nucleus Tyrosine Hydroxylase Neurons on Hedonic Food Intake

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Aim: Tyrosine hydroxylase (TH) neurons in the hypothalamic arcuate nucleus (ARC) are involved the regulation of food intake and energy consumption. It is well-known that manipulation of these neurons increases the amount of food consumption in mice. However, how these neurons affect hedonic food intake has not been elucidated. In the present study, we investigated the possible hedonic food intake alterations that may occur in mice as a result of the chemogenetic activation or inhibition of the TH neurons.

Methods: Sixteen female and male transgenic TH-Cre mice were used. For chemogenetic (chronic) manipulations, hM3D receptor (for activation) and hM4D receptor (for inhibition) genes were injected intracranially into the hypothalamus using adeno-associated virus. Chronic stimulation/inhibition was performed by administering Clozapine-n-Oxide intraperitoneally before the experiment. Mice were fed either with standard diet (chow) or high-fat in the experiments and 4-hour food consumption after activation and inhibition of ARC TH neurons was measured. Alterations in the electrical activity of neurons were recorded by using electrophysiology patch clamp technique. Data were analyzed either by One-way ANOVA followed by Tukey's multiple comparison test or Student's t-test. $P<0.05$ was considered statistically significant.

Results: Upon activation of TH neurons, amount of food consumption significantly increased ($p<0.05$), while the inhibition of these neurons did not change the amount of food consumption. The firing frequency of TH neurons significantly increased upon fasting ($p<0.05$). Activation or inhibition of these neurons did not significantly affect the hedonic food intake.

Conclusion: This is the first study investigating the role of TH neurons in the hypothalamic control of hedonic food intake. Our findings suggest that TH neurons do not play a role in the regulation of hedonic food intake as manipulations of TH neurons in the ARC alter the consumption of chow diet, but not that of high-fat diet in the transgenic mice.

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Keywords: Tyrosine Hydroxylase Neurons, Hypothalamus, Arcuate Nucleus, Chemogenetics, Electrophysiology, Food Intake.