Neuroendocrinology

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New in-vivo model and revised concept

In the two following studies from the same group, the authors used an interesting and sophisticated design of genetically modified mice that express diphtheria toxin upon Cre recombination, the expression of which itself is driven by the endogenous regulatory sequences of a target gene. This strategy thereby results in specific ablation of an entire cellular population, like kisspeptin or KISS1R neurons. What is intriguing, and certainly more unexpected, is that instead of observing at least similar or even worse effects than in single gene knockout mice, the authors observed a much milder phenotype, suggesting a greater physiologic plasticity when a cell population is theoretically missing rather than a gene. Overall, these two studies bring very challenging results, which have to be cautiously interpreted and will need further confirmations.

Embryonic gonadotropin-releasing hormone signaling is necessary for maturation of the male reproductive axis

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Proc Natl Acad Sci U S A 2010;107:16372–7.

Background: Gonadotropin-releasing hormone (GnRH) signaling regulates reproductive physiology in mammals by controlling synthesis and secretion of gonadotropins by gonadotrope cells. However, the role of GnRH signaling on embryonic gonadotrope cells development is not well established.

Methods: The authors developed transgenic mice in which GnRHR-expressing cells were selectively ablated by the expression of the diphtheria toxin. They developed another model in which diphtheria toxin gene was replaced by a gene encoding the yellow fluorescent protein (YFP) to study the timing of GnRHR expression.

Results: At embryonic day 16.75, GnRHR is only expressed in luteinizing hormone (LH)-expressing gonadotropes. The maturation of follicle-stimulating hormone (FSH)-expressing gonadotropes requires the increase of embryonic LH secretion. The lack of GnRHR-expressing cells does not alter migration of GnRH neurons toward the hypothalamus, but increases the number of GnRH neurons in the anterior hypothalamus.

Conclusion: Embryonic GnRH signaling is involved in the maturation of gonadotropes cells in males.

The central developmental control of reproduction by GnRH is now well established. The hypothalamo-pituitary-gonadal axis is functional during embryonic development. To study the precise role of GnRH signaling on the embryonic reproductive axis, the authors developed genetic strategies to ablate GnRHR-expressing cells in mice and to follow GnRHR expression with YFP. Their results show that GnRH signaling is required for gonadotrope cell maturation. At E16, only LH-expressing gonadotropes express GnRHR. LH secretion is increased in response to GnRH, inducing the development and maturation of FSH-expressing cells and LH receptor. These cells start to express GnRHR after E17. At E16, FSH cells transiently express thyroid-stimulating hormone (TSH), suggesting that they represent an intermediate gonadotrope cell type expressing TSH whose differentiation depends on GnRH signaling. Ablation of GnRHR cells did not affect migration of GnRH neurons, indicating that this phenomenon is independent of GnRH signaling. However, in mice lacking GnRHR cells, the number of GnRH neurons in the anterior hypothalamus increased by 30%. This observation is not in agreement with previous data showing that GnRH neurons expressed GnRHR and with analyses of mice expressing inactive GnRHR which are not associated with an increase in GnRH neurons. This set of data suggests that the number of GnRH neurons depends on GnRHR-expressing cells, but not on

GnRH signaling. These differences could be due to the model used in the present study in which one cell population is abated.

Female reproductive maturation in the absence of kisspeptin/GPR54 signaling

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Nat Neurosci 2011;14:704–10.

Background: The onset of puberty is triggered by an increase of GnRH secretion from a subpopulation of hypothalamic neurons. This reactivation of GnRH neurosecretion depends on the integration of several activating signals, but the neural network governing the timing of puberty remains largely unknown. The recent discovery of the kisspeptin neuropeptide as the most potent GnRH secretagogue suggests that kisspeptin neurons may be the key regulators of puberty onset.

Results: To test this hypothesis, the authors designed genetically modified female mice in which kisspeptin-expressing neurons are missing, and show that these animals undergo puberty at a normal age. They are also fertile although they have smaller ovaries. The complementary approach, consisting of ablating GPR54 neurons, also resulted in fertile animals. However, when kisspeptin neurons were ablated in adult mice, these became infertile, suggesting the existence of a time window during development when the kisspeptin/GPR54 system can be compensated.

Conclusion: Overall, these data indicate that puberty can be normally triggered and achieved in the absence of kisspeptin/GPR54 signaling.

In this surprising study, the authors show that in animals lacking kisspeptin or GPR54 neurons, puberty undergoes normally and adults are fertile, which is in contrast with knockout animals which are infertile. To explain this intriguing difference, the authors propose that a quiescent compensatory mechanism replaces the missing neural network, thereby driving normal reproductive function and that this mechanism would be under the influence of inhibitory signals coming from kisspeptin or GPR54 neurons. This interesting hypothesis is however challenged by the fact that contrary to knockout approaches, where kisspeptin neurons are present but the gene is not expressed, in the toxin strategy a few percent of kisspeptin or GPR54 neurons remain and could express some levels of a protein. With kisspeptin being by far the most potent GnRH secretagogue, it would not be unlikely that the few remaining cells could be sufficient to trigger puberty and maintain fertility. This hypothesis has to be tested because instead of undermining the importance of the kisspeptin/GPR54 system in puberty onset, it would highlight its extreme potency.

Using an alternative approach, the authors also acutely ablated kisspeptin or GPR54 neurons by injecting diphtheria toxin in adult animals expressing the diphtheria receptor instead of the toxin itself. Ablation of kisspeptin neurons in adults and in 20-day-old juvenile animals resulted in infertile and acyclic animals, suggesting that a compensatory network can only occur during a time-defined window during development. Very interestingly, the authors did not detect any kisspeptin-positive neurons, meaning that the inducible deletion of target neurons is more efficient than the genetic toxin strategy and may account for the loss of fertility in one case and not in the other. However, efficiently ablating GPR54 neurons resulted in a loss of 93% of GnRH neurons but did not totally impair fertility, suggesting that very few GnRH neurons are sufficient to maintain an active gonadotropic axis.

New concept

How estradiol influences pubertal timing

Timing and completion of puberty in female mice depend on estrogen receptor- α signaling in kisspeptin neurons

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Proc Natl Acad Sci U S A 2010;107:22693–8.

Background: Although poorly known, the mechanisms that trigger puberty probably rely on a dynamic of both inhibitory and activating mechanisms of GnRH neurosecretion. These maintain low levels of GnRH during the juvenile period but then increase it in order to achieve puberty and sexual maturation of the brain. Estrogen receptor- α (ER α) is known to participate to both signals, but the underlying neural network is poorly known.

Results: In this study, the authors hypothesized that kisspeptin-expressing neurons are the mediators of ER α signaling toward GnRH neurons. They designed a genetically modified mouse in which ER α expression is conditionally ablated in kisspeptin neurons, and show that it resulted in a dramatically advanced age of puberty onset in females. Although precariously activated, the gonadotropic axis failed to reach full maturation as the animals could not acquire normal ovulatory cyclicity.

Conclusion: The authors propose that two different populations of kisspeptin neurons in the hypothalamus respond to estrogens and are responsible for both the juvenile restraint and the pubertal activation of GnRH neurosecretion.

Estrogen and ER α play a major role in the regulation of the gonadotropic axis but given the fact that GnRH neurons do not express ER α , there had to be interneurons as signaling intermediates. The discovery of kisspeptin as the most potent GnRH secretagogue and the fact that all kisspeptin neurons expressed ER α naturally led to the hypothesis that these interneurons were kisspeptin neurons. Here, the authors give compelling evidence that it actually is the case, at least in females, thereby confirming what was suspected for several years. Surprisingly, onset of puberty and sexual maturation in males was not as dramatically impaired by the selective invalidation of ER α in kisspeptin neurons as in female mice, which may suggest that androgen receptor may compensate for the loss of ER α . The authors confirm what was previously known from studies of gonadectomized animals that kisspeptin neurons are upregulated (positive feedback) by estradiol in the anteroventral periventricular nucleus (AVPV) and downregulated (negative feedback) in the arcuate nucleus (ARC). In the context of puberty, they therefore hypothesize that ARC neurons provide the juvenile restraint on GnRH neurosecretion, which is supplanted by AVPV neurons at the end of the juvenile period. Overall, this study is extremely important because it shows the necessity of ER α expression within kisspeptin neurons for normal puberty onset in females.

Concept revised

Leptin does not act on the reproduction via kisspeptin neurons

Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons

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J Clin Invest 2011;121:355–68.

Background: A minimum amount of stored energy is required for normal pubertal onset. Leptin is a key metabolic signal to the neuroendocrine reproductive axis. Humans and mice lacking leptin or the leptin

receptor (LepR) fail to enter puberty. Leptin administration to leptin-deficient subjects and leptin-deficient mice (ob/ob) induces puberty and restores fertility, but the exact site of leptin action is unclear. Kisspeptins were recently described as the major regulator of GnRH secretion at the pubertal onset.

Methods and Results: In this study, the authors showed that selective invalidation of LepR in hypothalamic kisspeptin neurons in mice had no effect on puberty or fertility. Direct leptin signaling in kisspeptin neurons is therefore not required for these processes. However, bilateral lesions of the ventral premammillary nucleus (PMV) of ob/ob mice blunted the ability of exogenous leptin to induce sexual maturation but did not prevent leptin's effect on food intake. Moreover, unilateral re-expression of LepR in PMV neurons was sufficient to induce puberty and improve fertility in female LepR-null mice.

Conclusion: These data show that leptin does not directly act on kisspeptin neurons. They suggest that the PMV is a key site for leptin's permissive action at the onset of puberty and support the hypothesis that the multiple actions of leptin to control metabolism and reproduction are anatomically dissociated.

This article is interesting as it reports the first molecular study showing that leptin's function on reproduction differs from its function on metabolism. The role of leptin on pubertal onset and reproduction status has largely been described but the exact hypothalamic sites of action of this peripheral hormone were lacking. Since the description of kisspeptins in pubertal onset, leptin was proposed by several groups as a possible candidate to trigger activation of the kisspeptin system at the end of the juvenile period. As the leptin receptor is expressed in kisspeptin neurons, it was justified to test the hypothesis whether leptin receptor invalidation in these neurons interferes with reproduction status and pubertal onset. This paper clearly shows that this is not the case. Moreover, this paper reports that leptin action on the reproduction status is mediated through neurons located in the ventral premammillary nucleus. These PMV neurons do not relay leptin action on the metabolism. Additional work is now needed to determine how leptin-sensitive PMV neurons act on reproduction. The authors also have observed a sexual dimorphism of leptin action on PMV neurons that represents an additional complexity to the understanding of the link between nutrition and reproduction.

New concerns

Brain differentiation and endocrine disruptors

Endocrine disruption of brain sexual differentiation by developmental PCB exposure

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Endocrinology 2011;152:581–94.

Background: Adult reproductive functions critically rely on the sexual differentiation of the hypothalamus, which depends on sexually dimorphic secretion of sex hormones during perinatal development. The authors tested the hypothesis that polychlorinated biphenyl (PCB) exposure during the perinatal period may lead to permanent impairment of the sexual differentiation of the rat preoptic area (POA) through disruption of hormonal pathways.

Results: In order to test this hypothesis, the authors injected 16- or 18-day pregnant rats with several PCB mixtures, estradiol or a vehicle. They then analyzed the expression of known sexual hormones targets in the POA of the adult offspring using immunohistochemistry and a PCR-based expression array. They also monitored somatic and reproductive development of the pups. Overall, their results show that pubertal onset was delayed in PCB-exposed males and advanced in females and led to abnormal estrous cyclicity. Interestingly, PCB exposure induced in females a masculinization of the neuroendocrine system, as shown by expression of kisspeptins and estrogen receptor- α in the sexually dimorphic anteroventral periventricular (AVPV) nucleus. Using a PCR-based array, the authors show that the expression of genes encoding IGF-1, NMDA receptor subunit NR2b, androgen receptor and TGF- β_1 is strongly reduced in the female POA, suggesting a major impact of PCB exposure on sexual maturation of the brain during development of female rats.

Conclusion: PCB exposure during the perinatal period in rats leads to severe and permanent dysregulation of genes involved in puberty onset and estrous cycle in female adults.

PCBs are environmental endocrine-disrupting chemicals which are suspected of causing numerous reproductive impairments. They are present in nearly all humans and can be transferred from the mother to the baby through the placental barrier and milk. This study is important because it brings molecular insight into the dramatic effect of perinatal PCB exposure on female sexual maturation and shows that kisspeptin and ER α expression are impaired in the AVPV. Furthermore, PCB exposure masculinizes the female brain and induces a female-specific decrease of IGF-1, AR, NR2b and TGF- β_1 expression in the POA, suggesting that these genes are of particular importance in the establishment of the neural circuitry that leads to the sexual differentiation of the brain.

New mechanism **Nesfatin-1 and puberty onset**

The anorexigenic neuropeptide, nesfatin-1, is indispensable for normal puberty onset in the female rat

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J Neurosci 2010;30:7783–92.

Background: Nesfatin-1 is a peptide derived from the NEFA/nucleobindin 2 (NUCB2) precursor expressed in the hypothalamus. It was recently identified as an anorexigenic peptide, but other biological functions gated by body energy status remain unexplored. The authors studied the involvement of nesfatin-1 on the control of female puberty.

Methods and Results: ISH and ICC studies indicated that NUCB2/nesfatin mRNA and protein were detected at the hypothalamus of pubertal female rats, with prominent signal at lateral hypothalamus (LHA), paraventricular (PVN), and supraoptic (SON) nuclei. Quantitative analysis showed that expression of NUCB2/nesfatin increased threefold between late-infantile and peripubertal periods. 48 h fasting or sustained subnutrition of pubertal females induced a decrease in the expression of hypothalamic NUCB2 mRNA and/or protein levels. Central administration of nesfatin-1 induced an increase of circulating gonadotropins which was further enhanced upon food deprivation. Inhibition of NUCB2 in the hypothalamus by continuous intracerebroventricular infusion of antisense morpholino oligonucleotides along pubertal maturation was associated with a delay of vaginal opening and a decrease of ovarian weight and serum luteinizing hormone levels. In contrast, suppression of hypothalamic NUCB2 in adult female rats had no effect on gonadotropins levels and did not alter preovulatory gonadotropin surges.

Conclusion: NUCB2/nesfatin-1 plays a role in the control of puberty in female rat, a function that may contribute to the functional coupling between reproduction and energy homeostasis.

The link between energy balance and reproduction is crucial. In negative energy balance, reproduction function is aborted, but the mechanisms coupling energy balance and reproduction remain unclear. This study reports the effect of the anorexigenic peptide NUCB2/nesfatin-1 on the onset of puberty in female rat. The authors provide a neuroanatomical characterization of NUCB2/nesfatin-1 neurons distribution in the hypothalamus at puberty. Two populations of NUCB2/nesfatin-1 neurons seem to be present and have a different sensibility to development and metabolic cues. However, no mature nesfatin-1 peptide has been detected in the hypothalamus.

The role of nesfatin-1 on puberty was studied by pharmacological studies and in-vivo gene invalidation. This peptide stimulates gonadotropin release, with an enhanced effect in negative energy balance conditions. It is also involved in the timing of puberty but has no effect in adult female rat. One surprising observation is that suppression of nesfatin-1 in the hypothalamus does not affect food intake and body weight in peripubertal and adult female rats. Several hypotheses could explain this

result. First, redundant mechanisms of energy balance control could occur during puberty. Second, the anorexigenic effect of nesfatin-1 has been demonstrated in male rats, suggesting a possible sexual dimorphism of nesfatin-1 action. Third hypothesis, the decrease of hypothalamic nesfatin-1 expression by antisense morpholino oligonucleotides is not sufficient to abolish the anorexigenic effect of this peptide or another unknown source of peptide exists. Fourth, the hypothalamic sites of nesfatin-1 action differ between reproduction and regulation of food intake. This study is the first to highlight the role of the anorexigenic peptide nesfatin-1 on the onset of puberty. It will be interesting to investigate the molecular mechanisms of nesfatin-1 action in order to have a better knowledge of the link between reproduction and energy balance.

New concept

How maternal care influences estradiol receptor function in adulthood

Sex differences in epigenetic regulation of the estrogen receptor- α promoter within the developing preoptic area

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Endocrinology 2010;151:2297–305.

Background: Sexual dimorphism of the central nervous system is mainly organized by a postnatal testicular hormone surge that is absent in females. Although very brief, this hormone surge induces permanent changes in terms of sexual behavior and brain sexual differences. Because these largely depend on the brain-derived testosterone metabolite estradiol, which acts via the receptor ER α , the authors have investigated the hypothesis that a difference in DNA methylation of the ER α gene promoter region exists between male and female mice.

Results: The authors show that ER α promoter methylation is higher in males than females in the preoptic area of the hypothalamus, thereby decreasing transcription of the ER α gene. Furthermore, they found that simulating grooming of the pups' anogenital region by the mother resulted in an increase of ER α promoter methylation in females to reach the same level as in males. Finally, the authors show that females perinatally treated with estradiol have a male-like methylation pattern of the ER α gene promoter.

Conclusion: The authors show that the methylation level of the ER α gene promoter is sexually dimorphic and influenced by both the perinatal surge of male sexual hormone and maternal grooming.

Growing evidence is emerging from the literature that the sexual differentiation of the brain results, at least in part, from epigenetic modifications driven by environmental and social factors. Interestingly, this study strongly suggests that normal differences in maternal care, such as grooming of the anogenital region, can induce extremely tight and precise molecular modification of certain CpG sites in the ER α gene promoter in the preoptic area of the hypothalamus (although the authors did not examine methylation levels in other brain regions). The perinatal surge of estradiol has a lasting impact on the brain that will drive sexual behaviors but the underlying molecular mechanisms are unclear. Part of this effect, as shown by the authors, may result from a sexually dimorphic methylation pattern of CpG sites of the ER α gene promoter region in the preoptic area of the hypothalamus that leads to local transcriptional variations. The molecular link between a somatosensory stimulus and the methylation of a very specific genomic region remains unknown, but overall, evidences that such early social interactions as well as early estradiol exposure can influence the methylation of ER α promoter gives a better understanding on how variations in maternal care can have permanent effects on the sexual behavior during adulthood. Entire methylome studies, which are becoming more and more available, will certainly help to get further insight on the fascinating influence of the environment on sexual behaviors.

Pituitary growth hormone network responses are sexually dimorphic and regulated by gonadal steroids in adulthood

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Proc Natl Acad Sci U S A 2010;107:21878–83.

Background: Sex-specific gonadal steroid imprinting generates sex differences in many pituitary endocrine axes, notably the somatotrophic axis. The authors have previously shown that GH cells form functionally organized networks in the pituitary. In this study, they tested the hypothesis that the response of the GH pituitary network is sexually dimorphic.

Methods: Calcium spikes were monitored within GH cell networks in pituitary slices taken from 45-day-old male and female GH-eGFP transgenic mice after a GH-releasing hormone (GHRH) stimulus. These mice were sham-operated, gonadectomized or gonadectomized and treated with acute gonadal replacement. Experiments were carried out 15 days after surgery.

Results: GH cell network responses were sexually dimorphic: cell responses were higher in male mice. This sex difference was switched by postpubertal gonadectomy and reversed by hormone treatment.

Conclusion: The functional organization of the pituitary gland is involved in the sexually dimorphic responses in the GH axis and may explain some growth and metabolism differences between males and females.

GHRH exposure generates calcium spikes in the lateral wings of the pituitary, with significant differences in the proportion of GH-responsive cells between males (60%) and females (35%) in pituitary slices taken from male and female GH-eGFP transgenic mice. Repetitive waves of calcium spikes were observed in male pituitary slices whereas no waves were observed in female pituitary slices. These rhythmic calcium waves may influence a wide range of downstream processes. The authors did not observe any sexual dimorphism of the GHRH response when they tested isolated GH cells. They conclude that the difference in GHRH response between males and females is a property of the GH cell network in the pituitary.

The effects of these networks on GHRH responses seem to be regulated by gonadal steroid hormones in both adult females and males. After gonadectomy, steroid supplementation restored the proportion of GHRH-responsive cells back to what was seen in normal males and females. However, gonadectomy abolished the waves of activity in males after GHRH exposure and testosterone treatment did not restore it. Another interesting result is that masculinization of ovariectomized females increased to 60% the number of GHRH-responsive GH cells (instead of 80% in non-treated ovariectomized females), similar to the findings in intact males and generated repetitive waves of calcium spikes in lateral wings of the pituitary. Studies generally show that exposure to steroids during critical periods exert organizational effects on brain circuits. During the perinatal period, steroids permanently imprint a specific neuronal architecture that will drive behavioral and somatic traits. Gonadal steroid regulation of GH secretion has been largely attributed to their effect on the hypothalamic pulse generator: this study indicates that steroids also regulate GH response to GHRH at the pituitary level. The sexually dimorphic GH secretion observed in humans at puberty could therefore be related to hypothalamic and pituitary signals via steroid regulation.

Melanocortin signaling in the CNS directly regulates circulating cholesterol

Perez-Tilve D, Hofmann SM, Basford J, Nogueiras R, Pfluger PT, Patterson JT, Grant E, Wilson-Perez HE, Granholm NA, Arnold M, Trevaskis JL, Butler AA, Davidson WS, Woods SC, Benoit SC, Sleeman MW, DiMarchi RD, Hui DY, Tschöp MH

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Nat Neurosci 2010;13:877–82.

Background: Metabolic syndrome comprises obesity, hypertension, impaired glucose metabolism and dyslipidemia. The gut hormone ghrelin has been shown to increase adiposity and to promote hyperglycemia via brain circuits, the hypothalamic melanocortin system being the most important target. Melanocortin receptor mutations are strongly associated to human obesity and alterations in cholesterol transport are a common occurrence in the metabolic syndrome. In this study, the authors tested the hypothesis that a gut-brain axis regulates cholesterol metabolism via the central melanocortin system.

Methods and Results: Inhibition of the brain's melanocortin system by pharmacological, endocrine or genetic approaches reduced HDL uptake by the liver, resulting in an increase in circulating HDL cholesterol.

Conclusion: These data suggest that a neural circuit in the brain is directly involved in the control of cholesterol metabolism by the liver.

It is now well established that the gut hormone ghrelin is involved in the molecular energy balance regulation. This study indicates that a gut-brain axis including ghrelin and the melanocortin system directly regulates the HDL cholesterol level in rodents. The authors showed that the inhibition of the melanocortin system by antagonist treatment induced an increase of circulating HDL cholesterol associated with a decrease of its uptake by the liver. Peripheral administration of ghrelin had the same effect through an indirect inhibition of the melanocortin system. However, treatment with melanocortin receptor agonist or glucagon-like peptide 1 (GLP-1) had an opposite effect on circulating the HDL cholesterol level. This effect is mediated via melanocortin-4 receptor (MC4R) signaling and the vagal pathway, suggesting the involvement of the dorsal motor nucleus of the vagus and the lateral hypothalamus. The lack of MC4R signaling induces severe obesity, but is also associated with a decrease of HDL cholesterol uptake by the liver, leading to an increase of 'good' cholesterol. This paradox could be related to the fact that unlike in humans, in rodents the majority of circulating cholesterol is contained in HDL. The hypothalamic melanocortin system, which regulates the circulating cholesterol level, represents a new potent therapeutic target to treat hypercholesterolemia and metabolic syndrome.

Inflammation of the hypothalamus leads to defective pancreatic islet function

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J Biol Chem 2011;286:12870–80.

Background: The hypothalamus becomes inflamed and dysfunctional under diet-induced obesity and this could lead to type 2 diabetes. Because pancreatic function is partially regulated by the autonomic nervous system, the authors evaluated the role of hypothalamic inflammation in pancreatic islet function.

Methods: Intracerebroventricular injection of a low dose of tumor necrosis factor- α (TNF- α) was performed in two groups (control vs. 12-week high-fat diet) of 4-week-old rats. Alternatively, lean animals received stearic acid through intracerebroventricular injection, which activates inflammation through the tau-like receptor 4 and endoplasmic reticulum stress.

Results: TNF- α injection led to an increase of insulin secretion, whereas stearic acid produced an impairment of insulin secretion. Both TNF- α and stearic acid induced an increase of apoptosis marker expression in pancreatic islets. Insulin secretion after stearic acid injection was restored upon sympathectomy

or inhibition by a specific antisense oligonucleotide of the peroxisome proliferator-activated receptor- γ coactivator-1, PGC-1 α .

Conclusion: The present study provides evidence that the autonomic signals associated with hypothalamic inflammation induced by either TNF- α or a saturated fatty acid can impair pancreatic islet function. This phenomenon may explain the link between obesity and defective insulin secretion.

In animal models of obesity, the hypothalamus is one of the first affected tissues. Inflammation of the hypothalamus leads to its dysfunction associated with the development of leptin and insulin resistance as well as loss of coupling between energy expenditure and caloric intake. Insulinemia rises in proportion to the body mass. Then, with the appearance of type 2 diabetes onset, insulin production and secretion decline. The single injection of TNF- α in lean rats induces hypothalamic inflammation and an insulin-resistant phenotype. These functional changes observed in pancreatic islets and those observed in gene expression after intracerebroventricular TNF- α injection are similar to the ones found in obesity. Other studies have reported that some of the TNF- α upregulated genes are, at least in part, engaged in defective pancreatic islet function.

The authors suggest that the importance of sympathetic signals on the modulation of the pancreatic β -cell function could be a way to modulate pancreatic β -cell function via the classical cAMP or the alternative PGC-1 α pathways. For the first time, this study shows that inflamed dysfunctional hypothalamic tissues are connected with the pancreatic islets and are responsible for apoptotic gene activation and peripheral insulin resistance, notably via the sympathetic pathway. Considering this new mechanism, new therapeutic targets could eventually be developed to treat obesity and prevent the onset of diabetes.

New concept **Adolescents are more plastic to stress than adults**

Stress vulnerability during adolescent development in rats

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Background: Adolescence is a period of continued growth, sexual maturation, and behavioral and social development. The authors of this study therefore tested the hypothesis that adolescent development represents a time of increased susceptibility to stress.

Method: Male Sprague-Dawley rats were exposed (or not) to 14 days of chronic variable stress (CVS) paradigm at three different stages of life: early adolescence (35 days), late adolescence (50 days) and adulthood (80 days). Several items were analyzed to assess stress influence: depression-like behavior, somatic indices, hypothalamic-pituitary-adrenal axis activity, and hypothalamic neuropeptides expression.

Results: Increased immobility during the forced swim test, which is considered a depression-like behavior, was only observed in adult animals in response to CVS. Late adolescent animals however were selectively vulnerable to the somatic effects of stress, depicted by a disproportionate increase of corticosterone, thymic involution, a loss of body fat, and a decreased expression of oxytocin, a stress-protective peptide, in the paraventricular nucleus.

Conclusion: Despite the enhanced susceptibility to somatic and central effects of chronic stress, late adolescence represents a time period with unique protection from the effect of chronic stress on depression-like behavior.

The authors propose that adolescence is a window of unique vulnerabilities and protections to the effects of chronic stress. Previous studies showed that basal stress hormones do not change during puberty. However, adolescence is a period of intense brain maturation but has received less research attention than pre- and postnatal or adult life. The authors studied the effect of chronic stress on early/

late adolescent rats and adult rats. Although they observed somatic changes like increases in basal corticosterone, thymic involution, and loss of body fat in late-adolescent animals exposed to chronic stress, these animals were more resistant than adults to the effect of CVS on depression-like behavior. The mechanisms of this protection remain unknown. According to other studies, corticotrophin-releasing hormone (CRH) is also stress-sensitive, but no age differences of CRH hypothalamic expression were observed. On the contrary, vasopressin and oxytocin hypothalamic expression increased between early and late adolescence, suggesting an important role of these hormones. CVS during late adolescence prevents the normal increase of oxytocin, which is an important mediator of social behavior. This study shows the necessity to be more attentive to the possible link between unexplained somatic changes at the end of adolescence and a possible stress, considering that stress has major implications for health.

New mechanism **The importance of the perinatal photoperiod**

Perinatal photoperiod imprints the circadian clock

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Nat Neurosci 2011;14:25–7.

Background: It is well known that seasonal light input largely influences the activity of the mature biological clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. However, the impact of seasonal photoperiods during development on mature circadian clock remains largely unknown. In this study, the authors sought to analyze whether the perinatal photoperiod has a lasting effect on adult mouse circadian rhythms and behavior.

Methods and Results: The authors compared three groups of mice exposed to different seasonal developmental photoperiods: short day light/dark (LD) 8:16, long day, 16:8 or equinox day, 12:12 until weaning followed by 4 weeks of matching or counterbalanced continuation seasonal photoperiod. At 7 weeks of age, they analyzed SCN circadian clock by ex-vivo imaging or recorded wheel-running behavior in constant darkness. Their results show that in response to the continuation photoperiod, long days elicited SCN waveform broadening whereas short days elicited an increased degree of neuronal synchrony. When results were analyzed regardless of the ensuing continuation photoperiod, it appeared that SCN and neuron properties were solely determined by the perinatal photoperiod. The developmental imprinting was also reflected in circadian behavior.

Conclusion: The perinatal photoperiod has lasting effects on the circadian rhythms expressed by clock neurons as well as on mouse behavior, and sets the responsiveness of the biological clock to subsequent changes in photoperiod. These developmental gene/environment interactions may contribute to the influence of season on neurobehavioral disorders.

The circadian clock in mammals is located in the suprachiasmatic nucleus of the hypothalamus; it entrains a light-dark cycle and determines the phases of circadian rhythms onto behavior and physiology. The SCN consists of a number of oscillating cells in the circadian domain, which are independent but coupled to each other to produce coherent SCN output rhythms. The intracellular molecular machinery of circadian oscillation consists of interlocked transcriptional and translational auto-feed-back loops. It was well known that circadian rhythms in clock gene expression and their protein products change between long and short photoperiods. Differences in circadian activity of the SCN result in a change of the 24-hour hormonal profiles as well as behavior rhythms. The new finding reported in this paper is that the imprinting of the SCN circadian activity by perinatal photoperiods persists in mature mice. The molecular mechanism by which photoperiodic imprinting occurs and permanently modifies clock neuron activity is completely unknown. It may result from epigenetic effects of light on clock gene expression in the SCN. The medical application of such fundamental research is quite large and may be important to understand the link between perinatal environment and some psychiatric diseases. It may also be important for medical care and optimal environment of preterm babies in our NICU.

Human tears contain a chemosignal

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Background: Although tearing has been described as an eye protection or a mechanism for expelling toxic substances, the functional significance of emotional tearing remains unknown. This behavior is uniquely considered in humans. Tears are drops of liquid which contain proteins, lipids, electrolytes and traces of drugs, but the composition of emotional tears differs from that of reflexive eye-protective tears. In this paper, the authors decided to test whether emotional tears may also serve as a chemical signal.

Methods and Results: To analyze whether tears contain a chemical signal, the authors set up a clinical assay by collecting negative-emotion tears from adult women who watched sad films in isolation. They then exposed tears in different ways to young adult men. In the first test, they showed the absence of discernable odor in emotional tears. Then they tested whether tears contained a signal indicating sadness or information related to sociosexual behavior by viewing on screen ambiguous pictures of women's faces. This second test demonstrated that tears may contain a chemical signal decreasing the sexual attraction and arousal when compared to saline control. This decrease is correlated with a decrease of salivary testosterone levels in men sniffing tears when compared to saline. Finally, the authors showed by functional resonance imaging that sniffing tears significantly decreased the activity normally provoked by a sad film in the hypothalamus.

Conclusion: Emotional tears are odorless but contain a chemosignal which induces reductions in sexual arousal.

Emotional tears are specific to humans. In contrast to reflexive eye-protective tears, emotional tears are considered as an emotional signal but no specific function has been reported. In this paper, the authors report that emotional tears from sad women convey a chemosignal which seems to have an emotional impact on men sniffing those tears. Their main result is the reduced self-rated sexual arousal of men associated with reduced levels of salivary testosterone and reduced brain activity in specific regions implicated in sexual arousal such as the hypothalamus. This work is an example of how emotions can be transmitted through a chemical signal between two individuals close enough to each other. It also is an additional example of how the hypothalamus may relay emotions to the hormonal system. The active compound involved in this emotional effect remains to be described and it will be interesting to compare this chemical signal with the one conveying the emotions of 'tears of joy'.