Differential orderliness of the GH release process in castrate male and female rats

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Gevers, Evelien, Steve M. Pincus, Iain C. A. F. Robinson, and Johannes D. Veldhuis. Differential orderliness of the GH release process in castrate male and female rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R437–R444, 1998.—Male- and female-specific modes of episodic growth hormone (GH) release are presumptively imposed by sex steroid hormones, and, although typically evident visually, are vividly distinguished quantitatively via a regularity statistic, approximate entropy (ApEn), in both the rat and human. GH secretory patterns may determine GH-stimulated growth and specific hepatic and muscle gene expression in the rat. Consequently, it is important to discern mechanisms that underlie their regulation. Here we have examined the impact of prepubertal gonadal suppression (at 4 wk of age) via surgical or pharmacological (gonadotropin-releasing hormone [GnRH] agonist) intervention on the regularity (ApEn) of GH release in male and female rats (at 10–11 wk of age) sampled at 10-min intervals for 10 h (n = 60 points) during a lights-out (dark) period. We observed a graded hierarchy of mean disorderliness of GH release that was quantifiable by ApEn measures, with maximal to minimal disorderliness in the following rank order: intact female, GnRH agonist-treated female, ovariectomized female, orchidectomized male, GnRH agonist-treated male, and intact male. These observations suggest a continuum of sex steroid actions on the regularity of GH secretion and, by inference, on the interplay among GH-releasing hormone, somatostatin, and GH/insulin-like growth hormone I negative feedback.

entropy; rodent; pituitary; growth hormone; sex

UNDER PHYSIOLOGICAL conditions, growth hormone (GH) secretion in the human and experimental animals is episodic or pulsatile and sexually dimorphic, i.e., the pattern is influenced by gender and/or sex steroids (11, 33, 57, 70). Indeed, in the rodent, body growth and malelike versus female-specific gene expression in the liver are governed by the particular temporal mode of GH release (15, 68, 69, 71). For example, male rats have low interpeak GH secretion and distinct high-amplitude GH pulses occurring approximately every 3–3.3 h (14, 18, 61). This male GH pulse pattern is highly effective in inducing muscle insulin-like growth factor I (IGF-I) gene expression and body growth as well as certain hepatic reducing and steroid hydroxylating enzymes (15, 16, 56, 68). Females, on the other hand, have higher baseline serum GH concentrations and apparently more frequent, less visually conspicuous, and seemingly less regular GH pulse episodes (12). The female GH release pattern of nearly continuous secretion is more effective in inducing hepatic GH, epidermal growth factor, and low-density lipoprotein-receptor gene expression, as well as corticosteroid binding globulin and hepatic sulfatase (19, 20, 25, 26, 56, 68). Gender differences in mean serum GH concentrations and in the pulsatile pattern of GH release are also evident in humans (24, 44, 65).

Although one of the visual differences suggested between male and female rat GH profiles is the reduced apparent orderliness of GH secretion in the female, this intuitive characterization of hormone release profiles cannot be defined readily or completely by conventional methods of neurohormone pulse analysis. To elucidate distinct characteristics of hormone secretory dynamics further, we consider approximate entropy (ApEn), described below, a measure of serial irregularity that has yielded informative insights in both theoretical mathematical (39, 40, 47, 50) and biological settings, (e.g., Refs. 23, 48, 51, 64). In particular, ApEn evaluates both dominant and subordinate patterns in complex data; notably, pertinent to the above investigation of gender distinctions, this regularity measure will detect changes in underlying episodic behavior not reflected in the mean or variance of hormone concentrations or in peak occurrences or amplitudes (48).

We have recently shown via ApEn that females secrete GH consistently and, highly significantly, more irregularly than males, both in intact rats and humans (44). This strong gender contrast presumably reflects the ability of gonadal steroids to modulate the time structure of growth hormone (GH) secretion on a variety of both peak (pulsatile) and subordinate (nonpulsatile) levels of secretory activity. Sex steroids influence expression of the primary hypothalamic peptidic regulators of GH secretion, GH-releasing hormone (GHRH), and somatostatin (1, 28, 33, 36, 38, 70) and also modulate GH negative feedback (6). However, the role of sex steroids in defining and maintaining these gender distinctions in patterns of GH secretion is poorly understood. In the rat, both perinatal sex steroid imprinting and continued steroid exposure in adulthood are important in endowing the sexual dimorphism within the GH axis (27–29). Theoretically, as described below, ApEn changes can often be inferentially related to significant changes in within-axis regulatory interactions controlling neurohormone secretion. Such interactive changes cannot otherwise be noted and quantified readily in vivo without disrupting physiological linkages and thereby disturbing the interaction (41, 42).
Given the above perspective, we here investigate whether and the extent to which the orderliness of the GH release process is governed by prepubertal gonadal steroids. To this end, we have analyzed extended serum GH concentration profiles from intact and gonadally suppressed male and female rats. We employed both surgical and chemical (GHRH analog) castration in the two sexes to assess possible gradations in the orderliness of GH secretory activity and to define whether gender differences persist and are of similar magnitude in the partial versus complete absence of gonadal sex steroid secretion. The effects of these manipulations on growth and other GH-dependent parameters have been reported fully elsewhere (22).

MATERIALS AND METHODS

Animal protocols. Male and female rats from the AS strain bred at the National Institute of Medical Research (London, UK) were used. Rats were housed in a light and temperature-controlled room (23–25°C). To analyze the effect of gonadal steroids on the regularity of GH secretion, rats were ovarioctomized (n = 7) or orchidectomized (n = 6) at an age of 4 wk under O₂-N₂O-halothane anesthesia, whereas control rats were sham-operated (n = 23 for females and n = 14 for males). Other groups (n = 7 each) of male and female rats received treatment with triptorelin, a long-acting GnRH-analog (1 mg/kg sc every 3 wk) (Decapeptyl, Ferring, Malmo, Sweden), also beginning at 4 wk of age. This treatment resulted in selective suppression of the hypothalamic-gonadal axis with six- and eightfold reduction in serum testosterone and estradiol in the male and female animals, respectively (22). At the age of 10–11 wk, the animals were implanted with a cannula inserted into the jugular vein under O₂-N₂O-halothane anesthesia, as previously described (14, 22). Cannulas were kept patent by twice daily flushing with heparinized saline (20 U/ml). After 2 days of recovery, rats were connected to an automatic computer-controlled microsampling system. Rats not eating well were not studied further. Herein, we analyzed blood samples taken every 10 min during a 10-h dark period (n = 60 samples).

Assay. GH was measured in whole blood samples (i.e., 20 µl blood in a total volume of 100 µl heparinized saline), using the rat GH radioimmunoassay kit provided by the National Institute of Diabetes and Digestive and Kidney Diseases (National Institute of Health, Bethesda, MD), as reported earlier (14).

ApEn calculations. To quantify irregularity, we use ApEn, a model-independent statistic defined in Ref. 39, with further mathematical properties given in Refs. 40, 46, 47, and 50. In endocrine studies, ApEn has shown vivid distinctions (P < 10⁻¹⁰, nearly 100% sensitivity and specificity in each study) between normal and tumor-bearing subjects for GH (23), ACTH and cortisol (64), and aldosterone (54) release, with the tumors secreting markedly more irregularly. In addition, ApEn unmask a positive correlation between advancing age and greater irregularity of 1) GH (66) and 2) LH and testosterone (49) release.

ApEn assigns a single nonnegative number to a time series, with larger values corresponding to a greater apparent process randomness (serial irregularity) and smaller values corresponding to more instances of recognizable patterns or features in the data. Two input parameters, m and r, must be specified to compute ApEn. Briefly, ApEn measures the logarithmic likelihood that runs of patterns that are close (within r) for m contiguous observations remain close (within the same tolerance width r) on the next incremental comparisons; the formal mathematical definition is given in Ref. 39.

For this study, we calculated ApEn values for all data sets, m = 1 and r = 20% of the standard deviation (SD) of the individual subject time series. Normalizing r to each time series SD gives ApEn a translation and scale invariance to absolute serum concentration levels (43). ApEn is a relative measure of process regularity and can show significant variations in its absolute numerical value with changing background noise characteristics. Because ApEn generally increases with increasing process noise (and increasing intra-assay variation), it is appropriate to compare data sets with similar assay CVs, as we do here. Also, importantly, despite algorithmic similarities, ApEn(m,r) is not intended as an approximate value of the Kolmogorov-Sinai entropy (39, 46). It is essential to consider ApEn(m,r) as a family of parameters; comparisons are intended with fixed m and r.

Previous studies that included both theoretical analysis (39, 45, 46) and biological-clinical applications, such as the aforementioned endocrine studies, have demonstrated that the input parameters indicated above produce good statistical reproducibility for ApEn for time series of the lengths considered here (n = 60 data points). The ApEn application with m = 1 estimates the rate of entropy for a first-order (m = 1)-approximating Markov Chain to the underlying true process (40). Further technical discussion of mathematical and statistical properties of ApEn, including robustness to noise and artifacts, mesh interplay, relative consistency of m,r pair choices, asymptotic normality under general assumptions, statistical bias, and error estimation for general processes can be found elsewhere (45, 46). To develop a more intuitive, physiological understanding of the ApEn definition, a multistep description of its typical algorithmic implementation, with figures, is also developed in Ref. 45.

Statistics. All results shown are group means ± SE. Two-sided Student’s t-tests with unknown unequal variances and analysis of variance followed by Student-Newman-Keuls tests were used to compare means of two groups or three or more groups, respectively. We considered P < 0.05 as statistically significant.

RESULTS

Figure 1 shows group mean ± SE ApEn values for 10-h serum GH concentration profiles in both sexes obtained in intact animals during GnRH analog (triptorelin) treatment and following gonadectomy. Figure 2 illustrates representative serum GH concentration profiles during the dark from an intact male, a triptorelin-treated male, a gonadectomized male, an ovarioectomized female, a triptorelin-treated female, and an intact female. Figure 3 depicts scatterplots of individual ApEn values versus mean serum GH concentrations (Fig. 3A for intact males and female rats and Fig. 3B for surgically gonadectomized or pharmacologically neutered male and female rats).

ApEn was significantly higher, thus GH secretion more irregular, in intact female than intact male rats (P = 0.18 × 10⁻⁶), confirming earlier findings (44). There was an apparently graded variation in mean GH ApEn values, with maximal ApEn in intact females followed by progressively lower ApEn in ovarioectomized females, gonadectomized males, and intact males (Fig. 1). The mean GH ApEn of ovarioectomized females was significantly lower than that of normal females (P = 0.014), whereas gonadectomized male rats had slightly
but not significantly increased ApEn values compared with normal males. GH secretion was significantly more regular (lower ApEn) in gonadectomized males than in ovariectomized females ($P < 0.027$).

Treatment with triptorelin to reduce sex steroid levels produced less statistically vivid alterations compared with gonadectomy in the regularity of GH release. ApEn in triptorelin-treated males was similar statistically to that in gonadectomized males and not significantly different from that in intact males. GH ApEn in triptorelin-treated females was intermediate between values for intact and ovariectomized females, but not significantly different from that in either group.

Because there was no statistically significant difference in GH ApEn between surgically and chemically neutered rats for either sex, we pooled these two groups by sex. ApEn was highly significantly different between the pooled groups of neutered females [$\text{ApEn} = 1.090 \pm 0.035$ ($n = 14$)] and neutered males [$\text{ApEn} = 0.855 \pm 0.033$ ($n = 13$) ($P = 0.64 \times 10^{-2}$)]. This is of further note, because these secretory profiles are visually less distinct from one another than are the corresponding comparisons of intact male and female rats (Fig. 2).

As shown in scatterplots of ApEn versus mean serum GH concentrations in Fig. 3A, ApEn almost completely separated intact males and females. Importantly, in gonadectomized (both chemically and surgically) males and females, gender separation by ApEn remained quite pronounced: 10 of 13 neutered males had lower ApEn values for their GH secretory dynamics than any neutered female value (Fig. 3B).

![Fig. 1. Impact of surgical gonadectomy (Ovx or Gnx) and gonadotropin-releasing hormone (GnRH) analog triptorelin (Tripto)-induced downregulation of the gonadal axis in male and female (Fem) rats on the orderliness of growth hormone (GH) release profiles. Rats were submitted to 10 h of repeated (every 10 min) blood sampling in a dark period to define GH release patterns. Approximate entropy (ApEn) was used as a validated regularity statistic to quantify the orderliness of the GH release process. Higher ApEn denotes more irregularity. Data are means ± SE. $P$ values $< 0.05$ (*) and $0.01$ (**).

![Fig. 2. Representative serum GH concentration profiles from (in ascending order of ApEn values and hence increasing irregularity or disorderliness) an intact male (A), triptorelin-treated male (B), gonadectomized male (C), ovariectomized female (D), triptorelin-treated female (E), and intact female (F) rat sampled for 10 h in the dark. Serum GH concentrations (ng/ml) are standardized via National Institute of Diabetes and Digestive and Kidney Diseases rat pituitary GH-2 standard.](http://ajpregu.physiology.org/)}
to go beyond these first-order findings to evaluate whether and how gonadal steroids affect the regularity of GH release. Specifically, the present experiments employed both surgical and pharmacological castration of male and female rats to elucidate whether degrees of sex steroid hormone removal have a graded impact on the mean quantifiable regularity of GH release. Indeed, we now report an approximate hierarchy from maximum to minimum disorderliness (mean ApEn) of GH release as follows: intact females, GnRH analog-treated females, ovariectomized females, orchidectomized males, GnRH analog-treated males, and intact males. These observations suggest that 1) gonadal sex steroids have a major role in determining gender differences in the regularity of GH secretion; 2) the aforementioned incremental hierarchy of disorderliness to orderliness of GH release presumptively reflects a graded balance of estrogen-androgen actions on the GHRH-somatostatin-GH-IGF-I axis; and 3) ApEn detects gender differences in the orderliness of GH secretion that persist into adulthood, despite prepubertal gonadectomy or gonadal suppression. It is experimentally and statistically notable that \( n = 60 \) GH measurements per rat were sufficient to appreciate the above distinctions.

Elsewhere, we have analytically linked ApEn to mechanistic understanding via mathematical network analysis for general classes of networks (41, 48). Specifically, we demonstrated that ApEn changes with altered system coupling and/or varied external influences, hence providing an explicit barometer of autonomy in many coupled, complicated systems, including, for example, autoregressive moving average and graded superposition models and coupled stochastic differential equations. On the basis of that analysis, we suggest that the increased GH irregularity in the female indicates a more complex network directing GH regulation, either in the number of critical interacting factors and/or in the intensity of particular interactions. GH secretion is controlled by both a hypothalamic GHRH and an inhibiting peptide, somatostatin [somatostatin release-inhibiting factor (SRIF)], the molecular expression of both of which shows ultradian oscillations (75). The interplay between GHRH and SRIF is important in directing the episodicity of GH secretion (60), wherein, in the simplest schema, GHRH seems to be responsible for distinct GH pulses, but the ability of somatotrophs to respond to GHRH depends on the immediately prior and prevailing SRIF tone (17, 57). Higher SRIF concentrations render pituitary somatotrophs less immediately responsive to GHRH, but sensitize somatotrophs to subsequent GHRH stimuli (62). Indeed, in vivo in the rat, a GH pulse may be accompanied by a decrease in SRIF release and an increase in GHRH secretion (52); in sheep, a GHRH stimulus seems to be the primary antecedent event for a GH pulse (21). In the male rat, approximately every 3- to 3.3-h cyclic hypothalamic SRIF withdrawal appears to contribute to the rhythmicity of GH release, in part by allowing reciprocal GHRH release and by also sensitizing somatotrophs to GHRH (10, 62). These features of SRIF dynamics likely contrib-

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**DISCUSSION**

Quantifying the oftentimes visually subtle changes in the orderliness of neurohormone release patterns and the inferred complexity of feedback control of neuroendocrine axes in vivo has been very difficult to date. Nonetheless, for the GH axis, clear determinations of differences in GH release patterns (or temporal differences in exogenous GH treatment schedules) are very important to elucidate mechanisms of GH actions, because distinct GH release patterns evoke remarkably different growth rates and genomic responses (15, 16, 26) and activate separate signal transduction molecules and/or pathways (56, 68, 69). Therefore, quantifying GH secretory regularity and inferentially gaining insights into the physiological mechanisms that control the orderliness of GH release are important to our overall understanding of GH-regulated growth and GH pattern-dependent differential gene expression.

A recent study showed that ApEn, a regularity statistic, remarkably discriminates between male and female GH secretion patterns with an almost complete gender segmentation (44). This finding is confirmed in the current analyses (see scatterplots of ApEn vs. mean GH levels in Fig. 3A). In contrast to substantial overlap in intact male/female mean serum GH concentrations, there is highly significant male/female separation by ApEn. The primary objective of the present study was...
ute to a more regular GH release pattern in the male rodent. Moreover, secreted GH feeds back negatively on its own release after a time delay via decreasing hypothalamic GHRH and/or increasing SRIF release. This so-called autonegative feedback also occurs in a sexually dimorphic manner (6, 8, 13, 17, 30, 31, 35, 53). Such in vivo feedback relationships presumably collaborate to organize pulsatile GH secretion in the rat (2, 55, 58, 63, 75). Although gender differences in hypophysial portal blood SRIF and GHRH concentration profiles over time have not been studied directly, available indirect data suggest that reduced SRIF release and/or action in females (59) would allow more nearly uniform or continuous pituitary responsiveness to available GHRH (2, 38). Other investigations also suggest greater hypothalamic SRIF release and/or activity in males than females (9, 17, 32, 33, 70). As these findings would predict, female rats maintain GH release in response to repetitive GHRH stimuli, whereas male rats show a refractory period for 3 h after a GHRH pulse (6). Our observations using ApEn to estimate overall complexity/integration of GHRH-SRIF-GH feedback in vivo suggest that both castration and pharmacologically induced sex steroid withdrawal alter significantly and in a graded manner the orderliness of the GH release process and hence the strength of GHRH-SRIF interactions in both male and female animals.

A relevant secondary question is whether estrogen and/or testosterone determines this episodicity of GH secretion. In girls with gonadal failure due to Turner’s syndrome, short-term treatment with ethynylestradiol (100 ng·kg⁻¹·day⁻¹) significantly increases ApEn of GH release (67). Testosterone, but not 5α-dihydrotestosterone, injection in boys with constitutionally delayed puberty also increases ApEn and hence the quantifiable disorderliness of GH release over time (67). However, unlike the human, in the rat both aromatizable and nonaromatizable androgen treatment will stimulate a male pattern of GH release and increase expression of the hypothalamic periventricular SRIF and the arcuate nucleus GHRH genes (1, 9, 70). These experimental observations in the two species can be unified by the hypothesis that estrogen and aromatizable androgen increase ApEn and augment the irregularity of GH release in the human, whereas both aromatizable and nonaromatizable androgen lower ApEn and produce more orderly GH release in the rat. The basis of these species differences is not evident.

The present experiments reveal that the quantifiable regularity of GH release is affected by both ovariectomy and orchidectomy and possibly more by the former. As defined by discrete peak analysis, other measures of GH release (baseline, GH peak frequency and amplitude) are affected differentially by ovariectomy and orchidectomy: GH baseline levels are most evidently altered by ovariectomy, whereas GH peak amplitude is most changed by orchidectomy (22). Thus estrogen and testosterone (and/or nonsteroidal gonadal products) may differentially control pulsatile and basal serum GH concentrations. The current experiments support this inference from an irregularity perspective, because, as noted above, ApEn is an inferential marker of degree of feedback integration in many systems. In the GHRH-SRIF-GH axis, we propose that sex steroids likely alter the relative isolation of (or lack of coupling among) individual regulatory loci and/or the strength of key feedback and regulatory inputs.

Of considerable interest, ovariectomy did not convert ApEn values in females completely to male values or to the values of gonadectomized males. Mean ApEn in ovariectomized females was still significantly higher than that in orchidectomized males, indicating persisting disorderliness of GH release possibly due to either the genetic sex and/or the precastration sex steroid hormone milieu. This is evidenced in Fig. 3B, where ApEn values from only three gonadectomized male animals fall into the ovariectomized female range. Apparently, absence of gonadal estrogen from before the onset of sexual maturation in the rat is not sufficient to convert GH secretion (at 10–11 wk of age) entirely to a male GH release pattern. In contrast, the fact that ApEn values are similar in gonadectomized and intact male rats suggests that the short-term presence of testosterone is not absolutely required for the regular male pattern. Because we performed gonadectomy prepubertally at the age of 4 wk, it is possible that prenatal or early-life exposure to sex steroids and their imprinting effects are, in part, responsible for the gender differences in adult GH secretion patterns (2, 9, 27–29, 70). Alternatively, the presence of estrogen, independent of the age and sex of the animal, could be responsible for the more irregular female secretion pattern and higher ApEn values. To distinguish between these alternatives, one would need to determine if estrogen treatment of adult (castrate) male rats or testosterone treatment of adult (gonadectomized) female rats, respectively, will “feminize” or “masculinize” fully the orderliness of GH secretion. Estrogen treatment in male (adult) rats does raise and thus tends to feminize baseline serum GH levels (5) and feminizes GH-dependent hepatic enzyme expression (70), but ApEn analyses are not yet available in these experimental settings.

Available studies do not reveal the precise underlying mechanism(s) subserving the apparently greater effect of ovariectomy than orchidectomy on the regularity of GH secretion, as inferred here. However, gonadectomy lowers (and testosterone increases) hypothalamic SRIF mRNA content in both male and female rats (1, 9, 72). Testosterone, but not estrogen, treatment increases hypothalamic GHRH mRNA expression in the rodent (74). This suggests (but does not prove) that testosterone’s ability to elicit apparently more orderly patterns of GH release in the rat (lower ApEn) depends on coordinated interactions between highly expressed GHRH and SRIF peptidergic regulatory systems.

Another possible mechanism of sex steroid regulation, which does not exclude the above explanations, is sex hormone modulation of somatotroph responsiveness to either GHRH or SRIF. For example, a recent report indicates that SRIF-receptor mRNA in GH4C1 cells can be affected by gonadal steroids (73). However,
in this system, estrogen and testosterone exerted the same effect on SRIF-receptor expression. Although the GHRH-receptor gene has recently been cloned (37) and pituitary GHRH-receptor expression increases with sexual maturation (34), its expression is not sexually dimorphic or markedly affected by gonadal steroid administration (7).

Treatment with the GnRH analog triptorelin was not so effective in modifying the pattern of GH release, and hence ApEn, as gonadectomy, at least in females. In males this was more difficult to ascertain, because of the smaller effect of orchidectomy per se on ApEn. The latter may reflect the relatively small number of animals studied and/or the duration of the observation period available. Pertinently, GnRH agonist treatment also was not so effective as gonadectomy in affecting growth rate or other GH-dependent measures in these rats (22). It is likely that triptorelin does not completely suppress the pituitary-gonadal axis, or that some degree of gonadal steroid production may be maintained by factors other than GnRH-sensitive pituitary LH and FSH (3, 4), which are removed by surgical gonadectomy, but not by GnRH analog treatment. Another possibility is that (nonsteroidal) gonadal factor(s) that affect GH secretion are removed by gonadectomy but not by GnRH analog treatment. Given that serum estrogen and testosterone concentrations in rats treated with this dose of triptorelin were very low, but not undetectable (Robinson and Gevers, unpublished), we favor the interpretation that the orderliness of GH release is influenced by even the small amounts of sex steroids that remain after GnRH analog-induced downregulation of the gonadal axis.

Perspectives

From a more general perspective, a thematic accomplishment of this paper is to show that one can now quantify (by ApEn), and thus discriminate among, subtle gradations in hormonal secretory patterns, which may be less evident visually, even based on relatively few data points (here 60). Indeed, one can infer both physiological alterations and pathological derangements in neurohormone release patterns. For example, physiological GH neuroregulation is quantifiably distinct in males versus females and in puberty versus prepuberty, whereas pathophysiological or atypical GH release is evident in somatotrope tumors, aging, and (visceral) obesity. The present study highlights an enhanced irregularity of GH secretion in females than males (and in puberty versus prepuberty, etc.). From a mathematical viewpoint, this enhanced irregularity could arise from more (or novel) critical control nodes or regulatory factors present in the female than in the male (and/or at puberty compared with prepuberty, etc.) or, alternatively, from more intense or rapid interactions among feedback and feedforward elements (even without added control nodes) within the GH secretory network. We pose a number of possible mechanisms that further experiments, in conjunction with irregularity assessment, should clarify. First, biologically, a significant issue is whether prenatal, perinatal, and/or prepuberal programming alone can produce distinguishable gender-specific differences in adult GH secretory patterns independent of subsequent gonadal steroid secretion in adulthood and, if so, how and to what extent such imprinting arises. The present sampling and analysis methodologies could be conducted with suitably treated and/or castrated animals manipulated in utero, perinatally, or in adulthood to address this question directly. Second, greater understanding is needed of the cellular and molecular mechanisms mediating the actions of sex steroids on neuronal nuclei, their topographical and functional interconnections, and nodal feedforward and feedback interfaces within the GH axis/network. Accordingly, third, comprehension more formally of network- or systems-level control processes by biomathematical constructs of an intact neuroendocrine axis will be valuable not only within the present focus on the GHRH-somatostatin-GH-IGF-I axis, but also in broader contexts, such as the arginine vasopressin-corticotropin hormone-adrenocorticotropic hormone-cortisol stress-responsive axis the GnRH-lutenizing hormone-follicle-stimulating hormone-testosterone-inhibin axis, etc. Moreover, “open” and “closed” loop feedback-control networks will need to be studied in both experimental animals and the human. For example, appropriate studies could evaluate the orderliness of trophic and reproductive neurohormone release in estrogen-withdrawn (open loop) and again in estrogen-replaced (closed-loop) postmenopausal women and determine whether continuous versus pulsatile estradiol delivery is a more “physiological” feedback signal in restoring younger-adult GH, LH, or FSH secretory patterns considered singly or jointly (via cross-ApEn, cross-correlation, or pulse coincidence testing). Such studies would provide experimental paradigms to begin to dissect further the nature of sex steroid-sensitive neuroendocrine feedback-control mechanisms, of which ApEn is a practicable and sensitive measure.

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