

**Putative Growth Hormone (GH) Pulse Renewal: Periventricular Somatostatinergic Control
of an Arcuate-Nuclear Somatostatin and GH-Releasing Hormone Oscillator**

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ABSTRACT

Growth hormone (GH) pulsatility requires periventricular-nuclear somatostatin (SRIF_{PeV}), arcuate-nuclear (ArC) GH-releasing hormone [GHRH] and systemic GH autofeedback. However, no current formalism interlinks these regulatory loci in a manner that generates self-renewable GH dynamics. The latter must include in the adult rat: (i) infrequent volleys of high-amplitude GH peaks in the male; (ii) frequent discrete low-amplitude GH pulses in the female; (iii) disruption of the male pattern by severing SRIF_{PeV} outflow to ArC; (iv) stimulation of GHRH and GH secretion by CNS delivery of SRIF; (v) inhibition of GH release by central exposure to GHRH; and (vi) a rebound-like burst of GHRH secretion induced by stopping peripheral infusion of SRIF. The present study validates by computer-assisted simulations a simplified ensemble formulation that predicts each of the foregoing six outcomes, wherein: (a) blood-borne GH stimulates SRIF_{PeV} secretion after a long time latency; (b) SRIF_{PeV} inhibits both pituitary GH and ArC GHRH release; (c) ArC GHRH and SRIF_{ArC} oscillate reciprocally with brief time delay; and (d) SRIF_{PeV} represses and disinhibits the putative GHRH- SRIF_{ArC} oscillator. According to the present analytical construction, time-delayed feedforward and feedback signaling among SRIF_{PeV} , ArC GHRH and SRIF_{ArC} could endow the complex physiological patterns of GH secretion in the male and female.

INTRODUCTION

Frequent serial measurements of peripheral GH concentrations every 0.5, 2, 5, 7 and 10 min have unmasked complex patterns of gender-specific and developmentally regulated GH release in the rat, sheep and human (13;14;27;30;31;51;62;66;75;77;78). In particular, GH secretion evolves as infrequent volley-like clusters of prominent pulses in the adult male rodent, pubertal children, and young fasting or sleeping men and women, but unfolds as frequent, isolated low-amplitude bursts in the female rat and older, awake or nutrient-replete human. In a simplified view, GH pulse renewal requires (minimally) episodic drive by hypothalamic GH-releasing hormone (GHRH), intermittent repression by central-neural somatostatin (SRIF), and reversible negative feedback by systemic-CNS GH (9;29;55). An emergent concept is that time-delimited interactions among (rather than any single effect of) GHRH, SRIF and GH mediate the physiological dynamics and sexual dimorphism of pulsatile GH release (20;45;62). Regulatory and integrative mechanisms that control GH outflow are crucial to somatic growth, pubertal development and adult homeostasis. This is because the time-pattern of GH delivery to target tissues determines GH-receptor turnover, mode of second-messenger signaling, cell-specific gene expression and distinct metabolic responses (2;28;33;34).

Laboratory data in the male rat indicate that elevated systemic and CNS concentrations of GH act by way of time-delayed negative feedback to stimulate SRIF secretion from the periventricular nucleus (SRIF_{PeV}) into hypophyseal portal blood, which serves to antagonize GH release by the anterior pituitary gland (60;62). However, the mechanisms that generate individual high-frequency secretory bursts within more complex volleys in the male animal and sustain frequent single GH peaks in

the female are less clear. Recent hypotheses include an interactive (multiparameter) network that endows self-renewing GH pulses (69); an arbitrarily autonomous GHRH neuronal oscillator (79); rapid direct inhibition of arcuate-nucleus GHRH release by GH (24); intermittent GH-induced SRIF_{PeV} outflow (23); and, pituitary somatotrope store-dependent rebound-like GH secretion (25). None of the foregoing formulations is able to integrate presumptive connectivity among each of negative feedback by GH, PeV release of SRIF to ArC and into hypophyseal portal blood, presumptive coupling between SRIF_{ArC} and GHRH in the mediobasal hypothalamus, and release of GHRH to the pituitary gland (Methods: *Motivation*). The present effort is directed toward incorporating experimentally inferred linkages into a single, simplified interactive construct, and testing the relevance of this formalism to explicating known mechanisms of GH control.

METHODS

Motivation

The goal is to formulate a testable network-like construct that reproduces currently unexplained, but experimentally consistent, observations in the adult male and female rat. A premise is that intrahypothalamic interactions modulate an array of complex GH dynamics (9;9;16;29;55). First, intracerebroventricular (icv) infusion of SRIF and *in vitro* incubation of hypothalamic neuronal explants with SRIF stimulate GH and GHRH secretion, respectively (1;4;46;56). Second, anterolateral deafferentation of the mediobasal hypothalamus, which reduces SRIF immunoreactivity in the median eminence, markedly damps high-amplitude pulsatile GH release in the male rat

(37;54;71). Third, brief electrical stimulation of PeV triggers subsequent rebound-like GH secretion and hyperactivation of putative ArC GHRH neurons (19;57). Fourth, GH injection stimulates SRIF release and gene expression in both PeV and ArC (5;8;36;65). Fifth, icv delivery of specific antisense oligodeoxynucleotide directed to the SRIF receptor (subtype 1) suppresses high-amplitude GH pulses in the male animal (40). Sixth, icv injection of GHRH paradoxically inhibits GH secretion and stimulates SRIF release (48). Seventh, continuous systemic infusion of GHRH maintains the pulsatile mode of GH secretion (21;55;76;80). Eighth, successive systemic exposure to and withdrawal of SRIF evokes rebound-like release of both GHRH and GH (10;12;52;55;56;70;73). Ninth, repeated administration of a linear somatostatin-receptor antagonist peptide (unexpectedly) reduces body-weight and length gain in the immature male rat (6). And, tenth, ArC is accessible to certain blood-borne peptides. This set of observations has never been unified under a single simplified integrative construct.

Neuroanatomic connectivity

Studies in the rat, mouse and sheep provide extensive evidence of reciprocal signaling between SRIF (inhibitory) and GHRH (stimulatory) neuronal systems in the hypothalamus (29;55). Pivotal experimental observations, as yet unmodeled, include: (a) SRIF perikarya, axons and terminal nerve fields and cognate receptors located in both ArC and PeV (54;81); (b) SRIF receptors expressed by GHRH-positive perikarya in ArC and SRIF-positive perikarya in PeV and ArC (7); (c) GHRH-receptor gene transcripts identified in ArC, and synaptic contact of GHRH-positive nerve terminals with GHRH (see Perspectives) and SRIF-containing dendrites in ArC (32;72); (d) synaptic contact of SRIF_{PeV} and/or SRIF_{ArC} with GHRHergic neurons in ArC (16;44); (e) the

ability of central and systemic pulses of GH to act via GH-specific receptors on SRIF_{PeV} [and ArC neuropeptide Y (NPY)] neurons, stimulate SRIF_{PeV} release, and inhibit GHRH secretion and gene expression (8;54); (f) coupling of SRIF_{PeV} to GHRH in ArC and of GHRH in ArC to SRIF_{ArC} and GHRH directly or indirectly (see Perspectives) (9;19;37;53); and (g) connections between and autoinhibition by SRIF and SRIFergic neurons (46;61).

Proposed simplified network structure

In overview, the current network-like formulation assumes that: (a) time-delayed systemic GH-induced SRIF_{PeV} secretion suppresses both pituitary GH release and (ArC) GHRH secretion; (b) mediobasal hypothalamic, time-delayed, short-latency, reciprocal GHRH and SRIF_{ArC} interactions create a damped intraArC oscillator; and (c) sequential activation and quiescence of SRIF_{PeV} neurons serves to inhibit and amplify the amplitude of the GHRH-SRIF_{ArC} oscillator. The last concept is illustrated in **Figure 1 A**, and developed explicitly below.

Insert **Figure 1** here

The proposed mediobasal hypothalamic GHRH-SRIF_{ArC} oscillator arises by reciprocal coupling (i.e., bidirectional inhibitory and stimulatory linkages) and time delay (26). Viewed as a time-ordered pathway of interactions, increased GHRH outflow stimulates SRIF_{ArC} activity after a time delay, D_2 ; elevated SRIF_{ArC} activity suppresses GHRH outflow (7); and, both peptides (or their interneuronal effector signals) undergo elimination, diffusion, degradation and/or reuptake. These processes are encapsulated

in the following pair of coupled rate equations and corresponding Hill dose-response functions:

$$SRIF_{ArC}' = -k_2 SRIF_{ArC} + k_{r,2} \frac{(GHRH(t - D_2) / t_3)^{n_3}}{(GHRH(t - D_2) / t_3)^{n_3} + 1} \quad (1)$$

$$GHRH' = -k_3 GHRH + k_{r,3} \left[\frac{1}{([SRIF_{ArC} + P(t)] / t_4)^{n_4} + 1} \right] \quad (2)$$

In relation to terminology, the prime denotes the rate of change of concentration under feedback or feedforward control; (italicized) $SRIF_{ArC}$ and $GHRH$ define the concentration of each peptide; t is time; k_2 and k_3 give rate constants of $SRIF_{ArC}$ and $GHRH$ elimination; $k_{r,2}$ and $k_{r,3}$ signify rate constants driving and restraining release of $SRIF_{ArC}$ and $GHRH$, respectively; n_3 , and n_4 are slope (sensitivity or response-steepness) terms in the Hill functions; t_3 and t_4 designate half-maximally stimulatory or inhibitory concentrations (ED_{50} or ID_{50}), respectively, operating at $GHRH$ and $SRIF_{ArC}$ dose-response interfaces (+ and - in **Figure 1 A**); and, D_2 reflects the time-delay for $GHRH$'s stimulation of $SRIF_{ArC}$. The function $P(t)$ incorporates extra-ArC perturbation of $GHRH$ outflow by $SRIF_{PeV}$ (below).

An assumption is that the (unperturbed) $GHRH$ - $SRIF_{ArC}$ ArC oscillator is “damped”; i.e., high-amplitude oscillations are triggered by an on-off perturbation and decay gradually thereafter. Technically damped oscillatory behavior is readily explicated: **Appendix**. Biological damping will occur when volleys of GH induce $SRIF_{PeV}$ outflow. In addition, damping may be augmented by interneuronal signal depletion, receptor and/or post-receptor response downregulation and/or collateral ArC $SRIF$ receptor subtype-specific autoinhibition (9;40). **Figure 1 B** illustrates a waning

train of GHRH and $SRIF_{ArC}$ bursts generated from coupled Equations 1 and 2¹. In this example, the perturbation, $P(t)$, to the ArC oscillator is arbitrarily set to zero at all times, t , except $t \in [35,36]$ hr, within which 1-hr interval $P(t)$ increases to 20 and then decreases to zero. In physiological terms, outflow of $SRIF_{PeV}$ induced by initial GH pulses within a volley initiates the primary perturbation (thereby damping) and withdrawal of $SRIF_{PeV}$ due to the intervalley (nadir) removal of GH and depletion of $SRIF_{PeV}$ ends the perturbation (thus disinhibiting the oscillator). Specifically, $SRIF_{PeV}$ represses both GHRH release and GHRHergic stimulation of $SRIF_{ArC}$, which otherwise inhibits GHRH secretion; and, subsequent waning of $SRIF_{PeV}$ outflow releases GHRHergic drive of $SRIF_{ArC}$, thereby restoring GHRH- $SRIF_{ArC}$ interactions.

The present intrahypothalamic network-like construct is built around experimentally verified core model components developed earlier (23;24), which include: (i) GHRH release from ArC into the hypothalamo-pituitary portal circulation, which stimulates exocytotic GH secretion (42;54); (ii) GH secretion into systemic blood, which evokes SRIF release from PeV after a distinct time delay, D_1 (11;54;58); (iii) hypothalamic $SRIF_{PeV}$ neuronal outflow, which inhibits GHRHergic activity transsynaptically and/or internuncially (7;15;29); and (iv) $SRIF_{PeV}$ secreted into portal blood, which opposes GHRH-driven GH secretion (12;42). Each interface is represented algebraically via a corresponding dose-response (Hill) function (23;24). For simplicity, we do not include a term for GHRH-stimulated saturable accumulation of pituitary GH stores under reversible $SRIF_{PeV}$ inhibition of GH release but not synthesis (25).

¹ The coefficients used in this simulation are summarized in **Table 1** (discussed below).

According to the foregoing formulation, the four primary nodes (signaling junctions) of the putative pulse-generating network are GH, GHRH, SRIF_{ArC} and SRIF_{PeV}. The corresponding deterministic connections are depicted in **Figure 2**. Peptides (or interneuronal effectors) are subject to individual monoexponential elimination kinetics (not shown in **Figure 2**) (23;24). Feedforward by GH on SRIF_{PeV} and feedforward by GHRH on SRIF_{ArC} are delayed by distinct time lags (D_1 and D_2 , respectively). External input to the GHRH/SRIF_{ArC} oscillator is reversible GH-induced SRIF_{PeV} restraint and release of GHRH outflow [**Figure 1 B**]. Solely deterministic versus deterministic plus minor stochastic input to the GHRH/SRIF_{ArC} oscillator is achieved by disallowance and allowance, respectively, of variability in the sensitivity of GHRH neurons to total SRIF_{ArC} and SRIF_{PeV} inhibition. Specifically, the half-maximally inhibitory concentration of SRIF (t_4 in the Hill function in Equation 5 below) is fixed or varied by random, zero-mean, unit-normalized Gaussian noise imposed at a nominal 5% coefficient of variation (standard deviation/mean $\times 100\%$) at 30-min intervals.

Insert **Figure 2** here

Ensemble interactions are encapsulated in the following set of coupled, delayed ordinary nonlinear differential equations

$$GH' = -k_1 GH + k_{r,1} \left[\frac{(GHRH / t_1)^{n_1}}{(GHRH / t_1)^{n_1} + 1} \frac{1}{(SRIF_{PeV} / t_2)^{n_2} + 1} \right] \quad (3)$$

$$SRIF_{ArC}' = -k_2 SRIF_{ArC} + k_{r,2} \frac{(GHRH(t - D_2) / t_3)^{n_3}}{(GHRH(t - D_2) / t_3)^{n_3} + 1} \quad (4)$$

$$GHRH' = -k_3 GHRH + k_{r,3} \left[\frac{1}{([SRIF_{ArC} + SRIF_{PeV}] / t_4)^{n_4} + 1} \right] \quad (5)$$

$$SRIF_{PeV}' = -k_4 SRIF_{PeV} + k_{r,4} \frac{(GH(t - D_1) / t_5)^{n_5}}{(GH(t - D_1) / t_5)^{n_5} + 1} \quad (6)$$

Relevant additional definitions include GH and $SRIF_{PeV}$, which identify concentrations of the cognate peptides at time t ; k_1 and k_4 , rate constants of elimination of GH and $SRIF_{PeV}$, respectively; $k_{r,1}$ and $k_{r,4}$, rate constants of GH and $SRIF_{PeV}$ release; and n_1 , n_2 , n_5 , and t_1 , t_2 , t_5 , Hill coefficients (steepness term) and half-maximally effective concentrations (ID_{50} or ED_{50}) of GHRH, $SRIF_{PeV}$ and GH, respectively. As detailed in the core model representation (23;24), primary assumptions are that: (a) pituitary GH release requires simultaneous GHRH stimulation and submaximal competition by $SRIF$ [note term following $k_{r,1}$ in Eq. 1]; (b) GHRH secretion originates in ArC, and requires twofold withdrawal of inhibition by $SRIF_{ArC}$ and $SRIF_{PeV}$ [term after $k_{r,3}$ in Eq. 3]; (c) $SRIF_{ArC}$ inhibits ArC GHRH secretion [Eq. 2]; and (d) GHRH stimulates $SRIF_{ArC}$ [Eq. 4].

Nominal values of interactive constants are discussed in detail (23;24), and reviewed in **Table 1**. Lack of experimental data requires indirect estimation of the kinetics of unobserved $SRIF_{ArC}$; here, the criterion is a damped GHRH- $SRIF_{ArC}$ oscillator under intermittent inhibition and disinhibition by cycles of $SRIF_{PeV}$ outflow.

Insert Table 1 here

RESULTS

Reference model output

Insert **Figure 3** here

Figure 3 illustrates reference-model computer simulations of time-varying release of GH, GHRH, SRIF_{ArC} and SRIF_{PeV} in the male rat. For simplicity, all plots (except the one in the left bottom panel) depict deterministic model output without random variation in total SRIF-inhibited GHRH secretion. The left upper panel shows recurrent infrequent GH volleys driven by time-delayed feedback of systemic GH on SRIF_{PeV}. The right upper panel illustrates output of the intra-Arc mechanism generating high-frequency *intravolley* GH spikes under intermittent control by SRIF_{PeV}. The Arc oscillator performs at constant low amplitude unless perturbed by SRIF_{PeV} (METHODS, **Figure 1 B**; see also **APPENDIX**). Sequential outflow and withdrawal of SRIF_{PeV} trigger an amplitude-damped train of GHRH and SRIF_{ArC} spikes within a volley (**Figure 3**, right: top, middle and bottom).

Composite (volley and intravolley) GH oscillations in the adult male-like model arise as follows. In the evolution of infrequent compound peaks or cluster-like volleys, emergent GH pulses stimulate SRIF_{PeV} outflow after a relatively long systemic-CNS time delay. GH-induced SRIF_{PeV} secretion simultaneously antagonizes pituitary release of GH and suppresses Arc GHRHergic activity. The joint effect is to terminate the volley and enforce an intervalley nadir of (typically undetectable) GH secretion. The nadir interval persists until GH concentrations decay in plasma and interneuronal fluids, thereby relieving drive of SRIF_{PeV} output. Release of the GHRH-SRIF_{ArC} interaction from SRIF_{PeV} restraint disinhibits higher-amplitude GHRH and SRIF_{ArC} spikes

(**Figure 1 B**). The number of GHRH pulses in a given volley depends upon the *de facto* (potentially species, age or developmentally specific) damping properties of the ArC GHRH/SRIF oscillator (**APPENDIX**). Time constants here arbitrarily yield two intravolley bursts, thus emulating the normal male pattern. The degree of damping by $SRIF_{PeV}$ between consecutive volleys determines whether the ArC oscillator is repressed to low or undetectable amplitude (**Figure 3**, right lower panel). Low suppression would permit emergence of diminutive GHRH peaks (**Figure 3**, right upper panel), which may or may not elicit systemic GH pulses. Direct experimental data on this point are not available in the male rat, due to typically undetectable serum GH concentrations between successive volleys.

The left middle panel of **Figure 3** illustrates the projected dynamics of total SRIF ($SRIF_{ArC} + SRIF_{PeV}$) released into ArC based on strictly deterministic connections. Imposing small (5%) stochastic variability on GHRH neuronal sensitivity to total SRIF introduces greater nonuniformity of pulse amplitude, but does not disrupt overall model performance (**Figure 3**, left lower panel).

Diminished feedback of GH on $SRIF_{PeV}$ mimics the sex distinction in GH release

To test whether the current construct forecasts that relaxation of GH-on- $SRIF_{PeV}$ feedforward could account for the female-like GH profile, we increased the ED_{50} (lowered potency) for GH drive, (t_5), by 10-fold. This change substantially attenuates GH-driven $SRIF_{PeV}$ release. According to model simulations, sufficient muting of $SRIF_{PeV}$ outflow abolishes, and lesser restriction blunts, the emergence of recurrent GH volleys. The output thereby comprises discrete (single), high-frequency, low-amplitude

GH pulses, typical of the release pattern observed in the female rat [Introduction]:

Figure 4 A, right panel.

Insert **Figure 4** here

In view of the conceptual importance of the above outcome, as an alternative strategy to attenuate GH-induced SRIF_{PeV} drive, we reduced the rate of SRIF_{PeV} release ($k_{r,4}$) by 25 or 2.5-fold: **Figure 4 B**. In this experiment, low variability of GHRH sensitivity to SRIF was fixed at 5%. Reduced SRIF_{PeV} secretion generated epochs of variable-amplitude GH pulses as reported in the conscious female rat during intervals of extended (24-hr) and frequent (5-min) sampling (13). To examine the impact of stochastic variability in GHRH neuronal sensitivity to SRIF in the female model, the CV of t_4 (Methods) was increased from 5% (nominal realizations, above) to 18% over a brief time window: **Figure 4 A**, left panel. The latter adjustment yielded variable-amplitude GH pulses with no change in mean burst frequency. This notion may be relevant, if the SRIF_{PeV}-GHRH interface undergoes partial desensitization and resensitization over the day or night, accounting for the diurnal difference in the quantifiable irregularity in GH secretion patterns in the rat (59).

Anterolateral deafferentation of the mediobasal hypothalamus (ALD)

Anterolateral deafferentation of the mediobasal hypothalamus (ALD) restricts or eliminates SRIF influx into ArC from PeV, while ostensibly preserving GHRH and SRIF interactions within ArC. The outcome in the male rat is abolition or reduction of high-amplitude pulsatile GH release, irregular small GH pulses and retention or elevation of

basal GH secretion (35;37;43;54;71). We postulated that mechanistically ALD limits GH-driven SRIF_{PeV} outflow to GHRH, thereby abrogating cycles of inhibition and disinhibition of the GHRH- SRIF_{ArC} oscillator. This hypothesis was modeled by introducing a 100-fold increase in t_5 (GH ED_{50}) to limit GH inhibition via SRIF_{PeV} and 5-fold decrease in variability (CV) of GHRH sensitivity to SRIF: **Figure 5**. The resultant profiles are marked by irregular and diminutive, if any, GH pulses.

Insert **Figure 5** here

As an alternative (nonexclusive) mechanism of partial withdrawal of SRIF_{PeV} input (e.g., incomplete ALD), we increased the elimination rate (k_4) of SRIF_{PeV} across four arbitrary strata, thereby restricting SRIF_{PeV} availability to ArC GHRH; *viz.*, 3, 4, 10 and 20-fold reductions: **Figure 6 Panel A**. GHRH neuronal sensitivity to total SRIF was fixed at 5% variability at each gradation of SRIF_{PeV} outflow.

Insert **Figure 6 Panel A** here

In this simulated context, progressive SRIF_{PeV} depletion restricts the capability of GH feedback to trigger ArC GHRH/SRIF volleys and elevates intervalley release of GHRH and GH. The resultant output pattern emulates features of the female GH profile (**Figure 6 Panel A**, right lower plot). In corollary analyses, we increased t_5 (GH ED_{50}) to simulate GH-receptor downregulation in PeV. Up to 2.8-fold blunting of GH drive to SRIF_{PeV} augmented GH pulse amplitude, burst frequency and interpulse nadir GH

concentrations, thereby mimicking the time patterns shown in **Figure 6A** (top right). Comparable outcomes were reported by Pellegrini *et. al.* following partial (50%) depletion of CNS GH-receptor expression due to central infusion of GH-receptor antisense oligodeoxynucleotide in the adult male rat (58). A more marked increase in t_5 analytically in the male predicted feminization of the GH profile comparably to that shown in **Figure 6A** (lower). Deprivation of effectual GH drive of SRIF_{PeV} outflow is inferable by female resistance to exogenous GH autofeedback action [Introduction].

Intracerebroventricular (icv) SRIF infusion

Further simulations indicated that central delivery of SRIF serves to repress GH pulsatility only if the infused peptide is able to: (a) leave the CNS and inhibit the pituitary gland; and/or (b) enter ArC and inhibit the GHRH/SRIF_{ArC} oscillator: **Figure 6 Panel B**.

Insert **Figure 6 Panel B** here

In contrast to the inhibitory impact of peripheral SRIF infusion, central (icv) injection of SRIF in the anesthetized and conscious, freely moving adult male rat induces (paradoxical) GHRH and GH release (4;46;56). To simulate this experimental intervention, we add the term $Inf(t)$ to the right-hand side of Eq. [6] that incorporates a brief 1-hr impulse to mimic transient SRIF_{PeV} release (here centered on $t = 55.5$ hr). The impulse is defined algebraically by the piece-wise continuous function:

$$Inf(t) \begin{cases} = 0 & \text{if } t \leq 55 \\ \text{increases linearly} & \text{if } 55 < t < 55.5 \\ = 400 & \text{if } t \geq 56 \\ \text{decreases linearly} & \text{if } t = 55.5 \\ = 0 & \text{if } t \geq 56. \end{cases}$$

Figure 6 Panel C illustrates the prediction that time-delimited access of SRIF to ArC unleashes volley-like release of GH in both the male- and female-like constructs. Compared with the male-like network, the female-like model responds with more prolonged GHRH-ArC oscillations under a brief icv SRIF impulse. We are unaware of direct *in vivo* data that address this prediction in the female animal. The latter sex distinction arises in the current network perspective, inasmuch as the GH pulse (induced by GHRH rebound after SRIF infusion and decay) evokes less SRIF_{PeV} outflow to damp and disinhibit the GHRH-SRIF_{ArC} oscillator in the female than male construct [Methods].

Insert **Figure 6 Panel C** here

SRIF and GHRH interactions

Below we illustrate three additional pivotal model-based forecasts: (i) rebound-like GH release after systemic imposition and withdrawal of SRIF inhibition; (ii) sustained GH pulsatility during continuous peripheral GHRH stimulation; and (iii) attenuated GH release caused by continuous central GHRH delivery. These predictions are unique in applying to both the male and female models.

Insert **Figure 7** here

(i) Rebound-like GH secretion after systemic SRIF withdrawal

Figure 7 A illustrates the model prediction that abrupt cessation of systemic SRIF infusion *with access to ArC* will induce rebound-like GHRH and GH secretion [see *Motivation*]. The latter presumptive mechanism is complementary to, but distinct from, the nonexclusive hypothesis that SRIF can block somatotrope GH release (but not synthesis), thereby augmenting pituitary accumulation of subsequently GHRH-releasable GH stores as a mechanism to accentuate the amplitude of GH rebound (25). *In vivo* rebound secretion of GHRH (and GH) after systemic inhibition was documented directly in the conscious ram given a single intraperitoneal injection of the synthetic somatostatin agonist, octreotide, following initial repression of GHRH (and GH) (49). Direct facilitative actions of prior SRIF exposure on GHRH stimulation of GH secretion were also demonstrated *in vitro* (39). In the current model construct, ongoing peripheral SRIF infusion restrains GHRH release into portal block and within ArC by gaining access to and suppressing the GHRH/SRIF_{ArC} oscillator. Withdrawal of systemic and ArC SRIF disinhibits GHRH neurons and the GHRH-SRIF_{ArC} oscillator, thus inducing a burst of GHRH (and GH) secretion. Concomitant allowance for augmented pituitary GH stores during SRIF delivery would amplify the magnitude of GH rebound further (not shown).

(ii) Continuation of pulsatile GH release during constant peripheral GHRH delivery

Figure 7 B tests the impact of assuming that systemically infused GHRH cannot directly stimulate SRIF_{ArC}. In the current model, this assumption predicts that: (a)

continuous GHRH infusion will dose-dependently drive high-amplitude GH pulses of unchanged frequency; (b) minimal exogenous GHRH drive will be more effectual in the male than female feedback model (**Figure 7 B**, upper panels); and (c) higher (3-fold increased) constant GHRH stimulation will elicit male-like volleys of GH pulses in both sexes (**Figure 7 B**, lower panels), but only to the extent that elevated GH concentrations are allowed to trigger SRIF_{PeV} release in the female construct.

(iii) Repression of GH pulse amplitude by icv delivery of GHRH

To mimic GHRH infusion centrally, GHRH peptide was assumed to act on SRIF-secreting neurons in ArC, but not somatotropes in the anterior pituitary gland:

Figure 7 C. Under this assumption, both the male and female models predict attenuation of pulsatile GH secretion due to direct GHRH stimulation of SRIF_{ArC} secretion, therefore repressing endogenous GHRH release into portal blood. In the male model, diminished pulsatile GH secretion in turn reduces cyclic GH feedback-dependent outflow of SRIF_{PeV}, thereby suppressing individual GH pulses and volley-like GH release (left panel).

DISCUSSION

The present construct formalizes and tests the simplified network-like hypothesis that in the adult male rodent: (a) systemic GH pulses stimulate SRIF_{PeV}-dependent inhibition of all three of somatotrope GH release, GHRH secretion into portal blood and intrahypothalamic GHRH feedforward on SRIF_{ArC}, thereby enforcing an *intervolley* interval of reduced GH secretion; and (b) declining GH and hypothalamic SRIF_{PeV} concentrations during an interpulse trough disinhibit the putative GHRH-SRIF_{ArC}

oscillator, therein triggering a volley of high-frequency, amplitude-damped GHRH and GH secretory bursts. Amplitude-damped volleys are readily evident visually in published GH time series obtained by frequent (0.5-10 min) sampling of peripheral blood [Introduction]. The unequal feedback latencies required for systemic GH to stimulate $SRIF_{PeV}$ secretion (long) and for mediobasal hypothalamic GHRH to drive $SRIF_{ArC}$ inhibition (short) endow prolonged *intervolley* waiting times and short *intravolley* interpulse intervals, respectively. According to the present formulation, a reduction in GH-induced $SRIF_{PeV}$ release in the female animal would attenuate $SRIF_{PeV}$ -enforced suppression and disinhibition of coupled GHRH- $SRIF_{ArC}$ interactions. The combined outcome is discrete, high-frequency and low-amplitude GH pulses with no or only occasional escape of more complex volley-like events (13;14;59;62;66). This proposed model extension captures the foregoing elements (and below) and retains all basic GH pulsatility features recognized in the adult male and female (22-24).

A fundamental prediction of the current formalism is that depletion of $SRIF_{PeV}$ input to ArC would isolate the proposed GHRH- $SRIF_{ArC}$ oscillator from cycles of $SRIF_{PeV}$ -dependent repression and escape, which are timed by GH autofeedback. We show that modest and marked ArC oscillator isolation would accentuate and blunt GH pulse height, respectively. Oscillator sequestration (of variable degree) is inferable in at least three experimental settings: (i) the adult male rat with experimental partial or complete anterolateral deafferentation of the mediobasal hypothalamus (complete isolation); (ii) the adult female rodent with (moderate but not complete) sex-dependent attenuation of systemic GH drive of $SRIF_{PeV}$ release compared with the male; and (iii) the growing male rat administered a linear hexapeptide SRIF-receptor *antagonist*, which

presumptively blocks SRIF_{PeV} actions on GHRH and the pituitary gland and thereby feminizes the rate of somatic growth (6;35;37;43;50;54;71).

A second basic model forecast is that GH-induced SRIF_{PeV}-specific suppression and disinhibition of ArC GHRH release can explicate the capability of SRIF and octreotide to: (a) elicit GHRH release by hypothalamic explants *in vitro*; and (b) stimulate a burst of GHRH and GH secretion after icv or intraperitoneal injection (4;46;49;56). Specifically, the male-like construct predicts that in these experimental contexts: (i) *in vitro* effects of SRIF may reflect successive inhibition and disinhibition of GHRH neurons; (ii) icv actions of SRIF and CNS uptake of octreotide sequentially antagonize and disinhibit GHRH release due to the initial availability and subsequent metabolism and/or reuptake of exogenous peptide, thereby mimicking reversible GH-stimulated SRIF_{PeV} release (**Figure 6 Panel C**). In experimental support of SRIF_{PeV} and GHRH interactivity, brief electrical stimulation of PeV neurons in the male rat evokes rebound-like hyperactivation of putatively GHRH ArC neurons with attendant burst-like GH release (19;57).

A third expectation of the present feedback structure is that the effect of constant systemic infusion of SRIF will depend on relative accessibility of injected peptide to the GHRH-SRIF_{ArC} oscillator. In particular, uptake of peripheral SRIF into ArC forecasts repression of GHRH outflow during the infusion, and rebound-like GHRH secretion upon termination of GHRH stimulation (**Figure 7 A**). The latter biphasic response occurs in portal-venous blood of the conscious ram administered a single dose of a synthetic SRIF agonist (octreotide) intraperitoneally (49). The importance of the delayed GHRH burst in driving GH release after SRIF exposure and withdrawal is affirmed by the

capability of passive GHRH immunoneutralization to reduce rebound GH secretion by 50-83% in the adult male and female rat (12;52;73). Rebound GH secretion is amplified further in a model in which systemic infusion of SRIF blocks pituitary GH release, but not GH synthesis and accumulation in releasable stores (25;39). This interpretation requires that feedforward to somatotropes is maintained during systemic SRIF delivery by concomitant low-amplitude GHRH pulses, as predicted here (**Figure 3**, top right panel).

A fourth significant projection is that continuous systemic GHRH stimulation will evoke normally timed GH pulses, only under the assumption that infused peptide is excluded from ArC. With the latter exclusion, the male model forecasts that constant peripheral infusion of GHRH sustains recurrent high-amplitude GH volleys due to SRIF_{PeV}-induced cyclic suppression and disinhibition of GHRH-SRIF_{ArC} oscillations. On the other hand, significant uptake of circulating GHRH into ArC predictively represses high-frequency GHRH/SRIF_{ArC} oscillations by stimulating unabated SRIF_{ArC} outflow directly. Experimental data document that constant iv infusion of GHRH does maintain high-amplitude pulsatile GH secretion at a physiological mean event frequency in the male and female rat and human (21;55;76;80). Therefore, if the accompanying network model has validity, one may infer that systemically delivered GHRH exerts limited, if any, direct stimulatory effect on ArC SRIF neurons.

A fifth verifiable prediction of the proposed intrahypothalamic model is that GHRH will release SRIF by hypothalamic fragments *in vitro* and inhibit GH secretion following icv administration *in vivo*, as reported by others (4;48). In this regard, incubation with GHRH would directly stimulate SRIF_{ArC} release *in vitro* and icv delivery of GHRH would

force SRIF_{ArC}-dependent suppression of GHRH release (29;55). The foregoing experimental observations and model predictions require direct CNS actions by GHRH. The latter inference has not been proved, but is supported by: (a) hypothalamic expression of GHRH-receptor mRNA (72); (b) single-neuron recordings showing GHRH-induced cellular signaling (17;74); and (c) appetitive and somnifacient effects of central GHRH infusion, which are blocked by coadministration of a selective GHRH-receptor antagonist (47;68;82).

A sixth relevant implication is that resistance to GH-induced SRIF_{PeV} secretion in the female rat will mute the male-like mechanisms of: (i) GH-dependent, SRIF_{PeV}-mediated restraint of GHRH-SRIF_{ArC} coupling; and (ii) rebound-like release of GHRH/GH after SRIF_{PeV} withdrawal. The predicted result is a nearly continuous pattern of frequent, low-amplitude GH pulses, as reported experimentally (13;62). In support of the requirement for central GH action to sustain male-like GH pulsatility, molecular silencing of the CNS GH receptor decreases GH peak amplitude in the adult male rat (58).

And, seven, intrahypothalamic connectivity predicts that SRIF_{PeV} and GHRH pulses monitored in hypothalamo-hypophyseal portal blood should be linked over time: (a) reciprocally in the male rat; and (b) variably, if at all, in the female animal. The first prediction is affirmed by portal-venous sampling in the urethane-anesthetized male rat (60). The second forecast arises, because SRIF_{PeV} rather than SRIF_{ArC} is released into portal blood via nerve terminals in the median eminence (38). We are not aware of direct data on this expectation in the female rodent. However, 5-min blood sampling

has revealed a statistically random association between SRIF and GH concentrations in cavernous-sinus blood of the unanesthetized ewe (77).

Hypothalamic neuronal control involves complementary, parallel, sequential and intersecting signaling pathways (29;54;62). The current parsimonious formulation does not exclude plausible additional effectors, such as NPY, enkephalin, galanin, neurotensin, substance P and leptin *inter alia* (29;55). The generality of this basic formulation permits inclusion of other inputs, when clarified further. We subsume aggregate (unknown) collateral effects under a single stochastic term in the primary deterministic network; *viz.*, by way of allowable random variability in the sensitivity of GHRH to inhibition by total SRIF in ArC. Elevating stochastic input by up to 3.5-fold in simulated realizations establishes that the *primary timing properties* of GH volleys and GHRH-SRIF_{ArC} oscillations do not require formal inclusion of other (non-GHRH, non-SRIF) signals [see *Perspectives*, below]. This inference does not exclude important modulatory effects of one or more collateral or supplementary pathways. Indirect evidence to this end is illustrated by: (a) retention of physiological GH pulse frequency (despite profound reduction in burst amplitude) in rare patients harboring a truncational mutation of the GHRH-receptor gene (63); (b) unchanged GH pulse frequency and reduced GH and IGF-I concentrations in the female transgenic mouse with knock-down of the neuronal ghrelin-receptor gene (67); and (c) lack of feminization of somatic growth (and, therefore, presumptive retention of a masculine GH release profile) in the male transgenic mouse with enforced silencing of the somatostatin gene (45). The first observation could indicate that: (a) reported CNS actions of GHRH proceed via a receptor nonidentical to that transcribed in the pituitary or another GHRH-stimulatable

receptor in neurons [e.g. VIP (74)]; and/or (b) endogenous GHRH-SRIF_{ArC} interactions evolve via non-GHRH interneuronal signals; e.g., dopamine, galanin, neurotensin and/or other neurotransmitters expressed in GHRHergic neurons (18;22). The outcome of ghrelin-receptor silencing predicts an amplifying role of endogenous ghrelin in the female. Lastly, male-pattern somatic growth under transgenic somatostatin depletion points to possible contributions to GH pulse renewal by other neuronal systems and/or non-SRIF products of SRIFergic neurons, such as substance P, NPY and enkephalin peptides (16).

In summary, we construct and analyze an ensemble model of systemic-diencephalic and bipartite intrahypothalamic mediation of self-renewable GHRH and GH pulses, in which: (a) reversible GH negative feedback induces time-delimited SRIF_{PeV} secretion; (b) effectual SRIF_{PeV} outflow restrains each of ArC GHRH secretion, oscillatory GHRH feedforward on SRIF_{ArC}, and pituitary GH release, thereby enforcing *intervolley* quiescence; and (c) a cycle of inhibition and disinhibition induced by SRIF_{PeV} outflow in (a) restrains and unleashes the short-latency mediobasal hypothalamic GHRH and SRIF_{ArC} oscillator, which mediates rapid *intravolley* bursts of GHRH and GH release. The current network-like construction accounts for fundamental pulse renewal, viz.: (a) infrequent, high-amplitude GH pulse volleys in the adult male rat; and (b) frequent, low-amplitude individual GH secretory bursts in the adult female rat. Moreover, extended ensemble formalism explicates a set of previously unexplained experimental outcomes associated with each of: (i) anterolateral deafferentation of the mediobasal hypothalamus; (ii) central-neuronal delivery of SRIF or GHRH; (iii) constant

systemic GHRH infusion; (iv) post-SRIF rebound release of GHRH and GH; (v) electrical stimulation of PeV; and (vi) partial silencing of CNS GH-receptor gene.

PERSPECTIVES

A plausible proximate basis for recurrent burst-like release of GHRH and, thereby, GH emanates from reciprocal feedforward and feedback-coupling mechanisms linking blood-borne GH to PeV SRIF; PeV SRIF to ArC GHRH unidirectionally; and ArC GHRH and ArC SRIF bidirectionally. However, primary connectivity may be complemented by redundant or alternative interneuronal relationships. For example, neuroanatomic data do not exclude possible complementary linkages among GHRH, SRIF and GH/IGF-I: **Figure 8A**.

[Insert **Figure 8A** here]

Nonexclusively, first, GH feedback could *potentiate* SRIF_{PeV} release via GH receptor-stimulated NPYergic pathways, which originate in ArC and arborize in PeV on SRIF neurons (9;71). This circuit would predictively reinforce the reciprocal relationship between hypophysial portal-venous GHRH and SRIF pulses inferred in the adult male rat (11;60). Second, species, gender and age may determine inverse coupling between the outflow of SRIF_{PeV} and GHRH (or GH), as inferred indirectly in the human, rat, sheep and pig (9;29;54). Third, autoneuronal SRIFergic inhibition and facilitation by way of SRIF-receptor subtypes (SST)-1 and -2 could: (a) brake or amplify mediobasal hypothalamic GHRH-SRIF_{ArC} oscillations within a volley; (b) create an *intranuclear* SRIF

oscillator via reciprocal SST-1/SST-2 interactions in ArC; (c) endow an *internuclear* SRIF oscillator via connections between $SRIF_{PeV}$ and $SRIF_{ArC}$ (3;64); and (d) mediate an SRIF oscillator via autoinhibitory SST-1 in PeV (40;41;44). And, fourth, autoneuronal GHRHergic feedback, if operative, could in principle generate an ArC GHRH oscillator (72;81). Albeit nonexclusive, such examples highlight more subtle corollary pathways, which may provide adaptive flexibility in the course of development, aging and metabolic stress.

The current basic construct offers an analytically objective platform to evaluate novel mechanisms of GH regulation. Several examples are: (a) the experimentally observed synergy between ghrelin and GHRH; (b) ghrelin's inhibition of ArC SRIF (but not PeV SRIF) neuronal activation; and (c) GH autofeedback on ArC NPY neurons, which restrain PeV SRIF release.

Feedback time delays (D) constitute key requirements for automaticity. Here, D1 and D2 correspond to *two different feedback loops*, viz., long (GH on PeV SRIF) and short (GHRH on ArC SRIF), respectively. The long delay engenders infrequent volleys and the short delay mediates rapid pulses within volleys. In modeling terms, the algebraic sum of feedback latencies does not equal the total delay realized for the coupled ensemble. In fact, for $D1 = 72$ min and $D2 = 12$ min, the predicted intervalley interval is 3.3 hr (male formulation) and intravolley interval 0.88 hr (both male and female constructs). Sensitivity analyses in **Figure 8B** shows that: (i) the *intravolley* interpeak interval becomes prolonged for greater delays of ArC GHRH \leftrightarrow SRIF (D1) [top]; and (ii) the *intervalley* interval increases for longer latencies of GH \rightarrow PeV SRIF

(D2) [*bottom*]. These relationships highlight stability of the linked feedback oscillators over a physiologically relevant range of time delays (see *Appendix*).

[Insert Figure 8B here]

The precise numerical values of intrahypothalamic signal kinetics and dose-response constants are not known. Conceptually, the absolute value is not so central to primary inferences about the relevance of connections, when a range of parameters is explored (Figure 8B). Few models require *a priori* knowledge of all system constants to explore postulates of ensemble behavior (as done here). Nonetheless, empirical determination of species-, gender-, age- and context-sensitive signaling scales within the CNS should enlarge experimental insights further.

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APPENDIX

To illustrate dynamic stability of the GHRH-SRIF_{ArC} system, we demonstrate how GHRH oscillations are generated by perturbation of a system that does not have a periodic solution, but has an asymptotically stable fixed point (focus), which attracts all trajectories in the phase space.

The mathematical representation of GHRH-SRIF_{ArC} interactions is given in METHODS as:

$$SRIF_{ArC}' = -k_2 SRIF_{ArC} + k_{r,2} \frac{(GHRH(t - D_2) / t_3)^{n_3}}{(GHRH(t - D_2) / t_3)^{n_3} + 1} \quad (A1)$$

$$GHRH' = -k_3 GHRH + k_{r,3} \left[\frac{1}{([SRIF_{ArC} + P(t)] / t_4)^{n_4} + 1} \right] \quad (A2)$$

The coefficients appearing in (A1)-(A2) are listed in Table 1.

First, one performs stability analysis of (A1)-(A2) without perturbation: $P(t) = 0$.

Stability analysis

For simplicity, change the notations and rewrite the system (A1)-(A2) as follows:

$$\begin{aligned} x' &= -\alpha x + f(y(t - D)) \\ y' &= -\beta y + g(x(t)) \end{aligned} \quad (A3)$$

where $\alpha = 5, \beta = 8, D = 0.2, f(n) = 300 \frac{(n/390)^{1.7}}{(n/390)^{1.7} + 1}$, and $g(m) = 12000 \frac{1}{(m/20)^3 + 1}$. The

functions $x(t)$ and $y(t)$ replace $SRIF_{ArC}(t)$ and $GHRH(t)$, respectively. Below, we show that system (A3) has a unique fixed point, which is locally asymptotically stable.

The fixed points of (A3) are the solutions (m, n) of the system:

$$\begin{aligned} \alpha m &= f(n) \\ \beta n &= g(m) \end{aligned} \tag{A4}$$

The uniqueness of the solution (m_0, n_0) follows from the fact that f is monotonously increasing and g monotonously decreasing. A numerical solution gives an approximate value for the unique fixed point $(m_0, n_0) \approx (28.897, 373.483)$.

Illustrating the stability of the fixed point (m_0, n_0) , as shown elsewhere¹, reduces to determining the characteristic values of the linearized system (A3) about the fixed point. The latter are solutions to the equation:

$$\lambda^2 + (\alpha + \beta)\lambda + \alpha\beta = f'(n_0)g'(m_0)e^{-\lambda D} \tag{A5}$$

Note that (A5) has infinite number of solutions λ , which reflect the fact that the delayed system (A3) is infinite dimensional. However, only a finite number have a nonnegative real value. The stability of (m_0, n_0) follows, if one proves that for all solutions λ of (A5), $\Re\lambda < 0$. To this end, compare the corresponding real and imaginary parts of the right-hand and left-hand terms in (A5) under the assumption that $\Re\lambda \geq 0$.

Let $\lambda = a + ib$, where $i = \sqrt{-1}$ and $a \geq 0$. From (A5) and using the approximate value for (m_0, n_0) , one gets $\lambda^2 + 13\lambda + 40 = -\omega e^{-0.2\lambda}$, where $\omega \approx 79.4197$. Therefore

$$\begin{aligned} a^2 - b^2 + 13a + 40 &= -\omega e^{-0.2a} \cos(-0.2b) \\ b(2a + 13) &= -\omega e^{-0.2a} \sin(-0.2b) = \omega e^{-0.2a} \sin(0.2b) \end{aligned} \tag{A6}$$

Using $a \geq 0$, $2a + 13 > 13$, and from the second equation in (A6),

$b = \omega e^{-0.2a} \sin(0.2b) / (13 + 2a)$. Therefore, $|b| \leq \omega / (13 + 2a) < 6.1$. This yields

¹ For an application-oriented presentation see for example: Beretta E., Kuang Y. Modeling and analysis of a marine bacteriophage infection with latency period. *Nonlinear analysis: Real World Applications*. 2(2001): 35-74.

$|0.2b| < 1.22 < \pi/2$ and consequently $\cos(0.2b) > 0$. On the other side, $b^2 < 37.21 < 40$ and from the first equation in (A6),

$$0 < a^2 - b^2 + 13a + 40 = -\omega e^{-0.2a} \cos(0.2b) < 0.$$

Therefore, the assumption $a \geq 0$ yields a contradiction. This establishes the asymptotic stability of the fixed point (m_0, n_0) .

Equation (A5) does not have pure real solutions. Thus, all characteristic values λ have non-zero imaginary parts and, therefore, correspond to oscillating solutions to the linearized system (A3). On this basis (and supported by numerical experiments), we suppose that locally the fixed point (m_0, n_0) behaves like a stable focus. We speculate that the overall behavior of the *in vivo* system (A3) is dominated by the oscillating solution to the linearized system, corresponding to the two conjugate characteristic values λ_0 that have the largest real part. Numerical tests provide the values $\lambda_0^\pm = a_0 \pm ib_0 \approx -0.5346 \pm i6.97987$. The term $e^{a_0 t}$ eventually governs the rate of convergence (damping) of the trajectory to the fixed point, while the term $e^{ib_0 t}$ controls the periodicity of the trajectory as it “winds” around the focus. Hence, the above reasoning predicts the periodicity of emerging peaks to be $2\pi/b_0 \approx 0.900186\text{h}$, which also emerges in computer simulations: **Figure 1 B**.

Assuming (without proof, but supported by extensive numerical representations) that the fixed point (m_0, n_0) is a global attractor for the system (A3), the fixed point is viewed as a global asymptotically stable focus.

Oscillations generated by perturbations

To illustrate the generation of oscillations by perturbing the system (A3), we consider the system

$$\begin{aligned} x' &= -\alpha x + f(y(t-D)) \\ y' &= -\beta y + g(x(t) + p(t)) \end{aligned} \quad (\text{A7})$$

where $p(t)$ is a SRIF_{PeV} perturbation, which synergizes with endogenous SRIF_{ArC} to suppress GHRH. If the function $p(t)$ gets sufficiently large by exceeding endogenous SRIF_{ArC} levels, the former controls system behavior by inhibiting $x(t)$, which in turn suppresses endogenous SRIF_{ArC} release. Therefore, when the perturbation is removed, (A7) will be transformed to (A3) with an initial condition away from the fixed point. Thereafter, the system trajectory will wind around the globally attracting focus (m_0, n_0) . Thus, $x(t)$ oscillates around m_0 and $y(t)$ oscillates around n_0 . **Figure 1 B** depicts the response of the GHRH-SRIF system in the ArC to a compactly supported perturbation $p(t)$. The effect is comparable when the decay rate of $p(t)$ is similar to that of the endogenous elimination of SRIF. Simulations show that a perturbation imposed (but not released) for an extended interval would eventually damp the rebound effect.

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LEGENDS

Figure 1 A. Schema of reciprocal connectivity envisioned between SRIF and GHRH within the arcuate nucleus (ArC). The triangle, marked “D₂”, designates the time delay in feedforward drive of GHRH on SRIF_{ArC} outflow.

B. Model simulation of the impact of brief (1-hr) and reversible suppression by SRIF_{PeV} of GHRHergic activity. Termination of the time-delimited SRIF_{PeV} impulse (centered at $t = 35.5$ hr) triggers ArC GHRH-SRIF oscillations, which are subsequently damped (Appendix).

Figure 2 Schema of primary connections among hypothalamic SRIF and GHRH, the anterior pituitary gland, and systemic GH pulses. The vertical dashed line separates SRIFergic neurons residing in ArC and PeV. The horizontal line demarcates hypothalamic from pituitary loci of control.

Figure 3 Illustrative time series forecast by the male-like GH feedback model (Table 1). Plots depict simultaneously evolving release of GH (upper left), GHRH in ArC (upper right), SRIF_{PeV} + SRIF_{ArC} (middle left), SRIF_{ArC} (middle right), and SRIF_{PeV} (bottom right). For clarity, interactions proceed solely under deterministic connections (Figure 2), with the exception of 5% random variability imposed on the inhibitory sensitivity of GHRH neurons to total SRIF_{PeV} + SRIF_{ArC} (bottom left) [Methods].

Figure 4 A Left. Female-like GH release patterns generated by decreasing the sensitivity of SRIF_{PeV} neurons to stimulation by GH feedback in the primary male model. The demarcated 10-hr interval illustrates the additional effect of elevating

variability in GHRH sensitivity to total SRIF inhibition from a nominal coefficient of variation of 5% to 18%. Right. Expanded view of GH output during the baseline time interval 6-20 hr.

B. Female-like GH feedback model simulated by a decrease in systemic-CNS GH-driven SRIF_{PeV} release. The decrease is 2.5-fold during the interval 30-40 hr, and 25-fold at all other times. Representations assume superimposed 5% random variability in ArC sensitivity to $\text{SRIF}_{\text{PeV}} + \text{SRIF}_{\text{ArC}}$ (see Figure 3)

Figure 5 Prediction of the regulatory impact of anterolateral deafferentation (ALD) of the mediobasal hypothalamus in the male model. ALD is assumed to deplete SRIF_{PeV} inflow to the GHRH- SRIF_{ArC} oscillator.

Figure 6 Panel A. Implications of varying degrees of putative SRIF_{PeV} depletion on pulsatile GH secretion in the male feedback formulation. Reduced SRIF_{PeV} availability is simulated by 3-fold (upper left panel), 4-fold (upper right panel), 10-fold (lower left panel) and 20-fold (lower right panel) more rapid elimination of SRIF_{PeV} . The last simulation mimics complete experimental deafferentation of the mediobasal hypothalamus (Figure 5).

Panel B. Projected outcome of central-neural SRIF-receptor activation in the male-like system induced by constant icv infusion of SRIF capable of access to GHRH in ArC, but not the pituitary gland. The reference output (left upper panel) changes progressively in response to graded elevation of SRIF_{ArC} . The latter is escalated arbitrarily to 5 ng/ml (right upper), 8 ng/ml (left bottom) and 10 ng/ml (right bottom).

Panel C. Representative model output in response to single icv injection of (metabolizable) SRIF in the male- (left panel) and female-like (right panel) feedback constructs. The arrows indicate the time of simulated SRIF delivery.

Figure 7 A. Forecast rebound-like secretion of GHRH and GH triggered by peripheral infusion and withdrawal of SRIF in the male (left) and female (right) feedback models. The solid bar signifies simulated constant *systemic* delivery of SRIF with access to both ArC and the pituitary gland.

B. Nature of inferred GH pulsatility driven by continuous *peripheral* delivery of a low (upper panels) and higher (lower panels) dose of GHRH in the male (left panels) and female (right panels) feedback models. GHRH is allowed to stimulate SRIF_{ArC}, *except* during the indicated interval (solid bar).

C. Attenuation of pulsatile GH release enforced by continuous *icv* delivery (time line 1000-1800) of GHRH, assuming access to SRIF_{ArC}, but not the pituitary gland. Output is shown for the male (left panel) and female (right panel) neuroregulatory networks. The solid bar identifies the interval of allowable ArC exposure to icv GHRH.

Figure 8 Feedback construct illustrating selected additional (unmodeled) interactions among GH, IGF-I, SRIF and GHRH [*Perspectives*]. Key distinctions from the current formulation include: (a) direct or indirect GHRHergic stimulation of SRIF_{PeV} [top left]; (b) SRIF receptor-subtype (SST) -1 and -2 facilitation and inhibition of cognate neurons in ArC [top right]; (c) SRIF_{ArC} SST-specific restraint of

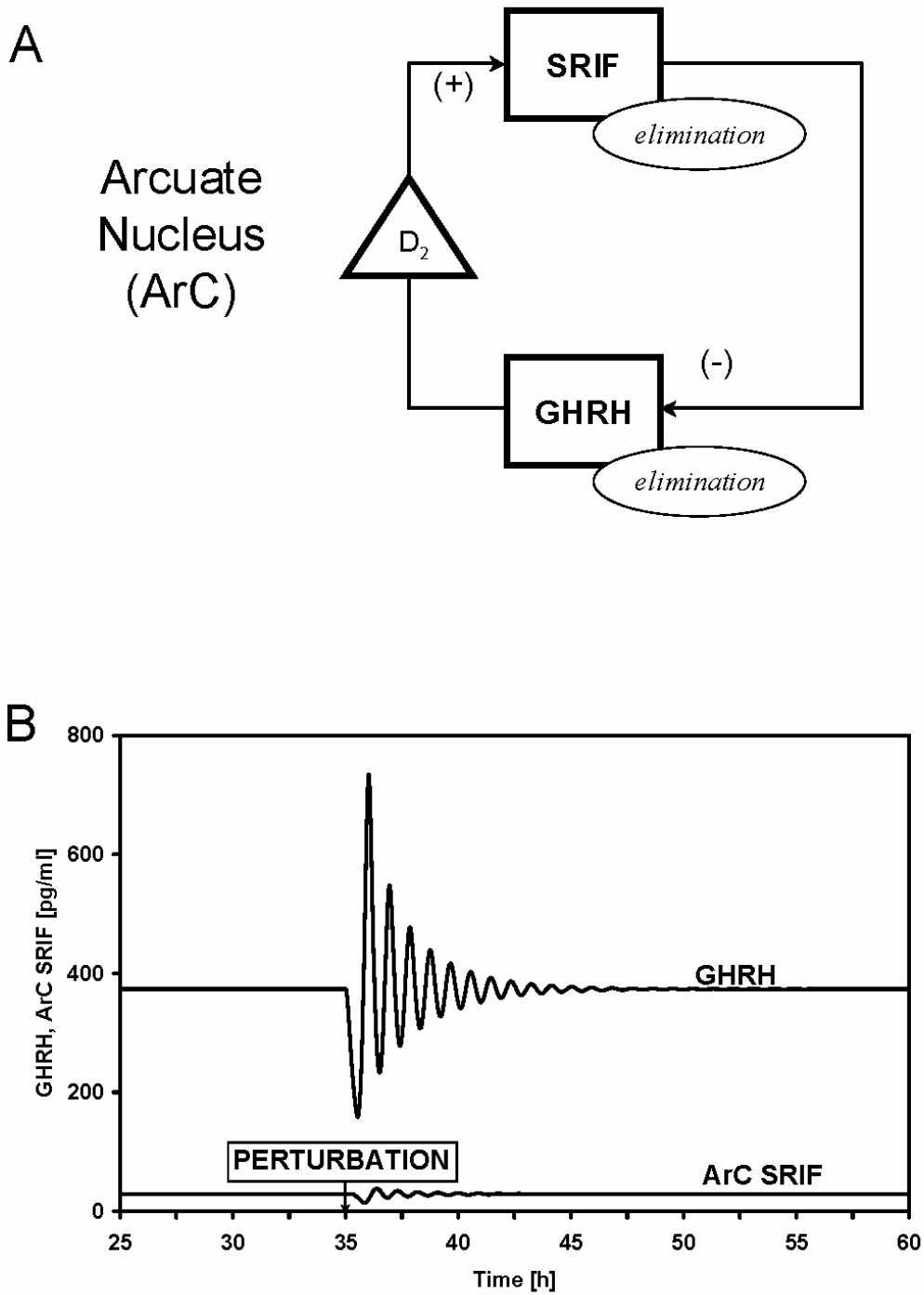
GHRH release [bottom left]; and (d) autofeedback via GH-generated IGF-I in the CNS [bottom right].

Table 1. *Summary of Core Interactive Constants in the GH Autofeedback Construct.*

	Rate Constant		Control (Hill) Functions		
	Elimination	Release	ED ₅₀ or ID ₅₀ *	Slope**	Delay
GH	$k_1 = 2.7 \text{ h}^{-1}$	$k_{r,1} = 5700 \text{ ng}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$	$t_5 = 112 \text{ ng}\cdot\text{ml}^{-1}$	$n_5 = 2$	$D_1 = 1.2 \text{ h}^\dagger$
SRIF _{ArC}	$k_2 = 5 \text{ h}^{-1}$	$k_{r,2} = 300 \text{ pg}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$	$t_4 = 20 \text{ pg}\cdot\text{ml}^{-1}$	$n_4 = 3$	
SRIF _{PeV}	$k_4 = 5 \text{ h}^{-1}$	$k_{r,4} = 600 \text{ pg}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$	$t_2 = 20 \text{ pg}\cdot\text{ml}^{-1}$	$n_2 = 4$	
GHRH	$k_3 = 8 \text{ h}^{-1}$	$k_{r,3} = 12000 \text{ pg}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$	$t_1 = 780 \text{ pg}\cdot\text{ml}^{-1}$ $t_3 = 390 \text{ pg}\cdot\text{ml}^{-1}$	$n_1 = 5$ $n_2 = 1.7$	$D_2 = 0.2 \text{ h}^\ddagger$

*ED₅₀ = half-maximally stimulatory concentration; ID₅₀ = half-maximally inhibitory concentration; **sensitivity or steepness of dose-response function; [†]D₁ = feedforward latency for systemic GH to drive SRIF_{PeV} release; and [‡]D₂ = feedforward time delay for GHRHergic outflow to stimulate SRIF_{ArC} neurons

Fig 1 A & B



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Fig 2

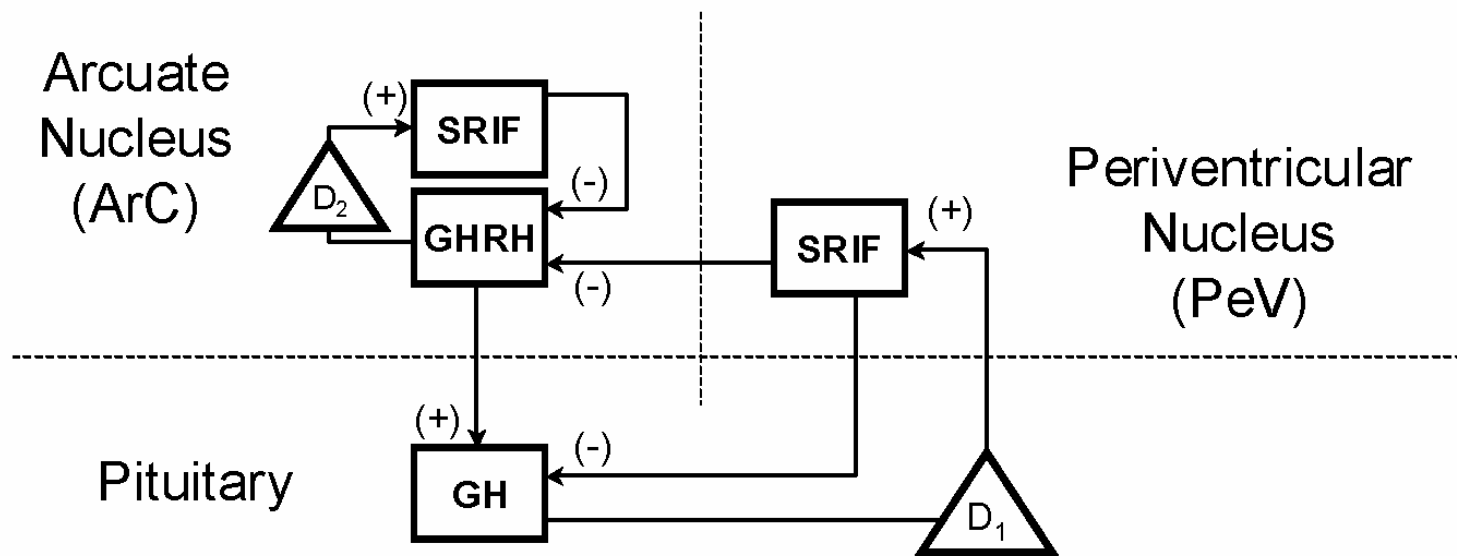


Fig 3

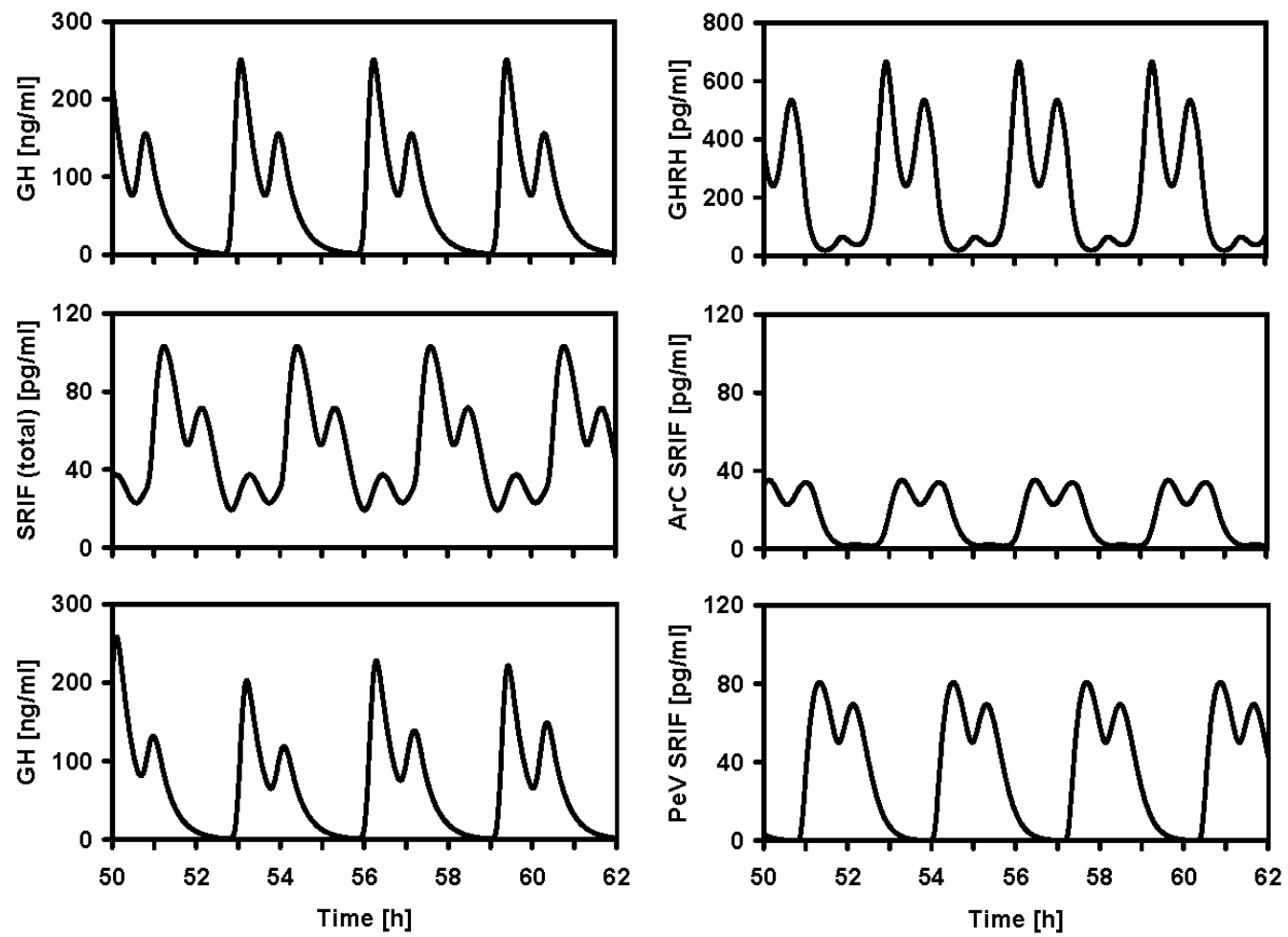


Fig 4 A & B

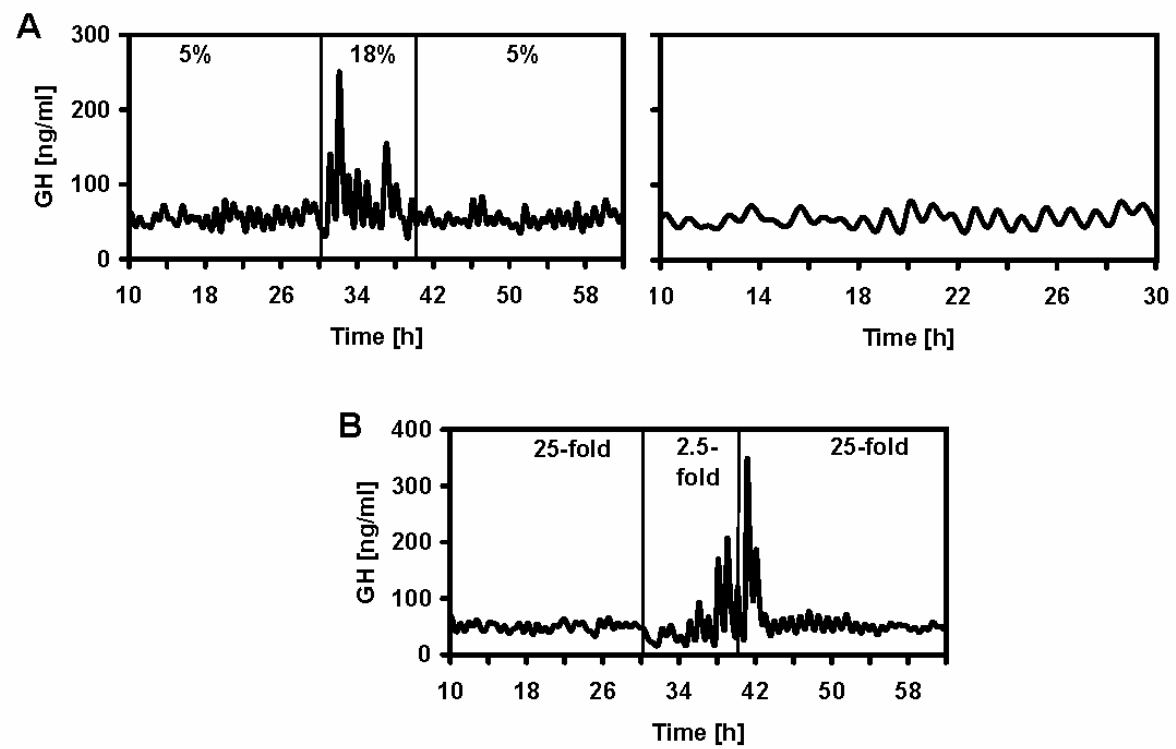


Fig 5

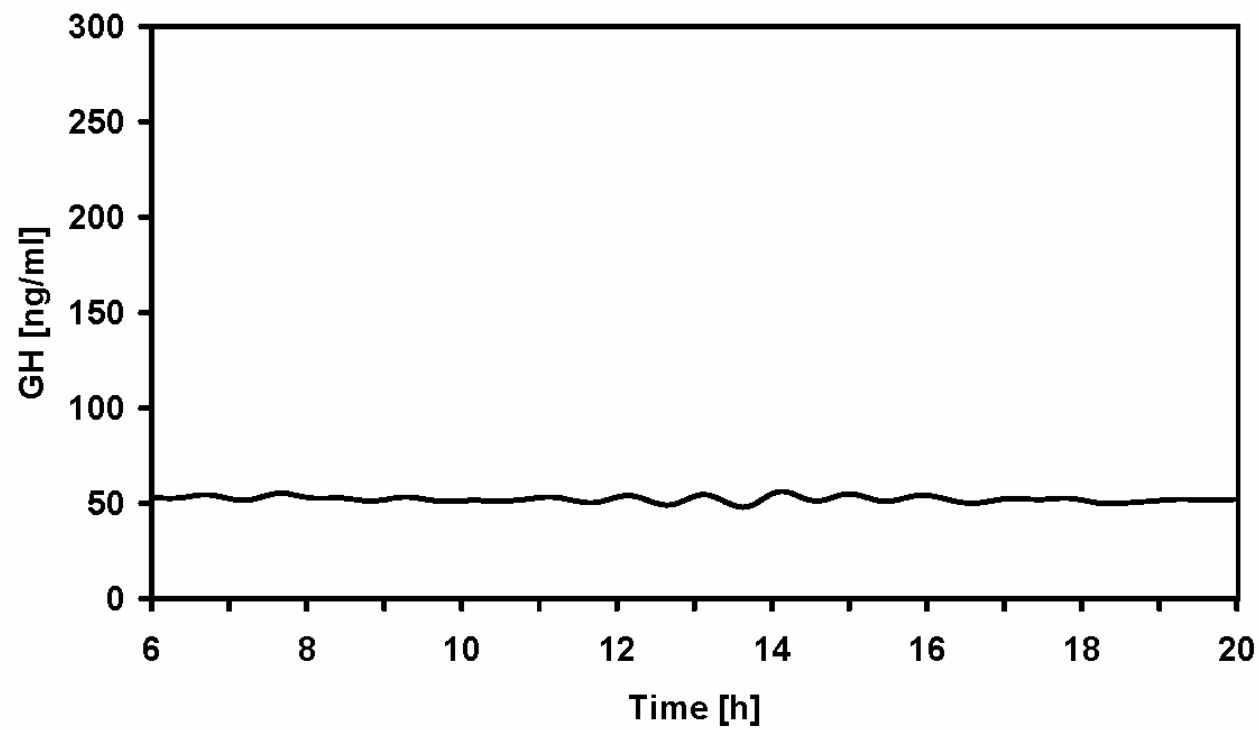


Fig 6A

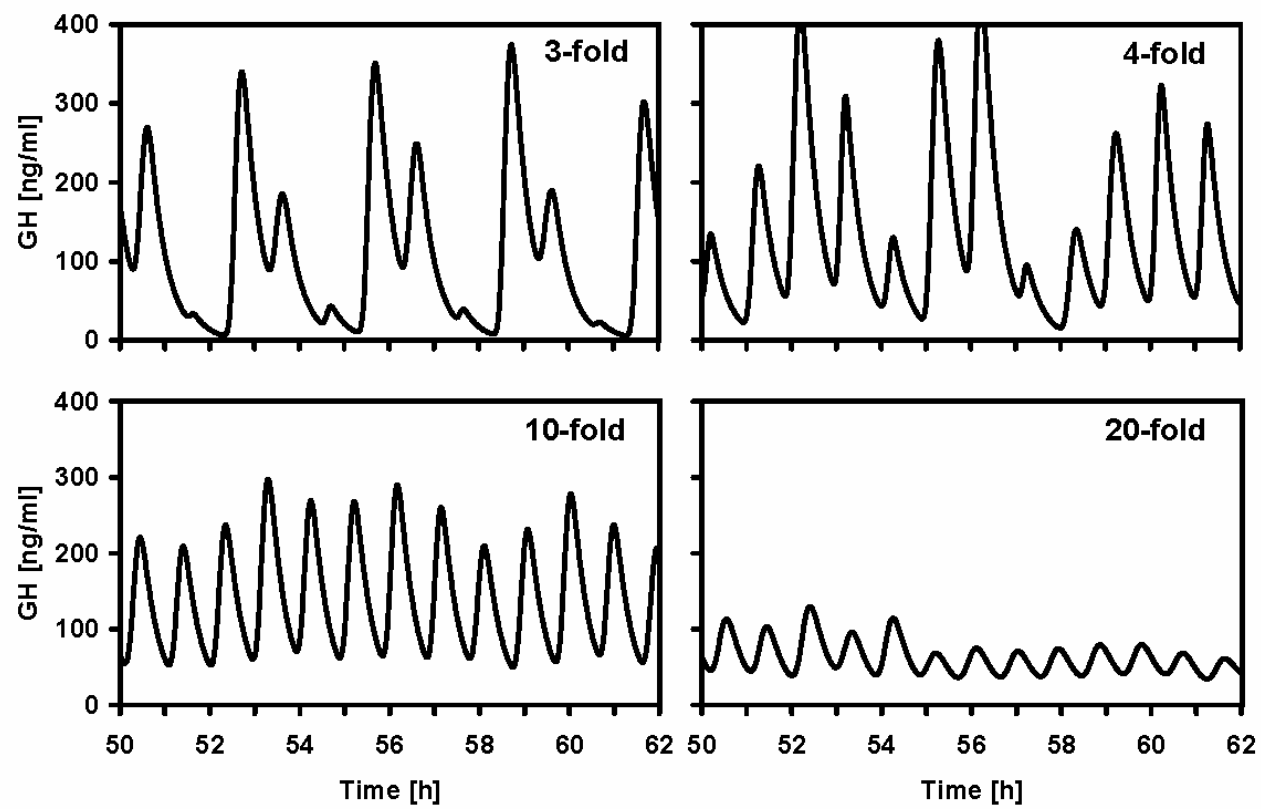


Fig 6B

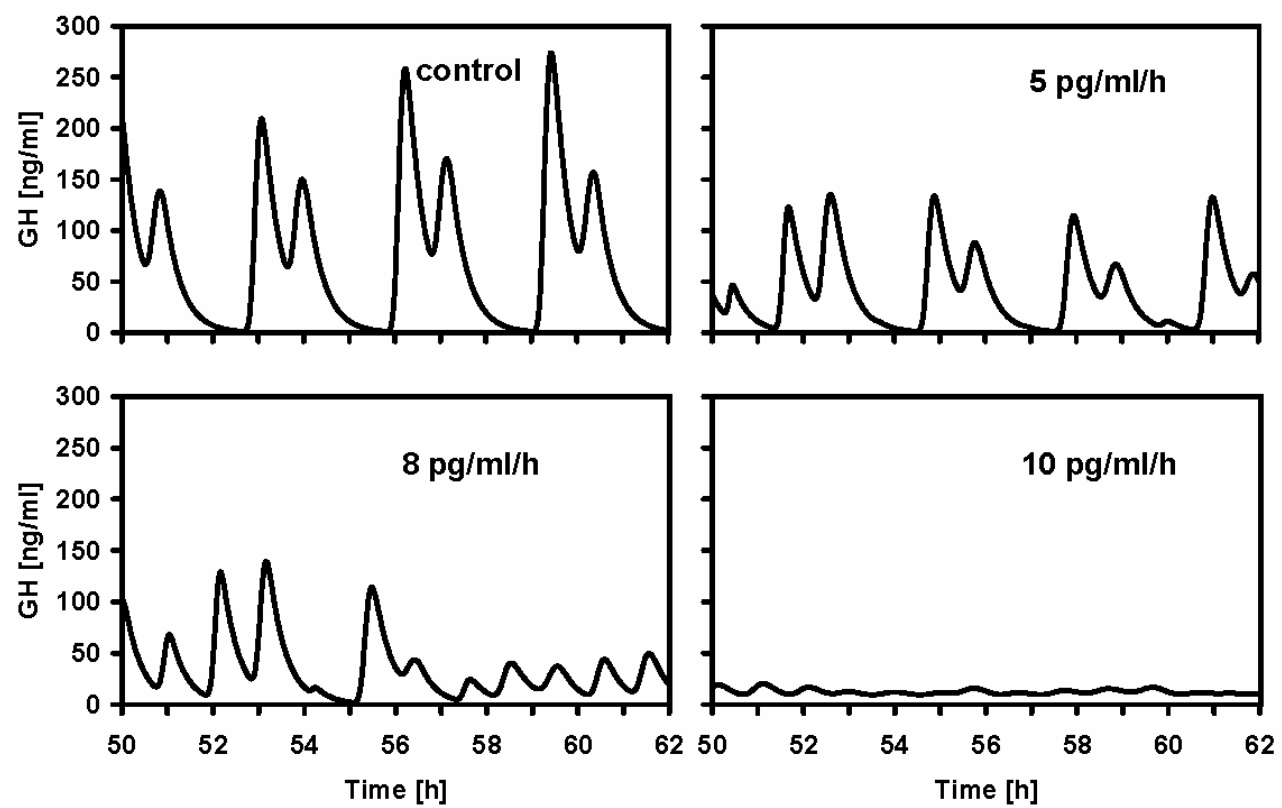


Fig 6C

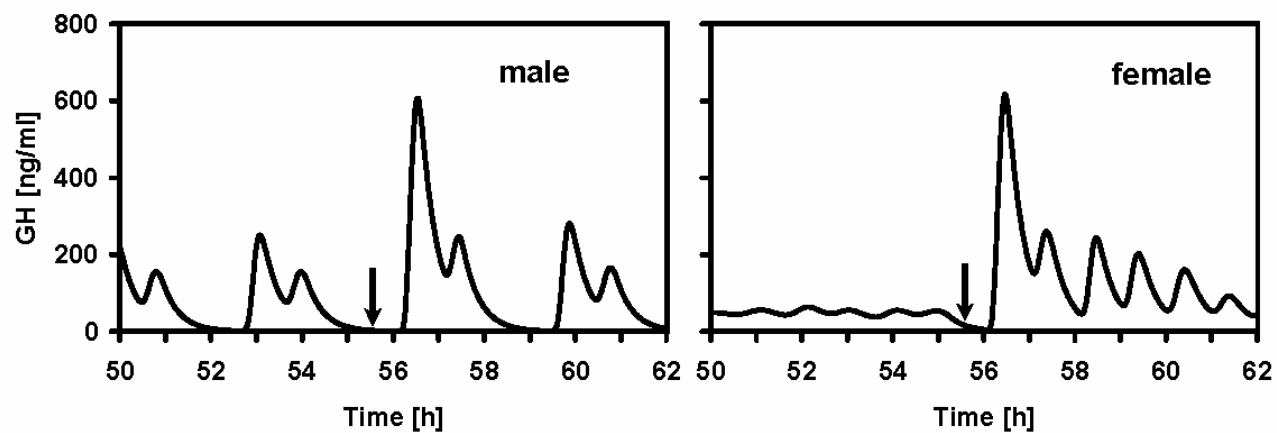


Fig 7 A,B & C

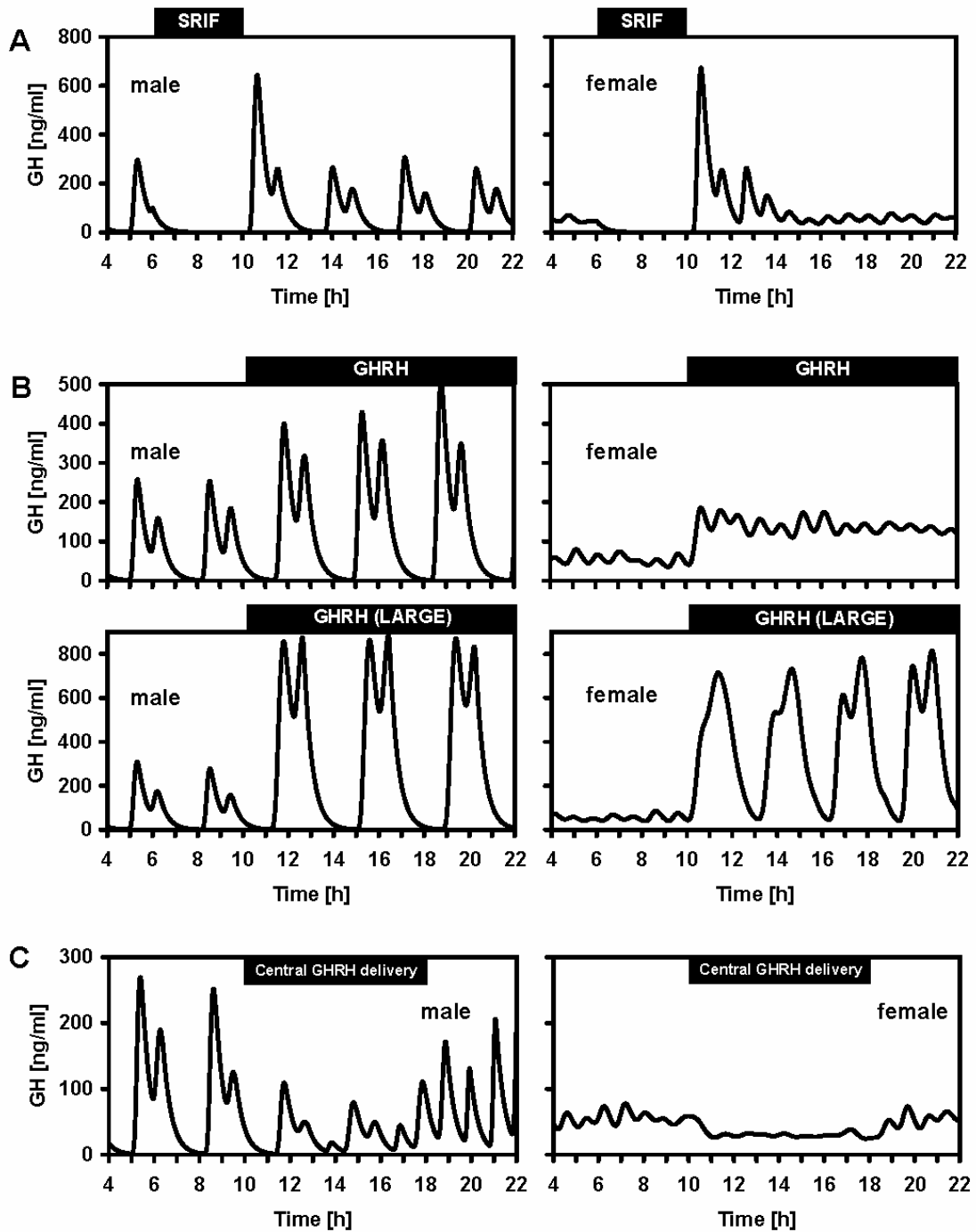


Fig 8A

Systemic-Diencephalic GH Autofeedback Complex

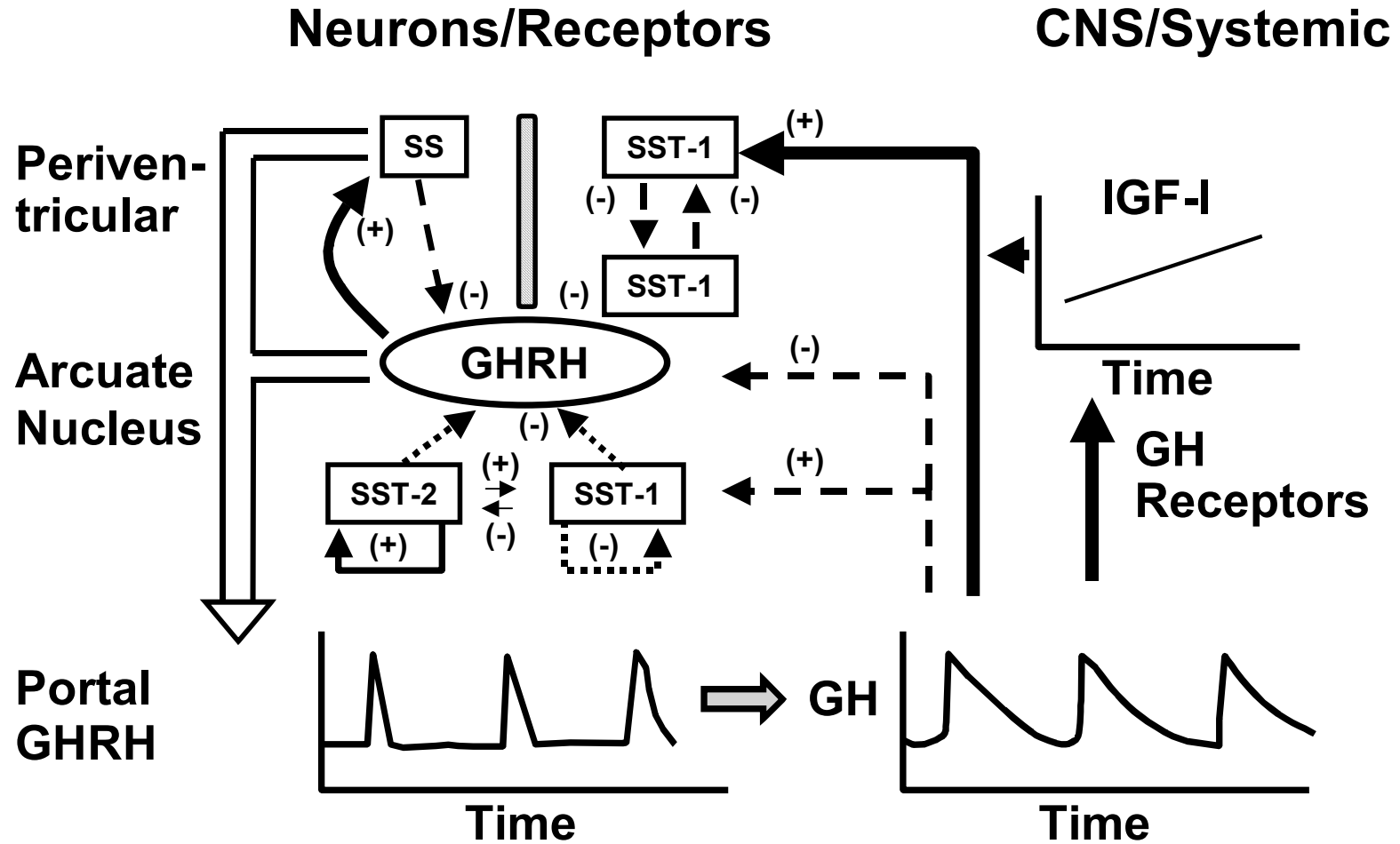


Fig 8B

Bipartite Oscillator: latency analysis

