Putative GH pulse renewal: periventricular somatostatinergic control of an arcuate-nuclear somatostatin and GH-releasing hormone oscillator

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Farhy, Leon S., and Johannes D. Veldhuis. Putative GH pulse renewal: periventricular somatostatinergic control of an arcuate-nuclear somatostatin and GH-releasing hormone oscillator. Am J Physiol Regul Integr Comp Physiol 286: R1030–R1042, 2004. First published February 26, 2004; 10.1152/ajpregu.00473.2003.—Growth hormone (GH) pulsatility requires periventricular-nuclear somatostatin (SRIFPeV), arcuate-nuclear (ArC) GH-releasing hormone (GHRH), and systemic GH autoregulatory feedback. 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 1) infrequent volleys of high-amplitude GH peaks in the male, 2) frequent discrete low-amplitude GH pulses in the female, 3) disruption of the male pattern by severing SRIFPeV outflow to ArC, 4) stimulation of GHRH and GH secretion by central nervous system delivery of SRIF, 5) inhibition of GH release by central exposure to GHRH, and 6) a reboundlike burst of GH 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 1) blood-borne GH stimulates SRIFPeV secretion after a long latency, 2) SRIFPeV inhibits both pituitary GH and ArC GHRH release, 3) ArC GHRH and SRIFArC oscillate reciprocally with brief time delay, and 4) SRIFPeV represses and disinhibits the putative GHRH-SRIFArC oscillator. According to the present analytic construction, time-delayed feedback and feedback signaling among SRIFPeV, ArC GHRH, and SRIFArC could endow the complex physiological patterns of GH secretion in the male and female.

FREQUENT SERIAL MEASUREMENTS of peripheral growth hormone (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 volleylike 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-central nervous system (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 (SRIFPeV) into hypophysial 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-nuclear GHRH release by GH (24), intermittent GH-induced SRIFPeV outflow (23), and pituitary somatotrope store-dependent reboundlike 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 hypophysial portal blood, presumptive coupling between SRIFArC 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 networklike 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, 16, 29, 55). First, intracerebroventricular 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 hypothala-
mus, 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 reboundlike 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, intracerebroventricular delivery of specific antisense oligodeoxynucleotide directed to the SRIF receptor (subtype 1) suppresses high-amplitude GH pulses in the male animal (40). Sixth, intracerebroventricular 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 reboundlike release of both GHRH and GH (10, 12, 52, 55, 60, 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 1) SRIF perikarya, axons, and terminal nerve fields and cognate receptors located in both ArC and PeV (54, 81); 2) SRIF receptors expressed by GHRH-positive perikarya in ArC and SRIF-positive perikarya in PeV and ArC (7); 3) 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); 4) synaptic contact of SRIF$_{PeV}$ and/or SRIF$_{ArC}$ with GHRHergic neurons in ArC (16, 44); 5) 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); 6) 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 7) connections between and autoinhibition by SRIF and SRIFergic neurons (46, 61).

**Proposed Simplified Network Structure**

In overview, the current networklike formulation assumes that 1) time-delayed systemic GH-induced SRIF$_{PeV}$ secretion suppresses both pituitary GH release and (ArC) GHRH secretion; 2) mediobasal hypothalamic, time-delayed, short-latency, reciprocal GHRH and SRIF$_{ArC}$ interactions create a damped intraArC oscillator; and 3) sequential activation and quiescence of SRIF$_{PeV}$ neurons serve to inhibit and amplify the amplitude of the GHRH-SRIF$_{ArC}$ oscillator. The last concept is illustrated in Fig. 1A and developed explicitly below.

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, $D_s$; 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 coupled pair of rate equations and corresponding Hill dose-response functions

$$SRIF'_{ArC} = -k_1SRIF_{ArC} + k_2 \left( \frac{GHRH(t - D_3)}{[GHRH(t - D_3)] + \epsilon} \right)^m$$

$$GHRH' = -k_4GHRH + k_3 \left( \frac{1}{[SRIF_{ArC} + P(t)] + \epsilon} \right)^m$$

In relation to terminology, the prime denotes the rate of change of concentration under feedback or feedforward control; 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; $k_{-1}$ and $k_{-3}$ 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 Fig. 1A); and $D_s$ 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 (see 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 postreceptor response downregulation, and/or collateral ArC SRIF receptor subtype-specific autoinhibition (9, 40). Figure 1B illustrates a waning train of GHRH and SRIF$_{ArC}$ bursts generated from coupled Eqs. 1 and 2. [The coefficients used in this simulation are summarized in Table 1 (discussed below).] In this example, the perturbation, $P(t)$, to the ArC oscillator is arbitrarily set to zero at all times, $t$, except $t = 35.5$ h, within which 1-h interval $P(t)$ increases to 20 and then decreases to zero. In physiological terms, outflow of SRIF$_{PeV}$ induced by initial GH pulses

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**Fig. 1. A:** Schema of reciprocal connectivity envisioned between somatostatin (SRIF) and growth hormone-related hormone (GHRH) within the arcuate nucleus (ArC). The triangle, marked “$D_s$” designates the time delay in feedforward drive of GHRH on SRIF$_{ArC}$ outflow. **B:** Model simulation of the impact of brief (1 h) and reversible suppression by SRIF$_{PeV}$ of GHRHergic activity. Termination of the time-delimited SRIF$_{PeV}$ impulse (centered at $t = 35.5$ h) triggers ArC GHRH-SRIF oscillations, which are subsequently damped (APPENDIX). PeV, periventricular-nuclear.

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**APPENDIX**

In this example, the perturbation, $P(t)$, to the ArC oscillator is arbitrarily set to zero at all times, $t$, except $t = 35.5$ h, within which 1-h 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 intervolley (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 networklike construct is built around experimentally verified core model components developed earlier (23, 24), which include 1) GHRH release from ArC into the hypothalamopituitary portal circulation, which stimulates eoscytic GH secretion (42, 54); 2) GH secretion into systemic blood, which evokes SRIF release from PeV after a distinct time delay, $D_1$ (11, 54, 58); 3) hypothalamic SRIF PeV neuronal outflow, which inhibits GHRHergic activity transsynaptically and/or internuncially (7, 15, 29); and 4) 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-driven GH secretion (42, 54); and $\gamma$ hypothalamic SRIF PeV neuronal outflow, which inhibits GHRHergic activity transsynaptically and/or internuncially (7, 15, 29); and 4) 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).

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 Fig. 2. Peptides (or interneuronal effectors) are subject to individual monoeponential elimination kinetics (not shown in Fig. 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 (Fig. 1B). Solely deterministic vs. 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. The half maximally inhibitory concentration of SRIF ($t_d$ in the Hill function in Eq. 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 × 100%) at 30-min intervals.

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

$$GH' = -k_1GH + k_{r,1} \left( \frac{[GHRH(t)]^{n_1}}{[GHRH(t)]^{n_1} + 1} \right)$$

$$SRIF_{ArC}' = -k_2SRIF_{ArC} + k_{r,2} \left( \frac{[GHRH(t - D_1)t_1^{n_2}]}{[GHRH(t - D_2)t_2^{n_2}]^{n_2} + 1} \right)$$

$$GHRH' = -k_3GHRH + k_{r,3} \left( \frac{1}{[[SRIF_{ArC} + SRIF_{PeV}]]^{n_3} + 1} \right)$$

$$SRIF_{PeV}' = -k_4SRIF_{PeV} + k_{r,4} \left( \frac{[GH(t - D_1)t_1^{n_4}]}{[GH(t - D_2)t_2^{n_4}]^{n_4} + 1} \right)$$

Relevant additional definitions include GH and SRIF PeV, which identify concentrations of the cognate peptides at time $t$; $k_1$ and $k_{r,1}$, 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 $n_1$, $n_2$, $n_3$, and $n_4$, 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 1) pituitary GH release requires simultaneous GHRH stimulation and submaximal competition by SRIF (note term after $k_{r,1}$ in Eq. 1); 2) 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); 3) SRIF ArC inhibits ArC GHRH secretion (Eq. 2); and 4) 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; hence, the criterion is a damped GHRH-SRIF ArC oscillator under intermittent inhibition and disinhibition by cycles of SRIF PeV outflow.

### RESULTS

### Reference Model Output

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 one in Fig. 3E) depict deterministic model output without random variation in total SRIF-inhibited GHRH secretion. Figure 3A shows recurrent infrequent GH volleys driven by time-delayed feedback of systemic GH on SRIF PeV. Figure 3B 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, Fig. 1B; see also APPENDIX). Sequential outflow and withdrawal

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**Table 1. Summary of core interactive constants in the GH autofeedback construct**

<table>
<thead>
<tr>
<th>Elimination</th>
<th>Release</th>
<th>ED$<em>{50}$ or ID$</em>{50}$</th>
<th>Slope</th>
<th>Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>$k_1 = 2.7$ h$^{-1}$</td>
<td>$k_{s,1} = 5,700$ ng·ml$^{-1}$·h$^{-1}$</td>
<td>$t_s = 112$ ng·ml$^{-1}$</td>
<td>$n_s = 2$</td>
</tr>
<tr>
<td>SRIF ArC</td>
<td>$k_2 = 5$ h$^{-1}$</td>
<td>$k_{s,2} = 600$ pg·ml$^{-1}$·h$^{-1}$</td>
<td>$t_s = 20$ pg·ml$^{-1}$</td>
<td>$n_s = 3$</td>
</tr>
<tr>
<td>SRIF PeV</td>
<td>$k_3 = 8$ h$^{-1}$</td>
<td>$k_{s,3} = 12,000$ pg·ml$^{-1}$·h$^{-1}$</td>
<td>$t_s = 390$ pg·ml$^{-1}$</td>
<td>$n_s = 1.7$</td>
</tr>
<tr>
<td>GHRH</td>
<td>$k_4 = 10$ h$^{-1}$</td>
<td>$k_{s,4} = 500$ pg·ml$^{-1}$·h$^{-1}$</td>
<td>$t_s = 780$ pg·ml$^{-1}$</td>
<td>$n_s = 5$</td>
</tr>
</tbody>
</table>

ED$_{50}$, half maximally stimulatory concentration; ID$_{50}$, half maximally inhibitory concentration; Slope, sensitivity or steepness of dose-response function; $^*D_1$, feedforward latency for systemic growth hormone (GH) to drive periventricular-nuclear somatostatin (SRIF PeV) release; and $^1D_2$, feedforward time delay for GH-releasing hormone (GHRH)ergic outflow to stimulate SRIF ArC neurons. ArC, arcuate-nuclear.
of SRIFPeV trigger an amplitude-damped train of GHRH and SRIFArC spikes within a volley (Fig. 3, B, D, and F).

Composite (volley and intravolley) GH oscillations in the adult malelike model arise as follows. In the evolution of infrequent compound peaks or clusterlike volleys, emergent GH pulses stimulate SRIFPeV outflow after a relatively long systemic-central nervous system (CNS) time delay. GH-induced SRIFPeV secretion simultaneously antagonizes pituitary release of GH and suppresses ArC GHRHergic activity. The joint effect is to terminate the volley and enforce an intervolley nadir of (typically undetectable) GH secretion. The nadir interval persists until GH concentrations decay in plasma and interneuronal fluids, thereby relieving drive of SRIFPeV output. Release of the GHRH-SRIFArC interaction from SRIFPeV restraint disinhibits higher-amplitude GHRH and SRIFArC spikes (Fig. 1B). The number of GHRH pulses in a given volley depends on 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 SRIFPeV between consecutive volleys determines whether the ArC oscillator is repressed to low or undetectable amplitude (Fig. 3B), 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.

Figure 3C illustrates the projected dynamics of total SRIF (SRIFArC + SRIFPeV) released into ArC based on strictly deterministic connections. Imposing small (5%) stochastic variability on GHRH neuronal sensitivity to total SRIFPeV restraint disinhibits higher-amplitude GHRH peaks and SRIFArC spikes (Fig. 1B). The number of GHRH pulses in a given volley depends on 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 SRIFPeV between consecutive volleys determines whether the ArC oscillator is repressed to low or undetectable amplitude (Fig. 3B), 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.

Diminished Feedback of GH on SRIFPeV Mimics the Sex Distinction in GH Release

To test whether the current construct forecasts that relaxation of GH-on-SRIFPeV feedforward could account for the femalelike GH profile, we increased the ED50 (lowered potency) for GH drive, t50, by 10-fold. This change substantially attenuates GH-driven SRIFPeV release. According to model simulations, sufficient muting of SRIFPeV 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 (see Introduction) (Fig. 4A, right).

In view of the conceptual importance of the above outcome, as an alternative strategy to attenuate GH-induced SRIFPeV drive, we reduced the rate of SRIFPeV release (k4) by 25- or 2.5-fold (Fig. 4B). In this experiment, low variability of GHRH neuronal sensitivity to SRIF was fixed at 5%. Reduced SRIFPeV secretion generated epochs of variable-amplitude GH pulses as reported in the conscious female rat during intervals of extended (24 h) and frequent (5 min) sampling (13). To examine the impact of stochastic variability in GHRH neuronal sensitivity to SRIF in the female model, the coefficient of variance (CV) of t4 (METHODS) was increased from 5% (nominal realizations, above) to 18% over a brief time window (Fig. 4A, left). The latter adjustment yielded variable-amplitude GH pulses with no change in mean burst frequency. This notion may be relevant, if the SRIFPeV-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

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\textsubscript{PeV} outflow to GHRH, thereby abrogating cycles of inhibition and disinhibition of the GHRH-SRIF\textsubscript{ArC} oscillator. This hypothesis was modeled by introducing a 100-fold increase in k\textsubscript{s} (GH ED\textsubscript{50}) to limit GH inhibition via SRIF\textsubscript{PeV} and 5-fold decrease in variability (CV) of GHRH sensitivity to SRIF (Fig. 5). The resultant profiles are marked by irregular and diminutive, if any, GH pulses.

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

In this simulated context, progressive SRIF\textsubscript{PeV} depletion restricts the capability of GH feedback to trigger ArC GHRH/SRIF volleys and elevates intervolley release of GHRH and GH. The resultant output pattern emulates features of the female GH profile (Fig. 6A, bottom right). In corollary analyses, we increased k\textsubscript{s} (GH ED\textsubscript{50}) to simulate GH-receptor downregulation in PeV. Up to 2.8-fold blunting of GH drive to SRIF\textsubscript{PeV} augmented GH pulse amplitude, burst frequency, and interpulse nadir GH concentrations, thereby mimicking the time patterns shown in Fig. 6A, top right. Comparable outcomes were reported by Pellegrini et al. (58) after 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 k\textsubscript{s} analytically in the male predicted feminization of the GH profile comparably to that shown in Fig. 6A, bottom. Deprivation of effectual GH drive of SRIF\textsubscript{PeV} outflow is inferable by female resistance to exogenous GH autofeedback action (Introduction).

Intracerebroventricular SRIF Infusion

Further simulations indicated that central delivery of SRIF serves to repress GH pulsatility only if the infused peptide is able to 1) leave the CNS and inhibit the pituitary gland, and/or 2) enter ArC and inhibit the GHRH/SRIF\textsubscript{ArC} oscillator (Fig. 6B).

In contrast to the inhibitory impact of peripheral SRIF infusion, central (intracerebroventricular) 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 righthand side of Eq. 6 that incorporates a brief 1-h impulse to mimic transient SRIF\textsubscript{PeV} release (here centered on t = 55.5 h). The impulse is defined algebraically by the piece-wise continuous function

\[
\text{Inf}(t) = \begin{cases} 
0 & \text{if } t \leq 55 \\
55.5 + (t - 55.5) & \text{if } 55 < t < 55.5 \\
400 & \text{if } t \geq 56 \\
0 & \text{if } t \geq 56 
\end{cases}
\]

Fig. 4. A, left: femalelike GH release patterns generated by decreasing the sensitivity of SRIF\textsubscript{PeV} neurons to stimulation by GH feedback in the primary male model. Demarcated 10-h interval illustrates the additional effect of elevating variability in GHRH sensitivity to total SRIF inhibition from a nominal coefficient of variation of 5–18%. Right: expanded view of GH output during the baseline time interval 6–20 h. B: femalelike GH feedback model simulated by a decrease in systemic-central nervous system GH-driven SRIF\textsubscript{PeV} release. Decrease is 2.5-fold during the interval 30–40 h and 25-fold at all other times. Representations assume superimposed 5% random variability in ArC sensitivity to SRIF\textsubscript{PeV} + SRIF\textsubscript{ArC} (see Fig. 3.)

Fig. 5. Prediction of the regulatory impact of anterolateral deafferentation (ALD) of the mediobasal hypothalamus in the male model. ALD is assumed to deplete SRIF\textsubscript{PeV} inflow to the GHRH-SRIF\textsubscript{ArC} oscillator.
Figure 6. A: implications of varying degrees of putative SRIF<sub>PeV</sub> depletion on pulsatile GH secretion in the male feedback formulation. Reduced SRIF<sub>PeV</sub> availability is simulated by 3-fold (top left), 4-fold (top right), 10-fold (bottom left), and 20-fold (bottom right) more rapid elimination of SRIF<sub>PeV</sub>. Last simulation mimics complete experimental deafferentation of the mediobasal hypothalamus (Fig. 5). B: projected outcome of central-neural SRIF-receptor activation in the malelike system induced by constant intracerebroventricular infusion of SRIF capable of access to GHRH in ArC, but not the pituitary gland. Reference output (top left) changes progressively in response to graded elevation of SRIF<sub>ArC</sub>. Latter is escalated arbitrarily by 5 pg·ml<sup>-1</sup>·h<sup>-1</sup> (top right), 8 pg·ml<sup>-1</sup>·h<sup>-1</sup> (bottom left), and 10 pg·ml<sup>-1</sup>·h<sup>-1</sup> (bottom right). C: representative model output in response to single intracerebroventricular injection of (metabolizable) SRIF in the male (left)- and femalelike (right) feedback constructs. Arrows indicate the time of simulated SRIF delivery.

Figure 6C illustrates the prediction that time-delimited access of SRIF to ArC unleashes volleylike release of GH in both the male- and femalelike constructs. Compared with the malelike network, the femalelike model responds with more prolonged GHRH-ArC oscillations under a brief intracerebroventricular 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<sub>PeV</sub> outflow to damp and disinhibit the GHRH-SRIF<sub>ArC</sub> oscillator in the female than male construct (METHODS).
SRIF and GHRH Interactions

Below we illustrate three additional pivotal model-based forecasts: 1) reboundlike GH release after systemic imposition and withdrawal of SRIF inhibition, 2) sustained GH pulsatility during continuous peripheral GHRH stimulation, and 3) attenuated GH release caused by continuous central GHRH delivery. These predictions are unique in applying to both the male and female models.

Reboundlike GH secretion after systemic SRIF withdrawal. Figure 7A illustrates the model prediction that abrupt cessation of systemic SRIF infusion with access to ArC will induce reboundlike 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 after 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/SRIFArC oscillator. Withdrawal of systemic and ArC SRIF disinhibits GHRH neurons and the GHRH-SRIFArC 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).

Continuation of pulsatile GH release during constant peripheral GHRH delivery. Figure 7B tests the impact of assuming that systemically infused GHRH cannot directly stimulate...
SRIF_{ArC}. In the current model, this assumption predicts that 1) continuous GHRH infusion will dose dependently drive high-amplitude GH pulses of unchanged frequency; 2) minimal exogenous GHRH drive will be more effectual in the male than female feedback model (Figure 7B, top); and 3) higher (3-fold increased) constant GHRH stimulation will elicit maledlike volleys of GH pulses in both sexes (Fig. 7B, bottom), but only to the extent that elevated GH concentrations are allowed to trigger SRIF_{PeV} release in the female construct.

Repression of GH pulse amplitude by intracerebroventricular 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 (Fig. 7C). 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 GH 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 volleylike GH release (Fig. 7C, left).

**DISCUSSION**

The present construct formalizes and tests the simplified networklike hypothesis that in the adult male rodent 1) systemic GH pulses stimulate SRIF_{PeV}-dependent inhibition of somatotrope GH release, GHRH secretion into portal blood, and intrahypothalamic GHRH feedforward on SRIF_{ArC}, thereby enforcing an interinterval of reduced GH secretion; and 2) 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 volleylike 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: 1) the adult male rat with experimental partial or complete anterolateral deafferentation of the mediobasal hypothalamus (complete isolation); 2) 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 3) 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 1) elicit GHRH release by hypothalamic explants in vitro, and 2) stimulate a burst of GHRH and GH secretion after intracerebroventricular or intraperitoneal injection (4, 46, 49, 56). Specifically, the maledlike construct predicts that in these experimental contexts: 1) in vitro effects of SRIF may reflect successive inhibition and disinhibition of GHRH neurons; and 2) intracerebroventricular 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 (Fig. 6C). In experimental support of SRIF_{PeV} and GHRH interactivity, brief electrical stimulation of PeV neurons in the male rat evokes reboundlike hyperactivation of putatively GHRH ArC neurons with attendant burstlike 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 reboundlike GHRH secretion on termination of GHRH stimulation (Fig. 7A). 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 GH pulses, as predicted here (Fig. 3D).

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 intravenous 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 after intracerebroventricular administration in vivo, as reported by others (4, 48). In this regard, incubation with GHRH would directly stimulate SRIF_{ArC} release in vitro and intracerebroventricular 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 1) hypothalamic expression of GHRH-receptor mRNA (72); 2) single-neuron recordings showing GHRH-induced cellular signaling (17, 74); and 3) 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 malelike mechanisms of 1) GH-dependent, SRIF_{PeV}-mediated restraint of GHRH-SRIF_{ArC} coupling; and 2) reboundlike 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 malelike 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 1) reciprocally in the male rat; and 2) 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 additional effectors, such as NPY, enkephalin, galanin, neuropeptide Y, substance P, and leptin among others (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; namely, 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 1) 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); 2) unchanged GH pulse frequency and reduced GH and IGF-I concentrations in the female transgenic mouse with knockdown of the neuronal ghrelin-receptor gene (67); and 3) 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 1) 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 2) endogenous GHRH-SRIF_{ArC} interactions evolve via non-GHRH interneuronal signals; e.g., dopamine, galanin, neuropeptide Y, 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 modulation of self-renewable GHRH and GH pulses, in which 1) reversible GH negative feedback induces time-delimited SRIF_{PeV} secretion; 2) effectual SRIF_{PeV} outflow restrains each of ArC GHRH secretion, oscillatory GHRH feedback-forward on SRIF_{ArC}, and pituitary GH release, thereby enforcing intervolley quiescence; and 3) a cycle of inhibition and disinhibition induced by SRIF_{PeV} outflow in point 1 above restrains and unleashes the short-latency medio basal hypothalamic GHRH and SRIF_{ArC} oscillator, which mediates rapid intravolley bursts of GHRH and GH release. The current networklike construction accounts for fundamental pulse renewal, namely, 1) infrequent, high-amplitude GH pulse volleys in the adult male rat; and 2) frequent, low-amplitude individual GH secretory bursts in the adult female rat. Moreover, extended ensemble formalism explicated a set of previously unexplained experimental outcomes associated with 1) anterolateral deafferentation of the medio basal hypothalamus; 2) central-neuronal delivery of SRIF or GHRH; 3) constant systemic GHRH infusion; 4) post-SRIF rebound release of GHRH and GH; and 5) electrical stimulation of PeV; and 6) partial silencing of CNS GH-receptor gene.

Perspectives

A plausible proximate basis for recurrent burstlike 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 (Fig. 8A).

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
The current basic construct offers an analytically objective platform to evaluate novel mechanisms of GH regulation. Several examples are 1) the experimentally observed synergy between ghrelin and GHRH; 2) ghrelin’s inhibition of ArC SRIF (but not PeV SRIF) neuronal activation; and 3) GH autofeedback on ArC NPY neurons, which stimulate PeV SRIF release.

Feedback time delays (D) constitute key requirements for automaticity. Here, D1 and D2 correspond to two different feedback loops, namely, long (GH on PeV SRIF) and short (GHRR 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 inter volley interval is 3.3 h (male formulation) and intravolley interval 0.88 h (both male and female constructs). Sensitivity analyses in Fig. 8B shows that 1) the intravolley interpeak interval becomes prolonged for greater delays of ArC GHRH ↔ SRIF (D1) (top); and 2) the interval increase increases for longer latencies of GH ↔ PeV SRIF (D2) (bottom). These relationships highlight stability of the linked feedback oscillators over a physiologically relevant range of time delays (see APPENDIX).

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 (Fig. 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.

**APPENDIX**

To illustrate dynamic stability of the GHRH-SRIFArC 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-SRIFArC interactions is given in methods as

\[
SRIF_{ArC} = -k_1SRIF_{ArC} + k_2 \left[ \frac{GHRH(t-D)}{H_1} \right]^{n} + 1
\]

(A1)

\[
GHRH' = -k_2GHRH + k_3 \left[ \frac{1}{[SRIF_{ArC} + P(t)H_2]^{n} + 1} \right]
\]

(A2)

The coefficients appearing in Eqs. A1 and A2 are listed in Table 1.

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

**Stability analysis.** For simplicity, change the notations and rewrite the systems A1 and A2 as follows

\[
x' = -\alpha x + f[y(t-D)]
\]

\[
y' = -\beta y + g(x(t))
\]

where \(\alpha = 5, \beta = 8, D = 0.2, f(n) = 300 \left[ (n/390)^{-1/7} + (n/39)^{-1/7} + 1 \right] \) and \(g(m) = 12,000 \left[ (m/20)^{-1/7} + 1 \right] \). 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

\[ \alpha m = f(n) \]
\[ \beta n = g(m) \] (A4)

The uniqueness of the solution (m0, n0) 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 (m0, n0) \( \approx (28.987, 373.483) \).

Illustrating the stability of the fixed point (m0, n0), as shown elsewhere, reduces to determining the characteristic values of the linearized system A3 about the fixed point. The latter are solutions to the equation

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

Note that A5 has infinite number of solutions \( \lambda \), 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 (m0, n0) follows, if one proves that for all solutions \( \lambda \) of A5, \( \text{Re} \lambda < 0 \). To this end, compare the corresponding real and imaginary parts of the right-hand and lefthand terms in A5 under the assumption that

\[ \text{Re} \lambda \geq 0. \]

Let \( \lambda = \alpha + ib \), where \( i = \sqrt{-1} \) and \( b \geq 0 \). From A5 and using the approximate value for (m0, n0), one gets \( \lambda^2 + 13\lambda + 40 = -\omega e^{-0.2a} \), where \( \omega = 79.917 \). Therefore

\[ a^2 - b^2 + 13a + 40 = -\omega e^{-0.2a} \cos(-0.2b) \]
\[ b(2a + 13) = -\omega e^{-0.2a} \sin(-0.2b) = \omega e^{-0.2a} \sin(0.2b) \] (A6)

Using \( \alpha \approx 0 \), \( 2a + 13 > 13 \), and from the second equation in A6, \( b = \omega e^{-0.2a} \sin(0.2b)/(13+2a) \). Therefore, \( |b| = |\omega|/(13+2a) \). This yields \( |0.2b| < 1.22 < \frac{\pi}{2} \) 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 \( \alpha \approx 0 \) yields a contradiction. This establishes the asymptotic stability of the fixed point (m0, n0).

Equation A5 does not have pure real solutions. Thus all characteristic values \( \lambda \) 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 (m0, n0) 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 \( \lambda_0 \) that have the largest real part. Numerical tests provide the values \( \lambda_0 = a_0 \pm ib_0 = -0.5346 \pm 10.2203 \). The term \( e^{\alpha t} \) eventually governs the rate of convergence (damping) of the trajectory to the fixed point, whereas the term \( e^{\beta t} \) controls the periodicity of the trajectory as it “winds” around the focus. Hence, the above reasoning predicts the periodicity of emerging peaks to be \( 2\pi\sqrt{b_0} \approx 0.900186 \), which also emerges in computer simulations (Fig. 1B).

Assuming (without proof, but supported by extensive numerical representations) that the fixed point (m0, n0) 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

\[ x' = -\alpha x + f[y(t - D)] \]
\[ y' = -\beta y + g[x(t) + p(t)] \] (A7)

where p(t) is an SRIF_{pA} perturbation, which synergizes with endogenous SRIF_{pAC} to suppress GHRH. If the function p(t) gets sufficiently large by exceeding endogenous SRIF_{pAC} levels, the former controls system behavior by inhibiting x(t), which in turn suppresses endogenous SRIF_{pAC} 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 (m0, n0). Thus x(t) oscillates around m0 and y(t) oscillates around n0. Figure 1B 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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