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The minimal model of the hypothalamic–pituitary–adrenal axis

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Abstract This paper concerns ODE modeling of the hypothalamic–pituitary–adrenal axis (HPA axis) using an analytical and numerical approach, combined with biological knowledge regarding physiological mechanisms and parameters. The three hormones, CRH, ACTH, and cortisol, which interact in the HPA axis are modeled as a system of three coupled, nonlinear differential equations. Experimental data shows the circadian as well as the ultradian rhythm. This paper focuses on the ultradian rhythm. The ultradian rhythm can mathematically be explained by oscillating solutions. Oscillating solutions to an ODE emerges from an unstable fixed point with complex eigenvalues with a positive real parts and a non-zero imaginary parts. The first part of the paper describes the general considerations to be obeyed for a mathematical model of the HPA axis. In this paper we only include the most widely accepted mechanisms that influence the dynamics of the HPA axis, i.e. a negative feedback from cortisol on CRH and ACTH. Therefore we term our model the minimal model. The minimal model, encompasses a wide class of different realizations, obeying only a few physiologically reasonable demands. The results include the existence of a trapping region guaranteeing that concentrations do not become negative or tend to infinity. Furthermore, this treatment guarantees the existence of a unique fixed point. A change in local stability of the fixed point, from stable to unstable, implies a Hopf bifurcation; thereby, oscillating solutions may emerge from the model. Sufficient criteria for local stability of the fixed point, and an easily applicable sufficient criteria guaranteeing global stability of the fixed point, is formulated. If the latter is fulfilled, ultradian rhythm is an impossible outcome of the minimal model and all realizations thereof. The second part of the paper concerns a specific realization of the minimal model in which feedback functions are built explicitly using receptor dynamics. Using physiologically reasonable parameter values, along with the results of the general case,

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it is demonstrated that un-physiological values of the parameters are needed in order to achieve local instability of the fixed point. Small changes in physiologically relevant parameters cause the system to be globally stable using the analytical criteria. All simulations show a globally stable fixed point, ruling out periodic solutions even when an investigation of the 'worst case parameters' is performed.

Keywords HPA axis · CRH · Cortisol · ACTH · Dynamical system · Mathematical modeling

Mathematics Subject Classification (2000) 92c45 · 92b99 · 34d23 · 34d20 · 34c23 · 34c25

1 Introduction

The HPA axis is a biological system consisting of the hypothalamus, pituitary, and adrenal glands. The interactions between these are mainly constituted by three hormones.

The HPA axis plays an important role under stressed conditions in raising the concentration of the HPA axis hormones, which leads to energy directed to the organism (Savic and Jelic 2006). The return to basal hormone levels after a while is an important feature of the system when it is working properly.

Corticotropin releasing hormone (CRH) is secreted in the hypothalamus and released through the blood supply into the anterior pituitary, where it stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH moves within the bloodstream, and when reaching the adrenal glands, stimulates the secretion of cortisol. It is commonly known that cortisol inhibits the secretion of CRH through glucocorticoid receptors (GR) situated in the pituitary (Wilson and Foster 1992). In addition, cortisol also performs a negative feedback on the secretion of ACTH through GR situated in hypothalamus (Tortora and Derrickson 2006). This is illustrated in Fig. 1.

Stress causes the body to alternate (more frequently increase than decrease) the level of cortisol. Keeping cortisol concentration within a certain range is important. Too high a level of cortisol (hypercortisolism) can cause depression, diabetes, visceral obesity or osteoporosis (Conrad et al. 2009). Too low a concentration is also not desirable, since it can result in a disturbed memory formation or life-threatening adrenal crises (Conrad et al. 2009).

The cortisol concentration has a circadian rhythm and is typically low between 8 p.m. and 2 a.m. and rises to a peak in the period between 6 a.m. and 10 a.m. (Jelic et al. 2005). CRH is secreted in a rhythm with a frequency of one to three secretory periods per hour (often referred to as ultradian rhythm) (Chrousos 1998). Throughout the literature (Griffin and Ojeda 2004; Carroll et al. 2007) we see circadian as well as ultradian rhythms in the hormone concentration of ACTH and cortisol.

The frequency of ultradian rhythm is rather insensitive to stress, whereas the amplitude increases (Chrousos 1998). Examples of data showing circadian and ultradian rhythms are illustrated in Figs. 2 and 3.

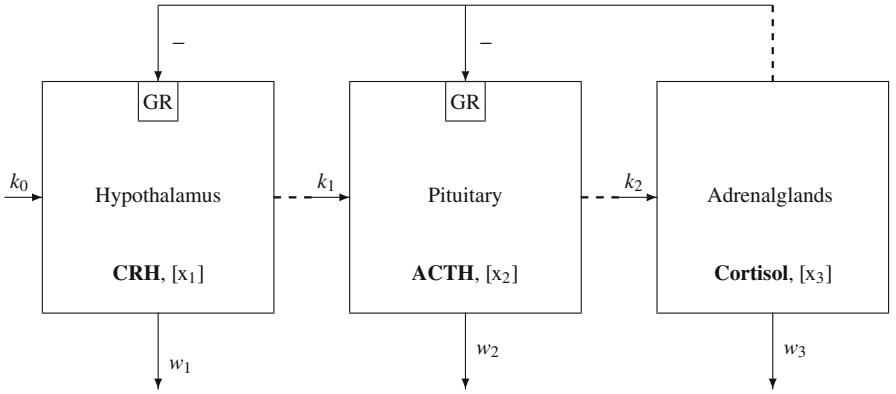

Fig. 1 Compartment diagram of the HPA axis. As illustrated above, there is a positive stimulus, through the rate constant k_0, on CRH partly controlled from hippocampus through neural pathways which is sensitive to the environment, e.g. daylight. CRH stimulates the production of ACTH through the reaction rate constant k_1 which stimulates the production of cortisol through the reaction rate constant k_2. Furthermore, cortisol performs negative feedback on CRH and ACTH through GR receptors. CRH, ACTH, and cortisol is eliminated through w_1, w_2 and w_3, respectively

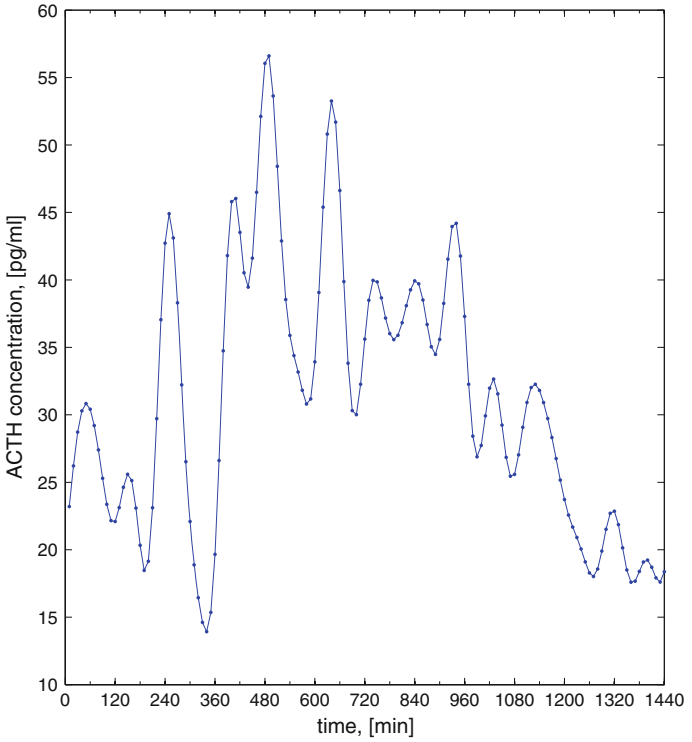

Fig. 2 Example of filtered ACTH data. Time $t = 0$ corresponds to midnight. Data was sampled every tenth minutes through a 24-h period

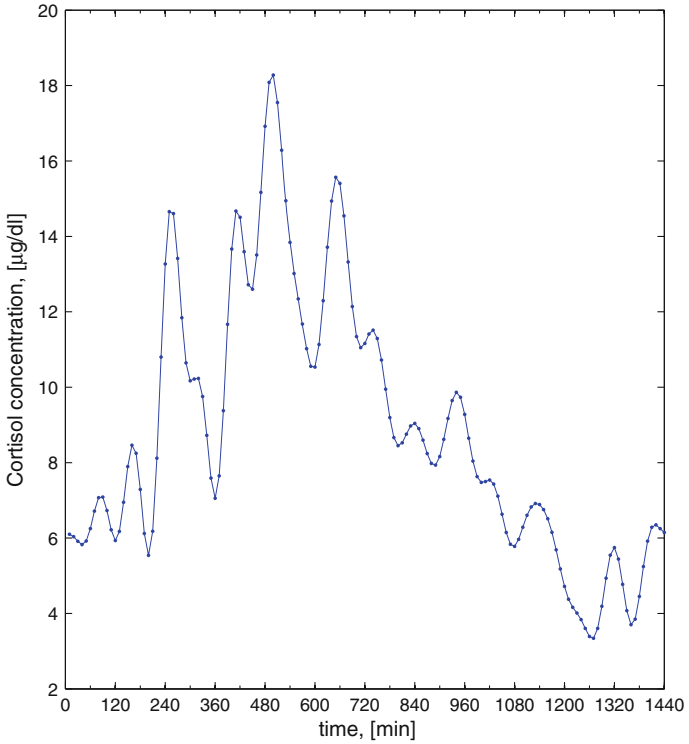

Fig. 3 Example of filtered cortisol data corresponding to the individual represented in Fig. 2. Time $t = 0$ corresponds to midnight. Data was sampled every tenth minutes through a 24-h period

Since several feedback mechanisms work simultaneously in the HPA axis, cause and effect may be hard to distinguish. A mathematical model may help in understanding this and can serve as an important tool for revealing the different ways in which a malfunction could occur. Known structures that a model should reflect are

- Feedbacks of cortisol on ACTH and CRH.
- Circadian rhythm of hormone concentrations.
- Ultradian rhythm of hormone concentrations.

Previous mathematical models of the HPA axis have been developed (Kyrylov et al. 2004, 2005; Jelic et al. 2005; Savic and Jelic 2005, 2006; Liu et al. 1999; Bingzhenga et al. 1990; Conrad et al. 2009; Veldhuis et al. 1989; Ben-Zvi et al. 2009; Gupta et al. 2007; Bairagi et al. 2008; Keenan et al. 2001; Keenan and Veldhuis 2003). However, the aim and assumptions of these models are different. The models presented by Gupta et al. (2007) and Ben-Zvi et al. (2009) consist of four, nonlinear differential equations and concern the existence of several stable steady states caused by the homodimerization of GR. Several stable steady state solutions are believed to explain illness due to hypocortisolism. The other models attempt to explain the circadian and diurnal rhythm seen in data. Separation of the circadian and ultradian rhythm is a common feature of these studies, where the ultradian rhythm appears to be considered

an inherent behaviour of the HPA axis, whereas the circadian rhythm is treated as external input to the axis, e.g. caused by daylight. The origin of the ultradian rhythm split these models mainly into two different categories. The models made by Veldhuis et al. (1989), Keenan et al. (2001) and Keenan and Veldhuis (2003) assume that the ultradian rhythm emerges from the bursting of hormones. The authors of Kyrylov et al. (2004, 2005), Jelic et al. (2005), Savic and Jelic (2005, 2006), Liu et al. (1999), Bingzhenga et al. (1990) and Bairagi et al. (2008) assume that the ultradian rhythm emerges from an unstable fixed point. Therefore, the overall aim of these analyses is to solve an autonomous system of nonlinear coupled differential equations and investigate the system for oscillating solutions corresponding to the ultradian rhythm of hormone levels.

If a stable fixed point turns unstable when perturbing a parameter then a Hopf bifurcation may occur. Thus, a limit cycle exists that may be interpreted as an ultradian rhythm. This is an important motivation for investigating the existence and stability of fixed points for the system.

The models presented in Kyrylov et al. (2004), Savic and Jelic (2005, 2006) and Bairagi et al. (2008) are systems of three differential equations with CRH, ACTH, and cortisol as variables. We would like to emphasize that the differential equations in Jelic et al. (2005) are based upon approximated stoichiometric relations between species. This is an important difference with respect to other models. The models presented in Kyrylov et al. (2005) and Liu et al. (1999) are systems of five differential equations with CRH, ACTH, corticosteroid-binding globulin (CBG) bound cortisol, albumin bound cortisol, and free cortisol as variables. Here, only the free cortisol is capable of performing negative feedback on the secretion of CRH and ACTH.

CBG binds approximately 90% of the cortisol. The binding and dissociation is extremely rapid (Wilson and Foster 1992). Saturation is visible for concentrations above 25 μg/dl, but this limit is above realistic concentration (Felig and Frohman 2001). 'Because CBG is the major cortisol-binding protein, the free cortisol in plasma is almost linearly related to the total cortisol at normal concentrations' (Felig and Frohman 2001). About 7% of plasma cortisol is bound to albumin (at 37°C). No saturation of albumin bound cortisol is present. Cortisol has a faster association and dissociation from albumin than from CBG (Felig and Frohman 2001). Using the above knowledge it is reasonable to consider the bound and the free form of cortisol to be in *quasi-equilibrium*. It is only free cortisol that is capable of interacting with the rest of the HPA-axis. The above reasoning means that the system of five differential equations, as performed by Kyrylov et al. (2005) and Liu et al. (1999), can be reduced to a system of three differential equations; thus making a system of three differential equations more appealing. Therefore, the rest of this paper will concern a system of three differential equations.

The model made by Bairagi et al. (2008) employed time delays to successfully obtain the desired Hopf bifurcation and thereby oscillating solutions. It is well known that time delays (if sufficiently large) may cause a Hopf bifurcation (Murray 2002). However, care must be taken to ensure that the delay is physiologically reasonable. Furthermore, Savic and Jelic (2005, 2006) also implement a time delay in their model but they reach the conclusion that the fixed point will remain stable. We will briefly return to this subject in Sect. 6.1.

Studies claiming to model the ultradian rhythm successfully without time varying input or time delay are Kyrylov et al. (2005) and Jelic et al. (2005). However, these may be criticized for lacking important physiological necessities. The work done by Christensen et al. (2007) shows that Jelic et al. (2005) have chosen parameter values that result in the concentration of ACTH deviating from measurements by a factor of 1,000. In order to obtain ultradian solutions, in Kyrylov et al. (2005), additional non-mechanistic non-linearities must be imposed on the system to ensure that hormone concentrations do not become negative or tend to infinity. It is our belief, that even the most basic model reflecting physiological reality should guarantee that solutions do not tend to infinity or become negative. Moreover, ultradian rhythm may be achieved for physiologically relevant parameter values.

In this paper we investigate the HPA axis with a mathematically parsimonious model, or more precisely, a family of models. This family, or class, of models we term 'the minimal model', since it involves only the most basic characteristics of the HPA axis. Neither time delay nor bound forms of cortisol are included in the model. The minimal model ensures realistic values of the solutions for a wide range of realizations consisting of three, coupled nonlinear differential equations obeying these few physiologically reasonable demands. Furthermore, this work reveals valuable information if future models are made that obey the demands of the minimal model.

A specific realization of the minimal model, based on receptor dynamics, is made to exemplify the general case. In this model, we choose parameters to be within a physiologically relevant interval. The model is not capable of showing the ultradian rhythm, which means that other specific realizations or further work are needed.

2 The minimal model

First, we will introduce the assumptions of the minimal model. In hypothalamus a positive stimulation, through the rate constant k_0, on the concentration of CRH, (x_1), is stimulated and partly controlled from hippocampus through neural pathways which are sensitive to the environment, e.g. daylight (de Kloet et al. 1998). CRH performs a positive stimulus on the concentration of ACTH, (x_2), through the rate constant k_1 and ACTH performs a positive stimulus on the concentration of free cortisol, (x_3), through the rate constant k_2. The hormone concentrations of CRH, ACTH and cortisol are depleted through w_1, w_2 and w_3. We model the negative feedback from the concentration of cortisol on the concentration of ACTH and the concentration of CRH as functions that decrease the positive stimulation on these hormones as cortisol concentration is increased. We consider the system without time varying input on CRH. Letting $g_1(x_3)$ denote the negative feedback from cortisol on CRH, and $g_2(x_3)$ denote feedback from cortisol on ACTH, we can generally formulate the system as Eq. (1):

$$\frac{dx_1}{dt} = k_0 g_1(x_3) - w_1 x_1 \tag{1a}$$

$$\frac{dx_2}{dt} = k_1 g_2(x_3) x_1 - w_2 x_2 \tag{1b}$$

$$\frac{dx_3}{dt} = k_2 x_2 - w_3 x_3. \tag{1c}$$

In order to ease the notation, we define the two functions $f_1(x_3) = k_0 g_1(x_3)$ and $f_2(x_3) = k_1 g_2(x_3)$. This produces the general system presented in Eq. (2):

$$\frac{dx_1}{dt} = f_1(x_3) - w_1 x_1 \tag{2a}$$

$$\frac{dx_2}{dt} = f_2(x_3) x_1 - w_2 x_2 \tag{2b}$$

$$\frac{dx_3}{dt} = k_2 x_2 - w_3 x_3, \tag{2c}$$

with elimination constants $w_1, w_2, w_3 > 0$, $f_1, f_2 : \mathbb{R}_+ \cup \{0\} \mapsto \mathbb{R}_+ \cup \{0\}$, $f_1, f_2 \in C^1$, $\sup(f_1(x_3)) = M_1, \sup(f_2(x_3)) = M_2, \inf(f_1(x_3)) = L_1, \inf(f_2(x_3)) = L_2, f_1(0) > 0$ and $f_2(0) > 0$. f_1 and f_2 have non-negative domains, since cortisol concentration is non-negative. The ranges of f_1 and f_2 are non-negative, since the positive stimulation of the hormones must not turn negative. The criteria that f_1 and f_2 are bounded reflects the saturation of receptors through which the feedbacks are realized. When no cortisol is present, the feedbacks must not shut down hormone production completely. This justifies $f_1(0) > 0$ and $f_2(0) > 0$. It is further assumed that the two feedbacks are negative, meaning that the derivatives $f_1'(x_3) < 0$, $f_2'(x_3) < 0, \forall x_3 \in \mathbb{R}_+ \cup \{0\}$. This class of models is very general since it is built upon very general constraints. It is hard to imagine a mathematical model of the HPA axis that includes the three hormones CRH, ACTH and cortisol not including feedback from cortisol on CRH and ACTH. However, based upon the approach of the mathematical model, the functional form of such feedback may be chosen differently. The results presented in this section will therefore be applicable to both present and future realizations of the minimal model stated in Eq. (2).

2.1 Existence and uniqueness of solutions

Since $f_1(x_3), f_2(x_3) \in C^1$, the system given in Eq. (2) fulfills the criteria for the existence and uniqueness of solutions for non-negative initial conditions of x_1, x_2, x_3.

2.2 All non-negative initial values lead to non-negative solutions

For $i \in \{1, 2, 3\}$ there is only one negative term in the expression for $\dot{x}_i$ and this negative term has x_i as a factor. Therefore, $x_i = 0$ and $x_j \geq 0$ implies that $\dot{x}_i \geq 0$ for $i \neq j$. This ensures that non-negative initial conditions lead to solutions that are non negative.

2.3 Existence of a unique fixed point

The fixed point condition of the Eq. (2) reduces to:

$$x_{1ss} = \frac{f_1(x_{3ss})}{w_1} \tag{3a}$$

$$x_{2ss} = \frac{f_1(x_{3ss})\, f_2(x_{3ss})}{w_1 w_2} \tag{3b}$$

$$x_{3ss} = \frac{k_2 f_1(x_{3ss})\, f_2(x_{3ss})}{w_1 w_2 w_3}. \tag{3c}$$

This means that, for each fixed point value x_{3ss}, steady state values x_{1ss} and x_{2ss} may be calculated using Eqs. (3a) and (3b). Equation (3c) may not be explicitly solvable for x_{3ss}. However, we can say something about existence of a solution of Eq. (3c) and approximate the solution numerically.

We define the functions

$$l(x_3) \equiv x_3, \quad \forall x_3 \geq 0, \tag{4}$$

and

$$r(x_3) \equiv \frac{k_2 f_1(x_3)\, f_2(x_3)}{w_1 w_2 w_3}, \quad \forall x_3 \geq 0. \tag{5}$$

Thus, finding x_{3ss} is equivalent to solving $l(x_3) = r(x_3)$. Since f_1 and f_2 are bounded, so is r

$$r(x_3) \leq \frac{k_2 M_1 M_2}{w_1 w_2 w_3} \equiv M_3, \quad \forall x_3 \geq 0. \tag{6}$$

Thus, $r(x_3) \leq M_3$ and $r(0) > 0$ guarantee that any non-negative fixed point value of x_3 is in the interval $(0; M_3] = (0; \frac{k_2 M_1 M_2}{w_1 w_2 w_3}]$. Then, any fixed point of Eq. (2) is in the set $(0; \frac{M_1}{w_1}] \times (0; \frac{M_1 M_2}{w_1 w_2}] \times (0; \frac{k_2 M_1 M_2}{w_1 w_2 w_3}]$. Since $f_1(x_3)$ and $f_2(x_3)$ are decreasing, so is $r(x_3)$. $l(x_3)$ increases linearly and $l(0) = 0$. Therefore, a unique fixed point exists.

2.4 Trapping region

A trapping region is a domain where a solution will never escape once there. It is a physiologically desirable property of the model, since this guarantees that reasonable initial values lead to reasonable hormone levels for all future time. A trapping region, U, exists for this model. Moreover, solutions outside this trapping region are attracted to the trapping region, which is the content of Proposition 1 (see Sect. 2.5). For $x_1 = \frac{M_1}{w_1}$ then $\dot{x_1} \leq 0$. For $x_1 = \frac{L_1}{w_1}$ then $\dot{x_1} \geq 0$. This means that $[\frac{L_1}{w_1}; \frac{M_1}{w_1}]$ is a 'trapping region' for x_1. Using this region for x_1 we can find a 'trapping region' for x_2, followed by one for x_3. For $x_1 \in [\frac{L_1}{w_1}; \frac{M_1}{w_1}] \equiv J_1$ and $x_2 = \frac{M_1 M_2}{w_1 w_2}$ then $\dot{x_2} \leq 0$. For $x_1 \in J_1$ and $x_2 = \frac{L_1 L_2}{w_1 w_2}$ then $\dot{x_2} \geq 0$. For $x_2 \in [\frac{L_1 L_2}{w_1 w_2}; \frac{M_1 M_2}{w_1 w_2}] \equiv J_2$ and $x_3 = \frac{k_2 M_1 M_2}{w_1 w_2 w_3}$ then $\dot{x_3} \leq 0$. For $x_2 \in J_2$ and $x_3 = \frac{k_2 L_1 L_2}{w_1 w_2 w_3}$ then $\dot{x_3} \geq 0$. This implies that $x_1 \in J_1$ and $x_2 \in J_2$ and $x_3 \in [\frac{k_2 L_1 L_2}{w_1 w_2 w_3}; \frac{k_2 M_1 M_2}{w_1 w_2 w_3}] \equiv J_3$ then $x_1(t)$, $x_2(t)$ and $x_3(t)$ are trapped in J_1, J_2 and J_3. This means we have the trapping region U

$$U \equiv J_1 \times J_2 \times J_3. \tag{7}$$

Note that any fixed point is contained in the trapping region.

2.5 Expansion of the trapping region

This section demonstrates that we may expand the trapping region, which is needed in Proposition 1. It is shown that any solution with non-negative initial conditions get arbitrarily close to U in finite time.

Define a larger box by adding an amount to each end point of J_i for each $i \in (1, 2, 3)$. Given $\varepsilon, \tilde{\varepsilon} \geq 0$ define $\varepsilon_2(\varepsilon)$, $\tilde{\varepsilon}_2(\tilde{\varepsilon})$, $\varepsilon_3(\varepsilon)$ and $\tilde{\varepsilon}_3(\tilde{\varepsilon})$ as:

$$\varepsilon_2(\varepsilon) \equiv 2\frac{M_2}{w_2}\varepsilon \tag{8}$$

$$\tilde{\varepsilon}_2(\tilde{\varepsilon}) \equiv 2\frac{L_2}{w_2}\tilde{\varepsilon} \tag{9}$$

$$\varepsilon_3(\varepsilon) \equiv 3\frac{k_2 M_2}{w_2 w_3}\varepsilon \tag{10}$$

$$\tilde{\varepsilon}_3(\tilde{\varepsilon}) \equiv 3\frac{k_2 L_2}{w_2 w_3}\tilde{\varepsilon}, \tag{11}$$

and define

$$\begin{aligned} W(\varepsilon, \tilde{\varepsilon}) \equiv & \left[\frac{L_1}{w_1} - \tilde{\varepsilon}; \frac{M_1}{w_1} + \varepsilon\right] \\ & \times \left[\frac{L_1 L_2}{w_1 w_2} - \tilde{\varepsilon}_2(\tilde{\varepsilon}); \frac{M_1 M_2}{w_1 w_2} + \varepsilon_2(\varepsilon)\right] \\ & \times \left[\frac{k_2 L_1 L_2}{w_1 w_2 w_3} - \tilde{\varepsilon}_3(\tilde{\varepsilon}); \frac{k_2 M_1 M_2}{w_1 w_2 w_3} + \varepsilon_3(\varepsilon)\right] \\ \equiv & I_1(\varepsilon, \tilde{\varepsilon}) \times I_2(\varepsilon, \tilde{\varepsilon}) \times I_3(\varepsilon, \tilde{\varepsilon}), \quad \forall \varepsilon, \tilde{\varepsilon} \geq 0. \end{aligned} \tag{12}$$

It is clear that $U = W(0, 0)$. By similar reasoning that U is a trapping region, $W(\varepsilon, \tilde{\varepsilon})$ is a trapping region $\forall \varepsilon, \tilde{\varepsilon} > 0$.

Proposition 1 *All solutions get arbitrarily close to U in finite time and then they stay close to U.*

Proof Given any initial condition, $\mathbf{x}(0)$, and any $\delta > 0$ we then need to show that there exists $T_0 < \infty$ such that $\text{dist}(\mathbf{x}(t), U) \leq \delta$ for $t \geq T_0$, where $\mathbf{x}(t) = (x_1(t), x_2(t), x_3(t))$.

If $\mathbf{x}(t) \in W(\varepsilon, \tilde{\varepsilon})$ for $t \geq T_0$ then $\text{dist}(\mathbf{x}(t), U) \leq \delta$ for sufficiently small $\varepsilon, \tilde{\varepsilon}$. Since $W(\varepsilon, \tilde{\varepsilon})$ is a trapping region for any $\varepsilon, \tilde{\varepsilon} \geq 0$ then we need to show that there exists $T_0 < \infty$ such that $\mathbf{x}(T_0) \in W(\varepsilon, \tilde{\varepsilon})$ for a given $\varepsilon, \tilde{\varepsilon} > 0$. If $x_1(0) \notin I_1(\varepsilon, \tilde{\varepsilon})$ then either $x_1 \in [x_1(0); \frac{L_1}{w_1} - \tilde{\varepsilon}]$ or $x_1 \in [\frac{M_1}{w_1} + \varepsilon; x_1(0)]$. Let us first consider the latter.

For any $\varepsilon > 0$, $\dot{x}_1 < 0$ for $x_1 \in [\frac{M_1}{w_1} + \varepsilon; x_1(0)]$. This ensures there exists $T_0' < \infty$, such that $x_1(T_0') \in I_1(\varepsilon, \tilde{\varepsilon})$. Notice that $\max\{\dot{x}_1 : x_1 \in [\frac{M_1}{w_1} + \varepsilon; x_1(0)]\} = -v_1 < 0$,

thus $\dot{x}_1 \leq -v_1 < 0$ on $[\frac{M_1}{w_1} + \varepsilon; x_1(0)]$. Hence, $x_1(T_0') = x_1(0) + \int_0^{T_0'} \dot{x}_1(t)dt < x_1(0) - v_1 T_0'$. Choose $T_0' = \frac{x_1(0) - M_1/v_1 - \varepsilon}{v_1}$ then $x_1(t) \in I_1(\varepsilon, \tilde{\varepsilon})$ for $t \geq T_0'$.

If $x_1(0) \in [x_1(0); \frac{L_1}{w_1} - \tilde{\varepsilon}]$, then for any $\tilde{\varepsilon} > 0$, $\dot{x}_1 > 0$. As before, there exists $T_0'' < \infty$ such that $x_1(T_0'') \in I_1(\varepsilon, \tilde{\varepsilon})$. $\min\{\dot{x}_1 : x_1 \in [x_1(0); \frac{L_1}{w_1} - \tilde{\varepsilon}]\} = \tilde{v}_1 > 0$, thus $\dot{x}_1 \geq \tilde{v}_1$ on $[x_1(0); \frac{L_1}{w_1} - \tilde{\varepsilon}]$. Choose $T_0'' = \frac{L_1/w_1 - \tilde{\varepsilon} - x_1(0)}{v_1}$ then $x_1(t) \in I_1(\varepsilon, \tilde{\varepsilon})$ for $t \geq T_0''$.

Then if $x_2(t) \notin I_2(\varepsilon, \tilde{\varepsilon})$ similar reasoning may be applied using $x_1 \in I_1(\varepsilon, \tilde{\varepsilon})$. After that similar reasoning may be applied on $x_3(t)$.

2.6 Stability of fixed points

It turns out that the unique fixed point of Eq. (2) is either stable or unstable with a set of complex conjugate eigenvalues with positive real parts and nonzero imaginary parts, the third eigenvalue being real and negative. This implies that if perturbation of a parameter causes the fixed point to change from stable to unstable then a Hopf bifurcation occurs. Thus, a limit cycle exists which may explain the ultradian rhythm of the observations.

A convenient way to make analytical results about the stability of a low dimensional system in terms of parameters is given by the Routh Hurwitz Criteria (Allen 2007).

Theorem 2 (Routh Hurwitz Criteria for a Nonlinear System)
Suppose

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}), \quad \mathbf{f} : \mathbb{R}^3 \mapsto \mathbb{R}^3, \quad \mathbf{x}(t_0) = \mathbf{x}_0. \tag{13}$$

Suppose $\mathbf{x}_{ss}$ *is a fixed point of Eq.* (13) *and the characteristic polynomial at the fixed point is:*

$$0 = \lambda^3 + \alpha_1\lambda^2 + \alpha_2\lambda + \alpha_3, \quad \alpha_1, \alpha_2, \alpha_1 \in \mathbb{R}. \tag{14}$$

If $\alpha_1 > 0$, $\alpha_3 > 0$ *and* $\alpha_1 \cdot \alpha_2 > \alpha_3$, *then the fixed point is asymptotically stable. If* $\alpha_1 < 0$, $\alpha_3 < 0$ *or* $\alpha_1 \cdot \alpha_2 < \alpha_3$, *then the fixed point is unstable.*

The Jacobian of Eq. (2), evaluated at the fixed point, is

$$\mathbf{J}_{ss} = \begin{pmatrix} -w_1 & 0 & f_1'(x_{3ss}) \\ f_2(x_{3ss}) & -w_2 & x_{1ss} f_2'(x_{3ss}) \\ 0 & k_2 & -w_3 \end{pmatrix}. \tag{15}$$

The characteristic polynomial has coefficients

$$\begin{aligned}\alpha_1 &= w_1 + w_2 + w_3\\ \alpha_2 &= w_1w_2 + w_1w_3 + w_2w_3 - k_2x_{1ss}f_2'(x_{3ss})\\ \alpha_3 &= w_1w_2w_3 - w_1k_2x_{1ss}f_2'(x_{3ss})\\ &\quad -k_2f_1'(x_{3ss})f_2(x_{3ss}).\end{aligned} \tag{16}$$

It is seen that $\alpha_1 > 0$ since $w_1, w_2, w_3 > 0$. Since $f_1'(x_{3ss}) < 0$ and $f_2'(x_{3ss}) < 0$, then $\alpha_2 > 0$ and $\alpha_3 > 0$. Note, there can be no non-negative, real root of Eq. (14) for $\alpha_1 > 0, \alpha_2 > 0$ and $\alpha_3 > 0$, i.e. there exists $\varepsilon > 0$ such that for any real root, λ_1, then $\lambda_1 < -\varepsilon$.

If $\alpha_1\alpha_2 - \alpha_3 < 0$, then one negative real root and a set of complex conjugate roots with positive real part and non-zero imaginary part exists.

The roots of a polynomial depend continuously on the coefficients and the coefficients in Eq. (14) depend continuously on the parameters. Therefore, if a parameter is changed continuously such that $\alpha_1\alpha_2 - \alpha_3$ goes from negative to positive values a Hopf bifurcation occurs.

2.7 Sufficient criteria for stable fixed point

Facilitating notation we introduce:

$$g(w_1, w_2, w_3) = (w_2 + w_3)(w_1w_2 + w_1w_3 + w_2w_3 + w_1^2). \tag{17}$$

Then $\alpha_1\alpha_2 - \alpha_3$ is calculated:

$$\begin{aligned}\alpha_1\alpha_2 - \alpha_3 &= g(w_1, w_2, w_3) - k_2\frac{w_2 + w_3}{w_1}f_1(x_{3ss})f_2'(x_{3ss})\\ &\quad +k_2f_1'(x_{3ss})f_2(x_{3ss}).\end{aligned} \tag{18}$$

The only negative term entering the right hand side is $k_2f_1'(x_{3ss})f_2(x_{3ss})$. Thus, a sufficient, easy applicable criterion for a stable fixed point may be formulated. First, note:

$$\alpha_1\alpha_2 - \alpha_3 \geq g(w_1, w_2, w_3) + k_2f_1'(x_{3ss})f_2(x_{3ss}). \tag{19}$$

Since the fixed point is located in the trapping region, denote:

$$m_2 = \max(f_2(x_3)), \quad \text{for } x_3 \in J_3. \tag{20}$$

Since J_3 is compact and f_2 is continuous the maximum $m_2 \leq M_2$ exists. Denote:

$$p_2 = \min(f_1'(x_3)), \quad \text{for } x_3 \in J_3. \tag{21}$$

Since J_3 is compact and $f_1'(x_3)$ is continuous the minimum exists. Then $p_2 \leq f_1'(x_{3ss}) < 0$. Inserting in (19):

$$\alpha_1\alpha_2 - \alpha_3 \geq g(w_1, w_2, w_3) + k_2 p_2 m_2. \tag{22}$$

Therefore if $g(w_1, w_2, w_3) + k_2 p_2 m_2 > 0$ then the fixed point is stable. This is a sufficient criterion for a stable fixed point that can be used without knowing the solution of the fixed point equation.

2.8 Sufficient criterion for globally stable fixed point

For specific feedback functions the fixed point equation may be used to make a better estimate guaranteeing the stability of the fixed point. However, the above sufficient estimate will typically be easier to apply. Note that if $g(w_1, w_2, w_3) + k_2 p_2 m_2 > 0$ then the fixed point is stable.

Analytical, relevant criteria for a globally stable fixed point are rarer. However, for this system it is possible to formulate such a criterion. Scaling of the differential equations facilitate this analysis. Equation (2) in dimensionless variables is:

$$\frac{dX_1}{d\theta} = F_1(X_3) - \tilde{w}_1 X_1 \tag{23a}$$

$$\frac{dX_2}{d\theta} = F_2(X_3)X_1 - \tilde{w}_2 X_2 \tag{23b}$$

$$\frac{dX_3}{d\theta} = X_2 - \tilde{w}_3 X_3, \tag{23c}$$

with dimensionless constants $\tilde{w}_1, \tilde{w}_2, \tilde{w}_3 > 0$, $F_1, F_2 : \mathbb{R}_+ \cup \{0\} \mapsto \mathbb{R}_+ \cup \{0\}$, $F_1, F_2 \in C^1$, $\sup(F_1(X_3)) = 1$, $\sup(F_2(X_3)) = 1$, $F_1(0) > 0$, $F_2(0) > 0$.

Using the notation:

$$H(X_3) \equiv \frac{F_1(X_3)F_2(X_3)}{\tilde{w}_1\tilde{w}_2\tilde{w}_3}, \tag{24}$$

An easily applicable theorem may be formulated guaranteeing global stability of the unique fixed point.

Theorem 3 *If* $H(X_3) > 0$, $H'(X_3) \in (-1; 1)$, $\forall X_3 \in [0; \frac{1}{\tilde{w}_1\tilde{w}_2\tilde{w}_3}]$, *then the fixed point of Eq.* (23) *is globally stable* (Andersen and Vinther 2010).

3 Specific realization of the minimal model

A realization of the minimal model serves to exemplify the general case. Another reason for showing this realization is to demonstrate that this theory works in practice. However, an important part of the behaviour of the system requires numerical values of depletion rates as well as choices of feedback functions. Therefore, the realization of the minimal model is interesting per se.

Feedbacks are realized through receptors. In terms of reaction kinetics this can be written as:

$$\alpha \check{x}_3 + \check{F} \xrightarrow{c_1} O \tag{25a}$$

$$\alpha \check{x}_3 + \check{F} \xleftarrow{c_{-1}} \check{O} \tag{25b}$$

$$\check{O} \xrightarrow{c_2} \check{F} + \beta \check{y} \tag{25c}$$

where α is the number of cortisol molecules ($\check{x}_3$) binding to a free receptor, $\check{F}$, thus becoming an occupied receptor ($\check{O}$). The occupied receptor either releases the cortisol molecules or transforms the cortisol molecules to β new products ($\check{y}$). These reactions happen at different rate constants denoted by c_1, c_{-1} and c_2.

Using the law of mass action (Landau and Lifshitz 2008), that the sum of occupied and free receptors is constant, the quasi-equilibrium assumption (e.g. caused by the receptors working at maximum capacity so that their occupancy rate is constant, and that no elimination of the concentration of substance $\check{y}$ takes place) it may easily be derived that:

$$\frac{dy}{dt} = c_{max} \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}, \tag{26}$$

where y is the concentration of substance $\check{y}$, x_3 denotes the cortisol concentration, $c_{max} = Rc_2\beta$, R is the sum of free and occupied receptors, and $c = \sqrt[\alpha]{(c_2 + c_{-1})/c_1}$. In biology, this function is termed the Hill function. A thorough deviation of the Hill function may be found in Appendix A.

We assume that substance y is capable of reacting with CRH and creating a new product A, with a rate constant c_3, which is no longer able to contribute to the dynamics of the HPA axis. Substance y is then produced and consumed according to

$$\frac{dy}{dt} = c_{max} \frac{x_3^\alpha}{x_3^\alpha + c^\alpha} - c_3 x_1 y. \tag{27}$$

Using the law of mass action the differential equation governing CRH may be formulated:

$$\frac{dx_1}{dt} = k_0 - c_1 x_1 y - w_1 x_1. \tag{28}$$

Assuming a quasi-steady state for substance y, we see that $c_1 x_1 y = c_{max} \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}$. Substituting this into Eq. (28), we get:

$$\begin{aligned} \frac{dx_1}{dt} &= k_0 - c_{max} \frac{x_3^\alpha}{x_3^\alpha + c^\alpha} - w_1 x_1 \\ &= k_0 \left(1 - \mu \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}\right) - w_1 x_1, \end{aligned} \tag{29}$$

where $\mu = c_{max}/k_0 \in [0; 1]$. Using a similar conclusion for the differential equations governing ACTH, we get:

$$f_1(x_3) = k_0 \left(1 - \mu \frac{x_3^\alpha}{c^\alpha + x_3^\alpha}\right) \tag{30}$$

$$f_2(x_3) = k_1 \left(1 - \rho \frac{x_3^{\alpha_1}}{c_1^{\alpha_1} + x_3^{\alpha_1}}\right). \tag{31}$$

Since the feedbacks of cortisol is realized through GR in the hypothalamus as well as in the pituitary, we assume $\alpha = \alpha_1$ and $c = c_1$. Thus Eq. (2) simplifies to:

$$\frac{dx_1}{dt} = k_0 \left(1 - \mu \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}\right) - w_1 x_1 \tag{32a}$$

$$\frac{dx_2}{dt} = k_1 \left(1 - \rho \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}\right) x_1 - w_2 x_2 \tag{32b}$$

$$\frac{dx_3}{dt} = k_2 x_2 - w_3 x_3. \tag{32c}$$

We denote the system given in Eqs. (32) a specific realization of the minimal model. One could argue that it would be more reasonable to assume that many receptors are involved in controlling each feedback at the same time. But if many individual receptors obey the same chemical reactions, and the receptors have different capacities, a_i, then the differential equation for CRH would look like:

$$\frac{dx_1}{dt} = k_0 \left(1 - \frac{\sum_i a_i}{k_0} \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}\right) - w_1 x_1$$

Denoting $\frac{\sum_i a_i}{k_0} = \mu$, we end up with the same differential equations as in (32). Therefore, nothing is lost in modeling all receptors as one 'big' receptor.

3.1 Description of the specific realization of the minimal model

Equation (32), with strictly positive k_0, k_1, k_2, w_1, w_2, w_3 and $\mu, \rho \in [0, 1]$ and α as an integer value, is in focus of the next section. The derivative of x_1 (CRH) has a positive term $k_0 \left(1 - \mu \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}\right)$, where a negative feedback from cortisol (x_3) inhibits the positive stimulation for increasing cortisol. There is a similar negative feedback mechanism from cortisol in the equation describing the derivative of x_2 (ACTH) where the positive term is 'proportional' to the CRH concentration. Again, an increasing concentration of cortisol inhibits the positive stimulation. The positive stimulation on the derivative of cortisol is linear in ACTH, thus, the more ACTH the more cortisol is produced. Changes in all of these hormone concentrations includes elimination depending linearly on the concentration itself. Equation (32) is thus a realization of (2), with physiologically interpretable parts.

3.2 Scaling the differential equations

Scaling differential equations may be a convenient way to reduce the number of controlling parameters by grouping the original parameters. We choose to reformulate the equations in non-dimensional form. In order to do this we apply the following scaling constants:

$$\begin{aligned}\zeta_0 &= \left(\frac{k_0 k_1 k_2}{c}\right)^{\frac{1}{3}} \\ \zeta_1 &= \left(\frac{c k_0}{k_1 k_2}\right)^{\frac{1}{3}} \\ \zeta_2 &= \left(\frac{c k_0 k_1}{k_2^2}\right)^{\frac{1}{3}} \\ \zeta_3 &= c.\end{aligned} \tag{33}$$

All the scaling constants are thus positive. Define θ, X_1, X_2, X_3 by

$$\begin{aligned}\theta &\equiv \zeta_0 t \\ x_1 &\equiv \zeta_1 X_1 \\ x_2 &\equiv \zeta_2 X_2 \\ x_3 &\equiv \zeta_3 X_3.\end{aligned} \tag{34}$$

From Eq. (32), we see that c has dimension of concentration, k_0 has dimension concentration divided by time and k_2, and k_3 have dimension inverse time. This means ζ_0 has dimension of inverse time and ζ_1, ζ_2, ζ_3 all have dimension of concentration.

Since the concentrations are all non-negative and the scaling constants are positive X_1, X_2, X_3 are non-negative. Time is scaled by a positive constant, thus an increase in time corresponds to an increase in θ. Note that X_1, X_2, X_3 and θ are all dimensionless by definition. Define positive parameters $\tilde{w}_1$, $\tilde{w}_2$, $\tilde{w}_3$ by:

$$\begin{aligned}\tilde{w}_1 &\equiv \frac{w_1}{\zeta_0} \\ \tilde{w}_2 &\equiv \frac{w_2}{\zeta_0} \\ \tilde{w}_3 &\equiv \frac{w_3}{\zeta_0}.\end{aligned} \tag{35}$$

Thus, $\tilde{w}_1$, $\tilde{w}_2$ and $\tilde{w}_3$ are dimensionless. Then we obtain the dimensionless system:

$$\frac{dX_1}{d\theta} = 1 - \mu \frac{X_3^\alpha}{1 + X_3^\alpha} - \tilde{w}_1 X_1 \tag{36a}$$

$$\frac{dX_2}{d\theta} = \left(1 - \rho\frac{X_3^\alpha}{1+X_3^\alpha}\right) X_1 - \tilde{w}_2 X_2 \tag{36b}$$

$$\frac{dX_3}{d\theta} = X_2 - \tilde{w}_3 X_3. \tag{36c}$$

Comparing with Eq. (32), we see that this version of the system has 6 parameters, which is 3 less than the original system. Therefore, the dimensionless form is suitable for finding the stability of the fixed point for different parameter values.

4 Analysis of stability of the unique fixed point

In this section, we will use the comprehensive notation:

$$Y \equiv \frac{X_3^\alpha}{1+X_3^\alpha}. \tag{37}$$

The fixed point equations, (3), can then be formulated:

$$X_{1ss} = \frac{1}{\tilde{w}_1}(1-\mu Y_{ss}) \tag{38a}$$

$$X_{2ss} = \frac{1}{\tilde{w}_1\tilde{w}_2}(1-\rho Y_{ss})(1-\mu Y_{ss}) \tag{38b}$$

$$X_{3ss} = \frac{1}{\tilde{w}_1\tilde{w}_2\tilde{w}_3}(1-\rho Y_{ss})(1-\mu Y_{ss}). \tag{38c}$$

The stability of the fixed point is determined by the sign of $\alpha_1\alpha_2 - \alpha_3$:

$$\begin{aligned}\alpha_1\alpha_2 - \alpha_3 = {} & \frac{\alpha\rho X_{1ss}Y_{ss}^2}{X_{3ss}^{\alpha+1}}(\tilde{w}_2+\tilde{w}_3) + g(\tilde{w}_1,\tilde{w}_2,\tilde{w}_3) \\ & + \frac{\alpha\mu Y_{ss}^2}{X_{3ss}^{\alpha+1}}(-1+\rho Y_{ss}).\end{aligned} \tag{39}$$

The only negative term is the last one. Throwing away the first term:

$$\begin{aligned}\alpha_1\alpha_2 - \alpha_3 \geq {} & g(\tilde{w}_1,\tilde{w}_2,\tilde{w}_3) \\ & + \frac{\alpha\mu Y_{ss}^2}{X_{3ss}^{\alpha+1}}(-1+\rho Y_{ss}).\end{aligned} \tag{40}$$

Considering the last term and using the definition of Y_{ss}:

$$\frac{\alpha\mu Y_{ss}^2}{X_{3ss}^{\alpha+1}}(-1+\rho Y_{ss}) = \frac{\alpha\mu Y_{ss}(1-Y_{ss})}{X_{3ss}}(-1+\rho Y_{ss}). \tag{41}$$

Inserting Eq. (38c) into Eq. (41):

$$\frac{\alpha\mu Y_{ss}^2}{X_{3ss}^{\alpha+1}}(-1+\rho Y_{ss}) = -\mu\tilde{w}_1\tilde{w}_2\tilde{w}_3\alpha Y_{ss}\frac{1-Y_{ss}}{1-\mu Y_{ss}} > -\alpha\tilde{w}_1\tilde{w}_2\tilde{w}_3. \tag{42}$$

Inserting in Eq. (40) gives:

$$\alpha_1\alpha_2 - \alpha_3 > g(\tilde{w}_1, \tilde{w}_2, \tilde{w}_3) - \alpha\tilde{w}_1\tilde{w}_2\tilde{w}_3. \tag{43}$$

Thus, a sufficient criterion for the fixed point to be stable is:

$$\alpha \leq \frac{g(\tilde{w}_1, \tilde{w}_2, \tilde{w}_3)}{\tilde{w}_1\tilde{w}_2\tilde{w}_3}. \tag{44}$$

Hence, by a thorough calculation it follows that the fixed point is stable for $\alpha \leq 4 + 2\sqrt{3} \approx 7.46$, $\forall\tilde{w}_1, \tilde{w}_2, \tilde{w}_3 > 0$, $\forall\mu, \rho \in [0; 1]$. Notice that this is a stronger statement than Eq. (22), because this result is independent of the value of the other parameters. This result supports the result from Murray (2002), where a three dimensional system of testosterone with only one negative feedback is present. However $\alpha > 7$ is considered unphysiological. Therefore, the fixed point of Eq. (32) is stable for all realistic values of the parameters and no Hopf bifurcation leading to limit cycles (oscillating solutions) is possible. This result encompasses the results of Savic and Jelic (2005), where the case $\alpha = 1$ is investigated and the unique fixed point is found to be stable.

5 Parameters for the model

We now know that the unique fixed point of the system (36) is stable. It remains unknown whether or not limit cycles co-exist within the trapping region away from the fixed point. To investigate the system, a closer estimation of parameters is required. The parameters will be given along with their standard deviation.

From the literature, we have an estimate of the elimination constants from the half-life, τ_i, of the concentrations. That is, we define $w_i = \ln(2)/\tau_i$: the half-life of CRH in human plasma is about 4 min (Felig and Frohman 2001); the half-life of ACTH is 19.9 ± 4.2 min (Carroll et al. 2007); and the half-life of cortisol is 76 min$\pm$16.2 min (Carroll et al. 2007). This produces the following values for w_i.

$$w_1 = 0.173\,\text{min}^{-1} \tag{45}$$
$$w_2 = 0.035\,\text{min}^{-1} \tag{46}$$
$$w_3 = 0.009\,\text{min}^{-1}. \tag{47}$$

The standard deviation of the half-life of ACTH and cortisol suggests that w_i can vary about 20%. There is no reliable estimate for the parameters μ and ρ but since these

are in the interval [0;1], we choose these to be 0.5 ± 0.5. The parameter α is neither well known. Values larger than 7 are unlikely (Murray 2002). If $\alpha = 1$, the feedback functions is described by Michaelis–Menten kinetics. This case has been investigated in Savic and Jelic (2005). We therefore choose $\alpha = 3 \pm 2$, which should cover most physiologically relevant values of α. In Felig and Frohman (2001) it is argued that GR is the most important receptor in regulating the HPA-axis. This suggests c^α has a value that ensures the receptor is around the inflection point of the Hill function for reasonable values of cortisol concentration. We denote the mean value of free cortisol as $\bar{x}_3$ and choose $c = \bar{x}_3$ as a first estimate.

The fixed point should be situated at reasonable levels of concentration. A first guess to three reasonable concentration levels could be the mean concentration of the hormones. Therefore we choose:

$$\dot{\mathbf{x}}|_{(\bar{x}_1,\bar{x}_2,\bar{x}_3)} = \mathbf{0}, \tag{48}$$

where $\bar{x}_i$ is the mean value of hormone i. The mean value of CRH is $\bar{x}_1 = 1.64$ pmol/ml (Felig and Frohman 2001) and the molecular weight of CRH is 4670 g/mol (Hashimoto et al. 1993).

$$\bar{x}_1 = 7.659 \text{ pg/ml}. \tag{49}$$

The mean value of ACTH and cortisol for healthy people are specified in Carroll et al. (2007), as[1]:

$$\bar{x}_2 = 21 \text{ pg/ml} \tag{50}$$

$$\bar{x}_3 = 3.055 \text{ ng/ml}. \tag{51}$$

Now, the mean values of the concentration and all parameters are estimated but the three k's are missing. Solving Eq. (48) produces the three default values of the k's. This leads to the following default parameter values for the unscaled system in Table 1, and the corresponding default values in the scaled system in Table 2.

Next, we will investigate how these parameter values correspond to the previously mentioned results concerning global stability. As explained in Sect. 4, the local stability of the unique fixed point is guaranteed since $\alpha < 8$.

5.1 Investigation of trapping region and global stability

For the system with the default parameters $(\tilde{w}_1\tilde{w}_2\tilde{w}_3)^{-1}\, L_1 L_2 = (4\tilde{w}_1\tilde{w}_2\tilde{w}_3)^{-1}$, from Proposition 1 $X_3(t)$ is guaranteed to enter the region $[(5\tilde{w}_1\tilde{w}_2\tilde{w}_3)^{-1}; (\tilde{w}_1\tilde{w}_2\tilde{w}_3)^{-1}] = W_{SM}$.

A sufficient criterion for global stability is $\max |H'(X_3)| < 1 : X_3 \in W_{SM}$. A numerical investigation of the system with the default parameter values is performed

[1] The amount given in Carroll et al. (2007) which is the total amount of cortisol (6.11 µg/dl), 5% of this corresponds to the free cortisol, which is given in Eq. (51).

Table 1 Default parameter values of the unscaled system

Parameter	Default value	Unit
k_0	1.7696	pg/(ml min)
k_1	0.12734	min^{-1}
k_2	0.0013198	min^{-1}
w_1	0.17329	min^{-1}
w_2	0.034832	min^{-1}
w_3	0.0090726	min^{-1}
ρ	0.5	
μ	0.5	
α	3	
c	3.055	ng/ml

Table 2 Default parameter values for the dimensionless system

Parameter	Default values
$\tilde{w}_1$	3.7669
$\tilde{w}_2$	0.75716
$\tilde{w}_3$	0.19722
ρ	0.5
μ	0.5
α	3

resulting in $\max |H'(X_3)| = 1.27$. Thus, we cannot guarantee global stability of the system with the default parameter values. Since the absolute value of the gradient of H is rather close to one, a perturbation of the parameters fulfill the sufficient criterion for global stability.

In the following, one parameter is allowed to change while the others are fixed at their default values. For instance, a decrease of the parameter α to 1 ensures global stability. Denoting a changed variable with a super-script hat along with the default parameter in standard notation we also see that global stability is guaranteed for $\hat{\tilde{w}}_i > 1.27\tilde{w}_i$ which is a little more than the standard deviation, $\hat{\mu} \leq 0.24$ and $\hat{\rho} \leq 0.24$. Thus, we are able to guarantee global stability for parameters within the subset of reasonable physiological parameters given by the estimates plus-minus their standard deviations. This pinpoints that this model is missing something, since ultradian rhythm is seen in all data.

We have investigated the system for what we consider the worst-case parameters,[2] that are still within a reasonable physiological range; these are given in Table 3. These parameters give $\max \left|H'(X_3)\right| = 5.186$. For these parameter values, a grid investigation is made. That is, we have made initial conditions with a grid masking of 0.5 in the region $[0; 4] \times [0; 4] \times [0; 4]$ and solved the system of differential equa-

[2] These are the parameters that make $\max \left|H'(X_3)\right|$ as large as possible.

Table 3 Worst case scenario of parameters

Worst case parameters
$\hat{\tilde{w}}_1 = 0.8\tilde{w}_1$
$\hat{\tilde{w}}_2 = 0.8\tilde{w}_2$
$\hat{\tilde{w}}_3 = 0.8\tilde{w}_3$
$\hat{\rho} = 1$
$\hat{\mu} = 1$
$\hat{\alpha} = 5$

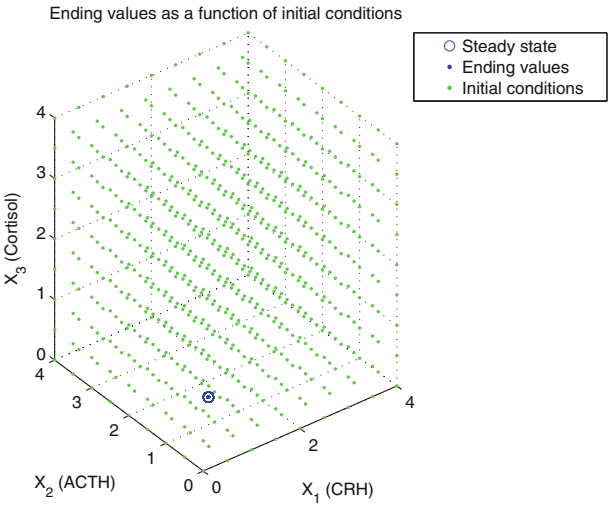

Fig. 4 Grid investigation of the scaled system. This figure shows the ending value as a function of initial conditions using the parameter values given in Table 3 corresponding to the 'worst-case scenario'. All initial conditions lead to solutions converging to the fixed point. The investigated region is $[0; 4] \times [0; 4] \times [0; 4]$ and the 'trapping region' is $[0; 0.3318] \times [0; 0.5478] \times [0; 3.4722]$

tions by means of numerical methods. As seen from Fig. 4, all solutions converge to the unique steady state marked by a ring. A similar investigation using a much finer mesh resulted in the same conclusion. Thus it seems unlikely that limit cycles exist, even within 'worst-case parameters'. Therefore, the realization of the minimal model, Eq. (36), cannot account for the ultradian rhythm.

6 Outlook

In this section we will briefly present the possibility of the ultradian rhythm coming from other mechanisms; primarily the inclusion of time delays in the differential equations.

6.1 Time delay

Time delay have been thoroughly studied in the literature (Savic and Jelic 2005, 2006; Bairagi et al. 2008). The most obvious reason for considering time delays is the physical distance between the brain, where the hypothalamus and pituitary are situated, and the kidney, where the adrenal glands are situated. This distance means that hormones are transported within the bloodstream.

The system studied in the papers (Savic and Jelic 2005, 2006) demonstrate a particular realization of the minimal model with the parameter α equal to one. Different time delays are included in the model and the solutions investigated for the possibility of oscillations. The conclusion of these papers is that the fixed point is stable. However, in Savic and Jelic (2005), a model with a single time delay is constructed and the criteria for limit cycles through a Hopf bifurcation is fulfilled for values of μ and ρ close to one (e.g. $\mu = 0.98$ and $\rho = 0.90$ and a scaled cortisol concentration attaining values between 2 and 17). In Savic and Jelic (2006), a more general model employing the model of Savic and Jelic (2005) is constructed and analyzed. Rouche's theorem is used and a sufficient criteria for a locally stable fixed point found

$$abZ_0^3 + (b\,(1+2a) - \mu)\,Z_0^2 + (a + 2b - \mu b)\,Z_0 + 1 > 0, \tag{52}$$

with $a = 1 - \mu$ and $b = 1 - \rho$. Z_0 is a scaled cortisol concentration which Savic and Jelic (2006) estimates to have the value 15. While Savic and Jelic (2006) consider inequality (52) to be fulfilled it is worth noticing the left hand side for the case $\mu = \rho = 1$ reduces to

$$-Z_0^2 + 1 > 0. \tag{53}$$

Thus, using the estimate for Z_0, sufficient criterion for a locally stable fixed point is not fulfilled for $\mu = \rho = 1$. The sensible approach of Savic and Jelic (2006) may therefore be used as sufficient criterion for a locally stable fixed point given by an elegant inequality (52) that may be fulfilled or not depending on 'real' parameters.

We have briefly investigated the possibility of time delays as capable of producing the ultradian rhythm. For simplicity, we assume that it takes the same time for cortisol to deliver its effect in the two areas of the brain and for ACTH to deliver its effect in the adrenal glands. Thus:

$$\dot{x}_1 = k_0 \left(1 - \mu \frac{(x_3(t-\tau))^\alpha}{c^\alpha + (x_3(t-\tau))^\alpha}\right) - w_1 x_1 \tag{54a}$$

$$\dot{x}_2 = k_1 \left(1 - \rho \frac{(x_3(t-\tau))^\alpha}{c^\alpha + (x_3(t-\tau))^\alpha}\right) x_1 - w_2 x_2 \tag{54b}$$

$$\dot{x}_3 = k_2 x_2(t-\tau) - w_3 x_3. \tag{54c}$$

Throughout this work we have gathered knowledge of the effect each parameter has on the stability of the system. Large values of α, μ and ρ are demonstrated as having

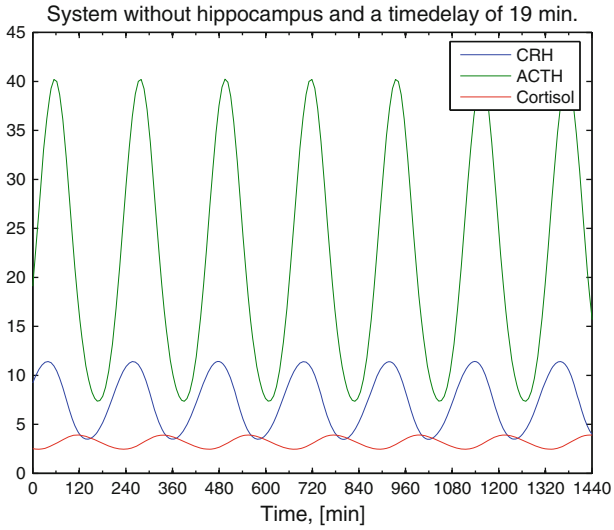

Fig. 5 The system given in Eq. (54) simulated with a time delay of 19 min and the parameters mentioned in this section. The figure shows oscillating solutions in all three hormones. The unit of CRH is pg/ml, the unit of ACTH is pg/ml, and the units of cortisol is ng/ml

a destabilizing effect. Therefore, we put $\alpha = 5$ and $\rho = \mu = 1$. w_i is not changed and k_i will be determined in the same way as in Sect. 5. This gives the following values of the parameters $k_0 = 2.6543$, $k_1 = 0.191$, $k_2 = 0.0013$ and $c = 3.055$. The steady state will be given as $\mathbf{x}_{ss} = (7.6508, 21, 3.055)$.

By simulating the system given by Eq. (54), along with a time delay ($\tau = 19$ min), oscillating solutions appear. These are seen on Fig. 5. Thus, it is possible to obtain an ultradian rhythm. In Bairagi et al. (2008) the sum of the two time delays needs to exceed 70 min in order to observe oscillating solutions. Thus, oscillating solutions exist in our model for a composite delay about half the size in comparison to the model of Bairagi et al. (2008). To investigate the splitting of circadian and ultradian rhythm the simulation shown in Fig. 5 is added a trigonometric function in the differential equation governing CRH. The solution curve of ACTH is shown in Fig. 6. This figure should then be compared to Fig. 2. The solution curve of cortisol is shown in Fig. 7. It has been scaled[3] to be compared to Fig. 3. As seen much of the desired dynamics is present. The concentration levels are reasonable. The circadian rhythm is present and both the amplitude and place of the peak is comparable to the data. The ultradian rhythm show a lower amplitude when the circadian rhythm is at its lowest and a higher amplitude when the circadian rhythm is at its highest. This is also observed in data.

Surely, a time delay of 19 min cannot be explained by the time it takes for the hormones to travel within the bloodstream. But the results show that oscillating solutions are possible. If one can give a reasonable explanation of why a delay of this magnitude is reasonable (e.g. the intrinsic receptor dynamic or the inter-cellular production and

[3] We have modeled the free cortisol as 5% of the total cortisol. The solution curve should therefore be scaled by a factor of twenty to be comparable to the total amount of cortisol shown in Fig. 3.

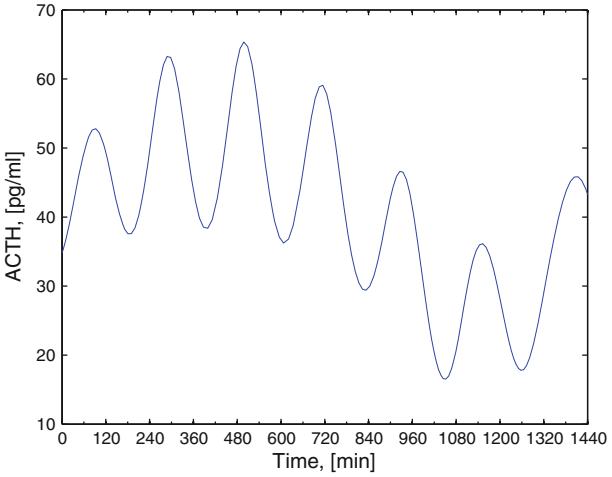

Fig. 6 The solution curve of ACTH from the simulation seen in Fig. 5 added a trigonometric in the differential equation governing CRH. This figure should be compared to the data seen in Fig. 2

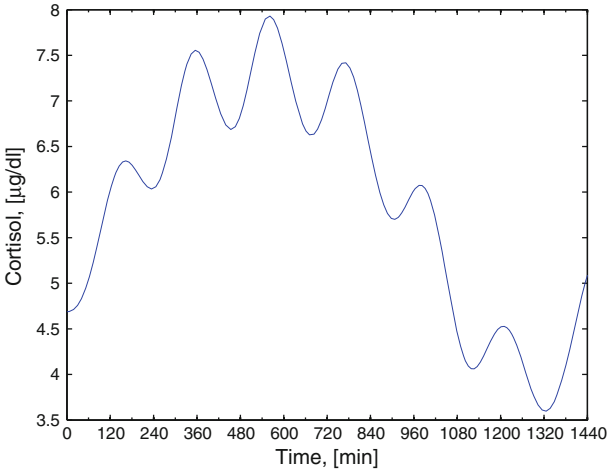

Fig. 7 The scaled solution curve of cortisol from the simulation seen in Fig. 5 added a trigonometric in the differential equation governing CRH. This figure should be compared to the data seen in Fig. 3

secretion of hormones), this could be an investigation to pursue. However there is no evidence for a time delay of that size. Thus, we have chosen not pursue along this line.

6.2 Other mechanisms that could lead to oscillating solutions

Other mechanisms that could lead to the observed ultradian rhythm might be found in the investigatory inclusion of mechanisms in hippocampus. The hippocampus stimulate the production of CRH and both positive and negative feedback mechanisms from

cortisol have been suggested as present in hippocampus (Jelic et al. 2005; Conrad et al. 2009).

Several different receptor types could lead to interesting dynamics. This could either be in the form of different feedback functions and/or different affinities for the various feedback functions.

A model where positive stimulation is considered as bursts of hormone could be beneficial. Stochastic noise imposed on the differential equations could lead to solutions similar to our data. Furthermore, a variable liver function implemented in the model as time varying half-life could lead to oscillating solutions.

7 Conclusion

In this paper, we have investigated the HPA axis analyzing a minimal mathematical model reflecting mechanisms found in literature. The results of this minimal model were divided into two sections. The first section presented the general results that apply to all models consisting of a system with three differential equations obeying the demands of continuous, differentiable and bounded feedback functions and that the feedback functions are different from zero at zero concentration of cortisol. These results include the existence of a unique fixed point. The solution curves have strictly positive values for non-negative initial conditions. This ensures that no negative concentrations can occur. The solution curves are shown to enter a trapping region in finite time. This ensures that concentrations tending to infinity cannot occur. Introducing the function $H(X_3)$ global stability is guaranteed if $\max|H'(X_3)|<1$, $H(X_3)>0$ for X_3 belonging to the smallest trapping region. This is a fast, easy, and elegant means of investigating whether or not oscillating solutions are possible.

The second section concerns the results for a specific realization of the minimal model. The feedback functions are constructed from receptor dynamics—the generalized Hill-function. This function introduces the parameter α. $\alpha = 1$ is a model using Michaelis–Menten kinetics and has been thoroughly investigated in Savic and Jelic (2005, 2006).

For the specific realization of the minimal model the unique fixed point is locally stable if $\alpha \leq 4 + 2\sqrt{3} \approx 7.46$, $\forall \tilde{w}_1, \tilde{w}_2, \tilde{w}_3 > 0$, $\forall \mu, \rho \in [0; 1]$. To the knowledge of the authors, $\alpha = 8$ is an un-physiological large number. Therefore, the specific realization of the minimal model is considered to have a locally stable fixed point. From the literature, values for the half-life parameters were obtained. The rest of the parameters were obtained using physiological reasoning. When keeping all but one parameter fixed at default values, a perturbation is made guaranteeing global stability of the fixed point. This is the case for:

- $\hat{\tilde{w}}_i > 1.27\tilde{w}_i$
- $\alpha = 1$
- $\mu, \rho < 0.24$.

Thus, when using these parameter values, no oscillating solutions are possible. Therefore, the model cannot account for the ultradian rhythm. For the default parameters, global stability is not analytically guaranteed. A grid investigation was performed by

means of numerical methods. Even when applying worst-case parameters in simulations, the solutions converged to the unique fixed point. Therefore, the existence of limit cycles seems unlikely. We conclude that the specific realization of the minimal model is not capable of producing the desired ultradian rhythm seen in the data, when the parameters are within physiologically relevant values.

Thus, some important mechanisms are missing in the standard description of the HPA axis. Such candidates could be (large) time delays, secretion of hormones in bursts by exocytosis, or oscillatory elimination rates (e.g. in the liver).

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Appendix A: Derivation of the Hill function

In this derivation we assume that the chemical law known as the *law of mass action* is valid (Landau and Lifshitz 2008). This law states that the rate at which a chemical reaction occurs is proportional to the product of concentration of reactants and the rate constant. Given the chemical stoichiometric balanced reaction scheme:

$$a\mathbf{A} + b\mathbf{B} \overset{k}{\rightarrow} \mathbf{C}, \tag{55}$$

where bold capital letters denote the reactants, small letters denote the number of reactants and k denote the rate constant. Thus, the rate of change of the concentration of product C will be given as:

$$\frac{dC}{dt} = kA^a B^b, \tag{56}$$

where the capital letters denote the concentration of the reactants.

Consider a system of a specific kind of receptors. In this system there is a concentration of free receptors, $F(t) \geq 0$ and occupied receptors, $O(t) \geq 0$. These receptors are not capable of leaving the system, meaning that $F(t) + O(t)$ is constant. Into the system is a flow of molecules with concentration, $x_3(t) \geq 0$. These are able to bind with the free receptors which then become occupied receptors with rate constant c_1. The occupied receptors should only be considered occupied by this single kind of molecule, which may be released to become a free receptor again at a different rate constant, c_{-1}. Furthermore, the occupied receptors shall be able to transform the incoming molecules to a new molecules with concentration, $y(t) \geq 0$, with rate constant c_2 and then to release it to leave the system. Hereby, the occupied receptor becomes unoccupied. In case of the HPA axis, the corresponding chemical reaction can be written as:

$$\alpha\check{x}_3 + \check{F} \overset{c_1}{\rightarrow} \check{O} \tag{57a}$$

$$\alpha \check{x}_3 + \check{F} \overset{c_{-1}}{\leftarrow} \check{O} \tag{57b}$$

$$\check{O} \overset{c_2}{\rightarrow} \check{F} + \beta \check{y}, \tag{57c}$$

where α is the number of incoming cortisol molecules ($\check{x}_3$) that react with one free receptor ($\check{F}$) and β is the number of new molecules ($\check{y}$) produced by the occupied receptor ($\check{O}$).

Using the law of mass action, we can produce differential equations describing the change in concentrations:

$$\frac{dx_3}{dt} = -c_1 x_3^\alpha F + c_{-1} O \tag{58a}$$

$$\frac{dF}{dt} = -c_1 x_3^\alpha F + c_{-1} O + c_2 O \tag{58b}$$

$$\frac{dO}{dt} = c_1 x_3^\alpha F - c_{-1} O - c_2 O \tag{58c}$$

$$\frac{dy}{dt} = \beta c_2 O. \tag{58d}$$

Using that the sum of the free and occupied receptors are constant, i.e. $F(t) + O(t) = R$, where R is a constant and substituting this into Eqs. (58), we end up with the following equations:

$$\frac{dx_3}{dt} = -c_1 x_3^\alpha R + \left(c_1 x_3^\alpha + c_{-1}\right) O \tag{59a}$$

$$\frac{dO}{dt} = c_1 x_3^\alpha R - \left(c_1 x_3^\alpha + c_{-1} + c_2\right) O \tag{59b}$$

$$\frac{dB}{dt} = \beta c_2 Y. \tag{59c}$$

In a biological system such as a gland or a cell, the number of incoming molecules is usually much larger than the number of receptors. Therefore, it is reasonable to think of receptors as working at maximum capacity, resulting in the occupancy rate as approximately constant ($dO/dt = 0$). This is known as the *quasi-equilibrium hypothesis* (Allen 2007). Solving $dO/dt = 0$ in Eq. (59) and isolating O we get:

$$O = \frac{c_1 x_3^\alpha R}{c_1 x_3^\alpha + c_2 + c_{-1}}. \tag{60}$$

Putting this expression into Eq. (58) we obtain the rate of outgoing molecules:

$$\frac{dy}{dt} = \beta c_2 O = \beta \frac{c_1 x_3^\alpha R c_2}{c_1 x_3^\alpha + c_2 + c_{-1}}. \tag{61}$$

Since the rate constants are positive we can simplify this to:

$$\frac{dy}{dt} = \beta \frac{x_3^\alpha R c_2}{x_3^\alpha + \frac{c_2 + c_{-1}}{c_1}} = \beta \frac{x_3^\alpha R c_2}{x_3^\alpha + \left(\sqrt[\alpha]{\frac{c_2 + c_{-1}}{c_1}}\right)^\alpha}. \tag{62}$$

Letting $c_{max} = \beta R c_2$ and $c = \sqrt[\alpha]{(c_2 + c_{-1})/c_1}$, we obtain the following expression for the rate of change of the product as a function of incoming molecules:

$$\frac{dy}{dt} = c_{max} \frac{x_3^\alpha}{x_3^\alpha + c^\alpha}. \tag{63}$$

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