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# Sufficient conditions for the existence of asymptotically stable homeostatic equilibrium points in negative feedback physiological systems

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ABSTRACT

While the adoption of mathematical models to represent and describe physiological phenomena is widely applied in bioengineering, medicine, and biochemistry research, the large variety of physio- and bio-logical systems, as well as the complex dynamics involved in homeostatic control, prevent the definition of a generalized biofidelic modeling approach for the analysis of systems' dynamics. Leveraging the formalism and principles of control theory, this manuscript lays the theoretical foundations for the study of self-regulatory mechanisms in multivariable negative feedback physiological systems. Starting from the definition of a system-theoretic oriented mathematical modeling framework, general assumptions and model-specific theorems, tailored to the order of the model, are derived and demonstrated to define sufficient conditions for the existence of unique and asymptotically stable equilibrium points within the system's operating regions. Then, the proposed methodological approach is translated into practice through application to relevant case studies representing physiological control systems at increasing degrees of complexity (from 2nd- to 4th-order models), namely: (i) the regulation of thyroid hormones circulation in the blood; (ii) the prey–predator model describing the dynamics of tumor progression; (iii) the cortisol dynamics in response of pain/stress stimuli. Finally, the capability of the proposed framework to effectively capture the behavior of additional physiological systems (from 2nd- up to 7th-order) available in the literature is also demonstrated, thus shaping a promising theoretical and methodological route for the analysis of uniqueness and stability of homeostatic equilibrium in both physiological and pathological conditions.

## 1. Introduction

The study of the dynamics of physiological control systems in healthy and disease encompasses the investigation of the behavior of a system nearby its equilibrium point, which guarantees its stability within the range of a particular set of parameter values at which it functions efficiently. This reflects the homeostastic nature of living systems, where physiological regulation aims to maintain key variables at constant equilibrium values [1]. Thus, investigating such self-regulating mechanisms can enhance understanding of biological processes and enable the development of strategies to restore homeostatic conditions when a pathology compromises them. This is one of the primary reasons why the concept of homeostasis has emerged as a crucial unifying and foundational principle in physiology research.

Homeostasis is typically achieved through negative feedback loops, which restore the system to the equilibrium if it deviates from it. From a system-theoretic perspective, this phenomenon can be studied by linearizing the system around the equilibrium points and utilizing eigenvalues to ascertain the system's stability, thereby establishing the conditions under which the existence and stability of equilibrium points can be assumed [2]. Investigating the stability of homeostatic equilibrium points is fundamental, since a strong deviation from it, or even instability are often related to a pathological condition.

For this reason, while some literature studies attempted to explain homeostatic mechanisms from a clinical and biological point of view (e.g. [3–5]), there has been an increasing number of papers trying to describe homeostatic processes through mathematical models (e.g., [6–9]), by leveraging the principles of control theory. However, these latter studies are often system-specific and propose dynamical models to describe the peculiar behavior of the physiological/biological process

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under examination, thus potentially neglecting multivariable interactions at different scales and lacking a more general and comprehensive mathematical representation of the negative feedback loop to reach homeostasis.

In this sense, a relatively recent study [10] proposed a general mathematical model covering different examples of negative feedback physiological systems and investigated the existence of equilibrium points for those systems characterized by two competing species (i.e., two system's state variables), one activating and one inhibiting, and provided three paradigmatic examples of physiological processes at different scales falling into the proposed general model. However, the situation of a single output to be controlled by a single regulating input is often far from what occurs in biological and physiological processes, which, more typically, have many outputs that need to be regulated by multiple inputs. To address this aspect, the authors in [11] proposed a further generalization of the original general two-state variable model into a unified multivariable framework, which takes into account negative feedback physiological systems with multiple state variables (i.e., multiple activation and inhibition species) and characterized by higher degree of complexity, inclusion of nonlinearities, and presence of possible time delays in both activation and inhibition channels of the model architecture.

In this context, the novel contribution of this paper is threefold: firstly, the work presents, defines, and theoretically describes the mathematical framework (assumptions and theorems) for establishing novel sufficient conditions for the existence, uniqueness, and stability of equilibrium points in negative feedback physiological control systems having the peculiar structure here considered; secondly, applications of the proposed approach to three real, relevant cases, representative of physiological systems at different scales and with increasing degree of complexity, are provided and discussed; thirdly, a comprehensive and in-depth theoretical discussion is devoted to gather the physiological systems provided in the existing literature and to investigate whether they are captured by our approach.

In particular, starting from the definition of a general reference model for physiological control systems, the manuscript delves into the theoretical proof of the sufficient conditions for the existence of an asymptotically stable equilibrium point for the case of a 2nd-order model characterized by only one activation and one inhibition variable. Then, the paper extends the findings to higher-order negative feedback physiological models, and determines the sufficient conditions by taking into account the peculiar structures of more complex multivariable systems. Based on the general conditions found for establishing the existence of asymptotically stable equilibrium points in different models' configurations, physiological case studies of homeostatic control are investigated, and case-specific solutions are inferred for each example by leveraging the previously theorized conditions. The examined cases refer to paradigmatic and biofidelic models of major multivariable homeostasis processes at increasing level of complexity in terms of both model formulation and number of involved state variables. Such case studies show how the proposed approach advances the previous research and provides a rigorous methodology to determine sufficient conditions for the existence of asymptotically stable homeostatic equilibrium points in a range of multivariable negative feedback physiological systems at different scales by adopting a system-theoretic-driven formalism.

More specifically, the following case studies are discussed: regarding the second-order model, (i) the case of homeostatic regulation of thyroid hormones is considered, which describes the negative-feedback mechanism governing the interplay between the thyroid gland stimulation and the circulation of thyroid hormones in the blood; as far as the third-order model, (ii) the prey–predator model describing the dynamics of tumor progression and related immune responses, accounting for two activation and one inhibition species; finally, concerning the fourth-order model architecture, (iii) the case of cortisol dynamics in response to pain/stress conditions is investigated, comprising two activating and two inhibiting species, and describing those biochemical interactions responsible for the regulation of the cortisol release and occurring at the level of the hypothalamus, the anterior pituitary gland, and the adrenal cortex.

Through both theoretical and case-specific investigations and discussion, the manuscript provides the necessary elements to achieve a formal yet substantial generalization of the proposed unified mathematical model for a wide range of negative feedback physiological control systems.

## 2. Methods

**Notation**: In the following, the space of $l$-tuple of real numbers is denoted by $\mathbb{R}^l$; the positive orthant of $\mathbb{R}^l$ is denoted by $\mathbb{R}^l_+$. The space of real $l \times p$ matrices is denoted by $\mathbb{R}^{l\times p}$. Given the vector-function $h : z \mapsto h(z)$, $\mathbb{R}^n \mapsto \mathbb{R}^m$, $h_z$ denotes the Jacobian matrix of $h$ versus $z$, that is

$$h_z := \begin{pmatrix} \frac{\partial h_1}{\partial z_1} & \frac{\partial h_1}{\partial z_2} & \dots & \frac{\partial h_1}{\partial z_n} \\ \frac{\partial h_2}{\partial z_1} & \frac{\partial h_2}{\partial z_2} & \dots & \frac{\partial h_2}{\partial z_n} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial h_m}{\partial z_1} & \frac{\partial h_m}{\partial z_2} & \dots & \frac{\partial h_m}{\partial z_n} \end{pmatrix};$$

note that when $m = 1$ and $n = 1$, $h_z$ recovers the classical derivative of $h$ wrt $z$; when $m = 1$, $h_z$ recovers the gradient of $h$ wrt $z$; finally, when $n = 1$, $h_z$ reduces to a column vector, whose components are the derivatives of the component of $h$ wrt $z$. For the sake of clarity, when $n = 1$ and $z$ is the time variable $t$, we use the classical notation $\dot{h}(t)$ to denote the derivative of $h$ with time. By $|H|$ we denote the determinant of the square matrix $H$.

### 2.1. Reference model for a physiological control system

We consider the following reference model to represent a general physiological control system, which has been introduced in [10–12]

$$\dot{y}(t) = A_a y(t) + B_a u^y(t) + f\left(y(t), x(t)\right) \tag{1a}$$

$$\dot{x}(t) = A_i x(t) + B_i u^x(t) + g\left(y(t), x(t)\right), \tag{1b}$$

where $A_a \in \mathbb{R}^{m\times m}$, $A_i \in \mathbb{R}^{n\times n}$, $B_a \in \mathbb{R}^{m\times u}$, $B_i \in \mathbb{R}^{n\times v}$.

In (1a), the input function $u^y$ represents the production rate of the inhibition species $y$, while the activation function $f(\cdot,\cdot)$ describes the mechanism of activation of the species $x$ against $y$. On the other hand, in (1b), $u^x$ indicates the rate of production of $x$, while the inhibition function $g(\cdot,\cdot)$ accounts for the mechanism of inhibition of $y$ against $x$.

In order to make the structure of model (1) a possible representation of a negative feedback physiological system, we will introduce, in the following, some specific assumptions which will require the development of a tailored approach to be addressed.

**Assumption 1.** Concerning model (1), the following holds.

(i) The matrices $A_a$ and $A_i$ are diagonal with nonpositive elements.
(ii) Both the activation and the inhibition functions, i.e $f(\cdot,\cdot)$ and $g(\cdot,\cdot)$, are piecewise continuously differentiable mappings from $\mathbb{R}^m \times \mathbb{R}^n$ to $\mathbb{R}^m$ and $\mathbb{R}^n$, respectively.
(iii) There exist a compact connected set $\mathcal{O}_y \subseteq \mathbb{R}^m$, and a compact connected set $\mathcal{O}_x \subseteq \mathbb{R}^n$, such that $f$ and $g$ are continuously differentiable in $\mathcal{O}_y \times \mathcal{O}_x$, and

- for any given $y \in \mathcal{O}_y$, $\frac{\partial f_i(y,x)}{\partial x_j} \geq 0$, $i = 1,\dots,m$, $j = 1,\dots,n$, for all $x \in \mathcal{O}_x$; moreover, there exist $i \in \{1,\dots,m\}$, and $h \in \{1,\dots,n\}$, such that $\frac{\partial f_i(y,x)}{\partial x_h} > 0$, for all $x \in \mathcal{O}_x$;
- for any given $x \in \mathcal{O}_x$, $\frac{\partial g_j(y,x)}{\partial y_i} \leq 0$, $j = 1,\dots,n$, $i = 1,\dots,m$, for all $y \in \mathcal{O}_y$; moreover, there exist $j \in \{1,\dots,n\}$, and $k \in \{1,\dots,m\}$, such that $\frac{\partial g_j(y,x)}{\partial y_k} < 0$, for all $y \in \mathcal{O}_y$.

(iv) The production rates are assumed constant in time, i.e. $u_y(t) = u^y \in \mathbb{R}^u_+$ and $u^x(t) = u^x \in \mathbb{R}^v_+$. ◊

Some comments are in order to clarify the rationale underlying Assumption 1.

**Remark 1.** Assumption 1-(i) is needed to guarantee the presence of a linear consumption term of the involved species, which has a stabilizing effect on the closed loop system; some eigenvalues of $A_a$ and/or $A_i$ may be located at the origin of the complex plane, due to the presence of integral actions. It is worth noting that in [11], a more general non-diagonal structure for $A_i$ and $A_a$ is considered; however Assumption 1-(i) does not affect the generality of the results stated in the following, since we can always reduce to diagonal $A_i$ and $A_a$ matrices by incorporating their possible extra-terms in the nonlinear part of the equations. ■

**Remark 2.** Assumption 1-(ii) guarantees the existence and uniqueness of the solution of model (1), see [13]. ■

**Remark 3.** Assumption 1-(iii) guarantees that the derivatives of the activation and inhibition functions are well defined at any point of $\mathcal{O}_y \times \mathcal{O}_x$; moreover, when a component of the activation vector $x$ increases, each component of the activation function $f$ is non-decreasing in every direction of the activation sub-space $\mathcal{O}_x$, and there is at least a component strictly increasing in some directions. This technical assumption takes into account the fact that, in some cases, the activation function $f$ could not depend on some activation species. In those cases the partial derivative of its components with respect to such species is identically zero; however *at least* one activation species has to activate $f$ and, consequently, the inhibition vector $y$. At the same time, when a component of the inhibition vector $y$ increases, each component of the inhibition function $g$ is non-increasing in every direction of the inhibition sub-space $\mathcal{O}_y$, and there is at least a component strictly decreasing in some directions. As we shall see in the sequel of the paper, the two opposite actions are necessary to ensure the stability of the homeostatic equilibrium point of the overall system. In the following we shall refer to the set $\mathcal{O} := \mathcal{O}_y \times \mathcal{O}_x$ as the operating envelope of model (1). ■

**Remark 4.** According to Remark 3, a consequence of Assumption 1-(iii) is that, in the simplest case of second order systems (one activation and one inhibition variable), both $f_x$ and $g_y$ must be strictly positive in the operating envelope. In the same way, for third order systems, with $m = 1$, $n = 2$, we have that either $\partial f/\partial x_1$ or $\partial f/\partial x_2$ has to be strictly positive, and either $\partial g_1/\partial y$ or $\partial g_2/\partial y$ needs to be strictly negative in the operating envelope. The dual considerations hold when $m = 2$, $n = 1$. ■

**Remark 5.** Model (1) differs from the one proposed in [11], since here we do not consider delay terms, being more focused on the investigation of the stability properties of the system structure assumed in (1).

**Remark 6.** A difference with respect to [10,11] is that we require the effectiveness of Assumption 1-(iii) to hold only in a subset of the state space of system (1). In the main results of the paper, we shall see that the set $\mathcal{O}$ is, in practice, a suitable neighborhood of the equilibrium point; when the set $\mathcal{O}$ coincides with the whole reachable state space (typically the positive orthant, since, in many cases, all state variables are intrinsically positive physical quantities) the equilibrium point, if existing, is unique. In presence of multiple equilibria, e.g. prey–predator models, where stable and unstable equilibria may coexist, the set $\mathcal{O}$ may reduce to a strict subset of the state space. Our case studies, discussed in the following, will better illustrate such point. ■

**Remark 7.** According to Assumption 1-(iv), in this paper, we shall assume that the production rates can have either an endogenous or exogenous nature and a constant value (to guarantee the existence of a homeostatic equilibrium). The possibility of external access to $u^y$ and/or $u^x$ is of great importance when we face the problem of artificially controlling a physiological system [14,15], which is not a goal of this paper and will be discussed in future work; in those cases the artificial control of the production rates can render $u^y$ and $u^x$ varying in time. ■

As explained in [11], a major difference between physiological and classical control systems is represented by the summation node, which is an essential part of the controller in the classical configuration and is missing in a physiological system; as a consequence, the error signal (the output of the summation node) cannot be defined in the physiological framework depicted in Fig. 1. Moreover, the reference signal is absent in a physiological control system. Indeed, control systems such as the one in Fig. 1 are sometimes referred to as *control system without error detection* [16].

Said $n$ and $m$ be the number of activation and inhibition variables, respectively, the main goal of this paper is to study the structural properties, with particular reference to the stability issues, of model (1) when $m + n \leq 4$, that is up to fourth-order systems.

### 2.2. Second order models

In [10], exploiting some paradigmatic examples, we have investigated the structure of a negative feedback physiological system, when only one activation and one inhibition variable are active. In this case, letting $A_a = -\alpha$, $A_i = -\beta$, with $\alpha$ and $\beta$ non-negative scalars, $B_i = B_a = 1$, model (1) can be rewritten as follows

$$\dot{y}(t) = -\alpha y(t) + u^y + f\,(y(t), x(t)) \tag{2a}$$

$$\dot{x}(t) = -\beta x(t) + u^x + g\,(y(t), x(t))\,, \tag{2b}$$

where $x$ and $y$ are scalar variables, and $\mathcal{O}_y$ and $\mathcal{O}_x$ (see Assumption 1-(iii)) reduces to closed intervals of $\mathbb{R}$.

We can state the following result, which is a condition for the existence and uniqueness of an asymptotically stable equilibrium point.

**Theorem 2.1** (*Uniqueness and asymptotic stability: second order case*)**.** *Consider system (2) under Assumption 1. Moreover, assume that*

*(i) at least one between $\alpha$ and $\beta$ is strictly positive;*
*(ii) for $(y\ x)^T \in \mathcal{O} := \mathcal{O}_y \times \mathcal{O}_x$,*

$$f_y(y, x) \leq 0\,, \quad g_x(y, x) \leq 0\,; \tag{3}$$

*then if an equilibrium point $(y_e\ x_e)^T \in \mathcal{O}$ exists, under the constant input $(u^y\ u^x)^T \in \mathbb{R}_+ \times \mathbb{R}_+$, it is asymptotically stable and unique in $\mathcal{O}$.*

**Proof.** Assume that an equilibrium point $(y_e\ x_e)^T \in \mathcal{O}$ exists, under the constant input $(u^y\ u^x)^T \in \mathbb{R}_+ \times \mathbb{R}_+$; then, the dynamical matrix of the linearized version of system (2), around such point, is given by

$$A := \begin{pmatrix} -\alpha + f_y(y, x) & f_x(y, x) \\ g_y(y, x) & -\beta + g_x(y, x) \end{pmatrix}\Bigg|_{\substack{y=y_e \\ x=x_e}}\,. \tag{4}$$

The characteristic polynomial associated to matrix $A$ is (for the sake of simplicity, the dependence of the derivatives on $(y_e, x_e)$ is omitted)

$$\begin{aligned} p(\lambda) &= \det(\lambda I - A) \\ &= \left|\begin{pmatrix} \lambda + \alpha - f_y & -f_x \\ -g_y & \lambda + \beta - g_x \end{pmatrix}\right| \\ &= \lambda^2 + (\alpha + \beta - g_x - f_y)\,\lambda + (\alpha - f_y)\,(\beta - g_x) - f_x g_y\,. \end{aligned} \tag{5}$$

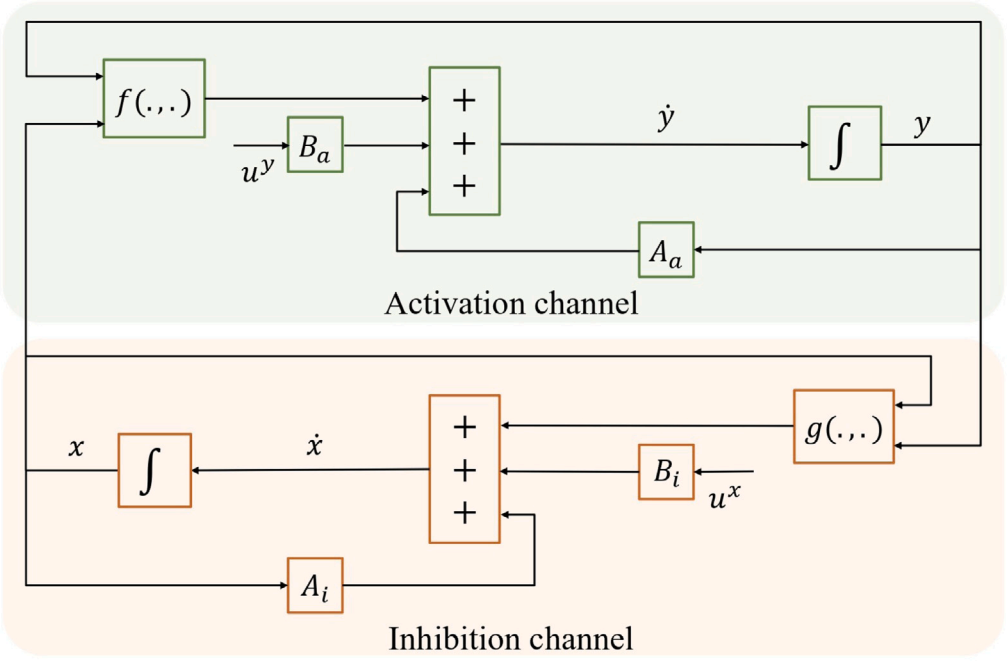



**Fig. 1.** Block-scheme implementation of model (1). Activation and inhibition channels operate reciprocally, creating a mirror topology. Unlike traditional control models, this system lacks a clear delineation between the controller and the plant: the control action is inherently integrated within the system to ensure homeostatic regulation.

By virtue of the hypothesis of the theorem and the considerations done in Remark 4, we have that at the equilibrium

$$\alpha + \beta - g_x - f_y \geq \alpha + \beta > 0 \tag{6a}$$

$$\left(\alpha - f_y\right)\left(\beta - g_x\right) - f_x g_y \geq -f_x g_y > 0\,. \tag{6b}$$

The left hand sides in (6) are exactly the coefficients of the polynomial $p(\lambda)$; since such polynomial is of degree two, positiveness of the coefficients implies that the roots have negative real part. Therefore, the linearized model is asymptotically stable; according to [17], Theorem 4.13, this guarantees asymptotic stability of the equilibrium point $(y_e\ x_e)^T$ of model (2).

Now, let us discuss the issue regarding uniqueness of the equilibrium. Given $(u^y\ u^x)^T \in \mathbb{R}_+ \times \mathbb{R}_+$, define

$$\xi(y,x) := -\alpha y + u^y + f(y,x) \tag{7a}$$

$$\psi(y,x) := -\beta x + u^x + g(y,x)\,. \tag{7b}$$

The equilibrium $(y_e\ x_e)^T$ then satisfies the pair of equations

$$\xi(y,x) = 0 \tag{8a}$$

$$\psi(y,x) = 0\,. \tag{8b}$$

Now assume, by contradiction, that there exists another equilibrium point $(\tilde{y}\ \tilde{x})^T \in \mathcal{O}$, with $(\tilde{y}\ \tilde{x})^T \neq (y_e\ x_e)^T$; by definition, even the point $(\tilde{y}\ \tilde{x})^T$ satisfies conditions (8). First, assume that $\tilde{y} > y_e$ and $\tilde{x} > x_e$. Since $\psi$ is a decreasing function of $y$, in view of Assumption 1-(iii) and Remark 4, and is a non-increasing function of $x$, in view of (3), we have

$$\begin{aligned}\psi(\tilde{y},\tilde{x}) - \psi(y_e,x_e) &= \psi(\tilde{y},\tilde{x}) - \psi(y_e,\tilde{x}) + \psi(y_e,\tilde{x}) - \psi(y_e,x_e)\\ &\leq \psi(\tilde{y},\tilde{x}) - \psi(y_e,\tilde{x})\\ &< 0\,.\end{aligned} \tag{9}$$

Condition (9) contradicts the fact that, since $\psi(\tilde{y},\tilde{x}) = \psi(y_e,x_e) = 0$, we have

$$\psi(\tilde{y},\tilde{x}) - \psi(y_e,x_e) = 0.$$

Now assume that $\tilde{y} < y_e$ and $\tilde{x} > x_e$. In this case, since $\xi$ is a non-increasing function of $y$, in view of (3), and an increasing function of $x$, in view of Assumption 1-(iii) and Remark 4,

$$\begin{aligned}\xi(\tilde{y},\tilde{x}) - \xi(y_e,x_e) &= \xi(\tilde{y},\tilde{x}) - \xi(y_e,\tilde{x}) + \xi(y_e,\tilde{x}) - \xi(y_e,x_e)\\ &\geq \xi(y_e,\tilde{x}) - \xi(y_e,x_e)\\ &> 0\,.\end{aligned} \tag{10}$$

Condition (10) contradicts the fact that, since $\xi(\tilde{y},\tilde{x}) = \xi(y_e,x_e) = 0$, we have

$$\xi(\tilde{y},\tilde{x}) - \xi(y_e,x_e) = 0.$$

Following the same guidelines, the other two cases namely $\tilde{y} > y_e$, $\tilde{x} < x_e$, and $\tilde{y} < y_e$, $\tilde{x} < x_e$, leads to similar contradictions.

We can conclude that the equilibrium point $(\tilde{y}\ \tilde{x})^T$ has necessarily to coincide with the equilibrium $(y_e\ x_e)^T$. Since the choice of $(\tilde{y}\ \tilde{x})^T \in \mathcal{O}$ is arbitrary, we can conclude that the equilibrium $(y_e\ x_e)^T$ is unique in $\mathcal{O}$. ■

**Remark 8.** In Theorem 2.1, we investigate local asymptotic stability, instead of the global one, since physiological control systems operate under fundamentally different assumptions from those typically adopted in control theory. Rather than evolving over unbounded state spaces, biological regulation is intrinsically constrained to finite and viability-limited ranges of operation. Variables such as hormone concentration, treated in our work, or others such as blood pressure, may vary only within specific intervals compatible with cellular and system integrity. Outside these intervals, the underlying biochemical and structural processes, that sustain regulation, begin to fail. Moreover, physiological control mechanisms are characterized by nonlinearities that reflect physical and biological constraints. Saturation effects, and threshold phenomena are common, since actuators, such as hormonal secretion rates or neural firing frequencies, have finite limits. For example, regulatory responses may activate only once a critical deviation is exceeded, as when glucose exceeds a certain value triggering the production of insulin. When perturbations exceed certain values in magnitude or duration, the system may undergo pathological or

irreversible states. In such cases, the notion of returning to a pre-existing equilibrium loses meaning. As a consequence, in the context of physiological systems, stability is inherently local, i.e. convergence to a homeostatic regime occurs only for initial conditions lying within a bounded region that preserves the integrity of the organism. Outside this region, trajectories may diverge toward qualitatively different outcomes, including pathological steady states or terminal conditions such as coma or death. ■

**Remark 9.** Theorem 2.1 guarantees the existence of a unique equilibrium point; an open question concerns the computation of an estimate of the domain of attraction (DA) of such equilibrium [17]. In general, this turns out to be a difficult task, which requires the optimization over the set of Lyapunov functions, and the computation of the corresponding level curves; moreover, its resolvability strongly depends on the structure of the nonlinear system under investigation (see, among others, [18–23]). It is worth noting that the set $\mathcal{O}$ introduced in Assumption 1 and Remark 3, does not coincide, in general, with an estimate of the DA; rather, it is a compact subset of the state space where, if the assumptions are satisfied, the homeostatic equilibrium point is located. The problem of estimating the DA of such points will be investigated in future works. ■

**Remark 10.** Note that, in Theorem 2.1, further conditions involving the cross derivatives, that is the derivative of the activation function $f$ versus the inhibition variable $y$ and vice versa, are required. We shall see that similar conditions will be encountered when more complex physiological systems are considered; we prefer to set apart such conditions rather than include them in the initial Assumption 1, which has a physical interpretation and is shared by all negative feedback physiological systems; more on this point is discussed in Section 4. ■

**Remark 11.** The thesis of Theorem 2.1 still holds when $\alpha$ and $\beta$ are both zero. In those cases, however, at least one of the conditions in (3) needs to be strictly negative (see condition (6a)). ■

It is interesting to note that a geometrical proof for the first part of Theorem 2.1 can be obtained through an approach based on the Implicit Function Theorem. Such proof helps to give a more physical and intuitive interpretation of the assumptions on the functions $f$ and $g$ to guarantee the stability of the equilibrium.

To this end, assume that $(y_e \ x_e)^T \in \mathcal{O}$ is an equilibrium point under the constant input $(u^y \ u^x)^T \in \mathbb{R}_+ \times \mathbb{R}_+$. From classical system theory, we can conclude that it is a solution of equation

$$\dot{y} = \xi(y, x) = 0\,, \tag{11}$$

where $\xi$ is defined in (7a).

Note that Eq. (11) represents the projection of $\dot{y}$ on the $(y, x)$-plane; the application of the theorem for implicit functions guarantees that there exists a neighborhood $\mathcal{I}_{y_e,x_e}$ of $(y_e \ x_e)^T$, contained in $\mathcal{O}$, such that the equation $\xi(y, x) = 0$ implicitly defines the function

$$y = \Xi(x)\,, \quad \begin{pmatrix} y & x \end{pmatrix}^T \in \mathcal{I}_{y_e,x_e},$$

and, at the point $(y_e \ x_e)^T$,

$$\Xi_x = -\frac{\xi_x}{\xi_y}\,. \tag{12}$$

From (7), we obtain that

$$\xi_x(y_e, x_e) = f_x(y_e, x_e) > 0,$$

and, by virtue of the assumptions in Theorem 2.1,

$$\xi_y(y_e, x_e) = -\alpha + f_y(y_e, x_e) \leq 0.$$

From (12), we can conclude that $\Xi_x$ is positive at $x_e$, therefore $\Xi(x)$ is increasing in $I_{y_e,x_e}$.

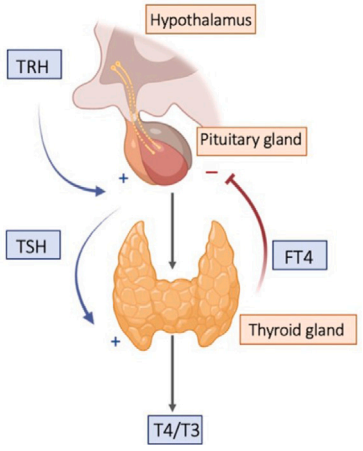



**Fig. 2.** Illustration of the homeostatic regulation of thyroid hormones.

On the other hand, the equilibrium $(y_e \ x_e)^T$ has also to satisfy

$$\dot{x} = \psi(y, x) = 0\,, \tag{13}$$

where $\psi(y, x)$ is defined (7b).

The equation $\psi(y, x) = 0$ implicitly defines the function

$$x = \Psi(y)\,, \quad \begin{pmatrix} y & x \end{pmatrix}^T \in \mathcal{I}_{y_e,x_e},$$

and, at the point $(y_e \ x_e)^T$,

$$\Psi_y = -\frac{\psi_y}{\psi_x}\,.$$

Since $\psi_y < 0$ and $\psi_x \leq 0$, we can conclude that $\Psi(y)$ is decreasing in $I_{y_e,x_e}$.

The projections of $\dot{y}$ and $\dot{x}$ onto the $(y, x)$-plane are then governed by the pair of functions $(\Xi, \Psi)$, which meets at the equilibrium point and exhibits a monotone opposite behavior, thus driving the trajectory $\big(y(t), x(t)\big)$ toward the equilibrium $(y_e \ x_e)^T$.

### 2.2.1. *Case study: Homeostatic regulation of thyroid hormones*

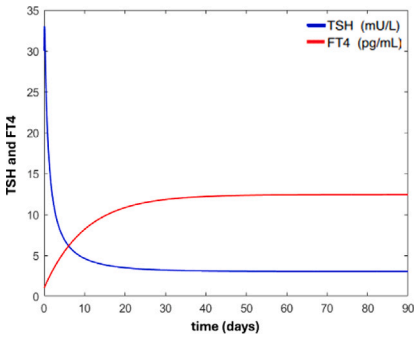
We consider the model that describes the homeostatic regulation of thyroid hormones, focusing on the dynamic interplay between thyrotropin, henceforth referred to as the concentration of thyroid-stimulating hormone ($TSH$), a pituitary hormone that stimulates the thyroid gland, and blood concentration of thyroxine (T4), which is the primary circulating thyroid hormone [24].

As depicted in Fig. 2, the model reproduces the characteristic negative feedback loop of the hypothalamic-pituitary-thyroid ($HPT$) axis, whereby the $TSH$, secreted by the anterior pituitary, stimulates the thyroid gland to produce T4. The free fraction of T4 in the blood ($FT4$) exerts the feedback control action: when the circulating $FT4$ level decreases, the secretion of $TSH$ increases to promote T4 synthesis; conversely, high values of $FT4$ suppress the production of $TSH$ by inhibiting the secretion of thyrotropin-releasing hormone ($TRH$) from the hypothalamus and $TSH$ from the pituitary.

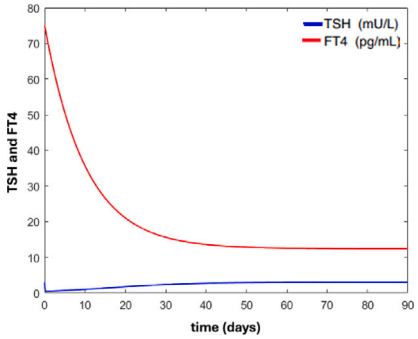
This regulatory mechanism establishes a homeostatic equilibrium for thyroid hormone levels. The system is analyzed under the assumption of a euthyroid state, corresponding to physiological conditions of the normal thyroid function. According to the discussion above, $FT4$ and $TSH$ play the role of the inhibition and the activation variables, respectively; therefore, the system can be modeled through a couple of differential equations, as follows

$$\dot{FT4} = -d_1 FT4 + \frac{TSH}{s_1 + TSH}\left(d_1 U\left(1 + \frac{d_2 s_1}{p}\right)\right) \tag{14a}$$

$$\dot{TSH} = -d_2 TSH + \frac{p(s_2 + U)}{s_2 + FT4}\,, \tag{14b}$$




(a) Dynamics of $TSH$ and $FT4$ starting from initial values $(FT4, TSH) = (1\,\text{pg/mL}, 30\,\text{mU/L})$, where $FT4$ is below and $TSH$ is above the normal range. The system returns to the euthyroid state within approximately 65 days.




(b) Dynamics of $TSH$ and $FT4$ starting from initial values $(FT4, TSH) = (75\,\text{pg/mL}, 3\,\text{mU/L})$, where $FT4$ is above and $TSH$ is below the normal range. The system also returns to the euthyroid state within approximately 65 days.

**Fig. 3.** Dynamics of $FT4$ and $TSH$ for a euthyroid individual under two different initial conditions. Each panel illustrates how the system evolves over time and asymptotically returns to equilibrium.

where $U$ denotes the maximal concentration of free thyroxine available for the pituitary–thyroid feedback loop, $d_1$ and $d_2$ represent the degradation rates of $FT4$ and $TSH$, respectively, $p$ is the basal production rate of $TSH$ in the absence of stimulation, $s_1$ and $s_2$ are sensitivity constants characterizing the stimulatory effect of $TSH$ on the $FT4$ release and the strength of the inhibitory feedback of $FT4$ on the $TSH$ secretion, respectively.

Referring to the general mathematical model described by (2), as said above, the concentration of free thyroxine $FT4$ corresponds to the inhibition variable, denoted by $y$, while the concentration of thyrotropin $TSH$ corresponds to the activation variable, denoted by $x$. In order to simplify the notation, we set

$$k = d_1 U \left(1 + \frac{d_2 s_1}{p}\right); \tag{15}$$

here, $k$ represents the rate of $FT4$ production in euthyroid individuals, capturing the balance between the $TSH$ stimulation and the negative feedback mechanisms. Thus, we can rewrite (14) in the form (2) as follows

$$\dot{y} = -d_1 y + f(x) \tag{16a}$$

$$\dot{x} = -d_2 x + g(y), \tag{16b}$$

where

$$f(x) = \frac{kx}{s_1 + x} \tag{17a}$$

$$g(y) = \frac{p(s_2 + U)}{s_2 + y}. \tag{17b}$$

In order to apply Theorem 2.1, first we have to show that Assumption 1 is satisfied; to this end note that

$$f_x = \frac{k s_1}{(s_1 + x)^2} > 0, \quad x \geq 0 \tag{18a}$$

$$g_y = -\frac{p(s_2 + U)}{(s_2 + y)^2} < 0, \quad y \geq 0. \tag{18b}$$

Moreover, the hypothesis of Theorem 2.1 are satisfied, since $\alpha = d_1$ and $\beta = d_2$ are strictly positive, and $f_y = g_x = 0$. Therefore, the thesis of Theorem 2.1 holds and, on the basis of (18), a suitable set $\mathcal{O}$ is any closed connected set in the positive orthant.

The application of Theorem 2.1 guarantees the possible existence of an asymptotically stable unique equilibrium point in $\mathcal{O}$. In order to find such point, we need to solve the system of equations

$$-d_1 y + \frac{kx}{s_1 + x} = 0 \tag{19a}$$

$$-d_2 x + \frac{p(s_2 + U)}{s_2 + y} = 0, \tag{19b}$$

where, according to [24], we assume that $d_1 = 0.099\, day^{-1}$, $s_1 = 0.0021\, mU \cdot L^{-1}$, $k = 1.237\, day^{-1} \cdot pg \cdot mL^{-1}$, $d_2 = 16.63\, day^{-1}$, $p = 50\, mU \cdot L^{-1} \cdot day^{-1}$, $s_2 = 0.0434\, pg \cdot mL^{-1}$ and $U = 12.5\, pg \cdot mL^{-1}$.

As expected, system (19) admits only one solution, with $y_e = 12.5\ pg \cdot mL^{-1}$, $x_e = 3\ mU \cdot L^{-1}$. In Fig. 3(a) and 3(b) the trajectories converging to the equilibrium point in the $(x, y)$-plane are depicted.

Note that, in this case study, an alternative way of exploiting Theorem 2.1 is that of defining

$$\tilde{f}(y, x) = -d_1 y + \frac{kx}{s_1 + x}$$

$$\tilde{g}(y, x) = -d_2 x + \frac{p(s_2 + U)}{s_2 + y}.$$

In this way, $\alpha = \beta = 0$, which does not satisfy the hypothesis of Theorem 2.1. However, according to Remark 11, the theorem thesis still holds, since $\tilde{f}_y$, $\tilde{g}_x$ are both strictly negative.

### 2.3. Third order models

The findings related to second-order systems can be extended to higher-order systems. In particular, in this section, our analysis will focus on third-order systems. For the latter, it is important to distinguish between the case where $m$=1 and $n$=2 (two activation variables and one inhibition variable) and the case where $m$=2 and $n$=1. To fix ideas, we will consider the case $m = 1$, $n = 2$; however, all the obtained results can easily be generalized to the other case.

Model (1) for third-order systems ($m$=1, $n$=2) can be rewritten as follows

$$\dot{y}(t) = -\alpha y(t) + B_a u^y + f\,(y(t), x(t)) \tag{20a}$$

$$\begin{bmatrix} \dot{x}_1(t) \\ \dot{x}_2(t) \end{bmatrix} = \begin{bmatrix} -\beta_1 & 0 \\ 0 & -\beta_2 \end{bmatrix} \begin{bmatrix} x_1(t) \\ x_2(t) \end{bmatrix} + \begin{bmatrix} b_{i_{11}} & b_{i_{12}} \\ b_{i_{21}} & b_{i_{22}} \end{bmatrix} \begin{bmatrix} u^{x_1} \\ u^{x_2} \end{bmatrix} + \begin{bmatrix} g_1(y(t), x(t)) \\ g_2(y(t), x(t)) \end{bmatrix} \tag{20b}$$

where $\alpha$, $\beta_1$ and $\beta_2$ are non-negative scalars, $y(t) \in \mathbb{R}$, $b_{i_{11}}, b_{i_{12}}, b_{i_{21}}$ and $b_{i_{22}}$ are real scalars, $(x_1(t)\ x_2(t))^T \in \mathbb{R}^2$, $B_a$ is a scalar (without loss of generality, we shall assume that $B_a = 1$).

As in the case of second-order systems, a sufficient condition can be established for the existence and uniqueness of an asymptotically stable equilibrium point.

**Theorem 2.2** (*Uniqueness and asymptotic stability: third order case, $m = 1$, $n = 2$*)**.**

*Consider system (20) under Assumption 1. Moreover, assume that*

*(i) at least one between $\alpha$, $\beta_1$ and $\beta_2$ is strictly positive;*
*(ii) in the set $\mathcal{O} := \mathcal{O}_y \times \mathcal{O}_x \subseteq \mathbb{R} \times \mathbb{R}^2$,*

$$f_y \leq 0\,, \quad \frac{\partial g_i}{\partial x_i} \leq 0\,, \quad i = 1,2 \tag{21a}$$

$$|g_x| > 0\,, \quad \left|g_{(y\,x_1)^T}\right| < 0\,, \quad \left|g_{(y\,x_2)^T}\right| > 0\,; \tag{21b}$$

*then if an equilibrium point $(y_e\ x_e)^T \in \mathcal{O}$ exists, under the constant input $(u^y\ u^x)^T \in \mathbb{R}_+ \times \mathbb{R}_+^2$, it is asymptotically stable and unique in $\mathcal{O}$.*

**Remark 12.** It is useful to make a comparison between the conditions stated in Theorem 2.1 and those ones given in Theorem 2.2. Both theorems require the satisfaction of Assumption 1; moreover, conditions (i) and (21a) are the generalization of conditions (i) and (ii) of Theorem 2.1 to the third order case. Finally Theorem 2.2 requires conditions (21b), involving second-order determinants. ■

**Proof.** The proof is long and technical and can be found in Appendix. ■

The dual result of Theorem 2.2 can be established if we have only one activation variable and two inhibition variables, i.e. $m = 2$, $n = 1$. In this case, model (1) can be rewritten as follows

$$\begin{bmatrix}\dot{y}_1(t)\\ \dot{y}_2(t)\end{bmatrix} = \begin{bmatrix}-\alpha_1 & 0\\ 0 & -\alpha_2\end{bmatrix}\begin{bmatrix}y_1(t)\\ y_2(t)\end{bmatrix} + \begin{bmatrix}b_{a_{11}} & b_{a_{12}}\\ b_{a_{21}} & b_{a_{22}}\end{bmatrix}\begin{bmatrix}u^{y_1}\\ u^{y_2}\end{bmatrix} + \begin{bmatrix}f_1(y(t),x(t))\\ f_2(y(t),x(t))\end{bmatrix} \tag{22a}$$

$$\dot{x}(t) = -\beta x(t) + B_i u^x + g\,(y(t), x(t))\,, \tag{22b}$$

where $\alpha_1$, $\alpha_2$ and $\beta$ are non-negative scalars, $x(t) \in \mathbb{R}$, $b_{a_{11}}, b_{a_{12}}, b_{a_{21}}$ and $b_{a_{22}}$ are real scalars, $(y_1(t)\ y_2(t))^T \in \mathbb{R}^2$, $B_i$ is a scalar (without loss of generality, we shall assume that $B_i = 1$).

The proof of the following theorem follows the same guidelines of those one of Theorem 2.2.

**Theorem 2.3** (*Uniqueness and asymptotic stability: third order case, $m = 2$, $n = 1$*)**.**

*Consider system (20) under Assumption 1. Moreover, assume that*

*(i) at least one between $\alpha_1$, $\alpha_2$ and $\beta$ is strictly positive;*
*(ii) in the set $\mathcal{O} := \mathcal{O}_y \times \mathcal{O}_x \subseteq \mathbb{R}^2 \times \mathbb{R}$,*

$$g_x \leq 0\,, \quad \frac{\partial f_i}{\partial y_i} \leq 0\,, \quad i = 1,2 \tag{23a}$$

$$|f_y| > 0\,, \quad \left|f_{(y_1\,x)^T}\right| < 0\,, \quad \left|f_{(y_2\,x)^T}\right| > 0\,; \tag{23b}$$

*then if an equilibrium point $(y_e\ x_e)^T \in \mathcal{O}$ exists, under the constant input $(u^y\ u^x)^T \in \mathbb{R}_+^2 \times \mathbb{R}_+$, it is asymptotically stable and unique.*

### *2.3.1. Case study: A prey–predator model to describe the dynamics of tumor progression*

The case study under examination focuses on the quadratic model for tumor growth. This model describes tumor progression as a prey–predator system. Tumor cells are represented as a prey population competing with a predator population composed of healthy cells within the framework of organic tissues.

The predators consist of cells involved in the immune response against the tumor, including T-lymphocytes, macrophages, and natural killer cells. These cells have the ability to engulf and destroy tumor cells. T-lymphocytes, in particular, can be categorized into two main types: regulatory and cytotoxic. Regulatory T-lymphocytes, also known as helper cells, coordinate the attack against tumor cells, even though they do not directly participate in their elimination. Nevertheless, they play a crucial role in promoting the growth of various populations of predator cells, such as macrophages and cytotoxic T-lymphocytes.

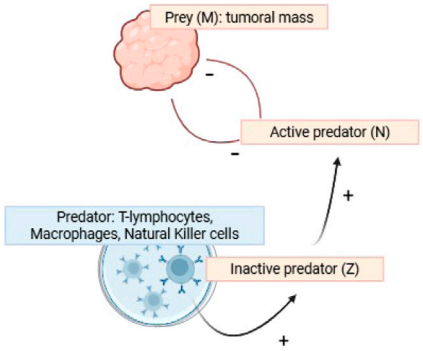



**Fig. 4.** Illustration of prey–predator dynamics of tumor progression.

Moreover, predator cells can exist in two functional states: active, when they are engaged in hunting, or inactive, when they are at rest. For instance, cytotoxic T-lymphocytes in their inactive state can be activated and transformed into effective predators (cytotoxic cells) upon receiving activation signals from helper T-lymphocytes.

Thereby, as depicted in Fig. 4, the model contains three state variables: the density of tumor cells $M$, the density of hunting predator cells $N$, and the density of resting predator cells $Z$. According to the discussion above, we can conclude that the inhibition variable is represented by the hunting predator cells, while the activation variables are the tumor cells and the resting predator cells.

The system model is described by the following set of differential equations [21]

$$\dot{M} = q + rM\left(1 - \frac{M}{k_1}\right) - \alpha M N \tag{24a}$$

$$\dot{N} = \beta N Z - d_1 N \tag{24b}$$

$$\dot{Z} = sZ\left(1 - \frac{Z}{k_2}\right) - \beta N Z - d_2 Z\,. \tag{24c}$$

First, letting

$$y = N\,, \quad x = \begin{pmatrix} M \\ Z \end{pmatrix} \tag{25a}$$

$$u^y = 0\,, \quad u^x = \begin{pmatrix} q \\ 0 \end{pmatrix} \tag{25b}$$

$$A_a = 0\,, \quad A_i = \begin{pmatrix} 0 & 0 \\ 0 & -d_2 \end{pmatrix} \tag{25c}$$

$$B_a = 0\,, \quad B_i = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} \tag{25d}$$

$$f(y,x) = -d_1 y + \beta y x_2\,, \quad g(y,x) = \begin{pmatrix} rx_1 - \frac{r}{k_1}(x_1)^2 - \alpha x_1 y \\ sx_2 - \frac{s}{k_2}(x_2)^2 - \beta y x_2 \end{pmatrix}, \tag{25e}$$

we rewrite system (24) in the form (1).

Therefore, based on the parameters in Table 1, we can rewrite system (24) as

$$\dot{y} = f(y,x) \tag{26a}$$

$$\dot{x} = \begin{pmatrix} 0 & 0 \\ 0 & -0.03 \end{pmatrix} x + \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} u^x + g(y,x)\,. \tag{26b}$$

The structure of system (26) is captured by the reference model (20); the satisfaction of the conditions stated in Assumption 1, and the application of Theorem 2.2 to investigate the existence of the homeostatic equilibrium will be discussed in the Results Section 3.

**Table 1**
Parameters and their numerical values.

| Parameter | Value | Description |
|---|---|---|
| $q$ | 10 | Rate of conversion of normal cells into malignant cells [$Number$ h$^{-1}$] |
| $r$ | 0.9 | Rate of tumor cell growth [h$^{-1}$] |
| $\alpha$ | 0.3 | Rate of tumor cell predation by hunting cells [$Number^{-1}$ h$^{-1}$] |
| $k_1$ | 0.8 | Maximum carrying capacity of the tumor cells [$Number$] |
| $\beta$ | 0.1 | Rate of conversion of the resting cells to the hunting cells [$Number^{-1}$ h$^{-1}$] |
| $d_1$ | 0.02 | Natural death of the hunting cells [h$^{-1}$] |
| $s$ | 0.8 | Growth rate of the resting predator cells [h$^{-1}$] |
| $k_2$ | 0.7 | Maximum carrying capacity of the resting cells [$Number$] |
| $d_2$ | 0.03 | Natural death rate of resting cells [h$^{-1}$] |

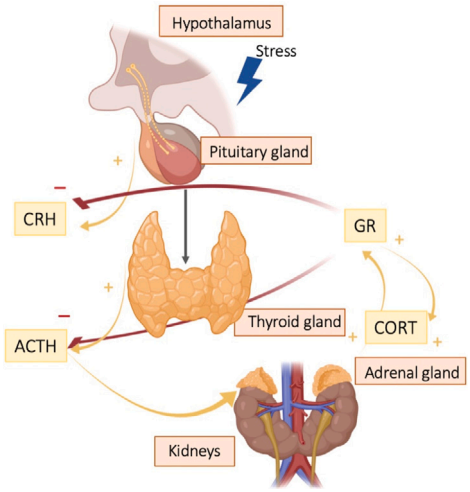



**Fig. 5.** Illustration of cortisol dynamics in response to stress/pain conditions.

### 2.4. Fourth order models: Cortisol dynamics in response to pain/stress conditions

In the case of fourth-order systems, there are various cases to be considered, namely $m = n = 2$, $m = 1$, $n = 3$, and $m = 3$, $n = 1$. Deriving conditions similar to those ones provided in Theorems 2.1–2.3 requires a formidable amount of computations and leads to conditions for stability that are not useful from a practical point of view. Therefore, in the following, we shall limit our analysis to investigating a complex negative feedback physiological system with four state variables, showing how its model is captured by (1); the numerical analysis of the existence of homeostatic equilibria is performed in Section 3.

The hypothalamic–pituitary–adrenal (HPA) axis is a central regulatory system in the human body, responsible for controlling the secretion of cortisol—a glucocorticoid hormone essential for the stress response and metabolic regulation. This axis involves a cascade of biochemical interactions between the hypothalamus, the anterior pituitary, and the adrenal cortex.

As depicted in Fig. 5, in response to stress, the corticotropin-releasing hormone ($CRH$) is secreted by the hypothalamus, stimulating the production of the adrenocorticotropic hormone ($ACTH$) in the pituitary, which subsequently induces cortisol synthesis in the adrenal cortex; therefore, in this case, $CRH$ and $ACTH$ are the activation species. Cortisol ($CORT$) exerts both feedforward and negative feedback actions within the HPA axis, with the latter being critical for maintaining homeostasis; hence, $CORT$ plays the role of inhibition species. The second inhibition variable is represented by $GR$ (glucocorticoid receptor complex): when cortisol binds to the glucocorticoid receptor, the GR complex is formed, which then dimerizes and takes part in the negative feedback mechanism on the hypothalamic–pituitary–adrenal axis, regulating the production of $CRH$ and $ACTH$.

In pathological conditions such as major depressive disorder and post-traumatic stress disorder (PTSD), the strength and effectiveness of the negative feedback loop are significantly altered, leading to hypercortisolemia or hypocortisolemia, respectively. To investigate these regulatory mechanisms, we construct a mathematical model based on a system of nonlinear ordinary differential equations incorporating Michaelis–Menten and Hill kinetics. This mechanistic framework allows for the analysis of how feedback sensitivity and stress intensity affect hormonal dynamics, providing insight into physiological transitions between healthy and diseased states.

Specifically, the system under investigation is defined by four state variables, $CRH$, $ACTH$, $CORT$, and $GR$, whose temporal evolution is governed by the following differential equations [25]

$$\begin{cases} \dot{CRH} = -k_{d_1} CRH - V_{S_3} \frac{CRH}{k_{m_1}+CRH} + k_{st} \frac{k_i^{n_2}}{k_i^{n_2}+GR^{n_2}} \\ \dot{ACTH} = -k_{d_2} ACTH - V_{S_4} \frac{ACTH}{k_{m_2}+ACTH} + k_{P_2} CRH \frac{k_i^{n_2}}{k_i^{n_2}+GR^{n_2}} \\ \dot{GR} = -k_{d_5} GR + V_{S_2} \frac{GR^{n_1}}{k_1^{n_1}+GR^{n_1}} + k_b CORT(G_{tot} - GR) \\ \dot{CORT} = -k_{d_3} CORT + k_{P_3} ACTH - V_{S_5} \frac{CORT}{k_{m_3}+CORT}, \end{cases} \tag{27}$$

where the parameter $G_{tot}$ is the total glucocorticoid receptor, related to $GR$ through the glucocorticoid receptor $G = G_{tot} - GR$. First, the system is rewritten in the form of the reference model (1); as said above the activation variables are the corticotrophin-releasing hormone $CRH$ and the aceto-corticotrophin hormone $ACTH$, while the inhibition variables are the complex $GR$ and the cortisol $CORT$. According to the notation adopted in this paper, we have that $m = 2$ and $n = 2$; moreover

$$y = \begin{pmatrix} GR \\ CORT \end{pmatrix}, \quad x = \begin{pmatrix} CRH \\ ACTH \end{pmatrix} \tag{28a}$$

$$u^y = \begin{pmatrix} 0 \\ 0 \end{pmatrix}, \quad u^x = \begin{pmatrix} 0 \\ 0 \end{pmatrix} \tag{28b}$$

$$A_a = \begin{pmatrix} -k_{d_5} & 0 \\ 0 & -k_{d_3} \end{pmatrix}, \quad A_i = \begin{pmatrix} -k_{d_1} & 0 \\ 0 & -k_{d_2} \end{pmatrix} \tag{28c}$$

$$f(y,x) = \begin{pmatrix} k_b y_2 (G_{tot} - y_1) + V_{S_2} \frac{y_1^{n_1}}{k_1^{n_1}+y_1^{n_1}}, \\ k_{P_3} x_2 - V_{S_5} \frac{y_2}{k_{m_3}+y_2} \end{pmatrix} \tag{28d}$$

$$g(y,x) = \begin{pmatrix} -V_{S_3} \frac{x_1}{k_{m_1}+x_1} + k_{st} \frac{k_i^{n_2}}{k_i^{n_2}+y_1^{n_2}} \\ -V_{S_4} \frac{x_2}{k_{m_2}+x_2} + k_{P_2} x_1 \frac{k_i^{n_2}}{k_i^{n_2}+y_1^{n_2}} \end{pmatrix}. \tag{28e}$$

Based on the parameters in Table 2, we can rewrite system (27) in the following form

$$\dot{y} = \begin{pmatrix} -0.0854 & 0 \\ 0 & -0.356 \end{pmatrix} y + f(y,x) \tag{29a}$$

$$\dot{x} = \begin{pmatrix} -0.00379 & 0 \\ 0 & -0.00916 \end{pmatrix} x + g(y,x). \tag{29b}$$

The structure of system (29) is captured by the reference model (1), with $m = n = 2$; the satisfaction of the conditions stated in Assumption 1, and the numerical analysis to investigate the existence of the homeostatic equilibrium will be discussed in the Results Section 3.

**Table 2**
Kinetic parameters and their numerical values.

| Parameter | Value | Description |
|---|---|---|
| $k_{stress}$ | 5 | Stress or pain induced parameter driving the CRH production [μg dL$^{-1}$ h$^{-1}$] |
| $k_i$ | 2 | Inhibition constant regulating the strength of the negative feedback loop [μg dL$^{-1}$] |
| $V_{S3}$ | 3.25 | Rates at which CRH is degraded enzymatically [μg dL$^{-1}$ h$^{-1}$] |
| $k_{m1}$ | 1.74 | Michaelis constant [μg dL$^{-1}$] |
| $k_{P2}$ | 8.30 | Rate of production of ACTH [h$^{-1}$] |
| $V_{S4}$ | 0.907 | Rates at which ACTH is degraded enzymatically [μg dL$^{-1}$ h$^{-1}$] |
| $k_{m2}$ | 0.112 | Michaelis constant [μg dL$^{-1}$] |
| $k_{P3}$ | 0.945 | Rate of production of CORT [h$^{-1}$] |
| $V_{S5}$ | 0.00535 | Rate at which CORT is degraded enzymatically [μg dL$^{-1}$ h$^{-1}$] |
| $k_{m3}$ | 0.0768 | Michaelis constant [μg dL$^{-1}$] |
| $k_{d1}$ | 0.00379 | Autonomous degradation constant [h$^{-1}$] |
| $k_{d2}$ | 0.00916 | Autonomous degradation constant [h$^{-1}$] |
| $k_{d3}$ | 0.356 | Autonomous degradation constant [h$^{-1}$] |
| $n_1$ | 5.43 | Hill constant [·] |
| $n_2$ | 5.10 | Hill constant [·] |
| $k_b$ | 0.0202 | Rate of production of GR [h$^{-1}$] |
| $G_{tot}$ | 3.28 | Total glucocorticoid receptor [μg] |
| $V_{S2}$ | 0.0509 | Rate constant regulating the strength of the saturation mechanism [μg dL$^{-1}$ h$^{-1}$] |
| $k_1$ | 0.645 | Michaelis–Menten constant [μg dL$^{-1}$] |
| $k_{d5}$ | 0.0854 | Autonomous degradation constant [h$^{-1}$] |

## 3. Results

### 3.1. Dynamics of tumor progression

In this section, exploiting Theorem 2.2, we investigate the stability properties of the equilibria of model (26). First, according to the paper [21], we define the set $\mathcal{O}$ as $[1,6]\times[2,3.5]\times[0.1,0.7]$, which is a region of the state space corresponding to a healthy behavior.

In order to verify the satisfaction of Assumption 1, we compute the partial derivatives of the activation and inhibition functions

$$\frac{\partial f}{\partial x_1} = 0 \tag{30a}$$

$$\frac{\partial f}{\partial x_2} = 0.1y \tag{30b}$$

$$\frac{\partial g_1}{\partial y} = -0.3x_1 \tag{30c}$$

$$\frac{\partial g_2}{\partial y} = -0.1x_2\,, \tag{30d}$$

which are readily seen to satisfy Assumption 1 in the set $\mathcal{O}$ defined above.

Concerning the assumptions of Theorem 2.2, we have that $\beta_2 = d_2$ is strictly positive. Moreover, evaluating the derivatives in the set $\mathcal{O}$, we obtain

$$f_y = -0.02 + 0.1x_2 \le 0 \quad x_2 \in [0.1\,,\,0.2] \tag{31a}$$

$$\frac{\partial g_1}{\partial x_1} = 0.9 - 2.25x_1 - 0.3y < 0 \tag{31b}$$

$$\frac{\partial g_1}{\partial x_2} = \frac{\partial g_2}{\partial x_1} = 0 \tag{31c}$$

$$\frac{\partial g_2}{\partial x_2} = 0.8 - 2.28x_2 - 0.1y < 0 \tag{31d}$$

$$\left|g_x\right| = (0.9 - 2.25x_1 - 0.3y)(0.8 - 2.28x_2 - 0.1y) > 0 \tag{31e}$$

$$\left|g_{(y\,x_1)^T}\right| = (0.9 - 2.25x_1 - 0.3y)(0.1y) < 0 \tag{31f}$$

$$\left|g_{(y\,x_2)^T}\right| = (-0.8 + 2.28x_2 + 0.1y)(0.3x_1) > 0\,. \tag{31g}$$

In order to accomplish (31a), we redefine the operating envelope as $\tilde{\mathcal{O}} := [1,6]\times[2,3.5]\times[0.1,0.2]$. In view of Theorem 2.2, we are guaranteed that, if existing, an equilibrium belonging to $\tilde{\mathcal{O}}$ is unique and asymptotically stable. In fact, the system of equations obtained by letting $\dot{y} = 0$ and $\dot{x} = 0$ in (26), admits a unique solution in $\tilde{\mathcal{O}}$ given by

$$\left(y_e\ x_{e1}\ x_{e2}\right)^T = (5.41\ 2.67\ 0.2)^T\,, \tag{32}$$

which is the asymptotically stable homeostatic equilibrium, corresponding to a physiological healthy behavior.

In Fig. 6, the behavior of the state variables, starting from various initial conditions around the equilibrium $\left(y_e\ x_{e1}\ x_{e2}\right)^T$, is shown; it is worth noting the asymptotic convergence to the equilibrium of each state variable.

It is worth noting that, according to [21], model (26) admits two additional equilibria, having the following parametric expressions

$$E_1 = \left(0\ \frac{k_1}{2}\left(1 + \sqrt{1 + \frac{4q}{rk_1}}\right)\ 0\right)^T \tag{33a}$$

$$E_2 = \left[0\ \frac{k_1}{2}\left(1 + \sqrt{1 + \frac{4q}{rk_1}}\right)\ k_2\left(1 - \frac{d_2}{s}\right)\right]\,. \tag{33b}$$

At equilibrium $E_1$, only malignant cells are present; thus, point $E_1$ represents a pathological case, where the concentration of malignant cells diverges. From a systemic point of view, this fact translates into the instability of $E_1$.

At equilibrium $E_2$, both malignant cells and resting predator cells are present, but no killer cells are observed; similar considerations, as for $E_1$, holds.

Let us focus on the equilibrium $E_1$; replacing in (33a) the values collected in Table 1, we have that $E_1 = (0\ 3.4\ 0)^T$. In Fig. 7 one trajectory starting from an initial condition close to $E_1$ is shown; as expected, it diverges, due to the instability of the equilibrium.

It is interesting to see that the conditions of Theorem 2.2 are not verified in correspondence of $E_1$; first, we look for an operating envelope containing $E_1$ and satisfying Assumption 1. We obtain

$$\frac{\partial f}{\partial x_1} = 0 \tag{34a}$$

$$\frac{\partial f}{\partial x_2} = 0.1y > 0\,, \quad y > 0 \tag{34b}$$

$$\frac{\partial g_1}{\partial y} = -0.3x_1 \le 0\,, \quad x_1 > 0 \tag{34c}$$

$$\frac{\partial g_2}{\partial y} = -0.1x_2 \le 0\,, \quad x_2 > 0\,. \tag{34d}$$

From (34), we can conclude that any closed connected set contained in the positive orthant, with $y > 0$, and either $x_1 > 0$ or $x_2 > 0$, would be a suitable operating envelope that satisfies Assumption 1; however, it is readily seen that there does not exist such a set containing $E_1$, since such point is characterized by $y = 0$, which does not satisfy (34b).

Therefore, Assumption 1 and, therefore, Theorem 2.2 is not satisfied; also, it is possible to show that some of the further conditions required by Theorem 2.2 are not satisfied. Similar considerations can be repeated for the equilibrium $E_2$ in (33b).

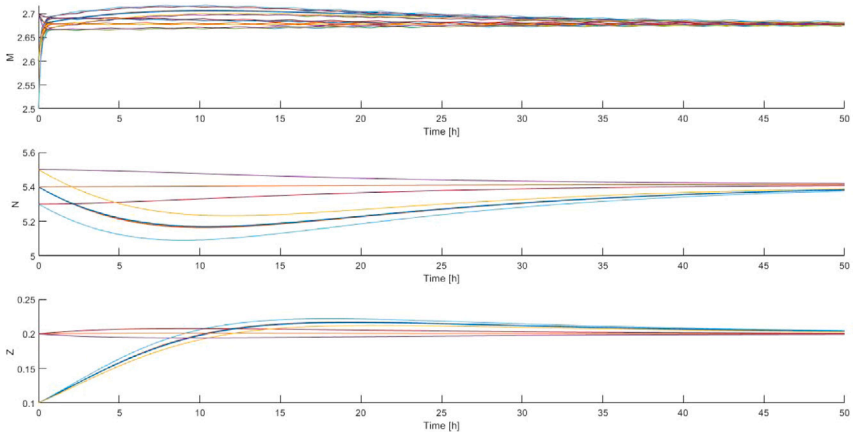



**Fig. 6.** Trajectories of the state variables of the tumor progression model as a function of time and at different initial conditions (ranges for initial conditions are set as: $y \in [5.3, 5.5]$; $x_1 \in [2.5, 2.7]$); $x_2 \in [0.1, 0.2]$).

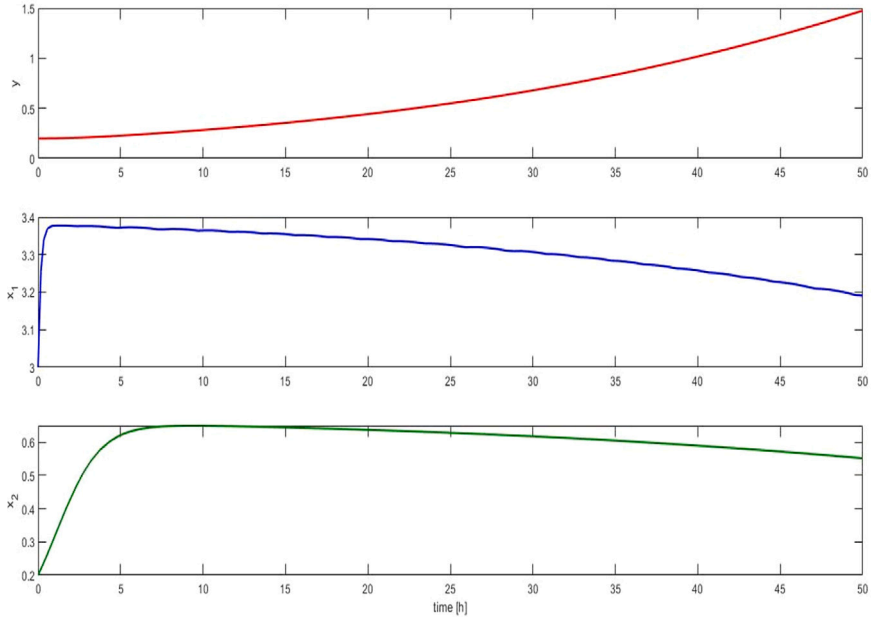



**Fig. 7.** Diverging trajectories of the state variables of the tumor progression model as a function of time (initial conditions are set close to the equilibrium point $E_1$: $y_0 = x_2 = 0.2$; $x_1 = 3$).

### 3.2. Cortisol dynamics in response to pain/stress conditions

First, we look for a suitable operating envelope, which satisfies Assumption 1; the derivatives of the activation and inhibition functions $f$ and $g$ yield

$$\frac{\partial g_1}{\partial y_1} = -\frac{874.5 y_1^{4.1}}{(34.29 + y_1^{5.1})^2} < 0\,, \quad y_1 \neq 0 \tag{35a}$$

$$\frac{\partial g_1}{\partial y_2} = 0 \tag{35b}$$

$$\frac{\partial g_2}{\partial y_1} = -\frac{1451.7 y_1^{4.1} x_1}{(34.29 + y_1^{5.1})^2} < 0\,, \quad y_1 \neq 0\,, x_1 \neq 0 \tag{35c}$$

$$\frac{\partial g_2}{\partial y_2} = \frac{\partial f_1}{\partial x_1} = \frac{\partial f_1}{\partial x_2} = \frac{\partial f_2}{\partial x_1} = 0 \tag{35d}$$

$$\frac{\partial f_2}{\partial x_2} = 0.945 > 0\,; \tag{35e}$$

therefore, Assumption 1 is satisfied in any compact connected set enclosed in the positive orthant.

Even if we do not have a specific theorem for fourth-order systems, generalizing the conditions in Theorems 2.1 and 2.2, it is arguable that a necessary condition to be satisfied in the operating envelope is

$$\frac{\partial f_i}{\partial y_i} \leq 0\,, \quad \frac{\partial g_i}{\partial x_i} \leq 0\,, \quad i = 1, 2\,. \tag{36}$$

According to (28d) and (28e), we have, in the positive orthant,

$$\frac{\partial f_1}{\partial y_1} \leq 0\,, \quad y_2 \geq \frac{V_{S2} n_1 k_1^{n_1} y_1^{n_1 - 1}}{k_b \left(k_1^{n_1} + y_1^{n_1}\right)^2} \tag{37a}$$

$$\frac{\partial f_2}{\partial y_2} \leq 0 \tag{37b}$$

$$\frac{\partial g_1}{\partial x_1} \leq 0 \tag{37c}$$

$$\frac{\partial g_2}{\partial x_2} \leq 0\,. \tag{37d}$$

Taking into account the constraint coming from the satisfaction of Assumption 1, constraints (37), and the parameter values contained in

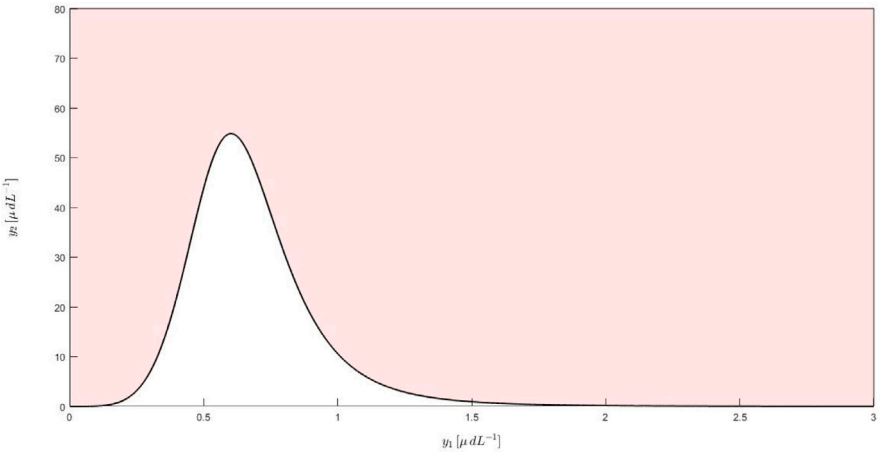



**Fig. 8.** 2D representation of the operating envelope $\mathcal{O}$ defined in (38) projected into the plane ($y_1$, $y_2$), i.e., the red region over the curve.

Table 2, we have that a suitable set $\mathcal{O}$ is any compact connected subset contained in the set

$$\left\{ \begin{pmatrix} y_1 & y_2 & x_1 & x_2 \end{pmatrix}^T : y_1 > 0, y_2 > \frac{0.0509 \cdot 5.43 \cdot 0.645^{5.43} \cdot y_1^{4.43}}{0.0202\left(0.645^{5.43} + y_1^{5.43}\right)^2}, x_1 > 0, x_2 > 0 \right\}. \tag{38}$$

Fig. 8 displays a bidimensional representation of the operating envelope $\mathcal{O}$ defined in (38) projected into the plane ($y_1$, $y_2$).

Therefore, we set to zero the derivatives in (29), and look for a numerical solution belonging to the set (38); a unique solution is found

$$\begin{pmatrix} y_{e1} & y_{e2} & x_{e1} & x_{e2} \end{pmatrix}^T = \begin{pmatrix} 2.7 & 15.6 & 0.65 & 5.9 \end{pmatrix}^T. \tag{39}$$

The eigenvalues of the dynamical matrix $A$ of the linearized system around the equilibrium point (39) are evaluated to assess the stability of the system; we have

$$A = \begin{pmatrix} -0.4 & 9.6 \times 10^{-3} & 0 & 0 \\ 0 & -0.35 & 0 & 0.94 \\ -1.17 & 0 & -0.99 & 0 \\ -1.26 & 0 & 1.26 & -0.012 \end{pmatrix}. \tag{40}$$

The eigenvalues are as follows

$$p_1 = -0.07 + 0.19i \tag{41a}$$

$$p_2 = -0.07 - 0.19i \tag{41b}$$

$$p_3 = -0.95 \tag{41c}$$

$$p_4 = -0.66, \tag{41d}$$

which all have negative real parts. Therefore, the homeostatic equilibrium point (39) is asymptotically stable, as also shown in Fig. 9, displaying the behavior of the state variables around the equilibrium.

## 4. Discussion

Despite the field of physiological and biological systems modeling has seen significant advancements over the last decades, and despite the presence of numerous studies proposing mathematical formulations and more sophisticated approaches to model several biological/physiological processes, all the available approaches provide systems-specific representations and lack generalization, thus preventing an integrated and comprehensive view able to recapitulate the behavior and dynamics of the variety of control systems that constitute complex living organisms. Indeed, in the related literature, most studies provide views and models tailored to specific physiological aspects and properties under investigation, thereby lacking a broader and more general system theory perspective, which is instead needed to the much wider problem of homeostasis control.

To address this research challenge, the present manuscript, through the formulation of the general model (1), provides a methodological route for establishing sufficient conditions for the existence, uniqueness, and stability of equilibrium points in negative feedback physiological control systems. The main achievements and scientific contributions of this study concern both theoretical and practical aspects of the research and are discussed in the following.

First of all, Assumption 1 summarizes in theoretical form the conditions and hypotheses under which a physiological control system can be represented through the model (1). Such conditions, as reported in the rationale throughout the Remarks 1–7, are sufficient to guarantee the existence and uniqueness of the solution of the differential Eqs. (1); moreover, they are shared by all case studies examined so far, as discussed later in this section.

A peculiar point of our approach is that the conditions listed in Assumption 1 must not necessarily hold in the whole state space of the system, since it is sufficient that they keep in a subset $\mathcal{O}$, referred to as the operating envelope of model (1), and practically corresponding to a (possible large) neighborhood of the equilibrium point, as illustrated, for instance, in Section 3.1, in the case regarding the 3rd-order model of tumor progression dynamics. Such a result is particularly relevant from both a theoretical and practical perspective: on the one hand, it suggests a practical approach to individuate the location of asymptotically stable homeostatic equilibria for an even complex nonlinear system; on the other, the definition of the operating envelope of the model can provide insights into the study of transitioning from stable to unstable behavior and/or drifting from physiological to pathological equilibria.

Moreover, model order-specific theorems, providing all the mathematical conditions for demonstrating the existence and uniqueness of an asymptotically stable equilibrium point in 2nd- and 3rd-order physiological control systems, are defined. Theoretical proof of the theorems as well as application to physiologically relevant case studies are provided. Taken together, Assumption 1 and model order-specific theorems allow demonstration of the uniqueness and stability of equilibrium points of a system properly represented under the mathematical formalism given in model (1).

Finally, the effectiveness and generalizability of the proposed methodological and mathematical framework has been additionally proved through three paradigmatic examples of physiological control systems at different scales and model orders, from 2nd- up to 4th-order. It is

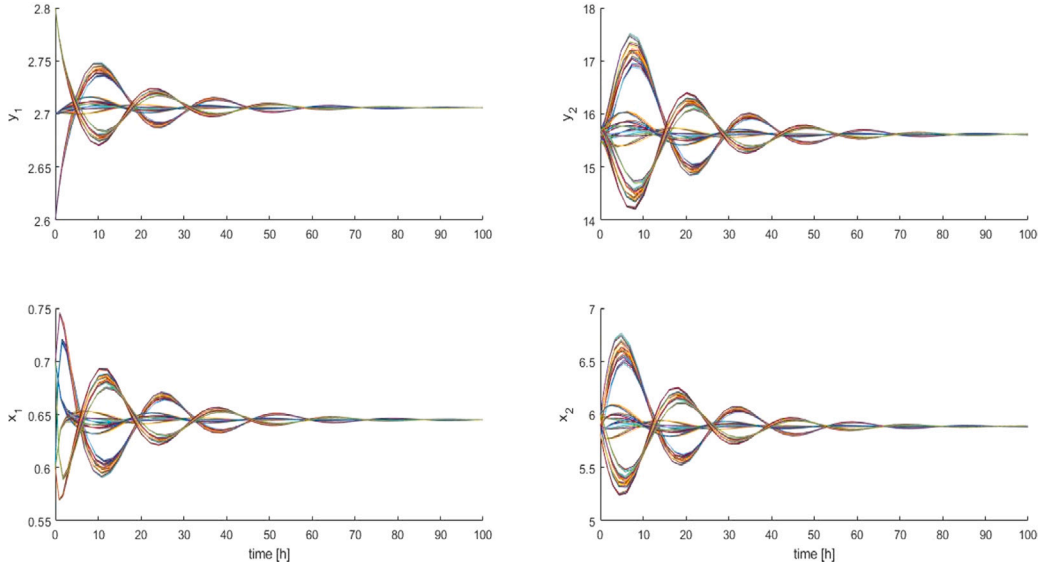



**Fig. 9.** Trajectories of the state variables of the cortisol dynamics model as a function of time.

**Table 3**
Compliance of negative feedback physiological control systems with the proposed general mathematical framework. Verification of Assumption 1 is checked, and the availability of model order-specific theorems is assessed.

| System | References | Order | $m$, $n$ | Ass. 1 | Theorem |
|---|---|---|---|---|---|
| Glucose regulation | [6,10,26–28] | 2 | 1 1 | yes | 2.1 |
| Ultrasensitivity | [10,16,29,30] | 2 | 1 1 | yes | 2.1 |
| Thyroid hormones | [24] | 2 | 1 1 | yes | 2.1 |
| Reflex arc | [10,31–33] | 2 | 1 1 | yes | 2.1 |
| Tumor dynamics | [21] | 3 | 1 2 | yes | 2.2 |
| Cortisol dynamics | [25] | 4 | 2 2 | yes | n.a. |
| Neuromuscular reflex | [11,34–36] | 4 | 1 3 | yes | ⋆ |
| $PI3K/AKT/mTOR$ | [37] | 7 | 4 3 | yes | n.a. |

n.a. stands for not available; ⋆ indicates that the theorem for linear systems is exploited.

important to remark that, while the statement of conditions for stability is only possible for lower order systems, the check of the satisfaction of Assumption 1, characterizing the belonging to the framework, can be performed for systems with an arbitrary number of activation and inhibition variables. In this regard, as a further proof of model generalizability, we will also discuss the physiological control system models available in the literature so far, in order to show that they are captured by the reference model given in (1); the results of this investigation are summarized and collected in Table 3, and are discussed in the following.

First, it is worth noting that having a reliable common framework for describing physiological systems is important both from the perspective of general scientific knowledge and for the strong impact it could have on future studies. In fact, when it comes to a new physiological system that can be framed within the proposed context, we can immediately apply the theory developed here to investigate its structural properties.

Let us start with the classical glucose regulation system, investigated in [6,26–28]; in [10], it is shown that such a model is described by the interaction of one activation and one inhibition variable, and is captured by the general representation (2). Denoted by $x$ the glucose concentration (the activation variable), and by $y$ the insulin concentration (the inhibition variable), it is simple to verify that Assumption 1 and Theorem 2.1 are satisfied for $y \geq 0$ and $x \geq 2.5\ mg/ml$; therefore a suitable set $\mathcal{O}$ can be defined as $\left\{(y\ x)^T\ :\ y \in [0, M]\,,\ x \geq 2.5\right\}$, with $M$ sufficiently large. The equilibrium point $(0.056\ 0.810)^T \in \mathcal{O}$ is found, which is asymptotically stable and unique according to Theorem 2.1.

In [16,29,30], the role of ultrasensitivity, which describes a common nonlinear characteristic of cellular systems for explaining the adaptive response dynamics observed in the yeast osmoregulatory response network, is investigated. In [10], it is shown that such a system is again captured by the general representation (2), where $x$ and $y$ denotes, as usual the activation and inhibition variables. Since, in this case, the activation and inhibition functions are increasing and decreasing Hill functions, respectively, it is readily seen that Assumption 1 and Theorem 2.1 are satisfied in any compact connected set in $\mathbb{R}^2$. The equilibrium point $(0.439\ 0.508)^T$ is found, which is asymptotically stable and unique according to Theorem 2.1.

The homeostatic regulation of thyroid hormones [24] discussed in this paper, is again a second order physiological control system. As shown in Section 2.2, Assumption 1 and Theorem 2.1 are satisfied in any compact connected set enclosed in the positive orthant. The unique, asymptotically stable equilibrium point is $(12.5\ 3)^T$.

The model of the muscle stretch reflex (reflex arc) [31,32], is a classical example of negative feedback physiological system at the whole body level. Denoting by $x$ the muscle length, playing the role of the activation variable, and by $y$ the efferent frequency, that is the inhibition variable, in [10] it is shown that such a 2nd-order system is captured by the general representation (2). As in the case of the ultrasensitivity dynamics described above, the functions $f$ and $g$ are increasing and decreasing, respectively, in the whole positive orthant; therefore, Assumption 1 and Theorem 2.1 are satisfied in any compact connected set in $\mathbb{R}^2_+$. The equilibrium point $(0.468\ 0.583)^T$ is asymptotically stable and unique according to Theorem 2.1.

The dynamics related to the tumor progression [21] have been investigated in the current paper within the context of negative feedback control systems. Such a model takes the general form (20), with two activation and one inhibition variables. As shown in Section 3.1, Assumption 1 and Theorem 2.2 are satisfied in the operating envelope $[1, 6]\times[2, 3.5]\times[0.1, 0.2]$. In this case, Theorem 2.2 guarantees uniqueness and asymptotic stability of the equilibrium point $(5.41\ 2.67\ 0.2)^T$. We also show that, in correspondence of unstable equilibria, the hypothesis of Theorem 2.2 are not satisfied; this fact further reinforces the reliability of the proposed approach.

The last case study considered in this paper is the one concerning the cortisol dynamics in the presence of stress [25]. Such a system is modeled through two activation and two inhibition variables, as discussed in Section 2.4. Here, we are able to verify the structural conditions established by Assumption 1, but there is no theorem at

our disposal; therefore we look directly for the existence of the equilibrium and verify its asymptotic stability in Section 3.2, exploiting the linearization technique. An estimate for the operating envelope is obtained by generalizing conditions (21a) to the fourth order case, see (36); such an estimate coincides with the set (38), and its projection on the $(y_1, y_2)$ plane is depicted in Fig. 8.

The neuromuscular stretch reflex [34–36], turns out to be a fourth order model, which has been investigated in the context of the physiological control systems in the paper [11]. Exploiting the notation of this paper, and neglecting the delays (which are not dealt with here), such a model can be rewritten in the form (1), with

$$y := M_0\,, \qquad x := \begin{pmatrix} \theta \\ \dot{\theta} \\ \ddot{\theta} \end{pmatrix} \tag{42a}$$

$$A_a := -\frac{1}{\tau}\,, \quad B_a := 0\,, \quad f(y,x) := \begin{pmatrix} \frac{\beta}{\eta\tau} & \beta & 0 \end{pmatrix} x \tag{42b}$$

$$A_i := \begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & -\frac{K}{J} & -\frac{K}{B} \end{pmatrix} \quad B_i := \begin{pmatrix} 0 \\ 0 \\ \frac{K}{BJ} \end{pmatrix}, \quad g(y,x) := \begin{pmatrix} 0 \\ 0 \\ -\frac{K}{BJ} \end{pmatrix} y \tag{42c}$$

$$u^y := 0\,, \qquad u^x := M_x\,, \tag{42d}$$

where $M_0$ is the torque produced by the muscular contraction, $\theta$ is the angular displacement of the forearm around the elbow, and $M_x$ is the torque exerted at the wrist; finally, $B$, $J$ and $K$ are bio-mechanical parameters. Model (42) does not satisfy Assumption 1-(i), since matrix $A_i$ is not diagonal. However, according to Remark 1, let us replace matrix $A_i$ and the inhibition function $g$ as follows

$$\tilde{A}_i := \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -\frac{K}{B} \end{pmatrix}, \quad \tilde{g}(y,x) := \begin{pmatrix} x_2 \\ x_3 \\ -\frac{K}{J}x_2 - \frac{K}{BJ}y \end{pmatrix}; \tag{43}$$

now, it is readily seen that Assumption 1 is satisfied. Note that this is a particular case, since the functions $f$ and $g$ are linear; this guarantees that the equilibrium point is unique. Moreover, to check asymptotic stability, it is necessary and sufficient to apply the classical theorem for linear systems.

In [37], the genic circuit, dealing with the interaction between the $PI3K/AKT$ and the $mTOR$ pathway, is considered. The paper [11] investigates such a model from the perspective of the current paper; the system is described by the general representation (2), with three activation and four inhibition variables. Looking at the expressions of $f$ and $g$, it is simple to verify that Assumption 1 is satisfied. Obviously, in this case, we do not have a theorem to investigate the existence and uniqueness of equilibria, therefore one has to resort to numerical optimization to find the homeostatic equilibrium point, and stability is checked via linearization.

In view of the discussion above, we can conclude that, not only is model (1) able to describe and effectively represent the dynamics of intrinsically different physio- and bio-logical control systems from the nanoscale to the macroscale, and at different degrees of complexity in terms of number of interacting species; but it also emerges how, even in the absence of model order-specific theorems (not yet available for systems greater than the 3rd order), Assumption 1 is kept verified for all the systems examined both in this manuscript and in the literature. As anticipated at the beginning of this section, such evidence is in line with the fact that Assumption 1 effectively recapitulates those sufficient conditions under which a physiological system can be represented by model (1).

The long-term goal of this study is to test the proposed model against other closed-loop negative feedback physiological control systems, to further validate and, if necessary, include further hypotheses to make model (1) as close to reality as possible, is the long-term goal of this study; thereby, this work must be seen as an encouraging step toward this objective. To this regard, to address the limitations of the current framework, future work will further extend the case study analysis by including additional higher-order physiological control systems, with a specific focus on 3rd- and 4th-order, to verify compliance with the theoretical assumptions and model fitting, thus expanding the library of unified model-compliant and physiologically relevant control mechanisms. Another issue to address (see Remark 9) will concern the quantitative estimation of the DA of homeostatic equilibrium points. Not least, framework adaptability to specific conditions, e.g., switching from healthy to pathological cases, presence of transmission delays in either activation or inhibition channels, as well as introduction of external artificial control dynamics through time-dependent production rates $u^y$ and $u^x$, will also be investigated by leveraging the formulation and major findings achieved in the present study.

## 5. Conclusions

In this work, a rigorous system-theoretic oriented approach is outlined to establish a general reference model for investigating the dynamics of multivariable negative feedback physiological control systems at different orders and scales. The principles and methodologies of control theory have been leveraged to define assumptions and sufficient conditions for the existence of asymptotically stable equilibrium points in homeostatic regulation mechanisms occurring in bio- and physio-logically relevant processes.

In particular, a methodological route has been derived to study physiological systems' behavior, and it has been then implemented through a biofidelic system-theoretic oriented formalism. Assumptions and related conditions to be checked and verified in order to properly represent a physiological system through the proposed formalism have been defined. The generalizability of such conditions is granted by the fact that they should not necessarily hold for the whole system, but it is sufficient that they are kept in a neighborhood of the equilibrium point. Furthermore, based on the above-mentioned assumptions and provided that the physiological system has been represented according to the proposed formalism, specific theorems, tailored to the order of the system under examination, have been derived to demonstrate the existence and uniqueness of asymptotically stable equilibrium points. Such theorems have been rigorously established and proved for both 2nd and 3rd order physiological systems.

Moreover, besides the theoretical demonstrations, the proposed mathematical framework has been successfully implemented for three case-specific applications regarding physiological systems from the 2nd up to the 4th order, namely: the homeostatic regulation of thyroid hormones; the prey–predator of tumor progression dynamics; the cortisol release dynamics in response to pain/stress. Not least, as further proof of the generalizability of the approach, the main assumption of the proposed mathematical framework has been proved not only for the examined case studies, but it has been also verified for additional existing literature models at increasing scale, complexity, and order. In this regard, it is worth noting that, even though model-order specific theorems are not yet available for systems above the 3rd order, we demonstrated that the main assumption of the framework is still kept in those cases.

In summary, the main findings of this study prove that the proposed framework is able to capture the dynamics of different homeostatic regulation mechanisms (in either healthy or pathological states) within a closed and theoretically-supported mathematical formalism, and that its assumptions are generalizable to higher order systems with increasing complexity, thus paving the way for further validation and adoption on a larger set of physio- and bio-logically relevant processes. Future research will move in several directions; a key issue will involve studying further physiological control systems to verify their compliance with the theoretical hypotheses developed in this paper, thereby expanding the library of unified model-compliant and physiologically relevant control mechanisms; other topics to be addressed will include the quantitative estimation of the DA of homeostatic equilibrium points and the artificial control of physiological systems through time-dependent production rates.

## CRediT authorship contribution statement

**Rita Granata:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis. **Fabrizio Lo Regio:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Anna Procopio:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Annarita Tedesco:** Writing – review & editing, Visualization, Methodology, Investigation. **Carlo Ricciardi:** Writing – review & editing, Visualization, Methodology, Investigation. **Carlo Cosentino:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Maria Romano:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Alfonso Maria Ponsiglione:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Francesco Amato:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix

*Proof of Theorem 2.2*

The dynamical matrix of the linearized version of system (20) around the equilibrium point $(y_e\ x_e)^T$ is as follows

$$A = \begin{pmatrix} -\alpha + f_y(y,x) & f_x(y,x) \\ g_y(y,x) & A_i + g_x(y,x) \end{pmatrix}\Big|_{\substack{x=x_e \\ y=y_e}}$$
$$= \begin{pmatrix} -\alpha + f_y(y,x) & \frac{\partial f}{\partial x_1}(y,x) & \frac{\partial f}{\partial x_2}(y,x) \\ \frac{\partial g_1}{\partial y}(y,x) & -\beta_1 + \frac{\partial g_1}{\partial x_1}(y,x) & \frac{\partial g_1}{\partial x_2}(y,x) \\ \frac{\partial g_2}{\partial y}(y,x) & \frac{\partial g_2}{\partial x_1}(y,x) & -\beta_2 + \frac{\partial g_2}{\partial x_2}(y,x) \end{pmatrix}\Big|_{\substack{y=y_e \\ x=x_e}}. \tag{44}$$

The characteristic polynomial associated to the matrix $A$ is (for the sake of simplicity, the dependence of the derivatives on $(y\,x)$ is omitted)

$$p(\lambda) = \det(\lambda I - A)$$
$$= \left| \begin{pmatrix} \lambda + \alpha - f_y & -\frac{\partial f}{\partial x_1} & -\frac{\partial f}{\partial x_2} \\ -\frac{\partial g_1}{\partial y} & \lambda + \beta_1 - \frac{\partial g_1}{\partial x_1} & -\frac{\partial g_1}{\partial x_2} \\ -\frac{\partial g_2}{\partial y} & -\frac{\partial g_2}{\partial x_1} & \lambda + \beta_2 - \frac{\partial g_2}{\partial x_2} \end{pmatrix} \right|_{\substack{x=x_e \\ y=y_e}}$$
$$= a_3\lambda^3 + a_2\lambda^2 + a_1\lambda + a_0\,. \tag{45}$$

Assumption 1-(iii) implies that at least one between $\partial f/\partial x_1$ and $\partial f/\partial x_2$ is strictly positive, and $\partial g_i/\partial y$, $i = 1,2$, are strictly negative; therefore, due to the hypothesis of the theorem, we have at the equilibrium

$$a_3 = 1 \tag{46a}$$
$$a_2 = \beta_1 + \beta_2 + \alpha - f_y - \frac{\partial g_1}{\partial x_1} - \frac{\partial g_2}{\partial x_2}$$
$$\geq \beta_1 + \beta_2 + \alpha > 0 \tag{46b}$$
$$a_1 = \beta_1\beta_2 + (\beta_1 + \beta_2)\alpha - \beta_1\frac{\partial g_2}{\partial x_2} - \beta_2\frac{\partial g_1}{\partial x_1} + |g_x|$$
$$- \alpha\left(\frac{\partial g_2}{\partial x_2} + \frac{\partial g_1}{\partial x_1}\right) + f_y\left(-\beta_1 - \beta_2 + \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2}\right)$$
$$- \frac{\partial f}{\partial x_2}\frac{\partial g_2}{\partial y} - \frac{\partial f}{\partial x_1}\frac{\partial g_1}{\partial y}$$
$$\geq |g_x| > 0 \tag{46c}$$
$$a_0 = (\alpha - f_y)\left(\beta_1\beta_2 - \beta_1\frac{\partial g_2}{\partial x_2} - \beta_2\frac{\partial g_1}{\partial x_1} + |g_x|\right)$$
$$+ \frac{\partial f}{\partial x_1}\left|g_{(yx_2)^T}\right| - \frac{\partial f}{\partial x_2}\left|g_{(yx_1)^T}\right| - \beta_1\frac{\partial g_2}{\partial y}\frac{\partial f}{\partial x_2} - \beta_2\frac{\partial f}{\partial x_1}\frac{\partial g_1}{\partial y}$$
$$\geq \frac{\partial f}{\partial x_1}\left|g_{(yx_2)^T}\right| - \frac{\partial f}{\partial x_2}\left|g_{(yx_1)^T}\right| > 0\,. \tag{46d}$$

However, when dealing with a 3rd-order polynomial, the positivity of the coefficients is not sufficient to guarantee that all roots have a negative real part. Therefore, the Routh–Hurwitz criterion is employed to ensure stability. For the Routh criterion to be satisfied, the following condition must hold

$$b_2 = \frac{a_1\,a_2 - a_3\,a_0}{a_2} = \frac{a_1\,a_2 - a_0}{a_2} > 0\,. \tag{47}$$

We know that $a_2 > 0$; moreover, due to the hypothesis of the theorem, we have

$$a_1a_2 - a_0$$
$$= \Big(\beta_1\beta_2 + (\beta_1 + \beta_2)\alpha - \beta_1\frac{\partial g_2}{\partial x_2} - \beta_2\frac{\partial g_1}{\partial x_1} + |g_x| - \alpha\left(\frac{\partial g_2}{\partial x_2} + \frac{\partial g_1}{\partial x_1}\right)$$
$$+ f_y\left(-\beta_1 - \beta_2 + \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2}\right) - \frac{\partial f}{\partial x_2}\frac{\partial g_2}{\partial y} - \frac{\partial f}{\partial x_1}\frac{\partial g_1}{\partial y}\Big)$$
$$\times \left(\beta_1 + \beta_2 + \alpha - f_y - \frac{\partial g_1}{\partial x_1} - \frac{\partial g_2}{\partial x_2}\right)$$
$$- (\alpha - f_y)\left(\beta_1\beta_2 - \beta_1\frac{\partial g_2}{\partial x_2} - \beta_2\frac{\partial g_1}{\partial x_1} + |g_x|\right)$$
$$- \frac{\partial f}{\partial x_1}\left|g_{(yx_2)^T}\right| + \frac{\partial f}{\partial x_2}\left|g_{(yx_1)^T}\right| + \beta_1\frac{\partial g_2}{\partial y}\frac{\partial f}{\partial x_2} + \beta_2\frac{\partial f}{\partial x_1}\frac{\partial g_1}{\partial y}$$
$$\geq (\alpha + \beta_1 + \beta_2)\,|g_x|$$
$$> 0\,.$$

Therefore, according to the Routh criterion, the equilibrium point $(y_e\ x_e)^T \in \mathcal{O}$ is asymptotically stable.

Now, under the assumptions of the theorem, it can be proven that the asymptotically stable equilibrium point is unique. For the sake of simplicity, let us assume that $g$ only depends on the inhibition variable, i.e. $g(y,x) = g(y)$.

To this regard, note that the set of the equilibrium points of model (20) under the constant input $(u^y\ u^x)^T \in \mathbb{R}_+ \times \mathbb{R}_+^2$ coincides with the set of the solutions of the system of algebraic equations

$$\alpha y = u^y + f(y,x) \tag{48a}$$
$$-A_i x = B_i u^x + g(y)\,; \tag{48b}$$

moreover, from (48b) we obtain

$$x = -A_i^{-1}\left(B_i u^x + g(y)\right). \tag{49}$$

Replacing (49) in (48a), yields

$$y = \alpha^{-1}\left(u_y + f\left(-A_i^{-1}\left(B_i u^x + g(y)\right), y\right)\right). \tag{50}$$

Letting

$$\phi(y) = \begin{pmatrix} \phi_1(y) \\ \phi_2(y) \end{pmatrix} = \begin{pmatrix} \phi_{1_1}(y) \\ \phi_{1_2}(y) \\ \phi_2(y) \end{pmatrix} := \begin{pmatrix} \frac{1}{\beta_1}\left(b_{i_{11}}u^{x_1} + b_{i_{12}}u^{x_2} + g_1(y)\right) \\ \frac{1}{\beta_2}\left(b_{i_{21}}u^{x_1} + b_{i_{22}}u^{x_2} + g_2(y)\right) \\ y \end{pmatrix},$$

we have, due to assumptions of the theorem,

$$f_y = f_\phi\phi_y$$

$$= \begin{pmatrix} \frac{\partial f}{\partial \phi_{1_1}} & \frac{\partial f}{\partial \phi_{1_2}} & \frac{\partial f}{\partial \phi_2} \end{pmatrix} \begin{pmatrix} \phi_{1_{1_y}} \\ \phi_{1_{2_y}} \\ \phi_{2_y} \end{pmatrix}$$
$$= \frac{\partial f}{\partial \phi_{1_1}} \phi_{1_{1_y}} + \frac{\partial f}{\partial \phi_{1_2}} \phi_{1_{2_y}} + \frac{\partial f}{\partial \phi_2} \phi_{2_y}$$
$$= \frac{1}{\beta_1} \frac{\partial f}{\partial \phi_{1_1}} \frac{\partial g_1}{\partial y} + \frac{1}{\beta_2} \frac{\partial f}{\partial \phi_{1_2}} \frac{\partial g_2}{\partial y} + f_y \leq 0 . \quad (51)$$

Eq. (51) implies that the right-hand side of (49) defines a plane with a non-increasing trend, while the left-hand side represents a line with an increasing trend. The opposite behavior of the plane and the line ensures that their intersection occurs at a unique point. Therefore, if an equilibrium point $(y_e\, x_e)^T$ exists, it is unique. ■

## Data availability

No data was used for the research described in the article.

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