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The Brain's Essential Role in Mediating Immune Responses: HPA Axis to Leverage Signals with a Systemic Approach

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Abstract: *While the role of the brain parts on mediating immune response is largely recognized, the neuroimmune pathways are difficult to be explored so they are still poorly understood. The animal knockout models are not always adapted to study the human biology. Because human neuroscience is very sensitive to the psychology, an appropriate way to federate various data and experiments is computational neuroscience. From this perspective our paper introduces an investigation methodology combining a complex landscape of biological signals: neuro-endocrine, lymphatic and neuro-electrical propagation. Having a hierachical HPA axis model as pivotal element of study, we show that previous results about the modulation of the immune response by the HPA axis obtained for murine models can be scrutinized using 'in silico' human models. This provide a new systemic computational schema to investigate neuroscience paradigms.*

Keywords: *neuroimunology; neuroelectricity; Hill cooperative model; hybrid automata.*

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1. Introduction

Past and recent studies show the implication of the brain parts and brain signaling to impact the immune system behavior (Lee & Tse, 1997). The brain source layers deliver messages to modulate the immune response in three structural different layers:

i) Neuro-Endocrine
ii) Lymphatic
iii) Electric Signals.

These systems are working in synergy, hard if not impossible to determine using exclusively up-to-date technological measurements.

Concerning the immune responses investigation, it is essential to consider mental, psychological, and biological factors that are implied to determine an increase, decrease, or lump of the immune function.

Experimental data required to engineer complex possible connections are often impossible to obtain. The available data have a limited extrapolation value, as related to a particular setup.

The major challenge is represented by Computational Neuroscience, emerging as a powerful 'in silico' investigation field.

If Computational Neuroscience is a field with huge potential as a companion for brain comprehension research, some precautions have to be taken. The spread of Machine Learning models, cumulated with the progress of molecular biology and functional imagery, made discoveries about brain connectivity and its chemogenomics. Mostly, the research done in computational neuroscience is a 'brick-by-brick approach' in the sense that knowledge from different areas is integrated, with ML models that are general, and adapted to the problem at hand. This can be considered a kind of 'blind' integration, as the modeling process is shaped to a predefined main computational technique.

In order to provide robustness of the model, one has to be sure the selected method follows biological consensual knowledge.

The power of our study is given by systemic model extension, using rigorous techniques, expressed by the following characteristics.

1. From a computational perspective, we develop a model using as much as possible exact analytical mathematical tools adapted to physiological processes, reducing the risk of inaccuracy given by ‘blind’ heuristic approaches.
2. The multidimensional characteristics of neuro-endocrine communication are considered in a systemic view, with contextual settings.
3. Direct and indirect links from the HPA axis to the immune response are represented in the model.
4. The immune response descriptors are selected to focus on some less understood mechanisms chosen from the plurality of immune system expression.
5. The explained mechanisms are much more complex than standard effects-cause automatic relationships.

We propose a computational approach schema to be explored in the future for investigating various brain-related conditions, via the implications from the HPA axis.

2. The Hypothalamic-Adrenal-Pituitary axis acts as an emitter and receptor

The HPA axis is composed of i) neuro-endocrine cell populations in the Hypothalamic Paraventricular Nucleus (PVN) whose non-myelinated axons have terminals in the median eminence (ME), which release Corticotropin Releasing Hormone (CRH), vasopressin (AVP) and other hormones into the hypophyseal portal venous system; ii) endocrine population cells (corticotrophs) at the anterior pituitary (AP), which secrete adrenocorticotropic hormone (ACTH); iii) cortisol producing population cells of the Adrenal Cortex (ACx); and iv) other neural and pituitary cell populations.

The main behavior observed from in vivo measurements shows that stimulatory AVP and output ACTH concentrations are strongly correlated, both under the hypertonic saline stimulus and in normal conditions, while CRH is largely uncorrelated with both AVP and ACTH concentrations in these conditions (Alexander, Irvine & Donald, 1996).

AVP and CRH elicit ACTH secretion using different pathways and produce different changes in ACTH concentration, CRH exhibits changes in hormone secretion per cell while AVP produces changes in the number of secreting cells) (Canny, Jia, & Leong, 1992). While CRH seems to mediate ACTH secretion in response to acute stress, AVP seems to mediate adaptation to chronic stress, probably mediating proliferative responses in the pituitary (La Rota, Vuza & Climescu-Haulica, 2019). CRH stimulates the secretion of ACTH by a factor of 8-fold in rat pituitary corticotrophs (Wynn et al., 1983) with half-maximum e_ective concentrations (EC50) _ 3 × 10−10M. The main regulator of ACTH secretion is GC, which seems to mainly regulate ACTH release (Sakakura, Yoshioka, & Kobayashi, 1982) by genomic and nongenomic pathways; while genomic pathways are relatively well known, nongenomic ones are much, less known and more controversial (Groeneweg, 2011).

The HPA shows circadian and ultradian oscillations or pulses. There are a bunch of models proposed in the literature, each one focusing on one aspect of the system behavior, which has shed some light on some of the mechanisms underlying them.

Opposite to a knockout bio-genetic method, the 'in silico' approach has to calibrate synthetic data with biological and mathematical knowledge, to create a correct choice alternative.

For this reason, the accuracy of re-creating a systemic HPA axis is an important feature of our study.

The greatest advantage is the possibility to study feedback relationships, which is not entirely available in 'in vivo' models. The main use of the computational model is the integration of strictly human modules, impossible to be considered in the model otherwise.

From a systemic view, the interest is to determine the HPA behavior and its underlying dynamics, for example, to investigate i) which is the origin of the ultradian pulses or oscillations, are they locally generated by the internal dynamics of a specific cell population, independently of the interactions among cell populations, do they come from an external source or do they result from the endogenous dynamics of the HPA cell population network? ii) what is the mechanism and the influence of the circadian rhythms and light in the HPA behavior? iii) which is or are the mechanisms leading to multistability and what is its physiological meaning? Some results in these directions are given in La Rota, Vuza & Climescu-Haulica (2019) and the references cited there.

For this reason, we consider the hierarchical HPA model described in La Rota, Vuza & Climescu-Haulica (2019) and we steer our investigation to show

1. how physical and psychological stress, as well as immunity challenges, influence HPA dynamics;
2. how HPA dynamics have an impact on the immunity challenge and psychological stress.

We also consider other systems that interact with the HPA system, so in its complexity, the HPA system is an emitter but also a receiver to/from connected systems.

A sequence of HPA subsystem neuro-endocrine models was developed by Keenan, Licinio, & Veldhuis (2001); Keenan, Roelfsema, & Veldhuis (2004); Keenan, Chattopadhyay & Veldhuis (2005), based on a previous model of the HPG (gonadal) subsystem presented in Keenan, Sun & Veldhuis (2000); Keenan & Veldhuis (1998). The initial model involves the synthesis, accumulation, and release mechanisms of hypothalamic CRH-AVP neurons, the ACTH in the AP, and the cortisol-secreting cells of the ACx. A more detailed model of the AP (ACTH)→ACx(cortisol) interaction, involving kinetics of exchange of plasma cortisol among free and protein-bound compartments was described in Keenan, Roelfsema, & Veldhuis (2004) and a still more detailed model of ACTH secretion based on these previous models was presented in Keenan, Chattopadhyay & Veldhuis (2005). An important feature of these models is the use of a stochastic pulsatory mechanism for the generation of CRH-AVP secretory pulses, which implicitly

assumes that pulse generation is independent of the network dynamics; in addition, no difference is made between CRH and AVP dynamics and between their effects on corticotroph activity. This model is capable of a very faithful reconstruction of hormone time series and allows estimation and detailed statistical analysis. However, it has many free parameters, it is not easily amenable to mathematical analysis, nothing is known about its general dynamics and behavior and some features and parameters (such as the pulse generation mechanism and parameters) lack a clear biological interpretation. Some of these modeling analyses suggest that ultradian oscillations are endogenously produced by the HPA system dynamics (Lenbury & Pornsawad, 2005; Kyrylov, Severyanova & Vieir, 2005; Jelic, Cupic & Kolar-Anic, 2005). Other models suggest a focus on homeostasis and long-term dynamical regime changes, suggesting putative mechanisms underlying both healthy and abnormal basal behavior (Conrad et al., 2009; Gupta et al., 2007).

However, none of these models is capable of retrieving the different behaviors observed at different time scales; each one of these models lacks some features that may be important in determining the dynamics of the system, such as intracellular secretion product accumulation before release, pulse-stimulated secretion, and others. Finally, a more physiologically plausible model of corticotroph electrophysiological activity has been developed by LeBeau et al., (1997); LeBeau et al., (1998); Shorten et al., (2000) to integrate it into a complete model of corticotroph secretion. This model is biologically plausible, mathematically tractable, and has relatively few free parameters; however, it is limited to phenomena occurring at the very new time and space scale of the cell membrane. Therefore, there is still the need for a more comprehensive model of the HPA system which includes clearly identifiable and biologically pertinent parameters, at different scales, and at the same time with a simple mathematical description that is amenable to analysis and simulation.

3. Hybrid Automata renders Complex Biological Modelling for connection into/with the HPA system

Important implementation problems found when modeling biological systems are solved by a Hybrid automata investigation engine:

i) the lack of knowledge about the precise mechanisms underlying dynamical behavior as well as the lack of precise values for many parameters of the system;

ii) the ubiquitous presence of nonlinearities and the complexity of the systems under study that make the analysis of their dynamics by mathematical methods very difficult;

iii) this same complexity hampers our ability to study the system by computational methods, because of the difficulty of tracking numerical errors, the computational burden that implies the numerical analysis of the parameter space and the behavioral space.

Hybrid automata is a relatively new modelling consisting of both real-valued state variables continuously changing and discrete control variables abruptly changing the dynamics of the system (Alur, 1993; Henzinger, 1996; Lynch, Segala & Vaandrager, 2003). This is suitable for the modeling of biological systems due to the existence of strong nonlinearities in biological mechanisms changing abruptly the dynamics of subsystems in response to small variations of their inputs or as a result of variations in their parameters due to changes in the behavior of other related systems.

Model checking, reachability analysis, time verification, and parameter scanning methods are companion tools for the analysis of complex systems with hybrid. Examples of applications in systems biology can be found in Piazza (2005); Fisher & Henzinger (2007); Barnat (2010), Mishra (2009); Collins et al., (2011); Dang, Le Guernic & Maler (2019).

The first layer of our model is the theoretical framework given by the mathematical theory of integrative physiology (MTIP) developed by G. Chauvet []. He proposes the notion of **functional interaction** inspired by the existence of molecules or signals emitted by one structural unit, the source, acting at a distance on another structural unit, the sink, in which a series of

transformations occur. Relevant examples are the propagation of electric potential along a nerve, the action of a hormone operating at a distance from its site of synthesis after being carried in the bloodstream, and the change produced in the shape of a molecule after it binds to another molecule. Roughly speaking this implies that a product emanating from one entity acts on another at a distance, without being physically in contact.

We model different temporal and spatial scales but retain the linking mathematical structure. Models of neural systems based on Chauvet's Mathematical Theory of Integrative Physiology (Chauvet, 2002) involving neurotransmitter release and synaptic phenomena have been proposed by Bennani (2009); Bennani, Chauvet & Dupont (2010); Chauvet & Chauvet (2002). Neural and hormonal systems share many features, such as the secretion of chemical substances by endocrine and neuroendocrine cell populations in order to send information to other cell populations, as well as the membrane excitability and the generation of action potentials. Figure 1 depicts some straightforward relations seen as functional interactions, in particular, related to the stress inputs. This is the foundation for the hybrid automata architecture.

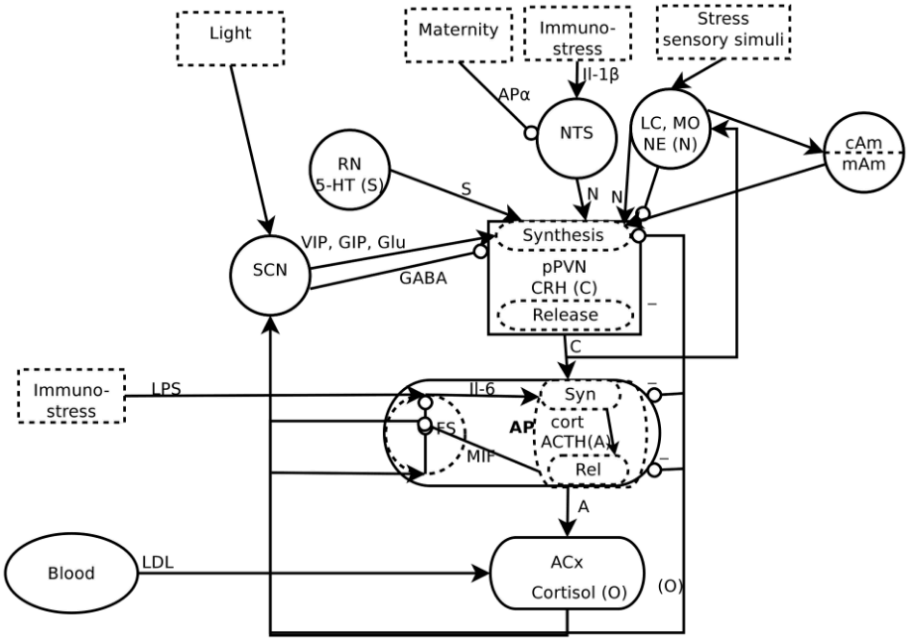

Figure 1. Example of functional interaction links of the HPA system and stress-related inputs

We focus on a detailed corticotroph model embedded in a simplified HPA network, trying to be faithful to current knowledge of corticotroph biology and electrophysiology (a bottom-up approach); as we aim to construct an integrated model of the HPA system, we take into account only dynamical behavior occurring from the minutes up to hours, in time scales. Our approach is modular and hierarchical, the complete model is composed of various categories of subsystems, each subsystem corresponding to a specific functional unit acting at a given time scale and living in a given structural unit at a given space scale. This complex system structure is implemented as a hierarchical set of hybrid automata and implemented in the SpaceEx tool (Frehse et al., 2011), which allows the composition of large, hierarchically organized sets of linear hybrid automata.

For the hormone release dynamics, we used the following considerations from La Rota, Vuza & Climescu-Haulica (2019) and the equations herein. The basic structural unit at the higher scale of interest is the homogeneous cell population. Cell populations can be either neural, neuro-endocrine, or endocrine. Every neuron is indeed a neuro-endocrine cell, because they use chemical signaling (secreting neurotransmitters, hormones, cytokines, etc), and endocrine cells show membrane excitability and electrical activities just as neurons do. There is another kind of

signaling between neuroendocrine cells, the electrical gap-junction which transfers information directly from cell to cell without the need of neurotransmitters; however, this kind of signaling is internal to the cell population, allowing cell fast communication and synchronization across cell populations.

Even if the molecular mechanisms underlying neuroendocrine signal processing vary among different cell populations, a stereotypical basic internal functional process exists. First, incoming signals are transformed and amplified internally by different signaling pathways then internal signals trigger fast and slow responses; fast responses control the immediate amount of secretion, which depends on hormone/neurotransmitter availability; slow responses control the parameters of the fast process, such as hormone availability or sensitivity to incoming signals.

Some hormones are stored on vesicles (slow process controlled) and released upon occurrence of internal events or in response to external signals (fast process). Other hormones (e.g. steroids), freely diffuse, but the synthesis rate is controlled by both fast and slow processes.
These detailed aspects are fundamental for the model's robustness. The complexity of the biological mechanism implied on the control relationship of the CRH about the ACTH secretion is presented, in a very simplified diagram in figure 2.

We use as input data provided by a knockout study by Poller et al. (2022), revealing the behavior of links between the HAP axis and the dynamics of leukocytes. Their study shows how specific neuron clusters from various regions of the brain cause big changes in leukocyte distribution and function. We use two distinct human 'in silico' models, male and female, making a distinction between their hormonal profiles.

As output we measure the impact of given system perturbations on the leukocytes number, representing them from a qualitative setup: location (lymph nodes, bone marrow or circulating), category (monocytes, lymphocytes, neutrophils), and the dynamic vector (stationary, sharp increase, sharp decrease, small increase, small decrease). These are parameters to determine immune surveillance as they decide the course of immunity or inflammation, the 'fight or flight' behavior.

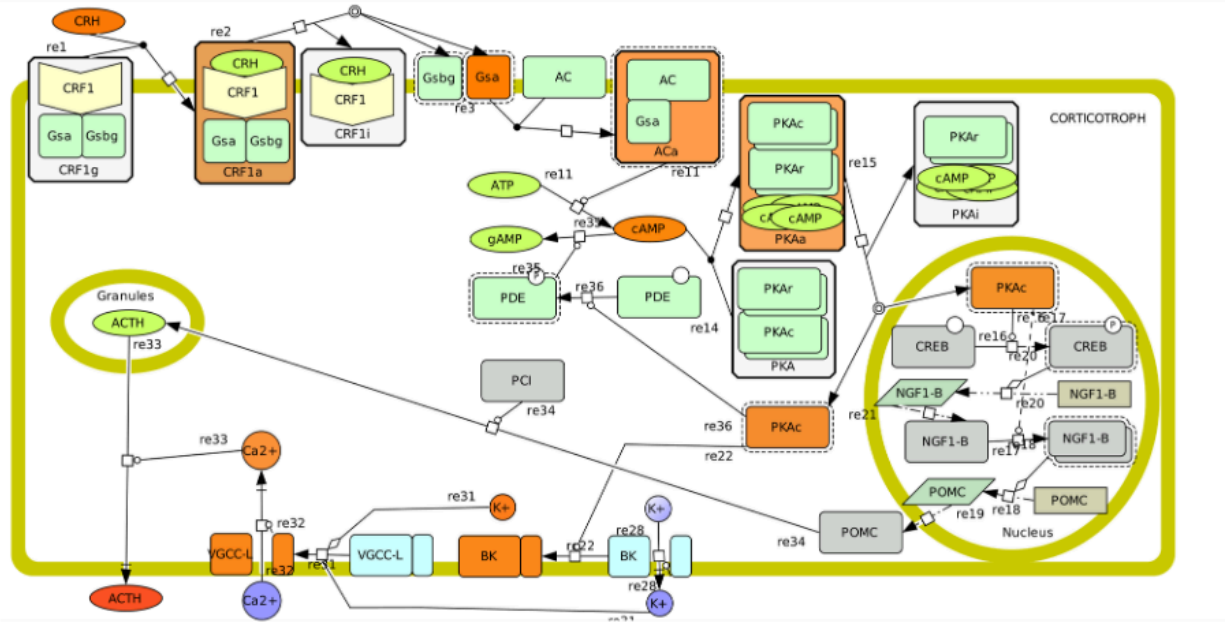

Figure 2. Diagram of reactions involved in the CRH control of ACTH secretion with a simplified view of the synthesis module

The hybrid automata implementation uses information from three concomitant mechanisms: neuro-endocrine secretion, hormone circulation (by blood/lymph), and neuro-electrical activity.

I. Neuro-endoctrine secretory mechanisms is assumed to follow a compact form expressed by the Hill cooperative secretion equation system:

$$\begin{aligned} \frac{d\mathbf{v}^+}{dt} &= \frac{K_{max}\mathbf{v}\zeta^n}{K^n+\zeta^n} - \lambda\mathbf{v}^+ \\ \frac{d\mathbf{v}^-}{dt} &= \lambda\frac{d\mathbf{v}^+}{dt} \end{aligned} \qquad (1)$$

where $\mathbf{v}^+$,$\mathbf{v}^-$, $\mathbf{v}$ are state variables, corresponding to the activated, inactivated (depleted), and ready available hormone vesicles concentrations, n is the Hill coefficient fixed for a given organism, ζ^n is the intracellular calcium concentration for a given organism, λ is the rate of vesicle depletion, Kmax is the exocytosis rate and K parameters is half maximal effective rate of exocytosis. These parameters are influencing the saturation and respectively, the increase of the Hill curve slope.

II. Blood hormone transport is modeled by a diffusion-decay partial differential equation expressing the dynamics of the hormone concentrations xh as a function of vessel length l at time t, formalized as

$$\frac{\partial x_h(l,t)}{\partial t} + \frac{\partial x_h(l,t)}{\partial l} = D_h\frac{\partial^2 x_h(l,t)}{\partial l^2} - \alpha_h x_h(l,t) \qquad (2)$$

where v is the velocity of the blood, Dhandαh is the diffusion and the degradation coefficients.

Therefore $x_h(l,t)$ as the solution of the above equation given by

$$x_h(l,t) = \frac{{x_h}^0}{\sqrt{4\pi D_h t}}\exp\left(-\frac{(l-vt)^2}{4D_h t} - \alpha_h t\right) \qquad (3)$$

with x_{h0} the initial impulse of hormone concentration. The availability of an analytical form providing a flow of the hybrid automata is increasing the accuracy and robustness of the model.

III. The dynamics of the membrane potential V is described by the following equation

$$c_m\frac{d\mathbf{V}}{dt} = -\left(\mathbf{I}_L(\mathbf{V}) + \mathbf{I}_T(\mathbf{V}) + \mathbf{I}_{BK}(\mathbf{V}) + \mathbf{I}_D(\mathbf{V})\right) \qquad (4)$$

where cm is the membrane conductance constant, IL is the current corresponding to the long-lasting Ca++ channel, IT is the current corresponding to the transient Ca++ channel, IBK is the current of the Big-K+ channel and ID is the Delayed Rectifier K+ current, taking in account also other types of current, less known. The higher impact on the action potential is given by the long-lasting Calcium channel. We used the constants observed by LeBeau(1997) for our implementation.

4. Results

The first iterations, used to infer stable system parameters, provided the following steady-state behavior for the HPA synthetic axis.

Four behaviors at different time scales have been observed in the HPA system:

1. fast changes in ACTH concentrations occurring in the 101 min scale
2. very fast irregular fluctuations (mean interpeak intervals ~ 5 min) have been observed when measuring at a high temporal resolution (t = 20 ~ 60 sec)
3. slow changes in the ~ hour scale (circadian oscillations lasting ~ 24 hours)
4. very slow, long-lasting changes in the day's scale under chronic stress or other sustained perturbations.

CRH basal concentrations are largely uncorrelated with those of ACTH, while AVP and ACTH show important temporal correlations. AVP seems therefore to be the main drive for high-frequency ACTH secretion in basal conditions while CRH is the main activator during acute stress, its activity being strongly repressed by cortisol in basal conditions but being sufficient to facilitate AVP action. Glucocorticoids negatively regulate HPA activity at multiple points with all CRH, ACTH, and AVP levels being modified, but not their rhythmicity. These outputs confirm the results from Alexander, Irvine & Donald (1996).

While performing different kinds of stresses on serial and parallel perturbations of the HAP system, we report the following biological comprehensible observations, some of them reported in the literature and serving as validation of our investigation model.

A. Physical immunological stressors (viruses) increase neural activity in Noradrenergic cell populations.
B. Psychological stress information acts directly on the PVN and indirectly on CRH neuron populations in the PVN.
C. While responses to immunological challenges seem to be strongly dependent on the noradrenergic projections from both the VLM and the NTS (Buller et al., 2001), psychological stress seems to be more strongly on the indirect path involving being less dependent on NE terminals, resulting in accordance with Dayas, Buller & Day (2001).
D. The strength of HPA responses to stress depends on the stage of the glucocorticoid ultradian pulses during which the stress occurs, active during the rising phase, and inhibited during the decrease phase (refractory period).
E. While ACTH concentrations increase during both acute and chronic stress, hypothalamic responses show important differences; both CRH and AVP increase in response to acute stress, whereas during chronic stress portal blood CRH concentrations and parvocellular mRNA diminish and those of AVP strongly increase. In addition, during chronic stress, the pulsatility strongly increases producing an apparent hyporesponsiveness to additional stress and attending the circadian rhythm (Lightman, 2008).
F. Il-1 applied directly to pPVN neurons facilitates pPVN cell activity apparently by interfering with GABA inhibitory activity (Ferri & Ferguson, 2005; Ferri & Ferguson, 2003).
G. NE exerts different influences on two different subpopulations of pPVN neurons: it facilitates Glu-mediated synaptic activity on some cells, via de L-adreno receptor, and it directly inhibits (hyperpolarizes) other subpopulations via the-adreno receptor Daftary, C Boudaba, and J G Tasker).
H. Bacterial endotoxin LPS directly stimulates interleukin IL-6 which induces ACTH secretion by corticotropes (Gloddek et al., 2001). MIF cytokine release by AP (corticotrophs) cells is stimulated by both LPS and glucocorticoids and inhibits glucocorticoid inhibition of IL-6 secretion; in corticotrophs, MIF secretion is also stimulated by CRH and the concentration required to release MIF is lower than that required to release ACTH (test inspired by results in Tierney et al., 2005).

I. Hormonal-induced stress, by progesterone, induces endogenous opioid enkephalin secretion by neurons of the NTS which inhibits NE secretion in neuron terminals in the PVN, suppressing responses to stressful stimuli.
J. For the female model, a physiological dose of estrogen can restrain cytokine and neuroendocrine responses, via a slowdown in cortisol production and a limitation of the neuro-electric propagation. This implied a decrease in the number of lymphocytes.
K. For the male model, the cut of the adrenal hormone implies an increase in the lymphocytes.
L. Simulation of joint psychological stress (restraint) and physical stress (virus) show an aggravation of the immune response compared with physical stress only.

The accuracy of the interpretation resides in the choice of hormone levels as entry points, ideally near the real values, based on a literature search. This shows a huge potential to obtain as output an 'almost real' bio-conformation, in order to deliver as priority hypotheses to be tested by conditionally focused studies.

5. Conclusions

This study brings a new 'in silico' investigation computational method for the impact of the brain, through the HPA axis, showing hormonal influence on the release of immune responses in various conditions. This is done as an example of the application of the method, but it is a framework to be used and developed for future research projects. Given the increase of scientific evidence about the brain's implication on biological states, the demands to clarify the intrinsic mechanisms are part of today's medical challenge. Because human-direct measures or 'in vivo/ in vitro' experiments are often not available and have to remain forbidden for ethical reasons, the duty is to provide accurate and inspiring computational neuroscience tools. The originality of our investigation is given by the systemic analysis of neuro-endocrine secretion with circulatory parameters and neuro-electricity patterns, in a computational mathematical mindset. From a synthetic biology point of view, the results of immune responses in our modelings highlight how different male and female organisms act in neuro-adaptive conditions.
This is a premise for future research examination, with an impact on healthcare standards.

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