IMFUFA tekst

- I, OM OG MED MATEMATIK OG FYSIK

Mechanism-based mathematical modelling of dynamics of the hypothalamic-pituitary-adrenal axis

Johanne Gudmand-Høyer
IMFUFA, Roskilde University
PhD thesis

nr. 508 – 2018

Roskilde University,
Department of Science and Environment, IMFUFA
P.O. Box 260, DK - 4000 Roskilde
E-mail: imfufa@ruc.dk

Mechanism-based mathematical modelling of dynamics of the hypothalamic-pituitary-adrenal axis

Johanne Gudmand-Høyer
IMFUFA, Roskilde University
PhD thesis

1^{th} edition, electronic version

IMFUFA tekst nr. 508/2018 — 46 sider — ISSN: 0106-6242

The level of the steroid hormone cortisol is controlled by a neuroendocrine system called the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis consists of complex interactions with both negative and positive feedbacks between corticotropin-releasing hormone (CRH) and vasopressin (AVP) secreted from the hypothalamus, adrenocorticotropic hormone (ACTH) secreted from the pituitary gland, and cortisol secreted from the adrenal glands. In the concentration levels of the HPA axis hormones both a circadian and faster approximately hourly ultradian oscillations are present.

Changed concentration levels of cortisol have been reported in a large percentage of major depressed patients. However, much is still unknown concerning the regulation of the HPA axis in both healthy individuals and in depressed patients.

In this thesis mechanism-based mathematical modelling of different parts of the HPA axis contributes to further knowledge building about the system. Three different modelling works are presented addressing different research questions related to the HPA axis. At first we state a new nonlinear ordinary differential equation model that is able to describe the oscillatory patterns in hormone concentration data from 29 depressed patients and healthy controls. Next we focus on cortisol in the blood and the prediction of how much is on free form. At last we model a network of key enzymes controlling cortisol synthesis. The results in the three works demonstrate in such the importance and the possibilities in applying a mechanism-based, but at the same time multilevel, modelling approach.

Mechanism-based mathematical modelling of dynamics of the hypothalamic-pituitary-adrenal axis

This thesis has been submitted to the Doctoral School of Science and Environment, Roskilde University,

by Johanne Gudmand-Hoeyer

August 2018

Supervisor: Professor Johnny T. Ottesen, IMFUFA
Department of Science and Environment, Roskilde University.

Roskilde University

0.1 Preface

This thesis has been submitted to the Doctoral School of Science and Environment, Roskilde University, as part of the requirements for obtaining the PhD degree in Mathematics.

The research project was supported by funding from Alternativfondet, The Danish Society for the Protection of Laboratory Animals, and Roskilde University. The overall objective of the project has been to contribute to the knowledge building in how mathematical modelling can be used in the reduction and/or replacement of animal experiments in the pharmaceutical industry. Since the PhD work is in Mathematics, the aim has been to contribute by developing mechanism-based mathematical models of an neuroendocrine system, namely the Hypothalamus-Pituitary-Adrenal(HPA) axis.

The research was primarily carried out at the Department of Science and Environment, Roskilde University under the supervision of Professor Johnny T. Ottesen, but included an external stay visiting Professor Michael C. Reed at Department of Mathematics, Duke University, North Carolina (August to December 2014).

The content of this thesis is structured as a synopsis consisting of an introduction and three scientific journal papers. At the time of writing, two of the journal papers have been published.

The thesis has been divided in part I containing the introduction and part II to IV containing each of the three papers.

I. Introduction: Modelling the Hypothalamus-Pituitary-Adrenal axis.

II. Gudmand-Hoeyer, J., Timmermann, S. & Ottesen, J. T. (2014). Patient-specific modeling of the neuroendocrine HPA-axis and its relation to depression: Ultradian and circadian oscillations, *Mathematical Biosciences* **257**: 23-32.

https://doi.org/10.1016/j.mbs.2014.07.013

III. Gudmand-Hoeyer, J. & Ottesen, J. T. (2018). Analysis and validation of a new extended method for estimating plasma free cortisol including neutrophil elastase and competition from other steroids, *The Journal of steroid biochemistry and molecular biology* **181**: 109-124.

https://doi.org/10.1016/j.jsbmb.2018.04.003

IV. Gudmand-Hoeyer, J. & Ottesen, J. T. (n.d.). Quasi-steady state approximations in mechanism-based modelling of the 3'-5'cyclic adenosine monphosphate-Protein kinase A signalling pathway.

The papers are not included in the web-version.

Roskilde, August 2018,
Johanne Gudmand-Høyer

0.2 Acknowledgements

First of all, I would like to thank my supervisor, Professor Johnny T. Ottesen, for his consistent support and competent advice throughout the entire PhD. We have had many interesting discussions on how to model biological systems, both related to this thesis and in general.

I also wish to give thanks to my friends and colleges at IMFUFA[1], especially, my office mates Lisa and Henriette for good company and a nice atmosphere during my years as a PhD student.

Furthermore, I wish to extend a warm thanks to professor Michael C Reed who I visited at Duke University, North Carolina, in the Fall of 2014. For taking his time to comment on my work, arranging interesting modelling seminars and discussing relations between models and data in general. A thank you to Lydia, Shalla, Erin, Ashley, and Josh for including me in all social and academic events and Erin, Ashley, and Poul for even welcoming me into their home at American Drive.

I would like to thank MD Carroll and MD Veldhuis for granting permission to use their clinical data for depressed patients and control individuals including time series measurements for corticotropin (ACTH) and cortisol concentrations.

The Danish Society for Protection of Laboratory Animals and Alternativfondet are gratefully acknowledged for their financial support and interest in mathematical modelling of biology.

Last, but not least, I would like to thank my family and friends for all their encouragement and patience. I would especially like to express my gratitude to my husband Eskild and our daughter Elin for their unconditional love and support.

[1] Imfufa is a research group in Mathematics and Physics at Roskilde University and an acronym for Indsatsområdet for studiet af Matematik og Fysik samt deres Funktioner i Undervisning, Forskning og Anvendelser, that is, Focus Area for the Study of Mathematics and Physics and Their Functions in Teaching, Research and Applications.

0.3 Abstract in English

The level of the steroid hormone cortisol is controlled by a neuroendocrine system called the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis consists of complex interactions with both negative and positive feedbacks between corticotropin-releasing hormone (CRH) and vasopressin (AVP) secreted from the hypothalamus, adrenocorticotropic hormone (ACTH) secreted from the pituitary gland, and cortisol secreted from the adrenal glands. In the concentration levels of the HPA axis hormones both a circadian and faster approximately hourly ultradian oscillations are present.

Changed concentration levels of cortisol have been reported in a large percentage of major depressed patients. However, much is still unknown concerning the regulation of the HPA axis in both healthy individuals and in depressed patients.

In this thesis mechanism-based mathematical modelling of different parts of the HPA axis contributes to further knowledge building about the system. Three different modelling works are presented addressing different research questions related to the HPA axis. The results in the three works demonstrate in such the importance and the possibilities in applying amechanism-based, but at the same time multilevel, modelling approach.

At first we state a new nonlinear ordinary differential equation (ODE) model that is able to describe the oscillatory patterns in hormone concentration data from 29 depressed patients and healthy controls. In our analysis of the model, we use an integrated methodology combining our ODE model with non-linear mixed effects modelling with statistical hypothesis testing. Thereby we identify three of the model parameters to be changed between groups with normal, high and low cortisol levels. Changes in these parameters influence the average levels as well as the ultradian frequencies and amplitudes of ACTH and cortisol. Since the model is mechanism-based, each of the parameters have a physiological interpretation and thereby indicate which mechanisms that have been changed.

Futher we focus on cortisol in the blood. Because of its hydrophobic properties most of cortisol in the blood is bound to transport proteins. Only free cortisol is considered bioactive, but in most clinical procedures only total cortisol is measured due to lower costs. Free cortisol is afterwards estimated. We state a new equilibrium model for predicting the distribution of cortisol in plasma on free and bound forms. The model include the activity of neutrophil elastase and competition from the steroids progesterone and testosterone.

Analysis shows that progesterone influences the distribution of cortisol during pregnancy and during the menstrual cycle and, hence, should be regarded when estimating free cortisol in women.

Neutrophil elastase cleaves the transport protein corticosteroid-binding globulin (CBG) thereby lowering CBG's affinity towards cortisol. When changing

the activity of neutrophil elastase in a physiological range the model is able to fit data excellently.

At last we zoom in on cortisol synthesis in the adrenal cortex cell. When ACTH stimulates the cell to synthesize cortisol, most of the intracellular signalling is orchestrated by the second messenger 3'-5'cyclic adenosine monophosphate (cAMP) and its effector enzyme protein kinase A (PKA).

PKA changes the activity of other enzymes by phosphorylating these. The reverse dephosphorylation is carried out by enzymes called phosphatases. A pair consisting of a kinase and a phosphatase can produce a so-called "ultrasensitive switch" (also denoted Goldbeter & Koshland switch) in the change in concentration of the enzyme being phosphorylated and dephosphorylated. The metabolism of cAMP is carried out solely by the type of enzymes phosphodiesterases (PDEs). The activity of some PDEs can increase after phosphorylation by PKA. This gives rise to a negative feedback loop on the cAMP-signaling, since the cAMP-dependent activation of PKA leads to an increase in the metabolism of the very same signalling molecule, cAMP. This could potentially lead to interesting dynamic behaviour.

When modelling enzyme kinetics the Michaelis-Menten expression derived from the standard Quasi-Steady State Approximation (sQSSA) is often used thereby reducing the number of parameters. However, the sQSSA is often problematic when modelling in vivo enzymatic reactions. An alternative is to use the total Quasi-Steady State Approximation (tQSSA). For our model of the cAMP-PKA signalling we state a full non-reduced, a sQSSA, and a tQSSA version of the model. We show that important dynamics may be lost in the sQSSA version of the model. For some parameter values, the tQSSA version of the model is able to depict the damped oscillations seen in the full model, while the sQSSA version of the model is not.

0.4 Dansk resumé (Abstract in Danish)

Niveauet af steroidhormonet cortisol styres af et neuroendokrint system, som kaldes hypotalamus-hypofyse-adrenal aksen (HPA). HPA-aksen består af komplekse interaktioner med både negative og positive feedbacks mellem corticotropin-frigivende hormon (CRH) og vasopressin (AVP), som udskilles fra hypothalamus, corticotropin (ACTH), som udskilles fra hypofysen og cortisol, som udskilles fra binyrerne. Der forekommer både langsomme diurnale og hurtigere ultradiane svingninger cirka pr. time i koncentrationsniveauerne af HPA-akse hormonerne.

Ændrede koncentrationsniveauer af cortisol er blevet målt hos en stor andel af deprimerede patienter. Imidlertid er meget stadig ukendt vedrørende mekanismerne bag HPA-aksens reguleringen hos både raske individer og hos deprimerede patienter.

I denne afhandling bidrager mekanismebaseret matematisk modellering af forskellige dele af HPA-aksen til yderligere videnopbygning om systemet. Tre forskellige modelleringsarbejder præsenteres for at adressere forskellige forskningsspørgsmål, som er relateret til HPA-aksen. Resultaterne af de tre arbejder demonstrerer tilsammen vigtigheden af og mulighederne i at anvende en mekanismebaserede og på den samme tid multilevel modellerings tilgang.

Først opstiller vi en ny ikke-lineær ordinær differentiallignings (ODE) model, der er i stand til at beskrive svingningsmønstre i hormonkoncentrationsdata fra 29 deprimerede patienter og sunde kontrolpersoner. I vores analyse af modellen bruger vi en integreret metode, der kombinerer vores ODE-model med non-linear mixed effects modelling med statistisk hypotesetestning. Derved identificeres tre af modelparametrene som værende ændret mellem grupper med normale, høje og lave cortisolniveauer. Ændringer i disse parametre påvirker såvel gennemsnitsniveauerne, som ultradiane frekvenser og amplituder af ACTH og cortisol. Da modellen er mekanismebaseret, har hver af parametrene en fysiologisk fortolkning, som derved indikerer hvilke mekanismer, der er blevet ændret.

Dernæst fokuserer vi på cortisol i blodet. På grund af dets hydrofobe egenskaber er det meste af cortisol i blodet bundet til transportproteiner. Kun frit cortisol betragtes som bioaktivt, men i de fleste kliniske procedurer måles kun total cortisol på grund af lavere omkostninger. Frit cortisol bliver efterfølgende estimeret. Vi angiver en ny ligevægtsmodel til forudsigelse af fordelingen af cortisol i plasma på frie og bundne former. Modellen omfatter aktiviteten af neutrofil elastase og konkurrence fra steroiderne progesteron og testosteron.

Analysen viser, at progesteron påvirker fordelingen af cortisol under graviditet og under menstruationscyklus og at dette derfor bør tages i betragtning ved estimering af frit cortisol hos kvinder.

Neutrofil elastase spalter transportproteinet corticosteroidbindende globulin (CBG) og sænker derved CBG's affinitet over for cortisol. Når man ændrer

aktiviteten af neutrofil elastase i en fysiologisk interval, er modellen i stand til at eftergøre data fremragende godt.

Til sidst zoomer we ind på cortisols syntese i binyrebarkcellen. Når ACTH stimulerer cellen til at syntetisere cortisol, bliver det meste af den intracellulære signalering orkestreret af den sekundære messenger 3'-5'-cyklisk adenosin monofosfat (cAMP) og dens effektorenzym proteinkinase A (PKA).

PKA ændrer aktiviteten af andre enzymer at phosphorylere disse. Den omvendte dephosphorylering udføres af enzymer kaldet fosfataser. Et par bestående af en kinase og en fosfatase kan producere en såkaldt "ultrasensitiv switch" (også betegnet Goldbeter - Koshland switch) i ændringen i koncentrationen af det enzym, der phosphoryleres og dephosphoryleres. Metabolismen af cAMP udføres udelukkende af enzymertypen fosfodiesteraser (PDE'er). Aktiviteten af nogle PDE'er kan øges efter fosforylering med PKA. Dette giver anledning til en negativ feedback sløjfe i cAMP-signaleringen, da den cAMP-afhængige aktivering af PKA fører til en stigning i metabolismen af det selv samme signalmolekyle, cAMP. Dette kunne potentielt føre til en interessant dynamik.

Ved modellering af enzymkinetik bruges ofte Michaelis-Menten-udtrykket, som er afledt af standard Quasi-Steady State Approximation (sQSSA), hvorved antallet af parametre reduceres. Imidlertid er sQSSA ofte problematisk ved modellering af in vivo enzymatiske reaktioner. Et alternativ er at bruge en total Quasi-Steady State Approximation (tQSSA). For vores model af cAMP-PKA signalering angiver vi en ikke-reduceret (fuld), en sQSSA og en tQSSA version af modellen. Vi viser, at vigtig dynamik kan gå tabt når sQSSA-versionen af modellen anvendes. For nogle parameterværdier udviser den fulde model og tQSSA-versionen af modellen dæmpede svingninger, mens sQSSA-versionen af modellen ikke er i stand til dette.

Contents

Part I

Introduction: Modelling the Hypothalamic-Pituitary-Adrenal axis

1 Introduction

1.1 The aim of this PhD work

The potentials of using mathematical modelling in biomedical and pharmaceutical research are significant. A mathematical model can be used to state or test hypothesises in medical research. Quantification and analysis of data in areas, where the biological mechanisms are well-known can be supported by mathematical models or a mathematical model can be used as a tool in the clarification of biological mechanism not yet understood. A mathematical model can be used in prioritizing which experiments are the most important in the further knowledge building and thereby help avoid unnecessary animal experiments. Some *in vivo* studies can be substituted with *in vitro* experiments in combination with a mathematical model. However, the usability stays speculative if not specific examples of mathematical modelling are analysed.

Cortisol is controlled by the neuroendocrine system called the hypothalamic-pituitary-adrenal (HPA) axis. The other three major hormones of the system are corticotropin-releasing hormone (CRH) and vasopressin (AVP), synthesised and secreted from the hypothalamus, and adrenocorticotropic hormone (ACTH), synthesised and secreted from the pituitary (see figure 1.1). CRH and AVP increase synergistically the secretion of ACTH. ACTH increases the synthesis and thereby secretion of cortisol from the adrenal cortex. Cortisol negatively feeds back on the secretion of CRH and ACTH (Ben-Shlomo & Melmed, 2011).

Both a circadian and a faster approximately hourly rhythm termed ultradian oscillations are present in the concentration levels of the HPA axis hormones (Gallagher et al., 1973). Much is still unknown regarding the origin and the function of these oscillations. In the mathematical modelling literature of the HPA axis, researchers have tried to investigate differences between normal individuals and patients with HPA axis related diseases (Belzung & de Villemeur, 2010; Gudmand-Hoeyer et al., 2014; Zarzer et al., 2013) as well as the origin of the ultradian oscillations (Andersen et al., 2013; Vinther et al., 2011; Walker et al., 2012, 2010a). In addition to the oscillations, the system will respond by change in hormone levels if the body is subjected to stress of either physiological or psychological nature (Tsigos & Chrousos, 2002).

Even though more and more is known about the individual elements of the HPA axis it is hard to predict the way the complex network that constitutes the

HPA axis regulation of cortisol will react as a whole (Gonzalez-Heydrich et al., 1994). Mathematical modelling of the system is needed in order to investigate the significance of the different elements (Stanojević et al., 2018b).

In this thesis, modelling of the HPA axis and its relation to major depression serves as a case on how mechanism-based mathematical modelling can contribute to the research in aetiology, pathogenesis, diagnosis and future treatments of such a disease.

Part I of the thesis is a general introduction to modelling of the HPA axis with focus on how the works of my PhD have contributed. Part I include a general description of the HPA axis in healthy individuals and changes observed in depressed patients. The three modelling works in part II to IV address different research questions related to the HPA axis. Part I includes motivation for these research questions and relates the results of the three modelling works to the general questions in the HPA axis research field.

The complete works of this PhD are conducted with what could be termed a mechanism-based and multilevel-modelling approach. Mechanism-based modelling refers to the model equations being built on underlying biological mechanisms so that every parameter has a physiological interpretation. However, this could potentially lead to very large incomprehensible models. Hence, following the principle of parsimony, the aim is to exclude non-essential elements and, consequently, some elements might be lumped together. Multilevel modelling refers to the approach that the selection of elements to include or lump together depends on the given modelling task. The three modelling works in part II to IV have different HPA axis related modelling tasks and consequently the included models are derived from different levels.

In part II a model for the overall HPA axis is stated to investigate differences between depressed patients and normal individuals in the HPA axis regulation of ACTH and cortisol. A novel patient-specific mechanistic non-linear ODE model for the HPA axis is suggested. This model shows both the fast ultradian and the slower circadian rhythm and can be fitted to data from healthy controls as well as depressed patients. Furthermore, an integrated modelling approach is suggested, where the non-linear ODE modelling is combined with non-linear mixed effects modelling with statistical hypothesis testing. This approach pinpoints parameters differing between high-, normo- and low-cortisolemic individuals, thereby informing us of HPA axis related physiological differences. Additionally, the method can be seen as a model validation tool.

In part III the goal is to investigate the influence of other steroids and the activity of the enzyme neutrophil elastase on the predictions for free cortisol. Hence, a zoom in on the level of cortisol's interaction with transport proteins in the blood is appropriate. A non-linear mechanistic model for the dynamics of cortisol in plasma is proposed. A static version of the model serves as a new formula for calculating plasma free cortisol concentration. This is of

clinical interest, since direct measurement of free cortisol in human plasma is time-consuming and labour-intensive and consequently often only total cortisol is measured. We investigate the influence of progesterone, testosterone and neutrophil elastase on plasma free cortisol concentration.

In part IV essential enzyme kinetic signalling structures in the cortisol-synthesizing cell is investigated. Looking at the cortisol-synthesizing cell, the signal of ACTH binding to its receptor on the cell membrane is passed onwards inside the cell by the second messenger cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A (PKA), which subsequently phosphorylate other enzymes in the cell. Some of the enzymes being phosphorylated by PKA are phosphodiesterases (PDEs). The metabolism of cAMP is carried out solely by PDEs. Models of this system is posed. The system is shown to be able to generate oscillations. Two different methods in reducing models of enzyme kinetics, the standard Quasi-Steady State Approximation (sQSSA) and the total Quasi-Steady State Approximation (tQSSA), are compared in their ability to not only model steady state situations, but also model the dynamical transition to steady state correctly.

Each of the three articles in part II to IV have minor reviews included of how each of them relates to the more specific fields of dynamical HPA axis models, equilibrium models for estimating free cortisol and dynamical models of 3'-5'-cyclic adenosine monophosphate signalling, respectively.

1.2 Research in major depression and the HPA axis

The classic diseases related to either increased levels of cortisol or lack of cortisol are Cushing's disease and Addison's disease, respectively. Low ACTH level combined with high cortisol level indicate an adrenal cortisol-synthesising tumour (primary hypercortisolism, Cushing's syndrome). High or normal ACTH level combined with high cortisol level indicate a pituitary or ectopic ACTH producing tumour (secondary hypercortisolism, called Cushing's disease or Cushing's syndrome, respectively) (Bornstein et al., 2010). High ACTH level combined with low cortisol level indicate a damage to the adrenal gland (primary hypocortisolism, Addison's disease), while low ACTH level combined with low cortisol level indicate diminished secretion of ACTH (secondary hypocortisolism) or in rare cases damage to hypothalamus (tertiary hypocortisolism) (Willenberg et al., 2010).

Together with alcohol abuse, insulin-resistant obesity, polycystic ovary syndrome, end-stage kidney disease, and some other neuropsychiatric disorder (Chabre, 2018), depression is amongst a group of diseases and conditions termed "pseudo-Cushing's" due to its clinical features sometimes being similar to Cushing's disease (Cunningham et al., 2002; Gold et al., 1986). Likewise, depression

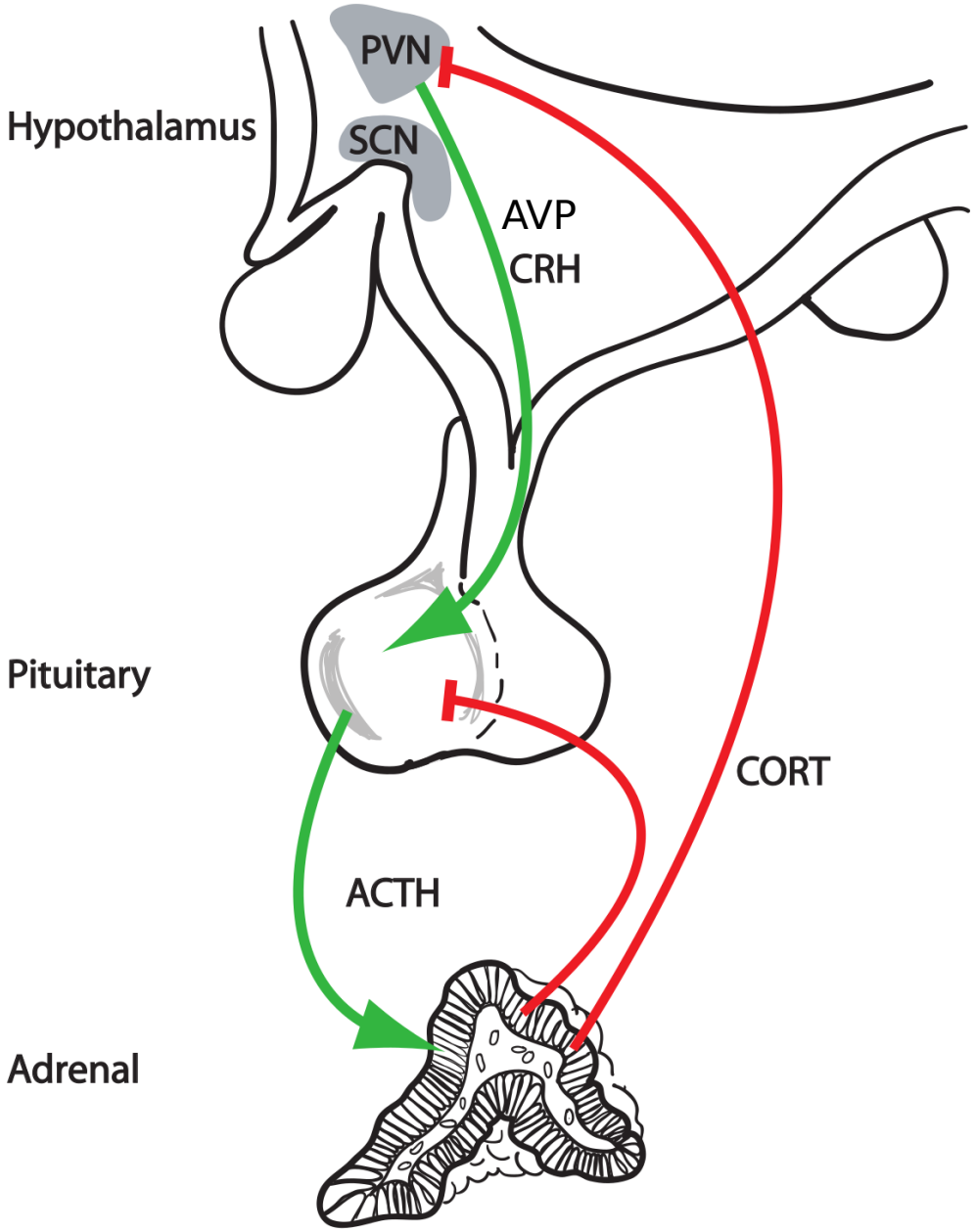

Figure 1.1: The Hypothalamic-pituitary-adrenal axis. CRH and AVP is secreted from the paraventricular nucleus (PVN) in the hypothalamus. CRH and AVP synergistically increase the secretion of ACTH from the pituitary gland (upper green arrow). ACTH affects the adrenal glands stimulating the synthesis and secretion of cortisol (lower green arrow). Cortisol negatively feedbacks on the other hormones of the axis (red dash-terminated lines). Figure modified after Gudmand-Hoeyer et al. (2014)

is considered a complication of Cushing's disease (Sonino et al., 1998).

Major depressive disorder is in the top tenth of causes of years lived with disability (YLDs) worldwide as reported by the Global Burden of Disease Study 2013 (Vos et al., 2015). Despite the treatments available today, depressed patients spend more than 40% of their time being ill (Forte et al., 2015). Diagnosis is done on the background of symptoms that are difficult to classify. The understanding of the underlying molecular pathways is insufficient, which has led to a standstill in the development of new treatments for psychiatric illnesses (Wesseling et al., 2014).

Increased levels of the steroid hormone cortisol have been reported in typically $25 - 30\%$ of major depressed patients (Young et al., 2001). In some of the remaining patients lower cortisol levels compared to control individuals have been reported (Bremmer et al., 2007; Carroll et al., 2007). These groups can be refereed to as high- and lowcortisolemic depressed or hypo- and hypercortisolemic depressed, respectively. Whether the changed hormone levels are a cause or a result of depression is still an open research question (Russell & Lightman, 2014). However, if the hormone levels during treatment do not normalize, the patient has a higher risk of relapsing (Holsboer, 2000).

The level of cortisol is controlled by the HPA axis. Research in depression has especially been focused on the negative feedback of cortisol on the superior hormones of the HPA axis, CRH and ACTH (Ben-Shlomo & Melmed, 2011; Pariante & Lightman, 2008), but much is still unknown in both the diseased and the normal state (Pariante & Lightman, 2008).

Most animal models of depression are either models of acute or of chronic stress (McGonigle, 2014; Razafsha et al., 2013). However, when looking at cortisol level a consensus of how wild animals react to chronic stress does not exist (Dickens & Romero, 2013).

As for humans behavioural symptoms are often used as measurements. The forced swimming test is the most often used (Kato et al., 2016), where immobility is often seen as a measure of "depression-like behaviour", though this is a simplification of this coping strategy that do not always equal behavioural despair (Commons et al., 2017; Kato et al., 2016).

Comparing different species there can be a great variability in the morphology of the adrenal cortex. In particular there is a lot of variability between different subspecies of mice with some having extra layers (Gallo-Payet & Battista, 2014). Cortisol is the most important glucocorticoid in humans where it in rodents are corticosterone.

Using in vitro models with adrenocortical cell lines poses some challenges as well. Gallo-Payet & Battista (2014) describe the paradox that ACTH administrated *in vivo* in both animal and in clinical studies increases the size and growth of the adrenal cortex, while it when administrated *in vitro* to either adreno-

cortical tumour cell lines or primary cultures of adrenocortical cells inhibits proliferation (Gallo-Payet & Battista, 2014).

Cortisol increases during pregnancy and especially in the weeks just before labour. El-Farhan et al. (2017) point on differences between animal models and humans as a hindering in further studying the relation between cortisol and pregnancy (El-Farhan et al., 2017).

According to Wesseling et al. (2014) there has been a focus on pathophysiologies identified in animal models in the development of drugs in psychiatric illnesses. However, the patophysiologies are not directly translational into human physiology (Wesseling et al., 2014). Moreover, Wesseling et al. (2014) attributes the standstill in development of novel drugs to a lack of knowledges of underlying molecular pathways of the not easily classified symptoms (Wesseling et al., 2014).

Using mathematical models combined with clinical human data is a direct way to model the human body where translation from animal into human physiology is not needed. The data available for measurement is of course limited when only looking at clinical data. However, many of the same limitations also apply to getting *in vivo* data from animals, e.g. getting time series measurements of substances in hypothalamus. The concentrations of ACTH and cortisol can be measured from blood samples (Carroll et al., 2007), but there are not yet any methods for direct measuring changes over time in CRH (Herbert, 2013). Neither CRH measured in blood or cerebrospinal fluid are good measures of CRH in the hypothalamus and thereby the HPA axis, since CRH production takes place elsewhere both peripheral (in testes, ovaries, the adrenal medulla, peripheral nerves and lymphocytes) and in the central nervous system (CNS) (prefrontal cortex and parts of the limbic system) (Stetler & Miller, 2011). Moreover, the concentration of CRH in the hypophysial-portal circulation is diluted in the general circulation to a degree that it might not be measurable at all (Keenan & Veldhuis, 2016).

However, by making a series of different clinical experiments one can perturb the system by different exogenous input and afterwards extract information about the unobserved substances from the observable (Keenan et al., 2012). Mechanism-based mathematical modelling can be used to increase the knowledge extracted from the data. Additionally, by making so-called *in silico* experiments, where input are given to the model variables or parameters of the model are perturbed, the model can assist the research in complex neuroendocrine diseases in search of physiological basis of the disease and potential new treatments.

1.3 The oscillations of the HPA axis

The origin of the oscillations in the concentration levels of hormones of the HPA axis as well as whether changes are present in diseases including major depression are widely asked research questions addressed by multiple research groups including us as seen in part II.

1.3.1 The circadian rhythm

A change in amplitudes rather than a change in frequencies of the ultradian oscillations introduces the circadian rhythms in the concentrations of ACTH and cortisol (Veldhuis et al., 1990, 1989). The circadian rhythm of the HPA axis is not a simple sinusoidal rhythm (Keenan & Veldhuis, 2016).

The general understanding is that the central clock or pacemaker of the body is situated in the suprachiasmatic nucleus (SCN) in hypothalamus (Balsalobre et al., 2000; Le Minh et al., 2001; Reppert & Weaver, 2002). The circadian rhythm of the glucocorticoids in rats vanishes if the SCN is damaged or disrupted by constant light (Waite et al., 2012). The SCN couples to the paraventricular nucleus (PVN) in the hypothalamus, where CRH is produced. CRH influences ACTH secretion, which afterwards influences cortisol production in a circadian manner. As a consequence much modelling work have introduced a circadian rhythm to the HPA axis through terms modelling the synthesis of CRH (e.g. (Lenbury, 1996; Lenbury & Pacheenburawana, 1991)).

There is not a one-to-one correspondence between the circadian oscillation of ACTH and cortisol. The circadian rhythm of cortisol is more pronounced than the circadian rhythm of ACTH (Russell et al., 2015). Evidence exists that the circadian signal from the SCN is also directed to the adrenal cortex through autonomic nervous system (ANS) innervation (Buijs et al., 1999; Chung et al., 2011; Ehrhart-Bornstein et al., 1995; Ishida et al., 2005). On top of this active clock genes are found in the adrenal cortex making it home for a peripheral clock (Oster et al., 2006).

Many tissues contains peripheral circadian pacemakers. These peripheral pacemakers are entrained by signals from the central pacemaker in the SCN. Glucocorticoids is one of these signals (Balsalobre et al., 2000; Le Minh et al., 2001; Reppert & Weaver, 2002). Change in food intake can change the phase of the peripheral oscillators, but glucocorticoids will to some degree antagonise this phase shift (Le Minh et al., 2001).

The major part of cortisol in the blood is bound to the transport proteins albumin and corticosteroid-binding globulin (Henley & Lightman, 2011; Pretorius et al., 2011) (see section 1.5 and part III Gudmand-Hoeyer & Ottesen (2018)). Some evidence exists in rodents (Hsu & Kuhn, 1988; Malisch et al., 2008) and humans (Lewis et al., 2006) of a circadian rhythm of the level of CBG in plasma. Though, as Malisch et al. (2008) writes, it might not be as consistent in pattern

across species as the circadian rhythms of glucocorticoids are (Malisch et al., 2008). Some reviews suggest the CBG levels have peak and nadir times opposite to those seen in glucocorticoid levels (Henley & Lightman, 2011; Spiga et al., 2014). The affinity of CBG for cortisol is decreased at higher temperatures in the physiological relevant range of 35 to 42 ^{o}C with the potential to influence the levels of free cortisol not only during fever, but also during the circadian variation in body core temperature (Cameron et al., 2010).

Hence, building on the knowledge of multiple circadian inputs, but from a common overall pacemaker, we in Gudmand-Hoeyer et al. (2014) chose to make four parameters circadian dependent, i.e. a parameter in the CRH dependent ACTH synthesis, a parameter in the ACTH-dependent cortisol synthesis and parameters in the elimination of ACTH and Cortisol. The model fits the ACTH and cortisol circadian profile of the individual data of 29 individuals very well (see the model fits of a hypocortisolemic depressed subject, a non-depressed subject, and a hypercortisolemic depressed subject in figure 7 in part II (Gudmand-Hoeyer et al., 2014)).

Mavroudis et al. (2014) and Sriram et al. (2012) obtain oscillations from their closed HPA axis model with a period corresponding to a circadian rhythm. As Mavroudis et al. (2014) mention themselves the negative feedback of cortisol has been ascribed to give rise to both the ultradian (Walker et al., 2012, 2010b) and the circadian rhythms (Mavroudis et al., 2014). In the later example a central entraining light signal influences the degradation of CRH (Mavroudis et al., 2014). Lu et al. (2013) analyse the earlier model of Gupta et al. (2007) by solving an inverse eigenvalue problem in search of bringing the system close to a Hopf bifurcation and there by gaining an oscillatory system. By this method they find parameter values that enable the model to show damped oscillations, but write that they by numerical bifurcation analysis near the found parameter values can get the system to oscillate (Lu et al., 2013). Lu et al. (2013) are not searching for ultradian or circadian oscillations, but oscillations of a period corresponding to cyclic Cushing syndrome, a condition where the cortisol levels are found to oscillate with a period of half to several days (Lu et al., 2013).

1.3.2 The ultradian oscillations

The ultradian oscillation pattern is characterised by approximately one pulse per 1-2 hour (Sarabdjitsingh et al., 2010). The mechanisms from where the ultradian oscillations origin and are controlled are still not fully understood (Terry et al., 2016). Both the biological and mathematical literature come with conflicting results on from where the ultradian oscillations origin (Engler et al., 1990; Mershon et al., 1992; Vinther et al., 2011; Walker et al., 2012, 2010a).

Tsigos & Chrousos (2002) ascribe all of the oscillations in the levels of ACTH cortisol to oscillations in the concentrations of CRH and AVP. However,

looking into their references they separately reference a study of Engler et al. (1989), where the concentrations of CRH and AVP are shown to oscillate in the hypophysial-portal circulation and to other studies of Horrocks et al. (1990) showing oscillations in the levels of cortisol and ACTH. However, a study by Mershon et al. (1992) have looked at CRH *in vitro* in isolated hypothalamus from the macaque. They found pulsative release of CRH with a very regular frequency. Hence, one hypothesis is that an ultradian pulse-generator located in the hypothalamus influences the CRH level (Mershon et al., 1992) and thereby the rest of the HPA axis.

Another hypothesis is that the negative feedback of cortisol/glucocorticoids on the ACTH production and secretion governs the ultradian rhythms. This view have gained substantial support since the modelling work by Walker et al. (2010a) where Walker et al. (2010a) stated a differential delay model capable of showing ultradian oscillations. The hypothesis is now stated as a fact even in biological research reviews discussing the multiple sources of circadian rhythms of the HPA axis hormones (see e.g. Tsang et al. (2016)).

Terry et al. (2016) argue from the results of their modelling work (Terry et al., 2016; Walker et al., 2010a) and from experiments with constant levels of CRH to rats in the nadir of their circadian phase (Terry et al., 2016) that constant levels at so-called intermediate levels of CRH can drive ultradian oscillations while higher or lower levels will not (Terry et al., 2016). A study in humans by Roelfsema et al. (2017), where constant infusion of CRH and AVP were given at maximally clinically acceptable concentrations and ACTH kept being secreted in a pulsatile fashion, can be seen as further support of the hypothesis by Walker et al. (2010a). However as pointed out by Roelfsema et al. (2017) the frequency of ACTH pulses were the same with and without the external infusion of CRH and AVP and the study does not exclude other pulsatile inputs influencing the system (Roelfsema et al., 2017).

As discussed by Vinther et al. (2011) many modellers of the HPA axis looking for so-called endogenous oscillations are investigating their models for Hopf bifurcations where the local stability of a fixed point goes from stable to unstable resulting in periodic solutions (Vinther et al., 2011). Some modellers achieve this by including time-delays in their model equations (see e.g. (Bairagi et al., 2008; Vinther et al., 2011; Walker et al., 2012, 2010a)).

Vinther et al. (2011) investigate whether ultradian oscillations can occur with the inclusion of time delays in their specific realization of what they name ”the minimal model“, which is a model that only include the textbook version of the HPA axis with positive feed forward of CRH on ACTH and ACTH on cortisol as well as negative feed back of cortisol on both CRH and ACTH. Vinther et al. (2011) include the same time delay for the positive feed forward of ACTH on the cortisol concentration and the negative feedback of cortisol on CRH concentration and ACTH concentration. Vinther et al. (2011) find that a time delay of 19

minutes give rise to oscillations of an ultradian character, but argue that they find time delays of that size unsupported in the literature (Vinther et al., 2011). If emphasis is put on the time it takes ACTH to travel from the pituitary to the adrenal and vice versa for cortisol by the general blood circulation only a couple of minutes is needed. However, the traditional receptors of cortisol is the intracellular glucocorticoid receptor (GR) and the mineralcorticoid receptor (MR), which have genomic effects and, hence, time delays could be argued with respect to these.

Another argument for time delays lays within the synthesis of cortisol. Cortisol is a steroid hormone and consequently can not be stored in vesicles ready for release like the peptide hormones CRH, AVP, and ACTH (Walker et al., 2010b). Hence, a time period from ACTH binds to its receptor on the cortisol-synthesising cell of the adrenal cortex and until cortisol is secreted, can be argued as by Walker et al. (2010b).

However, cortisol feedback happens through both slow and fast feedback mechanisms (Groeneweg et al., 2011) and the rise in glucocorticoids after an ACTH pulse is significant already 5 minutes after the pulse (Spiga et al., 2011a). Furthermore, behind the ACTH-induced synthesis of cortisol is a rich and complex network of enzymes and transport proteins directing the import of cholesterol to the mitochondria and the enzymatic conversion into steroids taking place in the mitochondria and endoplasmatic reticulum (Miller & Auchus, 2011). In this network of reactions, interesting dynamics influencing the oscillations of the HPA axis may be found. These dynamics are not included, when the synthesis is modelled by a time-delay (see section 1.4).

Whether the levels of the HPA axis hormones or the rhythms as well are changed under different pathological conditions is a frequently asked question. Aschbacher et al. (2014) ask the question whether a constant high level of cortisol is needed for making a change in adipose distribution and even insulin resistance as seen in Cushing's syndrome, or whether poorly controlled dynamics are enough and could, hence, be a underlying cause in people with the metabolic syndrome (Aschbacher et al., 2014). It has been hinted in the biological literature related to depression that there might be differences not only in the levels of the hormones, but also in the frequency of the ultradian oscillations (Russell & Lightman, 2014). In untreated obstructive sleep apnoea (OSA) and Cushing's disease altered ultradian rhythms have been observed (Henley & Lightman, 2014). Lightman & Conway-Campbell (2010) writes that changes in ultradian pulse amplitude, frequency and phase has been reported in patients with stress-related diseases and speculate that this might take part in the pathogenesis, but that further investigation is needed (Lightman & Conway-Campbell, 2010).

The mathematical modelling work in part II supports the notion of changed ultradian oscillations in depression, since parameters that influence not only the amplitude, but also the frequency of the ultradian oscillations, are shown to

improve the model significantly when set to vary between groups with high-, low- or normocortisolemic status (see part II (Gudmand-Hoeyer et al., 2014)).

In their original analysis of the data Carroll et al. (2007) did not find changes in the secretory burst frequencies of ACTH and cortisol in high- and low-cortisolemic depressed patient compared to normal individuals (Carroll et al., 2007). In Gudmand-Hoeyer et al. (2014) (part II) we used an integrated methodology by combining a mechanism-based non-linear ordinary differential equations (ODE) model with non-linear mixed effects (NLME) modelling with statistical hypothesis testing to identify the differences between the groups.

1.4 Modelling the cortisol synthesising cell

The lipophilic properties of glucocorticoids imply that they can not be stored in vesicles ready for release (Miller & Auchus, 2011; Spiga et al., 2011b) as is the case for the peptide hormones CRH, AVP, and ACTH of the HPA axis. However, the impact of ACTH on the synthesis of cortisol happens so fast that it can not be explained solely as an effect of the relatively slow (2 to 4 hours) transcription and synthesis of the enzymes involved in cortisol synthesis (de Jong, 2010). Hence, an ACTH-regulated mechanism responding very fast after the binding of ACTH to its receptor must be present (Spiga et al., 2011b).

The adrenal cortex is divided in three zones named zona glomerulosa, zona reticularis and zona fasciculata, respectively. Zona fasciculata and zona reticularis expresses the ACTH-receptor melanocortin receptor 2 (MC2R), but due to differences in the presence of certain enzymes and cofactors, cortisol is primarily synthesised in zona fasciculata (Miller & Auchus, 2011).

MC2R is a G-protein coupled receptor (GPCR) of the type, where its alpha subunit after activation stimulates the enzyme adenylyl cyclase (Roy et al., 2011). Adenylyl cyclase produces the second messenger 3'-5'-cyclic adenosine monophosphate (cAMP) from ATP. cAMP binds to and activates the enzyme protein kinase A (PKA), which can phosphorylate many different proteins and thereby change their activities (Cooray & Clark, 2011; Miller & Auchus, 2011; Roy et al., 2011).

ACTH affects the synthesis of steroid hormones on three time scales. In weeks to months an increase in the concentration of cAMP will lead to increased synthesis of insulin-like growth factor-II (IGF-II), fibroblast growth factor β (FGFβ) and epidermal-growth factor (EGF) by which the steroid synthesising tissue of the cortex will be increased both by hypertrophy and hyperplasia (Miller & Auchus, 2011). Likewise will less stimulation lead to atrophy of zona fasciculata and zona reticularis (Thomas et al., 2004). On the time scale of days cAMP will increase the transcription of enzymes and cofactors of the steroid synthesis (Miller & Auchus, 2011). ACTH upregulates the transcription of mRNA for

MC2R (Morita et al., 1995), for transcription of the transport protein steroid acute regulatory protein (StAR), enzymes P450scc, 3β-HSD (LaVoie & King, 2009), P450c17, P450c21, P45011β (Xing et al., 2010) and some of the enzymes in *de novo* synthesis of cholesterol (Vanparys et al., 2012).

The fastest effect of ACTH happens in minutes by non genomic action. StAR is phosphorylated and thereby increased in activity (Miller & Auchus, 2011), which may happen by protein kinase A (PKA) (Arakane et al., 1997). Cholesterol is transpoted from the outer mitochondrial membrane to the inner mitochondrial membrane by StAR. In the inner mitochondrial membrane cholesterol is cleaved into pregnenolone by the cholesterol side-chain cleavage enzyme P450scc. This first step in the synthesis is considered the rate-limiting step (Miller & Auchus, 2011).

Likewise, the activity of the enzyme hormon-sensitiv lipase (HSL) is increased by phosphorylation (Kraemer & Shen, 2002). pHSL releases existing cholesterol from lipid droplets of the cell, where it is stored on esterified form, thereby making it available for steroid synthesis (Kraemer, 2007; Miller & Auchus, 2011).

As described in section 1.3 modellers have used the need for de novo biosynthesis and release of cortisol in response to ACTH as an argument for introducing delays in their dynamical models of the HPA axis (see e.g. (Walker et al., 2010a)).

The fluctuations in ACTH and cortisol levels are approximately phase shifted 10 minutes (Aschbacher et al., 2012; Young et al., 2001). In a group consisting of 25 major depressed women and 25 controls with blood drawn every 10 minutes for 24 hours Young et al. (2001) finds the best correspondence of the pulsatile components of a cortisol sample and the ACTH sample one sample ahead, i.e. a lag time of approximately 10 min. However, in five of the 50 women cortisol and ACTH arises at the same time and in three women cortisol arises previously to ACTH (Young et al., 2001).

Goodman et al. (1994) investigates ACTH pulsatility on a time scale of minutes. They have ACTH concentration data from two subjects taken every 1-2 min over a three hour period. In these data they observe fluctuations in plasma ACTH concentrations on a time scale smaller than 10 minutes (Goodman et al., 1994). Gallagher et al. (1973) observe that for some pulses of ACTH a pulse in cortisol might be missing (Gallagher et al., 1973). Dissociation of ACTH and cortisol levels is according to Bornstein et al. (2008) a often seen phenomenon in either a physiological or pathophysiological setting (Bornstein et al., 2008). In an *in vivo* rat model with suppressed endogene ACTH and corticosterone secretion, Spiga et al. (2011b) showed that when given the same amount of ACTH (4 ng/hour) either with a constant infusion or by pulse lasting 5 minutes with a 55 minutes break afterwards, the corticosterone response was different. The pulses induced pulsative corticosterone secretion, while the constant infusion had very little effect (Spiga et al., 2011b).

The observations described above raise the interesting research question

whether interesting dynamics origins from the signalling structures of the cortisol synthesizing cell, which may be lost when modelling the synthesis by a time delay.

Mathematical modelling can be used to investigate how different elements of the steroidogenic pathway works together (Spiga et al., 2017). Since the activation of PKA by cAMP leads to much of the following activity of the cortisol synthesizing cell in response to ACTH stimulation, we have in part IV focused on the regulation of cAMP and PKA.

In part IV we state a model for the cAMP-PKA regulation. The model consists of non-linear ordinary differential equations derived from "the law of mass action", where the rate of a given chemical reaction is assumed proportional with the product of the concentrations of the reactants. However, when modelling enzyme kinetics with this approach the model incudes a multitude of parameters of which most are rate constants not easily established. Consequently, model reduction is critical for obtaining experimentally relatable parameters.

The standard Quasi-Steady State Approximation (sQSSA) is widely used in modelling enzyme kinetics and has been used in earlier models of the cAMP-PKA signalling system (see e.g. Garmendia-Torres et al. (2007); Gonzales et al. (2013); Wangorsch et al. (2011); Williamson et al. (2009)). However, as noted by others, use of the sQSSA is problematic when modelling *in vivo* enzymatic reactions where the validation criteria for sQSSA is often not fulfilled (Pedersen et al., 2008a). As an alternative another approximation named the total Quasi-State Approximation (tQSSA) may be used (Borghans et al., 1996). Hence, in part IV we derive sQSSA and tQSSA versions of our model and compare their performances to a non-reduced full model of the system.

The metabolism of cAMP is carried out solely by the type of enzymes phosphodiesterases (PDEs) (Cameron & Baillie, 2012; Conti & Beavo, 2007). PKA phosphorylates some PDEs thereby increasing there activity. Hence a feedback loop on the concentration of cAMP is formed through its activation of PKA (Conti & Beavo, 2007).

Enzymes called phosphatases runs the reverse dephosphorylation of proteins phosphorylated by PKA. A pair consisting of a kinase and a phosphatase can produce a so-called "ultrasensitive switch" in the change in concentration of the enzyme being phosphorylated and dephosphorylated. This was shown by Goldbeter & Koshland (1981) through a mathematical analysis using sQSSA.

In part IV we show that the model is able to generate damped oscillations. For some parameters these oscillations are present in the full version of the model and the tQSSA version of the model but not in the sQSSA version (see part IV).

Some of the earliest modelling of the HPA axis was concentrated on the cortisol synthesizing cell (Dempsher et al., 1984). In the recent years simultaneously with the work this PhD project other groups have suggested models with components of the cortisol synthesis (Spiga et al., 2017; Stanojević et al., 2018a, 2017; Walker

et al., 2015). In Walker et al. (2015) they by mathematical modelling suggest a rapid, intra-adrenal cortisol negative feedback loop, though further research is need to elucidate the specific biological mechanisms (Walker et al., 2015). Spiga et al. (2017) conclude that it is necessary to look at the coupling of the synthesis of cortisol to the immune mechanisms to investigate the dissociation of ACTH and cortisol responses during inflammatory stress (Spiga et al., 2017). Marković et al. (2016); Stanojević et al. (2018a, 2017) have included cholesterol as a variable in their HPA axis models (Marković et al., 2016; Stanojević et al., 2018a, 2017) and recently investigated the influence of external cholesterol input for modelling the effect of e.g. a meal (Stanojević et al., 2017). In the work in part IV we have focused on feedback mechanism in the regulation of PKA levels (see part IV).

Interesting research questions are related to which mechanisms are considered important in the cortisol synthesizing cell's response to ACTH and, hence, which regulatory components to include or alternatively term nonessential. Moreover, different research questions calls for different types of models.

Dempsher et al. (1984) used ODE stated from the knowledge they had at that time concerning the cortisol synthesis. Enzymatic reactions were mostly modelled by Michaelis-Menten expressions (Dempsher et al., 1984). Marković et al. (2016); Stanojević et al. (2018a, 2017) term their ODE models stochiometric network models and explain that their equations are derived from summarized outcomes of complex biological pathways (Čupić et al., 2017). Spiga et al. (2017); Walker et al. (2015) uses delay differential equations (DDEs). They term their model phenomenologically due to a limited knowledge of the regulatory processes (Spiga et al., 2017).

1.5 Cortisol in the blood

In the blood free cortisol quickly binds to the proteins corticosteroid-binding globulin (CBG) and albumin. These binding reactions are reversible. It is estimated that approximately 70% of total cortisol is bound to CBG, 20% is bound to albumin and only 10% of the total cortisol is on free form (Pretorius et al., 2011). CBG is a member of the family of serine protease inhibitors (SERPINS), but without being inhibitory (Lewis & Elder, 2014). The so-called native CBG protein is in a high-affine S-state, but may become cleaved to a less affine R-state, CBG*, by neutrophil elastase released by neutrophils (Chan et al., 2013; Lewis & Elder, 2011, 2014; Lin et al., 2009). This cleavage is assumed to happen at sites of inflammation (Nguyen et al., 2014), but even in normal individual the level of intact CBG is only approximately 65-70 % of total CBG (Lewis & Elder, 2013).

Only free cortisol is considered bioactive by being able to enter the cells

and bind to the intercellular glucocorticoid receptors (Kirchhoff et al., 2011; Mendel, 1989; Perogamvros et al., 2012; Pretorius et al., 2011). However, the available methods for direct measuring free cortisol are both time-consuming and labour-intensive (El-Farhan et al., 2017). In most clinical practise only the total concentration of cortisol is measured (Dorin et al., 2009; Nguyen et al., 2014; Perogamvros et al., 2012) though it has biological, physiological and clinical limitations that one need to hold in mind (Perogamvros et al., 2012). The equilibrium model Coolens equation (Coolens et al., 1987) is often used to estimate the free cortisol (Dorin et al., 2009; Nguyen et al., 2014; Perogamvros et al., 2012).

Equilibrium equations or models are algebraic equations models derived from mass action kinetics, assumptions of equilibrium of the binding reactions, potentially additional reactions, and conservation of the species (see part III (Gudmand-Hoeyer & Ottesen, 2018) as well as (Coolens et al., 1987; Dorin et al., 2009; Nguyen et al., 2014)). Coolens et al. (1987) included measurements of total cortisol and total CBG, but considered in their model the concentration of albumin as having a constant relation to its binding affinity (Coolens et al., 1987). Dorin et al. (2009) improved the predictions of their equilibrium model by including total albumin and showed that this extension was especially important in low albumin and low CBG cases (Dorin et al., 2009). Nguyen et al. (2014) included a distinction between high-affine CBG and low-affine CBG* further improving the model prediction (Nguyen et al., 2014).

Latest we in part III (Gudmand-Hoeyer & Ottesen, 2018) included the competition from progesterone and testosterone in binding to CBG and albumin as well as the reaction by which neutrophil elastase cleaves high-affine CBG to low-affine CBG*. The concentration of elastase and the kinetic constants describing the activity of elastase are collected in one single input parameter in the model. Our model extensions made it possible to evaluate the influence of these steroids as well as the activity of neutrophil elastase. The model fits data excellently, when the elastase activity is treated subject specific, and still performs better than earlier equilibrium models (Coolens et al., 1987; Dorin et al., 2009; Nguyen et al., 2014), when it is fitted collectively for more subjects. Furthermore, our model predictions showed that progesterone can be of influence not only during pregnancy, but also during the normal menstrual cycle of women. Furthermore, model comparison shows that the models (Coolens et al., 1987; Dorin et al., 2009; Gudmand-Hoeyer & Ottesen, 2018; Nguyen et al., 2014) differ considerably in their predictions for cortisol distribution on different forms, i.e. free cortisol and cortisol bound to albumin, intact CBG and elastase-cleaved CBG (see part III (Gudmand-Hoeyer & Ottesen, 2018)).

Our static equilibrium model in part III (Gudmand-Hoeyer & Ottesen, 2018) consists of five coupled second order algebraic equations in five variables. However, some of the terms of our full static model equations are much smaller than others

and can be ignored without changing the model predictions considerably. Hereby a fourth order polynomial equation is obtained. Only one physiologically relevant solution exists and can, accordingly, serve as a new and improved formula for calculating the plasma free cortisol concentration (Gudmand-Hoeyer & Ottesen, 2018) without the need for a numerical solution in line with the quadratic equation of Coolens et al. (1987), the cubic equation of Dorin et al. (2009) and the fourth order polynomial of Nguyen et al. (2014).

As pointed out by Perogamvros et al. (2012) a limitation with calculating free cortisol by Coolens et al. (1987); Dorin et al. (2009); Gudmand-Hoeyer & Ottesen (2018); Nguyen et al. (2014) and others are that the methods combine the imprecision, duration and cost of the assay for measuring each input variable, e.g. total cortisol, total albumin, total CBG, and intact CBG.

Cortisol bound to transport proteins is due to the fast reversible binding reactions generally considered a reservoir of glucocorticoids (Moisan, 2013) though not bioactive before it is released on free form (Mendel, 1989). Similar free cortisol concentrations, but very low total cortisol concentrations and decreased glucocorticoid bioactivities are found in subjects without functioning CBG, when comparing these to healthy controls and subjects heterozygote for the given CBG mutation (Perogamvros et al., 2011, 2010).

Interestingly in the study by Lewis et al. (2006) they find the mean CBG level in five men suffering from the metabolic syndrome to be significant lower than in five lean men (370 ± 65 vs 561 ± 105 nM, p=0.008) where as the total cortisol levels are not significant different (450 ± 88 vs 438 ± 158 nM). Hence, by use of the model in Coolens et al. (1987) higher levels of calculated free cortisol were found in the metabolic syndrome group (Lewis et al., 2006). In studies of depression and cortisol often only total cortisol is considered (Carroll et al., 2007). However, if the hypothesis of free cortisol is true, the interaction with transport proteins should be taken into account. Future clinical studies should at least include measurements of CBG and albumin levels and preferable measurements of inflammatory status and progesterone levels as well. Subsequently, these could be considered in future mathematical modelling studies.

As described above in section 1.3, the level of cortisol in the blood is highly dynamic. The level of cortisol exceeds that of CBG during the morning peak hours ultradian pulses, thus the corresponding binding-reaction becomes saturated (Cameron et al., 2010). Generally such saturation phenomenons are known to exhibit ultrasensitive dynamics, i.e. a small fold-change in the input to the concentration of one of the substances of the binding reaction can generate a large fold change in resulting concentrations in certain areas of concentrations of the substances (Buchler & Louis, 2008; Zhang et al., 2013). Hence, the amplitude of ultradian oscillations during low levels of cortisol in blood will intuitively be damped by the buffering effect whereas the amplitudes of the ultradian oscillations may not be during high levels of cortisol.

A few modellers have included CBG in their dynamic models of the HPA axis (e.g. (Kyrylov et al., 2005; Perrin et al., 1978; Yates & Urquhart, 1962; Yi-Wei et al., 1999)). Other modellers leave the binding to plasma proteins out either without mentioning it or by stating that the relation between free and total cortisol can be regarded as proportional (Bingzheng et al., 1990). Peters et al. (2007) writes that they tentatively had included CBG, but that it did not affect their mayor findings that concerned interaction between positive and negative feedback loops in the HPA axis mediated by the dynamics of the two types of cortisol receptors, i.e. mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). In an ongoing work not included in this thesis Johnny T. Ottesen and I are investigating a dynamical model of the HPA axis including dynamics with albumin and CBG to investigate the influence of the transport proteins on the oscillations in cortisol and ACTH levels.

Junkermann et al. (1982) suggest that a circadian rhythm observed in the levels of progesterone in pregnant women with an inverse relation to their levels of cortisol could be caused by the competition for binding to CBG (Junkermann et al., 1982). Hsu & Kuhn (1988) suggest that the circadian rhythm found in CBG is induced by the circadian rhythm of glucocorticoids (Hsu & Kuhn, 1988). A dynamical model of the HPA axis including synthesis and elimination of progesterone and CBG could investigate these hypothesises as well.

1.6 Concluding remarks and perspectives

In part II (Gudmand-Hoeyer et al., 2014) we investigated differences between normal individuals and hypo- and hypercorticosolemic depressed patients in a dynamic model of the HPA axis, in part III (Gudmand-Hoeyer & Ottesen, 2018) we investigated differences in predictions for free cortisol for different populations groups, e.g. sex differences and differences due to menstrual cycle phase and pregnancy in several equilibrium models, and in part IV (Gudmand-Hoeyer & Ottesen) essential enzyme kinetic signalling structures in the cortisol synthesising cell were investigated. Common for the works is a mechanism-based approach. However, as noted in section 1.1 our modelling approach is multi-level as well, i.e. decisions have been made on which of the HPA axis mechanisms to include and which to leave out or lump together.

As pointed out in the recent review by Stanojević et al. (2018b) the different HPA axis research modelling groups are still not in consensus on the mechanism important for the core feedback mechanisms of the HPA axis. Hence, the models can seem difficult to compare directly (Stanojević et al., 2018b).

Many modellers have used the model of Gupta et al. (2007) (e.g.Ben-Zvi & Lee (2009); Ben-Zvi et al. (2009); Chakrabarty et al. (2014); Hui & Żak (2013); Lu et al. (2013); Zak (2012)). Again others have made extensions of the model

(e.g. Kim et al. (2016); Walker et al. (2010a); Zarzer et al. (2013)). The model by Gupta et al. (2007) is a non-linear ODEs model. Beside having CRH, ACTH and cortisol as variables, Gupta et al. (2007) include the glucocorticoid receptors of the pituitary gland as a variable. When cortisol binds to the glucocorticoid receptors, it leads to up-regulation of the receptors and thereby inhibition of the ACTH secretion. Burstein & Coculescu (2012) terms it revolutionizing for mathematical modelling of the HPA axis that Gupta et al. (2007) included GR-cortisol dynamics in their model. Interestingly, Gupta et al. (2007) themselves state that including the receptor dynamic is merely an example of a putative mechanism leading to a needed bi-stability of the HPA axis system and that there could be other explanations (Gupta et al., 2007).

Hence, what to include or exclude does not have a given answer. It varies as explained earlier with the modelling task and, since some mechanisms differ between species, it varies if the questions are related to human or to animal physiology. Hopefully with each modelling work we will get one more brick in the puzzle to understand the complexity of the HPA axis.

Bibliography

Andersen, M., Vinther, F. & Ottesen, J. T. (2013). Mathematical modeling of the hypothalamic–pituitary–adrenal gland (HPA) axis, including hippocampal mechanisms, *Mathematical biosciences* **246**(1): 122–138.

Arakane, F., King, S. R., Du, Y., Kallen, C. B., Walsh, L. P., Watari, H., Stocco, D. M. & Strauss, J. F. (1997). Phosphorylation of steroidogenic acute regulatory protein (StAR) modulates its steroidogenic activity, *Journal of Biological Chemistry* **272**(51): 32656–32662.

Aschbacher, K., Adam, E. K., Crofford, L. J., Kemeny, M. E., Demitrack, M. A. & Ben-Zvi, A. (2012). Linking disease symptoms and subtypes with personalized systems-based phenotypes: a proof of concept study, *Brain, behavior, and immunity* **26**(7): 1047–1056.

Aschbacher, K., Rodriguez-Fernandez, M., van Wietmarschen, H., Tomiyama, A. J., Jain, S., Epel, E., Doyle, F. J. & van der Greef, J. (2014). The hypothalamic–pituitary–adrenal–leptin axis and metabolic health: a systems approach to resilience, robustness and control, *Interface focus* **4**(5): 20140020.

Bairagi, N., Chatterjee, S. & Chattopadhyay, J. (2008). Variability in the secretion of corticotropin-releasing hormone, adrenocorticotropic hormone and cortisol and understandability of the hypothalamic-pituitary-adrenal axis dynamics—a mathematical study based on clinical evidence, *Mathematical Medicine and Biology* **25**(1): 37–63.

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schütz, G. & Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling, *Science* **289**(5488): 2344–2347.

Belzung, C. & de Villemeur, E. B. (2010). The design of new antidepressants: can formal models help? A first attempt using a model of the hippocampal control over the HPA-axis based on review from the litterature, *Behavioural Pharmacology* **21**(8): 677–689.

Ben-Shlomo, A. & Melmed, S. (2011). Hypothalamic Regulation of Anterior Pituitary Function, *in* S. Melmed (ed.), *The Pituitary*, 3 edn, Elsevier, chapter 2, pp. 21–45.

Ben-Zvi, A. & Lee, J. M. (2009). Nonlinear dynamical analysis and optimization for biological/biomedical systems, *Methods in enzymology* **467**: 435–459.

Ben-Zvi, A., Vernon, S. D. & Broderick, G. (2009). Model-based therapeutic correction of hypothalamic-pituitary-adrenal axis dysfunction, *PLoS Comput Biol* **5**(1): e1000273.

Bingzheng, L., Zhenye, Z. & Liansong, C. (1990). A mathematical model of the regulation system of the secretion of glucocorticoids, *Journal of Biological Physics* **17**(4): 221–233.

Borghans, J. A., de Boer, R. J. & Segel, L. A. (1996). Extending the quasi-steady state approximation by changing variables, *Bulletin of Mathematical Biology* **58**(1): 43–63.

Bornstein, S. R., Engeland, W. C., Ehrhart-Bornstein, M. & Herman, J. P. (2008). Dissociation of ACTH and glucocorticoids, *Trends in Endocrinology & Metabolism* **19**(5): 175–180.

Bornstein, S. R., Gruber, M., Willenberg, H. S., Stratakis, C. A. & Chrousos, G. P. (2010). Cushing's Syndrome, Medical Aspects, *in* G. Fink (ed.), *Stress Science: Neuroendocrinology*, Academic Press, Elsevier, pp. 189–194.

Bremmer, M. A., Deeg, D. J., Beekman, A. T., Penninx, B. W., Lips, P. & Hoogendijk, W. J. (2007). Major depression in late life is associated with both hypo-and hypercortisolemia, *Biological psychiatry* **62**(5): 479–486.

Buchler, N. E. & Louis, M. (2008). Molecular Titration and Ultrasensitivity in Regulatory Networks, *Journal of Molecular Biololy* **384**: 1106–1119.

Buijs, R. M., Wortel, J., Van Heerikhuize, J. J., Feenstra, M. G., Ter Horst, G. J., Romijn, H. J. & Kalsbeek, A. (1999). Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway, *European Journal of Neuroscience* **11**(5): 1535–1544.

Burstein, G. & Coculescu, M. (2012). Integrative System Theory of Hippocampal-Hypothalamic-Pituitary-Adrenal Axis for Cortisol Feedback Dysfunctions and Feedback Drug Therapies, *Acta Endocrinologica (Buc)* **8**(3): 497–510.

Cameron, A., Henley, D., Carrell, R., Zhou, A., Clarke, A. & Lightman, S. (2010). Temperature-responsive release of cortisol from its binding globulin: a protein thermocouple, *Journal of Clinical Endocrinology & Metabolism* **95**(10): 4689.

Cameron, R. T. & Baillie, G. S. (2012). cAMP-Specific Phosphodiesterases: Modulation, Inhibation, and Activation, *in* L. M. Botana & M. Loza (eds), *Therapeutic Targets: Modulation, Inhibation, and Activation*, 1 edn, John Wiley and Sons, Inc., chapter 1, pp. 1–35.

Carroll, B., Cassidy, F., Naftolowitz, D., Tatham, N., Wilson, W., Iranmanesh, A., Liu, P. & Veldhuis, J. (2007). Pathophysiology of hypercortisolism in depression, *Acta Psychiatrica Scandinavica-Supplement* **115 (Suppl. 433)**: 90–103.

Chabre, O. (2018). The difficulties of pseudo-Cushing's syndrome (or "non-neoplastic hypercortisolism"), *Annales d'endocrinologie*, Vol. 79, Elsevier, pp. 138–145.

Chakrabarty, A., Buzzard, G. T., Corless, M. J., Zak, S. H. & Rundell, A. E. (2014). Correcting hypothalamic-pituitary-adrenal axis dysfunction using observer-based explicit nonlinear model predictive control, *Engineering in Medicine and Biology Society (EMBC), 2014 36th Annual International Conference of the IEEE*, IEEE, pp. 3426–3429.

Chan, W. L., Carrell, R. W., Zhou, A. & Read, R. J. (2013). How changes in affinity of corticosteroid-binding globulin modulate free cortisol concentration, *The Journal of Clinical Endocrinology & Metabolism* **98**(8): 3315–3322.

Chung, S., Son, G. H. & Kim, K. (2011). Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **1812**(5): 581–591.

Commons, K. G., Cholanians, A. B., Babb, J. A. & Ehlinger, D. G. (2017). The rodent forced swim test measures stress-coping strategy, not depression-like behavior, *ACS chemical neuroscience* **8**(5): 955–960.

Conti, M. & Beavo, J. (2007). Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling, *Annual Review of Biochemistry* **76**: 481–511.

Coolens, J.-L., van Baelen, H. & Heyns, W. (1987). Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin, *J. Steroid Biochem.* **26**(2): 197–202.

Cooray, S. N. & Clark, A. J. L. (2011). Melanocortin receptors and their accessory proteins, *Molecular and Cellular Endocrinology* **331**: 215–221.

Cunningham, J., Buxton, O. & Weiss, R. (2002). Circadian variation in Cushing's disease and pseudo-Cushing state by analysis of F and ACTH pulsatility, *J. Endocrinol. Invest.* **25**: 791–799.

de Jong, F. H. (2010). Steroid Hydroxylases, *in* G. Fink (ed.), *Stress science: Neuroendocrinology*, Academic Press, pp. 176–179.

Dempsher, D. P., Gann, D. S. & Phair, R. D. (1984). A mechanistic model of ACTH-stimulated cortisol secretion, *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **246**(4): R587–R596.

Dickens, M. J. & Romero, L. M. (2013). A consensus endocrine profile for chronically stressed wild animals does not exist, *General and Comparative Endocrinology* **191**: 177–189.

Dorin, R. I., Pai, H. K., Ho, J. T., Lewis, J. G., Torpy, D. J., Urban, F. K. & Qualls, C. R. (2009). Validation of a simple method of estimating plasma free cortisol: role of cortisol binding to albumin, *Clinical Biochemistry* **42**: 64–71.

Ehrhart-Bornstein, M., Bornstein, S. R., González-Hernández, J., Holst, J. J., Waterman, M. R. & Scherbaum, W. A. (1995). Symphathoadrenal regulation of adrenocortical steroidogenesis, *Endocrine Research* **21**(1&2): 13–24.

El-Farhan, N., Rees, D. A. & Evans, C. (2017). Measuring cortisol in serum, urine and saliva–are our assays good enough?, *Annals of clinical biochemistry* **54**(3): 308–322.

Engler, D., Pham, T., Fullerton, M. J., Ooi, G., Funder, J. W. & Clarke, I. J. (1989). Studies of the Secretion of Corticotropin-Releasing Factor and Arginine Vasopressin into the Hypophysial-Portal Circulation of the Conscious Sheep, *Neuroendocrinology* **49**: 367–381.

Engler, D., Pham, T., Liu, J. U. N. P., Fullerton, M. J., Clarke, I. J. & Funder, J. W. (1990). Studies of the regulation of the hypothalamic-pituitary-adrenal axis in sheep with hypothalamic-pituitary disconnection. II. Evidence for *in vivo* ultradian hypersecretion of proopiomelanocortin peptides by the isolated anterior and intermediate pituitary, *Endocrinology* **127**(4): 1956–1966.

Forte, A., Baldessarini, R. J., Tondo, L., Vázquez, G. H., Pompili, M. & Girardi, P. (2015). Long-term morbidity in bipolar-I, bipolar-II, and unipolar major depressive disorders, *Journal of affective disorders* **178**: 71–78.

Gallagher, T. F., Yoshida, K., Roffwarg, H. D., Fukushima, D. K., Weitzman, E. D. & Hellman, L. (1973). ACTH and Cortiol Secretory Patterns in Man, *The Journal of Clinical Endocrinology & Metabolism* **36**: 1058–1068.

Gallo-Payet, N. & Battista, M.-C. (2014). Steroidogenesis - Adrenal Cell Signal Transduction, *Comprehensive Physiology* **4**: 889–964.

Garmendia-Torres, C., Goldbeter, A. & Jacquet, M. (2007). Nucleocytoplasmic oscillations of the yeast transcription factor Msn2: evidence for periodic PKA activation, *Current Biology* **17**(12): 1044–1049.

Gold, P. W., Loriaux, L., Roy, A., Kling, M. A., Calabrese, J. R., Kellner, C. H., Nieman, L. K., Post, R. M., Pickar, D., Gallucci, W., Avgerinos, P., Paul, S., Oldfield, E. H., Cutler, G. B. & Chrousos, G. P. (1986). Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. Pathophysiologic and diagnostic implications., *The New England Journal of Medicine* **314(21)**: 1329–1335.

Goldbeter, A. & Koshland, D. E. (1981). An amplified sensitivity arising from covalent modification in biological systems, *Proceedings of the National Academy of Sciences of the United States of America* **78**(11): 6840–6844.

Gonzales, K., Kayıkçı, Ö., Schaeffer, D. G. & Magwene, P. M. (2013). Modeling mutant phenotypes and oscillatory dynamics in the Saccharomyces cerevisiae cAMP-PKA pathway, *BMC systems biology* **7**(1): 40.

Gonzalez-Heydrich, J., Steingard, R. & Kohane, I. (1994). A computer simulation of the hypothalamic-pituitary-adrenal axis., *Proceedings of the Annual Symposium on Computer Application in Medical Care*, American Medical Informatics Association, p. 1010.

Goodman, B. M., Carnes, M. & Lent, S. J. (1994). Model simulations of ACTH pulsatility, *Life Sciences* **54**(22): 1659–1669.

Groeneweg, F. L., Karst, H., de Kloet, E. R. & Joëls, M. (2011). Rapid non-genomic effects of corticosteroids and their role in the central stress response, *Journal of Endocrinology* **209**(2): 153–167.

Gudmand-Hoeyer, J. & Ottesen, J. T. (2018). Analysis and validation of a new extended method for estimating plasma free cortisol including neutrophil elastase and competition from other steroids, *The Journal of steroid biochemistry and molecular biology* **181**: 109–124.

Gudmand-Hoeyer, J., Timmermann, S. & Ottesen, J. T. (2014). Patient-specific modeling of the neuroendocrine HPA-axis and its relation to depression: Ultradian and circadian oscillations, *Mathematical Biosciences* **257**: 23–32.

Gupta, S., Aslakson, E., Gurbaxani, B. M. & Vernon, S. D. (2007). Inclusion of the glucocorticoid receptor in a hypothalamic pituitary adrenal axis model reveals bistability, *Theoretical Biology and Medical Modelling* **4**: 8.

Henley, D. E. & Lightman, S. L. (2011). New insights into corticosteroid-binding globulin and glucocorticoid delivery, *Neuroscience* **180**: 1–8.

Henley, D. E. & Lightman, S. L. (2014). Cardio-metabolic consequences of glucocorticoid replacement: relevance of ultradian signalling, *Clinical endocrinology* **80**(5): 621–628.

Herbert, J. (2013). Cortisol and depression: three questions for psychiatry, *Psychological Medicine* **43**: 449–469.

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression, *Neuropsychopharmacology* **23**(5): 477–501.

Horrocks, P., Jones, A., Ratcliffe, W., Holder, G., White, A., Holder, R., Ratcliffe, J. & London, D. (1990). Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females, *Clinical endocrinology* **32**(1): 127–134.

Hsu, B. R. & Kuhn, R. W. (1988). The role of the adrenal in generating the diurnal variation in circulating levels of corticosteroid-binding globulin in the rat, *Endocrinol.* **122**(2): 421–426.

Hui, S. & Żak, S. H. (2013). Stress estimation using unknown input observer, *2013 American Control Conference*, IEEE, pp. 259–264.

Ishida, A., Mutoh, T., Ueyama, T., Bando, H., Masubuchi, S., Nakahara, D., Tsujimoto, G. & Okamura, H. (2005). Light activates the adrenal gland: timing of gene expression and glucocorticoid release, *Cell metabolism* **2**(5): 297–307.

Junkermann, H., Mangold, H., Vecsei, P. & Runnebaum, B. (1982). Circadian rhythm of serum progesterone levels in human pregnancy and its relation to the rhythm of cortisol, *Acta endocrinologica* **101**(1): 98–104.

Kato, T., Kasahara, T., Kubota-Sakashita, M., Kato, T. & Nakajima, K. (2016). Animal models of recurrent or bipolar depression, *Neuroscience* **321**: 189–196.

Keenan, D. M. & Veldhuis, J. D. (2016). Pulsatility of hypothalamo-pituitary hormones: a challenge in quantification, *Physiology* **31**(1): 34–50.

Keenan, D. M., Wang, X., Pincus, S. M. & Veldhuis, J. D. (2012). Modelling the nonlinear time dynamics of multidimensional hormonal systems, *Journal of Time Series Analysis* **33**(5): 779–796.

Kim, L. U., D'Orsogna, M. R. & Chou, T. (2016). Onset, timing, and exposure therapy of stress disorders: mechanistic insight from a mathematical model of oscillating neuroendocrine dynamics, *Biology direct* **11**(1): 1.

Kirchhoff, F., Briegel, J. & Vogeser, M. (2011). Quantification of free serum cortisol based on equilibrium dialysis and isotope dilution-liquid chromatography–tandem mass spectrometry, *Clinical Biochemistry* **44**(10): 894–899.

Kraemer, F. B. (2007). Adrenal cholesterol utilization, *Molecular and Cellular Endocrinology* **265-266**: 42–45.

Kraemer, F. B. & Shen, W. J. (2002). Hormone-sensitive lipase: Control of intracellular tri-(di-)acylglycerol and cholesterol ester hydrolysis, *Journal of Lipid Research* **43**(10): 1585–1594.

Kyrylov, V., Severyanova, L. & Vieira, A. (2005). Modeling robust oscillatory behavior of the hypothalamic-pituitary-adrenal axis, *IEEE Transactions on Biomedical Engineering* **52**(12): 1977–1983.

LaVoie, H. A. & King, S. R. (2009). Transcriptional Regulation of Steroidogenic Genes: STARD1, CYP11A1 and HSD3B, *Experimental Biology and Medicine* **234**(8): 880–907.

Le Minh, N., Damiola, F., Tronche, F., Schütz, G. & Schibler, U. (2001). Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators, *The EMBO journal* **20**(24): 7128–7136.

Lenbury, Y. (1996). Parameter Space Classification of Solutions to a Mathematical Model for the Cortisol Secretion System in Normal Man, *Biomedical and Life Physics*, Springer, pp. 33–48.

Lenbury, Y. & Pacheenburawana, P. (1991). Modelling fluctuation phenomena in the plasma cortisol secretion system in normal man, *Biosystems* **26**(2): 117–125.

Lewis, J. G. & Elder, P. A. (2011). Corticosteroid-binding globulin reactive centre loop antibodies recognise only the intact natured protein: Elastase cleaved and uncleaved CBG may coexist in circulation, *The Journal of steroid biochemistry and molecular biology* **127**(3): 289–294.

Lewis, J. G. & Elder, P. A. (2013). Intact or "active" corticosteroid-binding globulin (CBG) and total CBG in plasma: Determination by parallel ELISAs using monoclonal antibodies, *Clinica Chimica Acta* **416**: 26–30.

Lewis, J. G. & Elder, P. A. (2014). The reactive centre loop of corticosteroid-binding globulin (CBG) is a protease target for cortisol release, *Molecular and cellular endocrinology* **384**(1): 96–101.

Lewis, J. G., Möpert, B., Shand, B. I., Doogue, M. P., Soule, S. G., Frampton, C. M. & Elder, P. A. (2006). Plasma variation of corticosteroid-binding globulin and sex hormone-binding globulin, *Hormone and Metabolic Research* **38**(4): 241–245.

Lightman, S. L. & Conway-Campbell, B. L. (2010). The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration, *Nature Reviews Neuroscience* **11**(10): 710–718.

Lin, H.-Y., Underhill, C., Gardill, B. R., YA., M. & Hammond, G. L. (2009). Residues in the human corticosteroid-binding globulin reactive center loop that influence steroid binding before and after elastase cleavage, *Journal of Biological Chemistry* **284**(2): 884–896.

Lu, J., August, E. & Koeppl, H. (2013). Inverse problems from biomedicine, *Journal of mathematical biology* **67**(1): 143–168.

Malisch, J. L., Breuner, C. W., Gomes, F. R., Chappell, M. A. & Garland, T. (2008). Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior, *General and Comparative Endocrinology* **156**(2): 210–217.

Marković, V. M., Čupić, v., Maćešić, S., Stanojević, A., Vukojević, V. & Kolar-Anić, L. (2016). Modelling cholesterol effects on the dynamics of the hypothalamic–pituitary–adrenal (HPA) axis, *Mathematical Medicine and Biology* **33**(1): 1–28.

Mavroudis, P. D., Corbett, S. A., Calvano, S. E. & Androulakis, I. P. (2014). Mathematical modeling of light-mediated HPA axis activity and downstream implications on the entrainment of peripheral clock genes, *Physiological genomics* **46**(20): 766–778.

McGonigle, P. (2014). Animal models of CNS disorders, *Biochemical pharmacology* **87**(1): 140–149.

Mendel, C. M. (1989). The free hormone hypothesis: a physiologically based mathematical model, *Endocr. Rev.* **10**(3): 232–274.

Mershon, J., Sehlhorst, C., Rebar, R. & Liu, J. (1992). Evidence of a corticotropin-releasing hormone pulse generator in the macaque hypothalamus, *Endocrinology* **130**(5): 2991–2996.

Miller, W. & Auchus, R. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders, *Endocrine Reviews* **32**(1): 81–151.

Moisan, M.-P. (2013). CBG: a cortisol reservoir rather than a transporter, *Nature Reviews Endocrinology* **9**(2): 78.

Morita, T. M., Imai, T., Murata, Y., Kambe, F., Funahashi, H., Takagi, H. & Seo, H. (1995). Adrenocorticotropic hormone (ACTH) increases the expression of its own receptor gene, *Endocrine Journal* **42**(4): 475–480.

Nguyen, P. T. T., Lewis, J. G., Sneyd, J., Lee, R. S. F., Torpy, D. J. & Shorten, P. R. (2014). Development of a formula for estimating plasma free cortisol concentration from a measured total cortisol concentration when elastase-cleaved and intact corticosteroid binding globulin coexist, *J. Steroid Biochem. Mol. Biol.* **141**: 16–25.

Oster, H., Damerow, S., Kiessling, S., Jakubcakova, V., Abraham, D., Tian, J., Hoffmann, M. W. & Eichele, G. (2006). The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock, *Cell metabolism* **4**(2): 163–173.

Pariante, C. M. & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments, *Trends in Neurosciences* **31**(9): 464–468.

Pedersen, M. G., Bersani, A. M. & Bersani, E. (2008a). Quasi steady-state approximations in complex intracellular signal transduction networks - a word of caution, *Journal of Mathematical Chemistry* **43**(4): 1318–1344.

Perogamvros, I., Kayahara, M., Trainer, P. J. & Ray, D. W. (2011). Serum regulates cortisol bioactivity by corticosteroid-binding globulin-dependent and independent mechanisms, as revealed by combined bioassay and physicochemical assay approaches, *Clinical Endocrinology* **75**(1): 31–38.

Perogamvros, I., Ray, D. W. & Trainer, P. J. (2012). Regulation of cortisol bioavailability—effects on hormone measurement and action, *Nature Reviews Endocrinology* **8**(12): 717.

Perogamvros, I., Underhill, C., Henley, D. E., Hadfield, K. D., Newman, W. G., Ray, D. W., Lightman, S. L., Hammond, G. L. & Trainer, P. J. (2010). Novel corticosteroid-binding globulin variant that lacks steroid binding activity, *Journal of Clinical Endocrinology & Metabolism* **95**(10): E142–E150.

Perrin, F., Normand, M. & Fortier, C. (1978). Analysis through mathematical models of the dynamics of the adrenal cortical response to ACTH in the rat, *Annals of biomedical engineering* **6**(1): 1–15.

Peters, A., Conrad, M., Hubold, C., Schweiger, U., Fischer, B. & Fehm, H. L. (2007). The principle of homeostasis in the hypothalamus-pituitary-adrenal

system: New insight from positive feedback, *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **293**(1): R83–R98.

Pretorius, C. J., Galligan, J. P., McWhinney, B. C., Briscoe, S. E. & Ungerer, J. P. (2011). Free cortisol method comparison: ultrafiltation, equilibrium dialysis, tracer dilution, tandem mass spectrometry and calculated free cortisol, *Clin. Chim. Acta* **412**(11): 1043–1047.

Razafsha, M., Behforuzi, H., Harati, H., Wafai, R. A., Khaku, A., Mondello, S., Gold, M. & Kobeissy, F. (2013). An updated overview of animal models in neuropsychiatry, *Neuroscience* **240**: 204–218.

Reppert, S. M. & Weaver, D. R. (2002). Coordination of circadian timing in mammals, *Nature* **418**(6901): 935.

Roelfsema, F., Aoun, P., Takahashi, P. Y., Erickson, D., Yang, R. & Veldhuis, J. D. (2017). Regulation of Pulsatile and Entropic ACTH Secretion Under Fixed Exogenous Secretagogue Clamps, *The Journal of Clinical Endocrinology & Metabolism* **102**(7): 2611–2619.

Roy, S., Roy, S. J., Pinard, S., Taillefer, L.-D., Rached, M., Parent, J.-L. & Gallo-Payet, N. (2011). Mechanisms of melanocortin-2 receptor (MC2R) internalization and recycling in human embryonic kidney (hek) cells: Identification of key Ser/Thr (S/T) amino acids, *Molecular Endocrinology* **25**(11): 1961–1977.

Russell, G. M., Kalafatakis, K. & Lightman, S. L. (2015). The Importance of Biological Oscillators for Hypothalamic- Pituitary-Adrenal Activity and Tissue Glucocorticoid Response: Coordinating Stress and Neurobehavioural Adaptation, *Journal of Neuroendocrinology* **27**: 378–388.

Russell, G. M. & Lightman, S. L. (2014). Can side effects of steroid treatments be minimized by the temporal aspects of delivery method?, *Expert opinion on drug safety* **13**(11): 1501–1513.

Sarabdjitsingh, R., Spiga, F., Oitzl, M., Kershaw, Y., Meijer, O., Lightman, S. & De Kloet, E. (2010). Recovery from Disrupted Ultradian Glucocorticoid Rhythmicity Reveals a Dissociation Between Hormonal and Behavioural Stress Responsiveness, *J. Neuroendocrinol.* **22(8)**: 862–871.

Sonino, N., Fava, G., Raffi, A., Boscaro, M. & Fallo, F. (1998). Clinical Correlates of Major Depression in Cushing's Disease, *Psychopathology* **31**: 302–306.

Spiga, F., Liu, Y., Aguilera, G. & Lightman, S. (2011a). Temporal effect of adrenocorticotrophic hormone on adrenal glucocorticoid steroidogenesis: Involvement of the transducer of regulated cyclic AMP-response element-binding protein activity, *Journal of neuroendocrinology* **23**(2): 136–142.

Spiga, F., Waite, E. J., Liu, Y., Kershaw, Y. M., Aguilera, G. & Lightman, S. L. (2011b). ACTH-Dependent Ultradian Rhythm of Corticosterone Secretion, *Endocrinology* **152**(4): 1448–1457.

Spiga, F., Walker, J. J., Terry, J. R. & Lightman, S. L. (2014). HPA axis-rhythms, *Comprehensive Physiology* .

Spiga, F., Zavala, E., Walker, J. J., Zhao, Z., Terry, J. R. & Lightman, S. L. (2017). Dynamic responses of the adrenal steroidogenic regulatory network, *Proceedings of the National Academy of Sciences* **114**(31): E6466–E6474.

Sriram, K., Rodriguez-Fernandez, M. & Doyle III, F. J. (2012). Modeling cortisol dynamics in the neuro-endocrine axis distinguishes normal, depression, and post-traumatic stress disorder (PTSD) in humans, *PLoS Computational Biology* **8**(2): e1002379.

Stanojević, A., Marković, V. M., Maćešić, S., Kolar-Anić, L. & Vukojević, V. (2018a). Kinetic modelling of testosterone-related differences in the hypothalamic–pituitary–adrenal axis response to stress, *Reaction Kinetics, Mechanisms and Catalysis* **123**(1): 17–30.

Stanojević, A., Marković, V. M., Čupić, v., Kolar-Anić, L. & Vukojević, V. (2018b). Advances in mathematical modelling of the hypothalamic–pituitary–adrenal (HPA) axis dynamics and the neuroendocrine response to stress, *Current Opinion in Chemical Engineering* **21**: 84–95.

Stanojević, A., Marković, V., Čupić, v., Vukojević, V. & Kolar-Anić, L. (2017). Modelling of the Hypothalamic-Pituitary-Adrenal Axis Perturbations by Externally Induced Cholesterol Pulses of Finite Duration and with Asymmetrically Distributed Concentration Profile, *Russian Journal of Physical Chemistry A* **91**(13): 2600–2607.

Stetler, C. & Miller, G. E. (2011). Depression and Hypothalamic-Pituitary-Adrenal Activation: A Quantitative Summary of Four Decades of Research, *Psychosomatic Medicine* **73**(2): 114–126.

Terry, J. R., Walker, J. J., Spiga, F. & Lightman, S. L. (2016). Dynamics of the HPA Axis: A Systems Modeling Approach, *Computational Neuroendocrinology* p. 252.

Thomas, M., Keramidas, M., Monchaux, E. & Feige, J.-J. (2004). Dual hormonal regulation of endocrine tissue mass and vasculature by adrenocorticotropin in the adrenal cortex, *Endocrinology* **145**(9): 4320–4329.

Tsang, A. H., Astiz, M., Friedrichs, M. & Oster, H. (2016). Endocrine regulation of circadian physiology, *Journal of endocrinology* **230**(1): R1–R11.

Tsigos, C. & Chrousos, G. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress, *Journal of psychosomatic research* **53**(4): 865–871.

Vanparys, C., Hectors, T. L. M., Blust, R. & De Coen, W. (2012). Mechanistic profiling of the cAMP-dependent steroidogenic pathway in the H295R endocrine disrupter screening system: New endpoints for toxicity testing, *Toxicology Letters* **208**(2): 174–184.

Čupić, v., Stanojević, A., Marković, V. M., Kolar-Anić, L., Terenius, L. & Vukojević, V. (2017). The HPA axis and ethanol: a synthesis of mathematical modelling and experimental observations, *Addiction biology* **22**(6): 1486–1500.

Veldhuis, J. D., Iranmanesh, A., Johnson, M. L. & Lizarralde, G. (1990). Amplitude, but not Frequency, Modulation of Adrenocorticotropin Secretory Bursts Gives Rise to the Nyctohemeral Rhythm of the Corticotropic Axis in Man*, *The Journal of Clinical Endocrinology & Metabolism* **71**(2): 452–463.

Veldhuis, J. D., Iranmanesh, A., Lizarralde, G. & Johnson, M. L. (1989). Amplitude modulation of a burstlike mode of cortisol secretion subserves the circadian glucocorticoid rhythm, *American Journal of Physiology-Endocrinology And Metabolism* **257**(1): E6–E14.

Vinther, F., Andersen, M. & Ottesen, J. T. (2011). The minimal model of the hypothalamic–pituitary–adrenal axis, *Journal of Mathematical Biology* **63**(4): 663–690.

Vos, T., Barber, R. M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., Charlson, F., Davis, A., Degenhardt, L., Dicker, D. et al. (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013, *The Lancet* **386**(9995): 743–800.

Waite, E. J., McKenna, M., Kershaw, Y., Walker, J. J., Cho, K., Piggins, H. D. & Lightman, S. L. (2012). Ultradian corticosterone secretion is maintained in the absence of circadian cues, *European Journal of Neuroscience* **36**(8): 3142–3150.

Walker, J. J., Spiga, F., Gupta, R., Zhao, Z., Lightman, S. L. & Terry, J. R. (2015). Rapid intra-adrenal feedback regulation of glucocorticoid synthesis, *Journal of The Royal Sociaty, Interface* **12**(102): 20140875.

Walker, J. J., Spiga, F., Waite, E., Zhao, Z., Kershaw, Y., Terry, J. R. & Lightman, S. L. (2012). The origin of glucocorticoid hormone oscillations, *PLoS-Biology* **10**(6): 1277.

Walker, J. J., Terry, J. R. & Lightman, S. L. (2010a). Origin of ultradian pulsatility in the hypothalamic–pituitary–adrenal axis, *Proceedings of the Royal Society B: Biological Sciences* **277**(1688): 1627–1633.

Walker, J., Terry, J., Tsaneva-Atanasova, K., Armstrong, S., McArdle, C. & Lightman, S. (2010b). Encoding and decoding mechanisms of pulsatile hormone secretion, *Journal of neuroendocrinology* **22**(12): 1226–1238.

Wangorsch, G., Butt, E., Mark, R., Hubertus, K., Geiger, J., Dandekar, T. & Dittrich, M. (2011). Time-resolved in silico modeling of fine-tuned cAMP signaling in platelets: feedback loops, titrated phosphorylations and pharmacological modulation, *BMC systems biology* **5**(1): 178.

Wesseling, H., Guest, P. C., Lago, S. G. & Bahn, S. (2014). Technological advances for deciphering the complexity of psychiatric disorders: merging proteomics with cell biology, *International Journal of Neuropsychopharmacology* **17**: 1327–1341.

Willenberg, H., Bornstein, S. & Chrousos, C. (2010). Adrenal Insufficiency, *in* G. Fink (ed.), *Stress Science: Neuroendocrinology*, Academic Press Elsevier, pp. 195–199.

Williamson, T., Schwartz, J.-M., Kell, D. B. & Stateva, L. (2009). Deterministic mathematical models of the cAMP pathway in Saccharomyces cerevisiae, *BMC systems biology* **3**(1): 70.

Xing, Y., Parker, C. R., Edwards, M. & Rainey, W. E. (2010). ACTH is a potent regulator of gene expression in human adrenal cells, *Journal of Molecular Endocrinology* **45**(1): 59–68.

Yates, F. E. & Urquhart, J. (1962). Control of plasma concentrations of adrenocortical hormones, *Physiological Reviews* **42**(3): 359–443.

Yi-Wei, L., Zhi-Hong, H., Peng, J.-H. & Liu, B.-Z. (1999). A dynamical model for the pulsatile secretion of the hypothalamo-pituitary-adrenal axis, *Mathematical and computer modelling* **29**(4): 103–110.

Young, E. A., Carlson, N. E. & Brown, M. B. (2001). Twenty-Four-Hour ACTH and Cortisol Pulsatility in Depressed Women, *Neuropsychopharmacology* **25**(2): 267–276.

Zak, S. (2012). *Stress estimation using an hypothalamic-pituitary-adrenal axis model*, Białystok: Printing House of Białystok University of Technology, pp. 283–294.

Zarzer, C. A., Puchinger, M. G., Köhler, G. & Kügler, P. (2013). Differentiation between genomic and non-genomic feedback controls yields an HPA axis model featuring Hypercortisolism as an irreversible bistable switch, *Theoretical Biology and Medical Modelling* **10**: 65.

Zhang, Q., Bhattacharya, S. & Andersen, E. (2013). Ultrasensitive response motifs: basic amplifiers in molecular signal networks, *Open Biol.* **3**(130031).