

# The Principle of Homeostasis in the Hypothalamus- Pituitary-Adrenal System: New Insight from Positive Feedback

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Feedback control, both negative and positive, is a fundamental feature of biological systems. Some of these systems strive to achieve a state of equilibrium or “homeostasis”. The major endocrine systems are regulated by negative feedback, a process believed to maintain hormonal levels within a relatively narrow range. Positive feedback is often thought to have a destabilizing effect. Here we present a “principle of homeostasis” which makes use of both positive and negative feedback loops. To test the hypothesis that this homeostatic concept is valid for the regulation of cortisol, we assessed experimental data in humans with different conditions (gender, obesity, endocrine disorders, medication) and analyzed these data by a novel computational approach. We showed that all obtained data sets were in agreement with the presented concept of homeostasis in the hypothalamus-pituitary-adrenal axis. According to this concept, a homeostatic system can stabilize itself with the help of a positive feedback loop. The brain mineralocorticoid and glucocorticoid receptors – with their known characteristics – fulfil the key functions in the homeostatic concept: binding cortisol with high and low affinities, acting in opposing manners, and mediating feedback effects on cortisol. This study supports the interaction between positive and negative feedback loops in the hypothalamus-pituitary-adrenal system and in this way sheds new light on the function of dual receptor regulation. Current knowledge suggests that this principle of homeostasis could also apply to other biological systems.

**Keywords:** homeostasis, mathematical modelling, positive feedback, mineralocorticoid receptor, glucocorticoid receptor, hypothalamus-pituitary-adrenal system

**Abbreviations:** ACTH, adrenocorticotropin; BDNF, brain-derived neurotrophic factor; CRH, corticotropin-releasing-hormone; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; MR, mineralocorticoid receptor

HORMONES, NEUROPEPTIDES, NEUROTRANSMITTERS, and growth factors help the organism to achieve a state of equilibrium, which is referred to as homeostasis (18). These messengers (e.g. glucocorticoids, neurotrophins) typically bind as ligands to at least two different kinds of receptors that display different affinities and in this way convey their stabilizing signals. The principle, however, that underlies such homeostatic processes and that may explain why there are two receptors, is a long-standing mystery. Therefore, we assessed experimental data and analyzed these by a novel computational approach, showing that the data were in agreement with a fundamental homeostatic principle in the hypothalamic-pituitary-adrenal (HPA) system. In addition, we provide evidence from the literature that this concept could apply to other biological systems.

Usually, equilibria are thought to be driven by negative feedback only, e.g. a deviated pendulum suffers a retrieving force to its stationary state. Biological systems, however, often display the typical components: a ligand, a low- and a high-affinity receptor. The presence of such components suggests a general “principle of homeostasis” which includes positive feedback in particular ranges of ligand concentrations. As a recent discovery, positive feedback has been shown to be a ubiquitous signal transduction motif that allows systems to convert graded inputs into decisive, all-or-none outputs (2; 13). Thus, focussing on positive feedback already has shed light on another class of processes, i.e. non-homeostatic “multistable” processes like cellular on-off switches. Here we elaborate a basic model of homeostasis and introduce positive feedback loops as key mechanisms of homeostasis (Box 1; Fig. 1a and b).

### **Box 1**

#### **The principle of biological homeostasis:**

A, B, and C are molecules: one ligand, two receptors.

*Rule 1:* The ligand A binds with high affinity to a receptor B, but with low affinity to a receptor C.

*Rule 2:* The two ligand-bound receptors act in opposing manners.

*Rule 3:* Ligand-bound B receptors increase\* while ligand-bound C receptors decrease† the concentration of A.

These interactions result in a homeostatic state of A.

\* Positive feedback; †negative feedback

Which components of the HPA system fulfil the functions of A, B, and C in the general rules (Box 1)? The brain-pituitary system via adrenocorticotropin (ACTH) stimulates the adrenal glands, increasing cortisol (Fig. 1c). In a feedback-loop, cortisol acts on the brain by binding as a ligand to cerebral glucocorticoid receptors: at low concentrations cortisol binds to the mineralocorticoid receptor (MR), and at higher concentrations cortisol binds to the glucocorticoid receptor (GR) (Fig. 1d). MR and GR descend from a gene duplication deep in the vertebrate lineage and function as dual cortisol sensors (14). Starting in the year 1968 with the milestone paper of Bruce McEwen (67), a large number of researchers have since managed to characterize the two brain receptor subtypes both biochemically and functionally. High densities of MR were found in the hippocampus, dentate gyrus, lateral septum, and amygdala, while abundant GR were detected in all brain regions (74).

It is known that the two corticosteroid receptors MR and GR operate in an opposing manner on several processes such as synaptic transmission (28), synaptic plasticity (4; 52; 53; 76; 77; 92) and cell survival (25). It is unknown, however, whether brain MR and GR also operate counteractively in the central feedback on the HPA system. Some scientist referred to pharmacological studies (3; 31; 35; 47; 73; 81; 89; 99) and concluded that MR and GR both exert inhibitory feedback in the HPA system. Ron de Kloet emphasized the “proactive” mode of MR and the “reactive” mode of GR, and that the balance between MR and GR is essential for cell homeostasis, mental performance, and health (28). Nevertheless, he did not bring up positive feedback in this context. Considering the opposing nature of MR and GR in other biological processes, we here assume that brain MR and GR make use of positive and negative feedback in the HPA-system, respectively. We therefore formulate the homeostatic rules with respect to the HPA system by replacing A, B, and C by cortisol, MR, and GR (Box 2).

**Box 2****The principle of homeostasis in the HPA system:**

*Rule 1:* Cortisol binds at low concentrations to the brain MR and only at high concentrations to the brain GR.

*Rule 2:* Activated brain MR and GR operate in an opposing manner.

*Rule 3:* Cortisol raises\* its own serum concentration via activated brain MR, while it reduces† it via activated brain GR.

These interactions result in a homeostatic state of cortisol.

\* Positive feedback; †negative feedback

In order to test the hypothesis that the "principle of homeostasis" applies to the regulation of cortisol, we applied a computational approach to experimental data (Fig. 2):

- We formulated the above mentioned three rules using two differential equations with known constants and up to five unknown parameters.
- We identified the unknown parameters for each individual by analyzing experimental data.
- With these identified parameters we solved the differential equations: the solution constitutes a predicted time-dependent concentration of ACTH and cortisol.
- We compared the theoretical prediction obtained with the experimental data and computed the prediction error.
- We tested the hypothesis (significant prediction error).

In order to test the hypothesis over a broad scope, we assessed experimental data under various physiological, pharmacological, and pathological conditions. We obtained data from healthy women, healthy men, and obese men during a corticotropin-releasing-hormone (CRH) challenge test (Fig. 3a and b); we also referred to data which we had already published earlier, obtained in healthy subjects during MR blockade (Fig. 3c) (96), as well as in patients with Addison's disease and in adrenalectomised patients with Cushing's disease during a cortisol-infusion challenge (Fig. 3d) (38).

## METHODS

### *Differential equations*

In order to mathematically analyze experimental data sets, we established a system involving two differential equations. The rationale for the choice of terms in the equations is based on the following aspects: Basically, the equations represent the three rules (Box 2). We used a limited number of extensions that take into account specific physiological features of the HPA system, i.e. ACTH as an intermediary signal posed in the brain-to-adrenal pathway, a clearance of hormones from blood, and a peripheral feedback at the adrenal level. As commented in the discussion, we deliberately abstained from adding extra compartments (regions of the brain) or extra regulatory processes (e.g. heterodimerization). For the denotations used in the equations see Table 1:

$$\boxed{\frac{dACTH(t)}{dt} = c_1 \cdot ACTH(t) + \frac{MR_{E\max}^{brain} \cdot CORT(t - \tau_1)}{CORT(t - \tau_1) + r \cdot MR_{EC50}} - \frac{GR_{E\max}^{brain} \cdot CORT(t - \tau_1)}{CORT(t - \tau_1) + r \cdot GR_{EC50}} + CRH_E^{exog} \cdot e_1(t)} \quad (1)}$$

The term  $\frac{dACTH(t)}{dt}$  denotes the derivative of plasma ACTH concentrations over a time  $t$ . This change of ACTH in time depends: firstly on ACTH and its 1<sup>st</sup>-order clearance  $c_1$  from plasma (see Table 1), secondly on a positive MR-mediated effect, which is a function of  $CORT(t - \tau_1)$  ( $\tau_1$  indicates a delay time) and the pharmacokinetic parameters  $MR_{E\max}^{brain}$ ,  $MR_{EC50}$  and  $r$ , thirdly on a negative central GR-effect, which is also a function of  $CORT(t - \tau_1)$  and the respective pharmacokinetic parameters  $GR_{E\max}^{brain}$ ,  $GR_{EC50}$ , and  $r$ , and finally on an external disturbance  $e_1(t)$  (which is a typical gamma-distribution (21)) acting on ACTH, e.g. an injection of CRH (with the parameter  $CRH_E^{exog}$ ).

$$\frac{dCORT(t)}{dt} = c_2 \cdot CORT(t) + ACTH_E^{adrenal} \cdot ACTH(t - \tau_2) - GR_E^{adrenal} \cdot CORT(t - \tau_1) + s \cdot e_2(t) \quad (2)$$

The term  $\frac{dCORT(t)}{dt}$  denotes the derivative of serum cortisol concentrations over time  $t$ . This change in cortisol over time depends: firstly on cortisol and its 1<sup>st</sup>-order clearance  $c_2$  from plasma, secondly on a positive ACTH-effect, which is a function of  $ACTH(t - \tau_2)$  ( $\tau_2$  indicates a delay time) and the parameter  $ACTH_E^{adrenal}$ , thirdly on a negative peripheral GR-effect, which is a function of  $CORT(t - \tau_1)$  and the parameter  $GR_E^{adrenal}$ , and finally on an external disturbance  $e_2(t)$  acting on serum cortisol, e.g. an infusion of cortisol (with the pharmacokinetic parameter  $s$ ).

Equation (1) and (2) are “delay differential equations” (9). In these equations, ACTH and cortisol function as “integral controllers” (34). In the forward pathway, there is first a delay time  $\tau_2$  (1.2 minutes) until ACTH has an effect on the *derivative of serum cortisol concentration* over time  $\frac{dCORT(t)}{dt}$ . Second, the full effect of ACTH on *serum cortisol concentration* per se is carried out time-lagged by this integral controller. This delayed effect individually arises from the constants and identified parameters in the differential equations. In order to estimate the total time lag in the forward pathway, we assessed the peak-to-peak phase shift between the ACTH and cortisol curve and found the lag to be 25.0 minutes (figure 2d). In the feedback pathway, there are similar time lags: a delay time  $\tau_1$  (2.0 minutes) until cortisol influences the *derivative of plasma ACTH concentrations*  $\frac{dACTH(t)}{dt}$ , and another time lag until cortisol measurably affects *plasma ACTH concentrations* per se. To estimate the total time lag in the brain corticosteroid feedback, we assessed the peak-to-nadir phase shift between the cortisol and ACTH curve and found the lag to be 38.0 minutes (supplementary figure S3). The differential equations were solved numerically using MATLAB 7.0.



### *Data collection*

We performed CRH challenge-tests in 10 normal men, 10 normal women, and 7 obese men. Exclusion criteria were chronic or acute physical and mental illness, alcohol or drug abuse, smoking, competitive sports, exceptional physical or psychological stress, and current medication of any kind. Group characteristics are listed in supplementary Table 1. All data collection procedures were reviewed and approved by the local ethics committee, and all subjects provided written informed consent.

The participants were instructed to abstain from alcohol, not to perform any kind of exhausting physical activity, and to go to bed no later than 11:00 p.m. on the day preceding the study. On the days of experiments, subjects arrived at noontime in the medical research unit and received a standardized meal. A cannula was inserted into an antecubital vein at 12:00 p.m. Afterwards, each participant remained fasted except for the free intake of water. Three baseline blood samples for determining plasma ACTH and serum cortisol were collected from 3:30 p.m. until 4:00 p.m. After the baseline period, CRH (CRH Ferring, Ferring Arzneimittel, Kiel, Germany) was intravenously injected at 4:00 p.m. at a dose of 1 $\mu$ g/kg adapted to body mass. Blood samples were subsequently drawn at short intervals of 5 minutes until 5:30 p.m., and at intervals of 15 minutes until the end of the test at 8:00 p.m.

All blood samples were immediately centrifuged and the supernatants stored at minus 24°C until assaying. Cortisol serum concentrations were determined by electrochemiluminescence immunoassay (ECLIA) in conjunction with the Elecsys 2010 (Roche Molecular Biochemical Diagnostics, Mannheim, Germany; intraassay CV < 1.3 %; interassay CV < 1.5%) and plasma ACTH concentrations were measured by an immunoluminometric two-step assay (Lumitest ACTH, Brahms Diagnostics, Berlin, Germany; intraassay CV < 6.0%; interassay CV < 8.0%). Corticosteroid-binding globulin (CBG) was measured by a highly sensitive RIA (IBL Immuno-Biological Laboratories, Hamburg, Germany; intraassay CV < 3.8% and interassay CV < 5.0%).

The experimental methods we had used in the study on MR-blockade and the study on patients with patients with Addison's and Cushing's disease are described in the supplementary methods.

### *Parameter identification*

The parameter identification method is a mathematical procedure for obtaining optimal parameters from the comparison of experimental data and a system of differential equations. The parameter identification procedure repeats three steps until an optimal parameter set has been found. We begin with a set of  $n$  parameters on an  $n$ -dimensional grid:

In the first step of the algorithm, approximate concentration profiles ACTH(t) and CORT(t) are created. For this purpose conventional numeric “delay differential-equation-solvers” are employed (9; 46). In a second step the algorithm compares the differences between the obtained concentration profiles with the actual experimental ACTH and cortisol data from a test subject. This difference provides a measure for the prediction error. The difference is calculated using the root-mean-square procedure. The third step of the algorithm modifies the parameter values so that the prediction error decreases. Here we employed what we refer to as direct exploratory methods of "numeric optimization" (39).

These three steps are repeated until the difference can no longer be reduced. Then we carry out this procedure for all points of the  $n$ -dimensional grid. The outcome of the parameter identification method is the parameter set that provides the lowest prediction error. A prediction error  $<0.05$  is considered as high agreement between experimental data and theoretic prediction. In the sense of the used error measure, an error  $<0.20$  can be regarded as satisfactory, a larger error as unacceptable agreement. We apply this procedure for every test individual separately. The parameter identification was done by MATLAB 7.0.

### *Statistical analysis*

In order to compare data among different groups of subjects, we used the following statistical tools: Dependent and independent student's T-tests were used for comparing characteristics and identified parameters between subject groups. One sample T-tests were employed to test whether the mean of

one variable differs from a constant. ANOVA was performed for comparing parameters among three groups. P-values < 0.05 were considered as significant. Numbers that follow  $\pm$  signs are standard errors (s.e.m.). The analysis was done with SPSS 12.0.

## RESULTS

### *Experiments in healthy humans*

When CRH was administered to healthy subjects (characteristics in supplementary Table 1), ACTH profiles in men and women were similar, although women had slightly higher cortisol concentrations in the recovery phase (Fig. 3a left). The ACTH curves reflect the combined effects of CRH and cortisol on the brain-pituitary system. The cortisol profiles reflect the combined effects of ACTH and cortisol on the adrenal gland. In women, cortisol concentrations were higher than in men, suggesting that the brain corticosteroid feedback was different between the sexes. When the parameter identification procedure was applied to these data, the parameter  $MR_{E\max}^{brain}$  was found to be higher ( $2.3\pm 0.1$  vs.  $2.0\pm 0.1$  fmol l<sup>-1</sup>s<sup>-1</sup>; P<0.05) and  $GR_{E\max}^{brain}$  lower ( $2.8\pm 0.1$  vs.  $3.2\pm 0.1$  fmol l<sup>-1</sup>s<sup>-1</sup>; P<0.05) in healthy women than it was in healthy men (Table 2). In order to interpret these results easier, we calculated the ratio between  $MR_{E\max}^{brain}$  and  $GR_{E\max}^{brain}$  as an estimate of the “MR : GR balance”: the balance was larger in healthy women (1 : 1.22) as compared to healthy men (1 : 1.60). Note that  $MR_{E\max}^{brain}$  quantifies a positive and  $GR_{E\max}^{brain}$  a negative feedback (see equation 1).

When the identified parameters were used to reconstruct the ACTH and cortisol profiles, these theoretic predictions were similar to the observed data (prediction error < 0.05; Fig. 3a right; Table 2). When analyzing how the variability of estimated parameters affected the predictions, we found that changes in the central parameters  $MR_{E\max}^{brain}$ ,  $GR_{E\max}^{brain}$ ,  $CRH_E^{exog}$  sensitively influenced the predicted ACTH and cortisol concentrations (Figure 4a,b), while changes in the peripheral parameters  $ACTH_E^{adrenal}$ , and  $GR_E^{adrenal}$  mainly affected predicted serum cortisol (Figure 4c,d,e). Systematic

variation of  $MR_{E_{\max}}^{brain}$  and  $GR_{E_{\max}}^{brain}$  revealed a high robustness of the prediction since there is only one parameter set representing the best model fit (Fig. 5).

When the identified parameters were used to analyse the dose-response function for the brain corticosteroid feedback, women had a weaker inhibitory GR-effect than men (insert in Fig. 3a). In both sexes, the maximum MR-mediated positive feedback effects were evident at a low serum cortisol concentration of 50 nmol/l (typical value at bedtime), at 300 nmol/l (typical value at wake-up time) the counteracting MR- and GR effects were of equal strength, while at 600 nmol/l (typical value during a stressful event) the negative feedback effect of GR predominated. The theoretically determined weaker central feedback inhibition in women reflects the observation that they had higher cortisol compared to men. In this experiment, we found that a model of the HPA system that makes use of positive and negative feedback loops is consistent with the data set.

### *Pharmacological blockade in healthy humans*

We analysed a data set derived from experiments using the MR antagonist canrenoate (96). When canrenoate was administered intravenously, serum cortisol concentrations were elevated as compared to placebo (Fig. 3c left). Similar results have been obtained in several other studies (3; 31; 35; 47; 99). Looking at the adrenal-to-brain feedback pathway, the fact that higher cortisol concentrations were observed suggests a different MR-GR feedback in the brain. Since canrenoate competes as a competitive antagonist with cortisol for binding to MR and (non-selectively to) GR, we identified the following parameters under canrenoate treatment: the affinities (described by  $MR_{EC50}$  and  $GR_{EC50}$ ) but not the efficacies ( $MR_{E_{\max}}^{brain}$  and  $GR_{E_{\max}}^{brain}$ ) (Table 1). When the parameter identification procedure was applied to the experimental data, canrenoate was found to increase the  $MR_{EC50}$  by around 7-fold ( $P < 0.005$ ), but the  $GR_{EC50}$  only by around 5-fold (Table 2).

When the identified parameters were used to reconstruct the ACTH and cortisol profiles, these theoretic predictions were similar to the observed data (prediction error  $< 0.05$ ; Fig. 3c right, Table 2).

When the identified parameters were used to analyze the brain corticosteroid feedback, the pharmacological MR blockade was plausibly found to shift the dose response curve to the right (Fig. 3c insert). Thus, we used the computational approach in the pharmacologically blocked HPA system and found results (i.e. increased  $EC_{50}$  values for the corticoid receptors) that are in agreement with what is known about the characteristics of competitive blockade.

In order to interpret the influences of canrenoate, we refer to the three rules (Box 2). Rule 1: In the presence of competitive blockade (which affects MR and non-selectively also GR), cortisol binds only at *medium* concentrations to the brain MR and at *very high* concentrations to the brain GR (Fig. 6; upper panel). Rule 2: Activated brain MR and GR operate in opposing manners. Rule 3: As long as brain MR are predominantly activated, a stimulatory effect prevails that acts to increase ACTH and consequently cortisol concentrations; as long as brain GR are predominantly activated, there is an inhibitory effect working that acts to decrease ACTH and so cortisol concentrations. In this way, the cortisol concentrations strive to a point of equilibrium where the stimulatory and the inhibitory effects of the brain corticosteroid receptors are of the same magnitude (Fig. 6; lower panel). These interactions would result in a homeostatic hypercortisolemic state under competitive blockade with canrenoate. Based on this reasoning, we can interpret that a blockade of the corticosteroid receptors elevates the brain setpoint of the HPA system (Fig. 3c insert): when cortisol has to compete with canrenoate for binding at the MR, it can only exert its full (MR-mediated) *stimulatory drive* in the HPA system at higher cortisol concentrations.

Looking in turn at the brain-to-adrenal forward pathway, the ACTH time courses were similar, but the cortisol concentrations were higher under canrenoate. We found canrenoate administration to decrease  $GR_E^{adrenal}$ , i.e. efficacy of adrenal GR feedback. This finding is in line with the view of other researches suggesting that MR blockade affects the sensitivity of the adrenal gland to ACTH (99).

Genetic MR inactivation within the central nervous system represents another approach for testing our model of homeostasis. Central MR mutant mice show lower serum corticosteroid concentrations than their control littermates (10). We applied this published data in the parameter identification procedure and found a 20% (albeit not complete) reduction in the  $MR_{E\max}^{brain}$ . Merely, it

remained unclear why the functional loss of MR efficacy was incomplete. Basically, our model of the HPA system also applies to this data set in which the MR was genetically inactivated in the brain.

### *Experiments in patients with Addison's and Cushing's disease*

We resorted to a data set that we had already published on patients with primary adrenal insufficiency, i.e. Addison's disease, and on patients with central Cushing's disease, whereby the latter had had adrenalectomy (ADX) for therapeutic purposes (38). This experimental condition now made it possible to systematically investigate the brain corticosteroid feedback on ACTH almost over the entire range of cortisol concentrations, particularly focussing the low-concentrations range. When hydrocortisone was infused intravenously, the plasma ACTH in patients with Cushing's disease/ADX was found to steeply rise in the beginning and then to continuously fall (Fig. 3d left), indicating both positive and negative feedback. Recently, we performed a replication study using up-to-date laboratory methods, and confirmed the stimulatory effect on ACTH at low cortisol concentrations (supplementary figure S1). In patients with Addison's disease, low cortisol is the result of adrenal insufficiency. However, low cortisol, as it occurred after replacement therapy was withheld for 24 hours, may also lead to secondary alterations in the MR : GR balance as can be expected from experiments in adrenalectomized animals (69). We found that plasma ACTH continuously declined from the beginning of hydrocortisone infusion, thus indicating a dominant negative feedback effect of cortisol. Of note, in a replication study on patients with Addison's disease we were able to detect an initial dose-dependent rise in plasma ACTH (albeit less pronounced than in patients Cushing's disease)(37). A similar initial and transient rise in plasma ACTH has also been reported in normal humans, when hydrocortisone was infused during oral metyrapone treatment (97). With Cushing's disease, the central MR efficacy is large compared to the MR efficacy seen in patients with Addison's disease/ADX ( $23.4 \pm 6.3$  vs.  $0.3 \pm 0.2$  fmol  $l^{-1}s^{-1}$ ;  $P < 0.005$ ; Table 2). Moreover, in patients with Cushing's disease the "MR : GR balance" was markedly larger (1 : 1.09) than in normal men (1 : 1.60).

When the identified parameters were used to reconstruct the ACTH and cortisol profiles, the agreement between experimental data and theoretical prediction was satisfactory (prediction error  $<0.20$ ; Fig. 3d right, Table 2). When the identified parameters were used to analyze the brain corticosteroid feedback, central feedback effects over the entire cortisol range were found to be mainly inhibitory in patients with Addison's disease and stimulatory in patients with Cushing's disease (insert in Fig. 3d). Our model therefore adequately explains why the homeostatic level of cortisol is pathologically raised in Cushing's disease.

Since some scientists have referred to experiments (3; 31; 35; 47; 73; 81; 89; 99) and concluded that the MR exerted a tonic *inhibition* at low cortisol concentrations, we tested the alternative system of differential equations that described an *inhibitory* feedback effect of MR - in addition to the inhibitory feedback of GR. When this alternative system was used to reconstruct the ACTH and cortisol profiles in Cushing's disease/ADX, the observed peak ACTH concentration was found to deviate unacceptably from the prediction (prediction error  $>0.60$ ; supplementary figure S2). Thus, our model had a wider scope of validity with the data sets examined here than a concept based on a MR tonic inhibition (Table 3).

### *Experiments in obese humans*

Is the brain corticosteroid-feedback altered in obesity? When corticotropin-releasing-hormone (CRH) was administered to obese male subjects (characteristics in supplementary Table 1), ACTH responses were 2-fold higher and the cortisol responses were slightly higher than in normal weight men (Fig. 3b left). The obese men revealed a higher MR efficacy ( $2.4 \pm 0.1$  vs.  $2.1 \pm 0.1$  fmol  $l^{-1}s^{-1}$ ;  $P<0.01$ ) and a lower GR efficacy ( $2.8 \pm 0.2$  vs.  $3.2 \pm 0.1$  fmol  $l^{-1}s^{-1}$ ;  $P<0.01$ ) compared to the normal controls. In the obese men the "MR : GR balance" was larger (1 : 1.17) as compared to healthy men (1 : 1.60). The peripheral adrenal parameters were of the same magnitude in the obese and control individuals.

The theoretical prediction shows a high agreement with the experimental observations (prediction error  $< 0.05$ ; Fig. 3b right, Table 2). In obese men central feedback inhibition was

markedly reduced, especially at the high cortisol concentrations (insert in Fig. 3b). The computational approach allows locating specific derangements in the HPA system in all situations that are characterized by disturbed ACTH/cortisol secretion.

One homeostatic system often conveys an input signal to another related homeostatic system in the organism. For example, energy homeostasis is closely linked to the HPA system (78). Our analysis shows that with obese men the function of the GR is decreased and that as a result the HPA system is less well-braked, i.e. inhibited. The two stress systems, i.e. the sympathetic nervous system and the HPA system, increase the energy supply of the brain. Recent studies have shown that in adrenalectomized animals the components of the HPA system are highly stimulated, and that an exogenous application of glucose can almost completely return the hyperfunctioning HPA system to normal activity (8; 56; 57). Glucose can therefore substitute for the GR-mediated braking function of the HPA system (27). In a challenged organism, the alteration of one homeostatic system (e.g. brain energy metabolism) may strongly affect the homeostasis of another system (e.g. the HPA). Thus, input signals from related homeostatic systems can be influential, but - as we have demonstrated in this paper - they are not required to generate homeostasis.

## DISCUSSION

The question addressed by this study was whether the presented “principle of homeostasis” (Box 2) is in agreement with data on the HPA system. The main finding of the study is that this model applies even for extreme conditions in the HPA system, like Addison’s and Cushing’s disease.

The discussion about homeostasis is closely linked to the question how setpoints are generated in biological systems. In this paper, we showed that the HPA system can be described by a “pn-system”, i.e. one that is driven by (p) positive MR-mediated and (n) negative GR-mediated feedback loops. We had also investigated an alternative “nn-system” that is driven by negative MR and negative GR feedback loops. The pn-systems are able to stabilize themselves with the help of a positive



feedback loop in order to achieve equilibrium at a given setpoint. In contrast, the nn-systems do require a positive signal input into their regulatory loop; otherwise they strive to equilibrium at zero values. Basically, generation of a setpoint requires a positive signal that originates either from the inside (positive feedback loop) or from the outside of a closed-loop system (external reference input). In humans, the HPA system is the major systemic stress system that normally stabilizes at a setpoint about midnight (ACTH 2 pmol/l, cortisol 50 nmol/l). It is important to maintain the low serum concentrations of cortisol during the slow wave sleep periods in the early night, because cortisol then predominantly binds to hippocampal MR, which is necessary for memory consolidation and underlying synaptic plasticity in these neuronal networks (95). If the HPA system were an nn-system, it would require a steady positive signal input from outside the system (e.g. from the isocortex) to maintain midnight concentrations of ACTH and cortisol. If such a second system also were an nn-system, a third system would be necessary, and so on. Adding further nn-systems does not solve the problem of how homeostatic setpoints are created. From this theoretical point of view, homeostasis is possible only when at least one pn-system exists in the organism. Our data support the view that the HPA system itself is a required pn-system that allows achieving homeostasis at a given setpoint.

The problem that one experimental data set can be in agreement with two distinct theoretical approaches has often prompted a scientific debate. This issue is often referred to as “inverse problem“ (87). Some researchers consider the HPA systems to be characterized by an nn-system, while we present the pn-system here. During the past decades, extensive empirical data accumulated that led researchers to assume an nn-system which includes MR-mediated tonic inhibition, i.e. negative feedback. The majority of these data has been obtained from experiments using more or less specific pharmacological MR blockade in humans (3; 31; 35; 47; 99) and animals (73; 81; 89). As a representative of such studies we selected one from our own group for a more detailed mathematical investigation. In the original publication on the collected data it was at first concluded that “MR blockade leads to a tonically increased cortisol secretion”, suggesting an nn-system regulation of cortisol. When the novel computational approach was used to reinvestigate the data, an nn-model was in fact found to fit with these experimental data, but in contrast to some expectations the pn-model which included MR-mediated positive feedback also did (Table 3). Such an ambiguity typically arises

from an “inverse problem”. At this point, we emphasize that “MR-mediated tonic inhibition” is not necessarily the only explanation in this data set, but “MR-mediated positive feedback” is a plausible possibility. The above mentioned data sets on canrenoate cannot help us to distinguish which of the alternative models is more accurate in making predictions. Neither can the data sets on CRH challenge tests in healthy or obese humans help to make this distinction (Table 3). To solve an “inverse problem” it might be helpful to investigate additional data sets. We therefore selected a data set that particularly focussed regulatory behavior of the HPA system in the low cortisol concentration range in adrenalectomized patients. The comparison between the two alternative models shows that the pn-model fits with this data set, while an nn-model fails. Thus, we conclude that our model that uses MR-mediated positive feedback components has a wider scope of validity as compared to a model with pure negative feedback (Table 3).

What are the underlying molecular mechanisms that could mediate positive and negative feedback in the HPA system? First, the brain mineralocorticoid and glucocorticoid receptors fulfil the key functions in the homeostatic rules (Box 2): binding cortisol with high and low affinities, acting in opposing manners, and mediating feedback effects on cortisol. The fact that the pair of MR and GR fulfils these required criteria supports MR and GR as one mechanism underlying positive and negative feedback in the HPA system, but we cannot rule out that other molecules are involved. Second, the fact that MR and GR fulfil the criteria (Box 2) does not allow inferring whether genomic and nongenomic molecular mechanisms underlie the HPA-system’s feedback. By genomic mechanisms, the corticosteroid receptors modify transcription of responsive genes, either through DNA binding or through protein–protein interactions with other transcription factors (7). Glucocorticoids influence de novo protein synthesis within a short, intermediate, and prolonged timeframe (41; 42). By nongenomic mechanisms, the glucocorticoids can exert fast effects on excitation-secretion coupling (48). Furthermore, MR were found indispensable for rapid nongenomic modulation of hippocampal glutamate transmission by corticosteroids (51). Corticosteroids also exert fast-onset inhibitory effects via membrane receptors (32; 93). There is the notion that the fast glucocorticoid actions which are mediated by membrane receptors are an ancient type of sterol/steroid-mediated effect, and that these may be the primordial glucocorticoid receptors (26). It is conceivable that brain corticosteroid

feedback consists of a *combination* of genomic and nongenomic MR and GR actions: while nongenomic MR and GR mechanisms modulate the early response, the late response is mainly influenced by genomic mechanisms — with the latter being more important in the homeostatic phase. Of note, the rules underlying our model (Box 2) do not include auxiliary conditions about time. Most relevant for our considerations are the balances assessed in the homeostatic state of equilibrium. Third, MR and GR display heterogenic distribution in the brain and they may be located on excitatory or inhibitory neurons. Thus, a diversity of specific neuronal pathways and molecular mechanisms is conceivable that underlies the brain corticosteroid feedback. In all, based on the current evidence we regard MR and GR as the major mechanisms that fulfil the functions in the homeostatic rules and underlie brain corticosteroid feedback.

Is our model of the HPA system oversimplified? One point of view might be that important control mechanisms escape mention here so that the true complexity of the HPA system is not delved into. We have in fact refrained from including a large number of mechanisms that might be relevant in the regulation, e. g. CBG, autoregulation of MR and GR, and heterodimerization. First, CBG binds cortisol with high affinity but low capacity, so that the extent to which it binds cortisol saturates with mid-level cortisol concentrations. In this way, CBG acts like a functional buffer. When we tentatively included CBG in the model, we found that CBG had minor influence on the binding kinetics of MR and GR (particularly with rising or falling cortisol), but the crucial characteristics of two intersecting receptor-kinetic curves (figure 1d, upper panel) were preserved. Second, autoregulation of MR and GR describes a short loop feedback process, by which both cerebral receptors influence their own gene expression. This process affects the efficacies of MR and GR. When we included autoregulation in the model, we observed that the system became more stable, but the essential features of two intersecting receptor-kinetic curves (figure 1d, upper panel) remained conserved. Third, heterodimerization of MR and GR mainly affects how the separate receptor effects are integrated (61; 72; 88). When heterodimerization was included in a preliminary model (22), the combined feedback effect of both receptors effects (figure 1d, lower panel) displayed an enhanced contrast between the positive and negative feedback effects, but again the critical aspects of the regulation remained unaffected. In all, there are several regulatory processes in the HPA system involved that might of course modify its

regulatory behavior. Since using the presented minimal model of the HPA system led to satisfactory approximation in all our data sets, we abstained from including extra biological mechanisms into the final model. We are aware that the restrictions proposed here might be the subject of debate, but we feel that the specificities of the model presented are less important than the general basic conception proposed here for homeostasis.

Our findings on the HPA-system regulation lead us to speculate that the outlined principle also applies to other biological systems. Almost all known ligands bind to at least two receptors (a selection is shown in Table 4). In most of these dual receptor systems the first and second rule (Box 2) has been demonstrated (17). In many dual receptor systems also the third rule has been established: these systems are the homeostatic ones. As a first example, the brain-derived neurotrophic factor (BDNF) binds to high and low-affinity receptors and exerts counteracting effects on the survival and apoptosis of cells (Table 4). BDNF acts as an autocrine survival factor in neurons supporting its own release at low BDNF concentrations and inhibiting it at high concentrations. Thus, there is experimental evidence that all three homeostatic rules also apply to BDNF. As a second example, insulin has been shown to act by a dual receptor systems with evidence for the existence of all three homeostatic rules (Table 4). The pancreatic  $\beta$ -cell secretes insulin molecules, which in an autocrine manner bind to high- or low-affinity insulin receptors located on the  $\beta$ -cell surface. Insulin acts on its own secretion either stimulatory at low insulin concentrations or inhibitory at high concentrations. In this way, insulin can stabilize its basal pancreatic secretion in a self-perpetuating process. As a third example, the three rules may also apply to the regulation of neuronal ATP (79). High and low-affinity ATP-sensitive potassium channels adjust the excitation of neurons, the neuronal energy provision, and neuronal energy consumption. Here, dual ATP-sensitive receptor systems exert a homeostatic influence on cellular energy metabolism. In summary, we can therefore find evidence for examples from most receptor classes which suggest that many biological systems have evolved positive feedback loops to create homeostasis.

In this paper, we did not intend to show that the outlined model of the HPA-system regulation represents reality or that any other possibilities are excluded. We did demonstrate, however, that this model could fit all our experimental data in a sensitive and robust manner and that among alternative

models, the “principle of homeostasis” was in agreement with the largest data set — representing the widest scope of validity.

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## Figure legends

**Fig. 1.** Schematic representation of the “principle of homeostasis”. **(a)** A biological homeostatic system stabilizes itself by producing ligands A, which exert both positive feedback via high-affinity receptors B, and negative feedback via low-affinity receptors C. **(b)** The efficacy of the B (green) and C (orange) receptors depend on the concentration of A; the combined feedback effect of both receptors (black) also depends on the concentration of A. The feedback effect is stimulatory at low concentrations of A, but inhibitory at high concentrations of A. **(c)** Flow chart of the HPA system. Five parameters are indicated (magenta), which describe the efficacies of action at the respective sites. The brain-pituitary system releases ACTH, which stimulates the adrenal glands to secrete cortisol. In a long feedback loop, cortisol exerts positive feedback via MR and negative via GR on the brain-pituitary system. In a short feedback loop, glucocorticoids bind to GR in the adrenal cortex and in so doing suppress adrenal glucocorticoid release (63; 75). External CRH administration stimulates the brain-pituitary system to release ACTH. **(d)** Brain MR (blue) and GR (red) efficacies depend on cortisol concentrations. We regard the brain MR as the major representative member of the nuclear receptor superfamily which binds cortisol with high affinity. For the sake of simplicity, we attribute the effects of all such high-affinity cortisol receptors to MR. Accordingly, we consider the brain GR as a major representative member binding cortisol with low affinity. The maximal efficacies  $MR_{E_{\max}}^{\text{brain}}$  and  $GR_{E_{\max}}^{\text{brain}}$  are indicated. These efficacies represent the integrated actions of all MRs and GRs localized in different brain regions (e.g. amygdala, hippocampus, hypothalamus, and pituitary). Thus, computing such global MR and GR efficacies which describe the actions on plasma ACTH does not allow localizing the specific site of action within the brain-pituitary system. We designate the ratio of global  $MR_{E_{\max}}^{\text{brain}}$  and  $GR_{E_{\max}}^{\text{brain}}$  as “MR : GR balance”.  $MR_{EC50}$  denotes the molar concentration of cortisol, which produces 50% of the maximum possible MR-mediated response, i.e. the change of ACTH in time.  $GR_{EC50}$  is the respective cortisol concentration for GR-mediated changes of ACTH in time. The summation curve (black) shows the combined feedback effect of both receptors on the

brain-pituitary system, which also depends on cortisol concentrations. The brain setpoint of the HPA system (the zero indicated by the black arrow) is characterized by equilibrium between positive and negative feedback.

**Fig. 2.** Flow-chart illustrating the working steps used in this paper. **(a)** We formulated the “principle of homeostasis” using two differential equations with known constants and up to five unknown parameters (magenta). **(b)** We obtained experimental data, here exemplified by data from a CRH challenge test in a healthy man (case C.H.). **(c)** We identified the unknown parameters (magenta) for each individual (e.g. Case C.H.) by analyzing his/her experimental data. **(d)** With these identified parameters we solved the differential equations: the solution constitutes a predicted time-dependent concentration of ACTH and cortisol (blue). We compared the theoretical prediction obtained (blue line) with the experimental data (black dots), computed the prediction error (violet), and tested the hypothesis (significant prediction error).

**Fig. 3.** Experimental data and theoretical predictions of ACTH and cortisol concentrations during various challenge tests. In all conditions, the experimental data (left panels) are similar to the theoretical predictions (right panels). **(a)** CRH challenge in healthy women (blue) and men (black). *Insert in right panel:* Feedback-analysis showing the dose-response curves for these women and men: The change of ACTH in time [ $\text{fmol l}^{-1} \text{s}^{-1}$ ] depends on serum cortisol [ $\text{nmol l}^{-1}$ ]. These functions are computed using the differential equations on the basis of the experimental data. The shaded area indicates the range of cortisol concentrations that is covered by experimental observations. The curves depicted outside this range are extrapolated with the help of data-based parameter identification and the differential equations. **(b)** CRH challenge in obese (red) and healthy men (black). **(c)** Treatment with canrenoate (turquoise) or placebo (black) in healthy men. *Insert in right panel:* The feedback-analysis illustrates that the biphasic dose-response curve (cortisol  $\rightarrow$  change in ACTH in time) is shifted to the right. With the right shift of the biphasic curve, the brain setpoint of the HPA system (black dotted arrow) is altered — and homeostasis is achieved only at higher cortisol concentrations. **(d)** Continuous hydrocortisone infusion (50 mg/h, 0-120min) in patients with Addison’s disease

(violet) or in patients with Cushing's disease/ADX (orange). *Insert in right panel:* Note the broad range of cortisol concentrations that is covered by experimental observations (shaded area). Error bars indicate standard errors (s.e.m.).

**Fig. 4.** Effect of parameter-modifications on the prediction of ACTH and cortisol concentrations in healthy men. For validation of a model it is mandatory to perform a sensitivity analysis (33; 34). Results of our sensitivity analysis are illustrated as follows: **(a)** Stepwise increasing the values of the central parameter  $MR_{E\max}^{brain}$  by 125%, 150%, and 175%, leads to marked elevations of predicted ACTH and cortisol profiles. Thus, the predictions are very sensitive to changes in this parameter. **(b)** Using stepwise increased values of the central parameter  $GR_{E\max}^{brain}$ , the predicted profiles of ACTH and cortisol are clearly lowered, indicating sensitivity to changes in this parameter. **(c)** When the parameter  $CRH_E^{exog}$  is altered, we also find the predicted ACTH and cortisol profiles sensitively influenced. **(d, e)** Modifying the peripheral parameters  $ACTH_E^{adrenal}$  and  $GR_E^{adrenal}$  mainly affects the predicted cortisol profiles in a sensitive manner.

**Fig. 5.** Effect of 2-parameter-modifications on the precision of prediction in healthy men. Precision is determined by the deviation between two predictions: first, the optimal prediction of data obtained in healthy men, second, the prediction calculated with the modified parameters  $MR_{E\max}^{brain}$  and  $GR_{E\max}^{brain}$ ; high precision (red), low precision (blue). If the central parameters  $MR_{E\max}^{brain}$  and  $GR_{E\max}^{brain}$  are slightly modified, predictions markedly deviate from the optimal predictions in healthy men. Note, that there is only one parameter set of  $MR_{E\max}^{brain}$  and  $GR_{E\max}^{brain}$  fulfilling the best model fit (dark red).

**Fig. 6.** *Upper panel:* Competitive blockade of the brain corticosteroid receptors and the right-shift of dose-response curves (arrows). Canrenoate predominantly affects the MR dose-response curve (blue) but also the GR dose-response curve in a non-selective manner (red). In this way, the intersection (crossed circle) of the MR and GR receptor-kinetic curves is shifted to higher cortisol concentrations. *Lower panel:* The summation curve of stimulatory MR and inhibitory GR feedback effects is also



shifted to the right under competitive blockade with canrenoate. Note that canrenoate shifts the MR dose-response curve closer to the GR dose-response curve, and in this way reduces the maximum of the biphasic feedback curve. The zero of the summation curve constitutes the brain setpoint of the HPA system (dotted arrow). At this setpoint, the brain's stimulatory and inhibitory effects on cortisol secretion are of the same magnitude – and the HPA system stabilizes in a homeostatic state. canrenoate can be viewed to shift the brain setpoint of the HPA system to higher cortisol concentrations.

Table 1. Functions, parameters, and constants used in the differential equations

	Identifier	Description	Value	Unit	Ref.
<b>Functions in time</b>	$ACTH(t)$	Concentration of plasma ACTH	Variable (dependent on time)	$\text{pmol l}^{-1}$	
	$CORT(t)$	Concentration of serum cortisol	Variable (dependent on time)	$\text{nmol l}^{-1}$	
<b>Parameters in subject</b>	$MR_{E \max}^{\text{brain} \ddagger}$	Maximal efficacy of cortisol-bound brain/pituitary MR on ACTH	* To be determined individually in respective subject	$\text{fmol l}^{-1} \text{s}^{-1}$	
	$GR_{E \max}^{\text{brain} \ddagger}$	Maximal efficacy of cortisol-bound brain/pituitary GR on ACTH	* To be determined individually in respective subject	$\text{fmol l}^{-1} \text{s}^{-1}$	
	$CRH_E^{\text{exog}}$	§ Efficacy of exogenous CRH on ACTH	* To be determined individually in respective subject	$\text{fmol l}^{-1} \text{s}^{-1}$	
	$ACTH_E^{\text{adrenal}}$	§ Efficacy of pituitary ACTH on cortisol	* To be determined individually in respective subject	$\text{pmol l}^{-1} \text{s}^{-1}$	
	$GR_E^{\text{adrenal}}$	§ Efficacy of cortisol-bound adrenal GR on cortisol	* To be determined individually in respective subject	$\text{pmol l}^{-1} \text{s}^{-1}$	
<b>Constants</b>	$c_1$	Clearance rate of plasma ACTH	$\frac{\log(2)}{60 \cdot 20}$	$\text{s}^{-1}$	(45; 91)
	$c_2$	Clearance rate of serum cortisol	$\frac{\log(2)}{60 \cdot 102}$	$\text{s}^{-1}$	(30)
	$\tau_1$	¶ Delay time in the feedback pathway	120	s	(26)
	$\tau_2$	¶ Delay time in the forward pathway	72	s	(26)
	$MR_{EC50}$	Concentration of free cortisol that	† 0.5	$\text{nmol l}^{-1}$	(82)

	evokes half-maximal MR-response on ACTH			
$GR_{EC50}$	Concentration of free cortisol that evokes half-maximal GR response on ACTH	<sup>†</sup> 5.0	nmol l <sup>-1</sup>	(82)
$q$	Conversion factor for adrenal inputs	1	pmol <sup>-1</sup> l	
$r$	Ratio of total to free serum cortisol	28	(none)	(28)
$s$	Supply factor of exogenous cortisol	1	nmol l <sup>-1</sup> s <sup>-1</sup>	
<b>Disturbances in time</b>	$e_1(t)$	External input acting on plasma ACTH	<sup>  </sup> Variable (dependent on time)	(none)
	$e_2(t)$	External input acting on serum cortisol	<sup>**</sup> Variable (dependent on time)	(none)

\* In each subject that was experimentally studied, we applied the parameter identification method; results see table 2.

<sup>†</sup> Only in the data-set on MR-blockade with canrenoate, we regard  $MR_{EC50}$  and  $GR_{EC50}$  as parameters, which have to be individually determined by the parameter identification method; results see table 2.

<sup>‡</sup> The first parameter describes the combined effects of MRs in different parts of the brain, which are integrated into one final signal (acting on ACTH secretion). The same is true with the second parameter which describes the integrated GR effects.

<sup>§</sup> To minimize the number of parameters, we used linear (i.e. non-sigmoid) dose-response relations for the effects at the adrenal and pituitary level.

<sup>¶</sup> Within this delay time, cortisol or ACTH are not active yet.

<sup>||</sup> Used in the data-set on CRH-Stimulation ( $e_1$  is a typical gamma-distribution), in the other conditions  $e_1 \equiv 0$ .

<sup>\*\*</sup> Used in the data-set on cortisol-infusion ( $e_2$  is a ramp function), in the other conditions  $e_2 \equiv 0$ .

**Table 2. Results from the parameter identification method**

	Identified Parameters							Pre-diction error
	$MR_{E\max}^{brain}$	$GR_{E\max}^{brain}$	$CRH_E^{exog}$	$ACTH_E^{adrenal}$	$GR_E^{adrenal}$	$MR_{EC50}$	$GR_{EC50}$	
	[fmol l <sup>-1</sup> s <sup>-1</sup> ]	[fmol l <sup>-1</sup> s <sup>-1</sup> ]	[fmol l <sup>-1</sup> s <sup>-1</sup> ]	[pmol l <sup>-1</sup> s <sup>-1</sup> ]	[pmol l <sup>-1</sup> s <sup>-1</sup> ]	[nmol l <sup>-1</sup> ]	[nmol l <sup>-1</sup> ]	
Women	2.3	2.8	550	79	1.0			0.046
Normal	± 0.1	± 0.1	± 50	± 4	± 0.1	††	‡‡	
Men	*2.0	*3.2	440	71	1.2			0.048
Normal	± 0.1	± 0.1	± 50	± 4	± 0.1	††	‡‡	
Men	†2.4	†2.8	440	73	1.1			0.049
Obese	± 0.1	± 0.2	± 110	± 47	± 0.1	††	‡‡	
Men					0.7			0.023
Placebo					± 0.1	††	‡‡	
Men					0.5	‡3.4	‡25.3	0.040
Canrenoate					± 0.1	± 0.8	± 4.1	
Addison's disease	0.3	3.1						0.106
	± 0.2	± 1.1		**	**	††	‡‡	
Cushing disease, Adrenalectomised	§23.4	§25.7						0.170
	± 6.3	± 6.5		**	**	††	‡‡	

\* P<0.050, men normal vs. women normal; ANOVA (women normal, men normal, and men obese) the other parameters as covariates

† P<0.010, men obese vs. men normal; ANOVA (women normal, men normal, and men obese) with the other parameters as covariates

‡ P<0.005, canrenoate vs. †† or ‡‡; one sample t-test

§ P<0.005, M. Cushing after adrenalectomy vs. M. Addison's disease; independent student's t-test

¶ Average prediction error based on distant function: (predicted value-observed value)/observed value

|| Adopted from normal men

\*\* Non-existent in open-loop system, i.e. in the absence of adrenal glands

††  $MR_{EC50} = 0.5 \text{ nmol l}^{-1}$  is regarded as a constant, value taken from reference (Table 1)

‡‡  $GR_{EC50} = 5.0 \text{ nmol l}^{-1}$  is regarded as a constant, value taken from reference (Table 1).

**Table 3. Agreement between two alternative models and different data sets**

		Models		
		pn-model <sup>*</sup>	nn-model <sup>†</sup>	
<b>Data sets</b> (cortisol range)	CRH challenge in healthy and obese humans	(50 - 500 nmol/l)	yes	yes
	Blockade with canrenoate in healthy humans	(130 - 500 nmol/l)	yes	yes
	Cortisol infusion in Addison and Cushing/ADX patients	(7 - 2000 nmol/l) <sup>‡</sup>	yes	no <sup>¶</sup>

<sup>\*</sup> A “pn-model” – as is proposed in the current paper - includes (p) positive MR-mediated and (n) negative GR-mediated.

<sup>†</sup> A “nn-model” – which we tested as an alternative model - includes (n) negative MR and (n) negative GR feedback loops. In case of positive agreement, we regard it dispensable to show the simulations.

<sup>‡</sup> Note that this data set particularly covers the “MR-sensitive” low cortisol concentration range.

<sup>¶</sup> See supplementary figure S2. The scope of validity of the nn-model is smaller than that of the pn-model, as it is only in agreement with a subset of the tested data.

**Table 4. Examples of biological systems and respective evidence from the literature that the rules of homeostasis apply**

Receptor Class	A	B	C	Rule 1 references	Rule 2 references	Rule 3 references	Open-Loop B-Action	Closed-Loop B-Action
Nuclear Receptors	Cortisol	MR	GR	(28; 54; 82)	(12; 24; 50; 85)	*	LTP and Cell-Survival	↑ Cortisol
Tyrosine kinase	BDNF	TrkB	P75	(6; 19)	(11; 98; 100)	(1; 94)	LTP and Cell-Survival	↑ BDNF
	Insulin	I-R	IGF-1-R	(29; 83)	(59)	(55; 90; 101)	Insulin Secretion	↑ Insulin
	Leptin	LR-L	LR-S	(20; 66)	(5; 70)		Satiety	
Serine kinase	TGF b	TGF-beta/ <sub>ALK1</sub>	TGF-beta/ <sub>ALK5</sub>	(40; 43)	(40; 43; 44)		Cell growth	
G protein coupled	Adrenaline	β <sub>2</sub>	α <sub>1</sub> ; β <sub>1</sub>	(68)	(16; 23; 58; 71)	(58)	Excitation	
Mixed (G protein/Channel)	Ach	Nicotinic -R	Muscarinic-R (M3)		(15; 62)	(60; 64; 65; 80)	Excitation	↑ Acetylcholine
K <sub>ATP</sub> -Channel	ATP	SUR1	SUR2	(49; 84)	(36; 86)	(79)	Excitation	↑ Neuronal ATP

\* Shown in this paper to be in agreement with the regulation of cortisol

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Figure 1

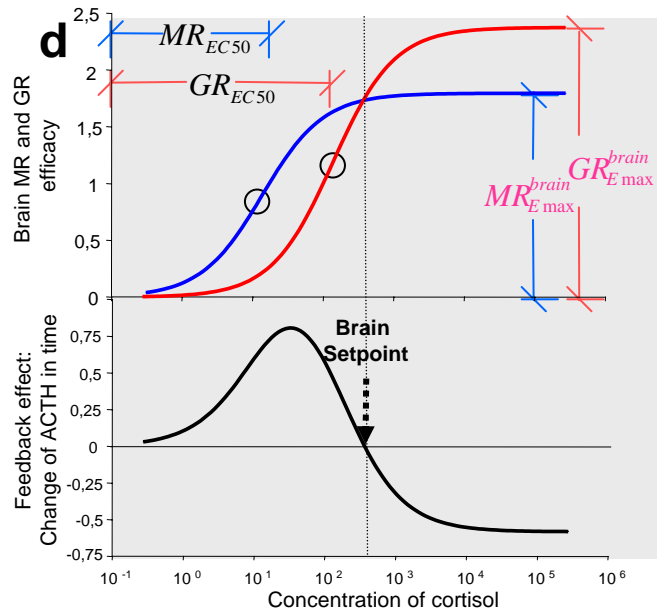
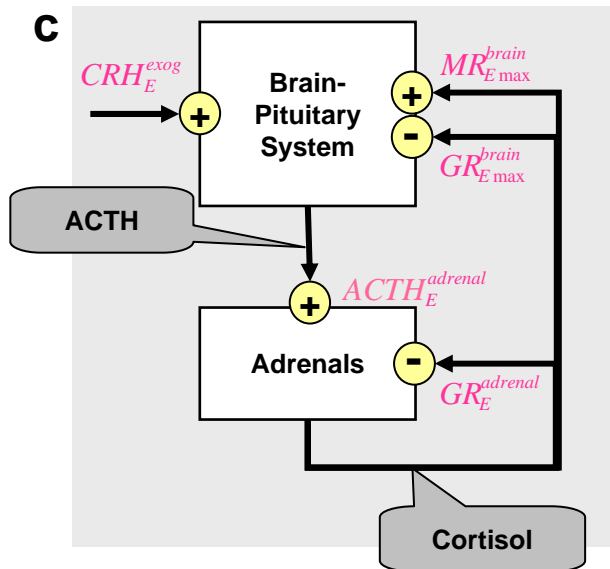
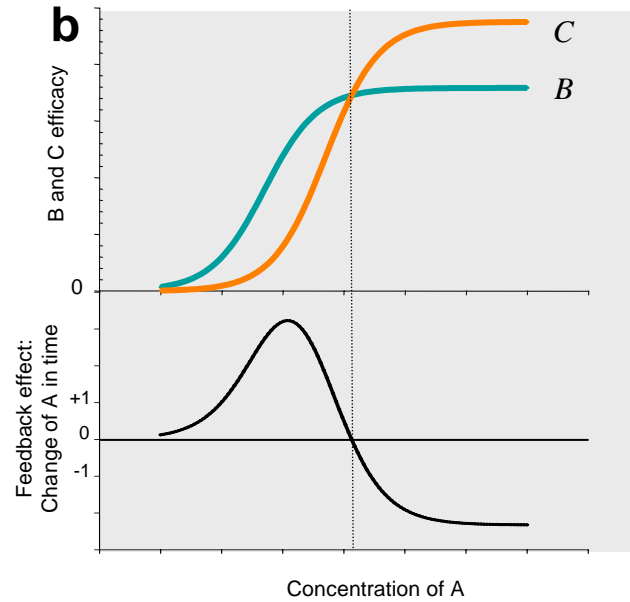
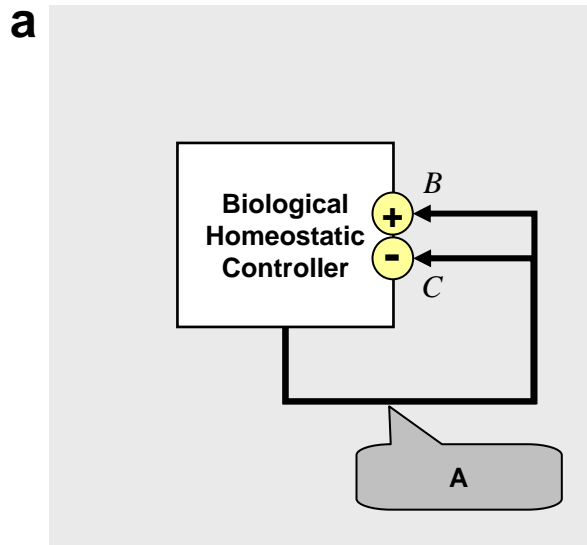
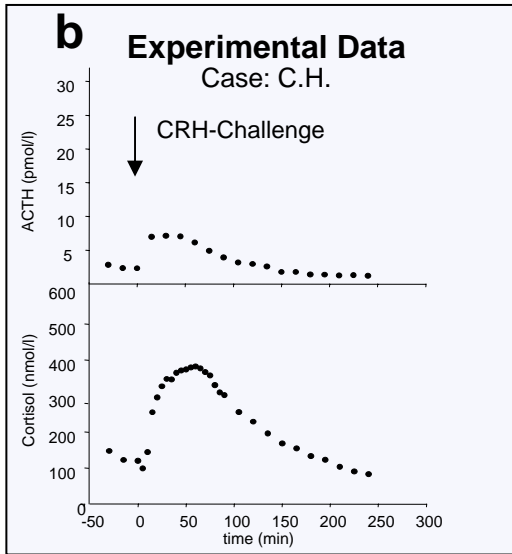


Figure 2

**a** **Differential Equations**

$$\frac{dACTH(t)}{dt} = c_1 \cdot ACTH(t) + \frac{MR_{E\max}^{brain} \cdot CORT(t - \tau_1)}{CORT(t - \tau_1) + r \cdot MR_{EC50}} - \frac{GR_{E\max}^{brain} \cdot CORT(t - \tau_1)}{CORT(t - \tau_1) + r \cdot GR_{EC50}} + CRH_E^{exog} \cdot e_1(t)$$

$$\frac{dCORT(t)}{dt} = c_2 \cdot CORT(t) + ACTH_E^{adrenal} \cdot ACTH(t - \tau_2) - GR_E^{adrenal} \cdot CORT(t - \tau_1) + s \cdot e_2(t)$$



**c** **Parameter Identification**  
Case: C.H.

Identified Parameters

$MR_{E\max}^{brain}$	$GR_{E\max}^{brain}$	$CRH_E^{exog}$	$ACTH_E^{adren}$	$GR_E^{adren}$
[fmol l <sup>-1</sup> s <sup>-1</sup> ]	[fmol l <sup>-1</sup> s <sup>-1</sup> ]	[fmol l <sup>-1</sup> s <sup>-1</sup> ]	[pmol l <sup>-1</sup> s <sup>-1</sup> ]	[pmol l <sup>-1</sup> s <sup>-1</sup> ]
2.0	2.5	500	49	0.7

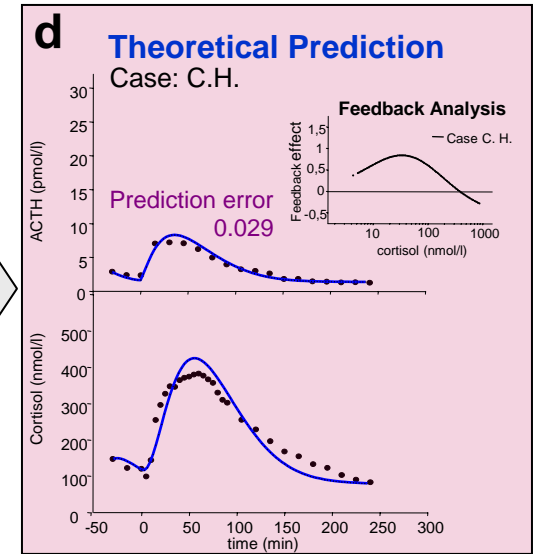


Figure 3a

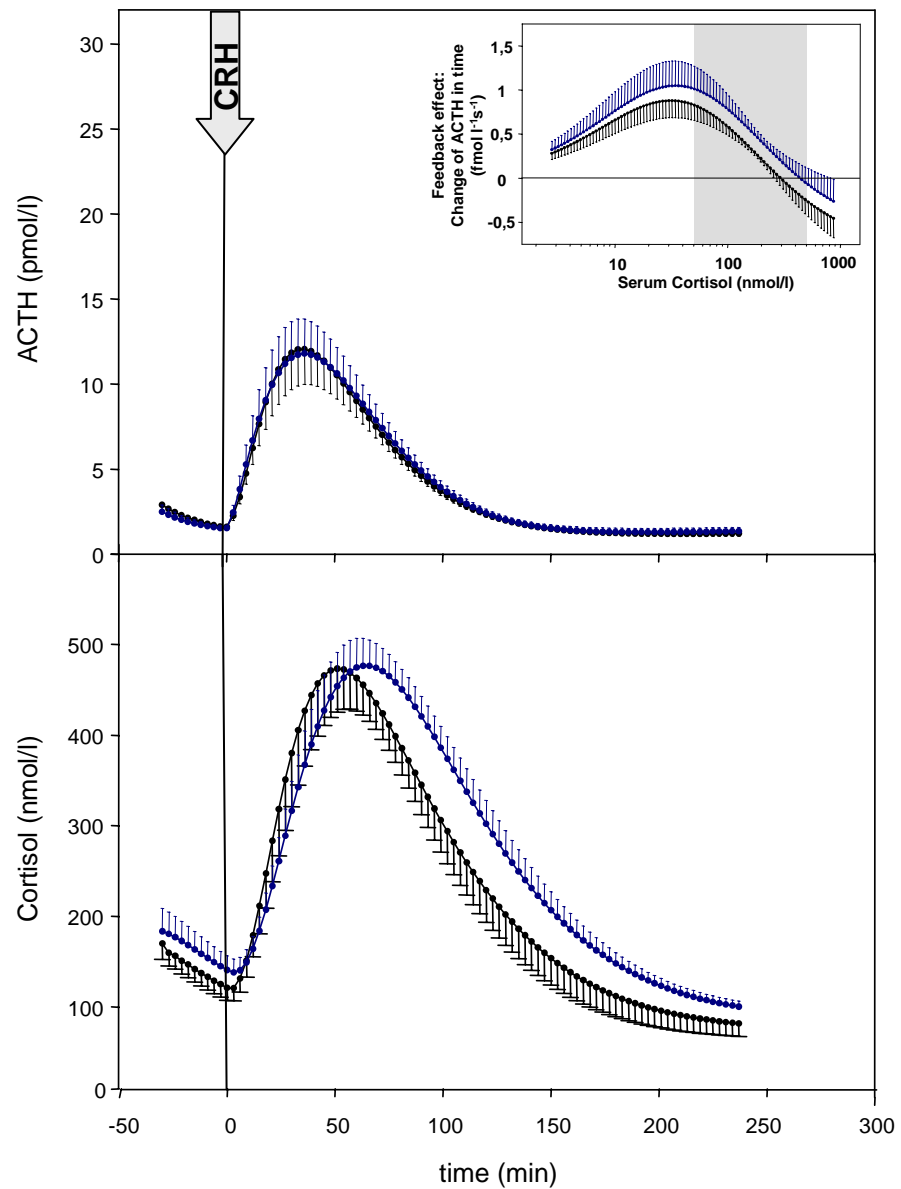
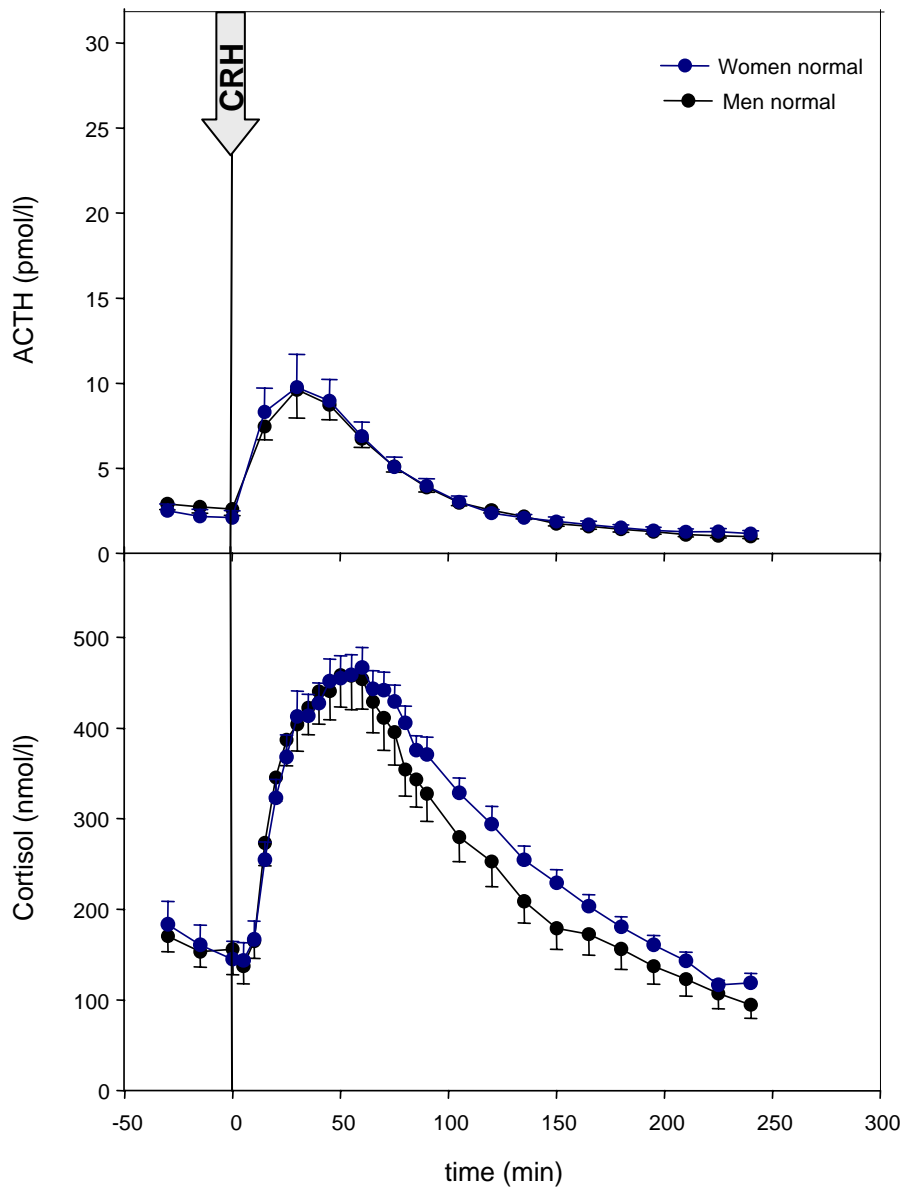


Figure 3b

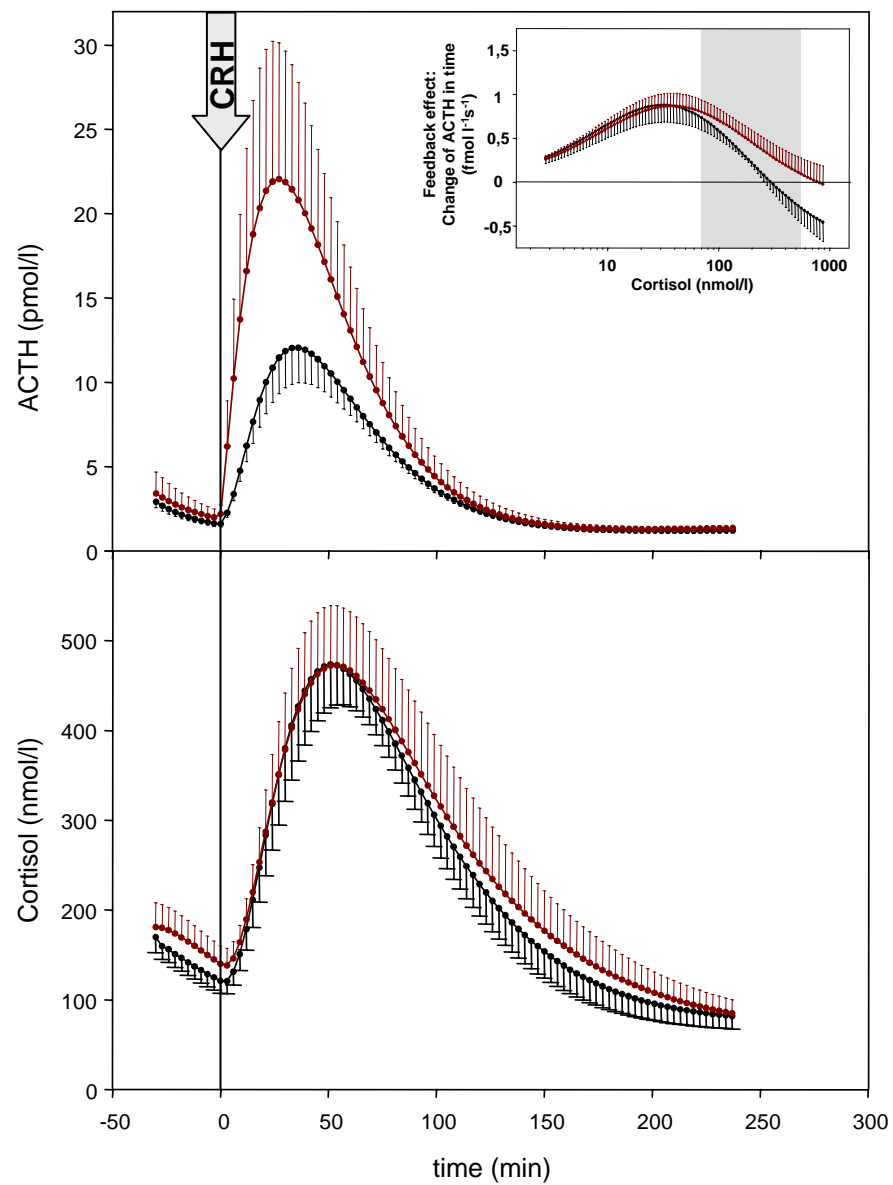
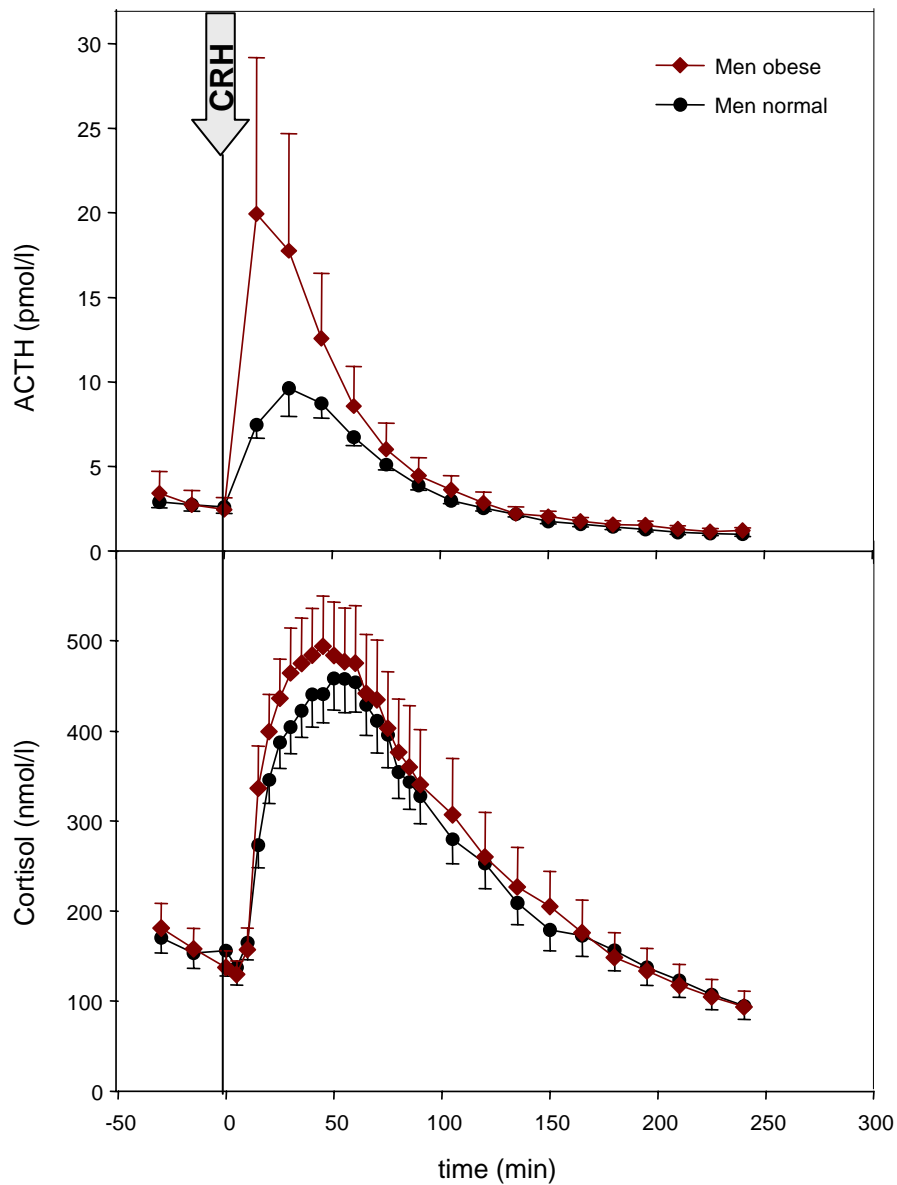


Figure 3c

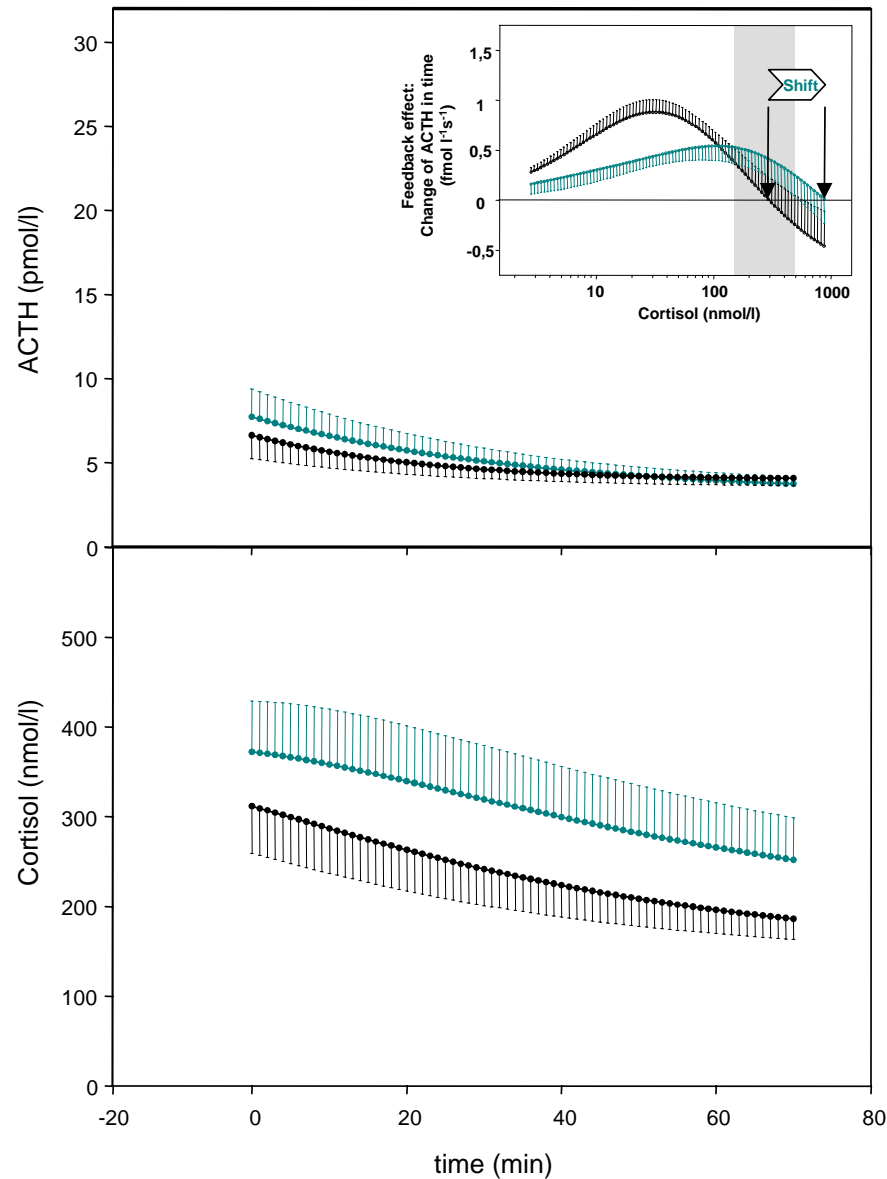
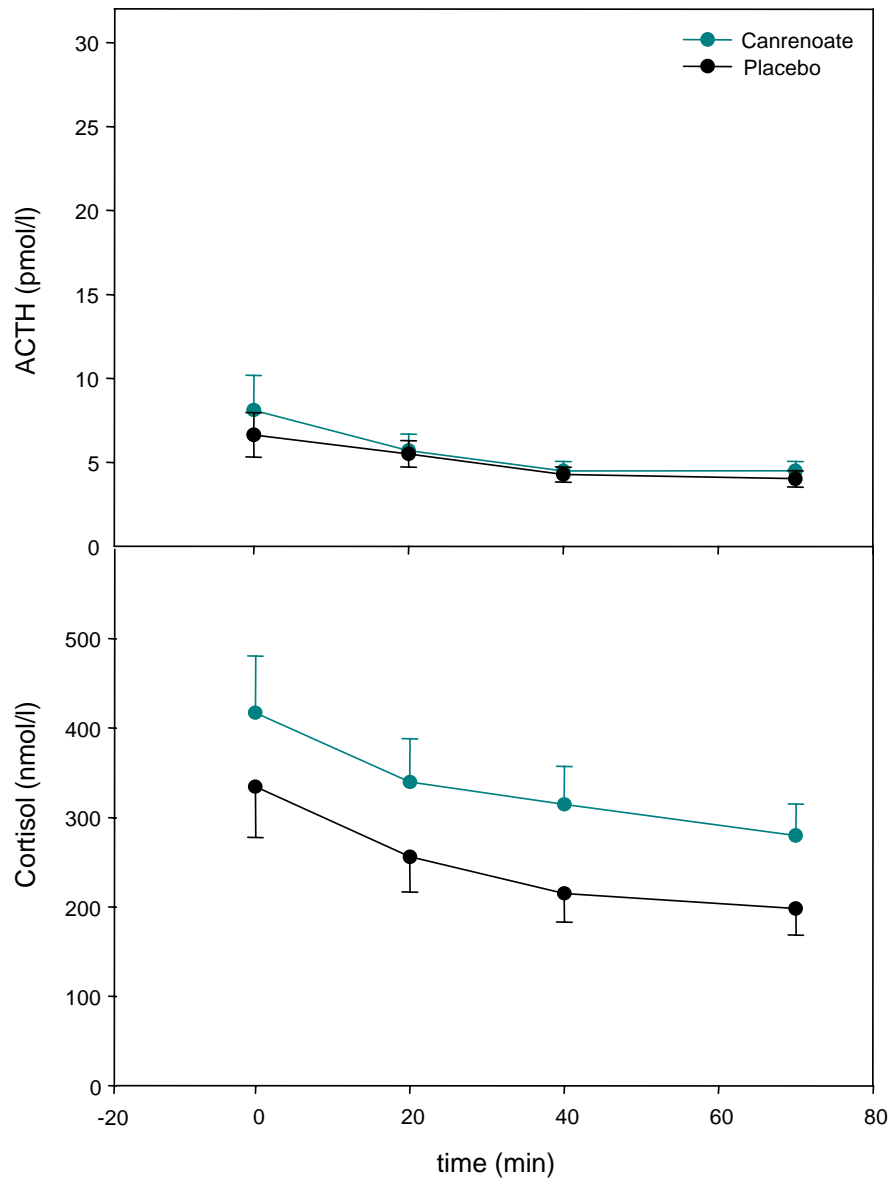


Figure 3d

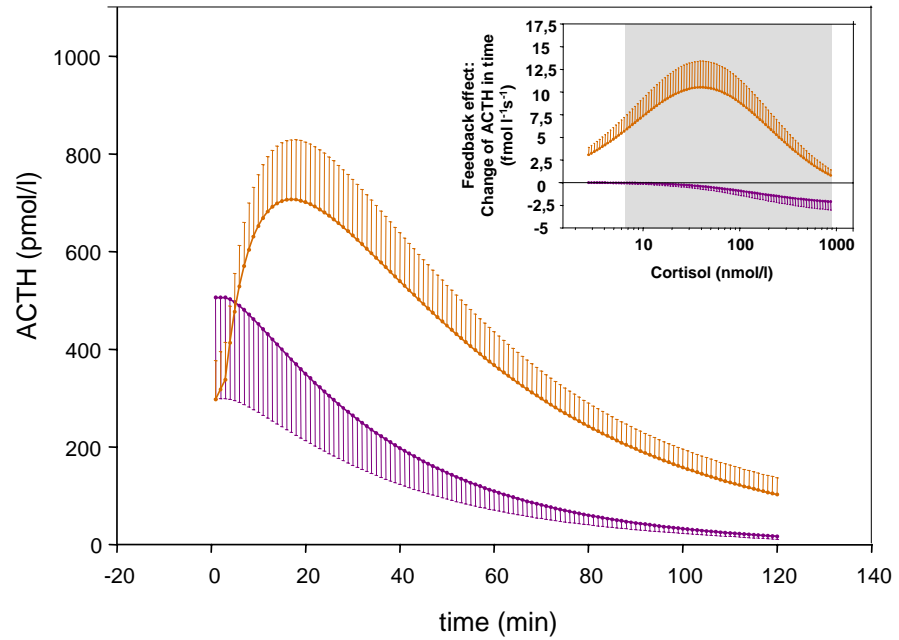
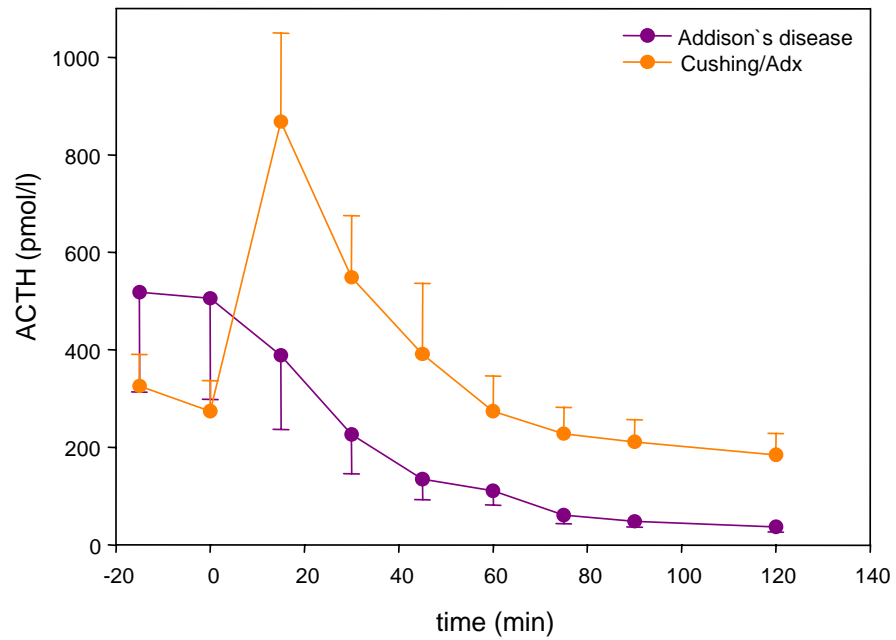


Figure 4a, b

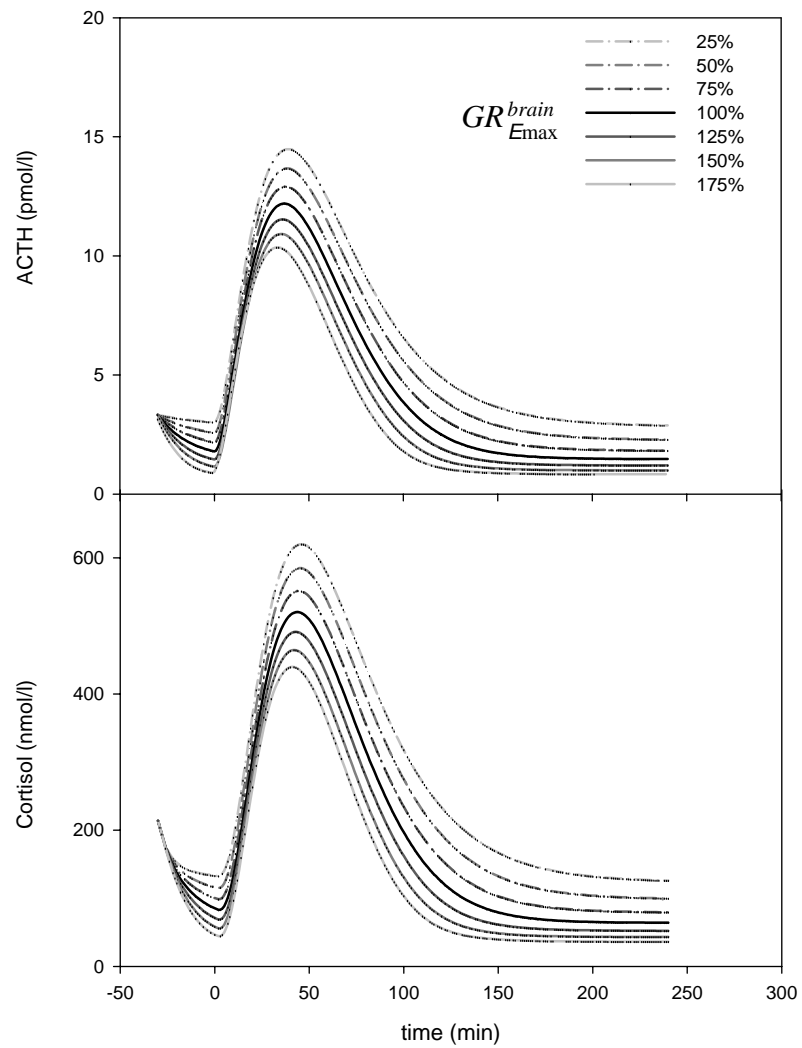
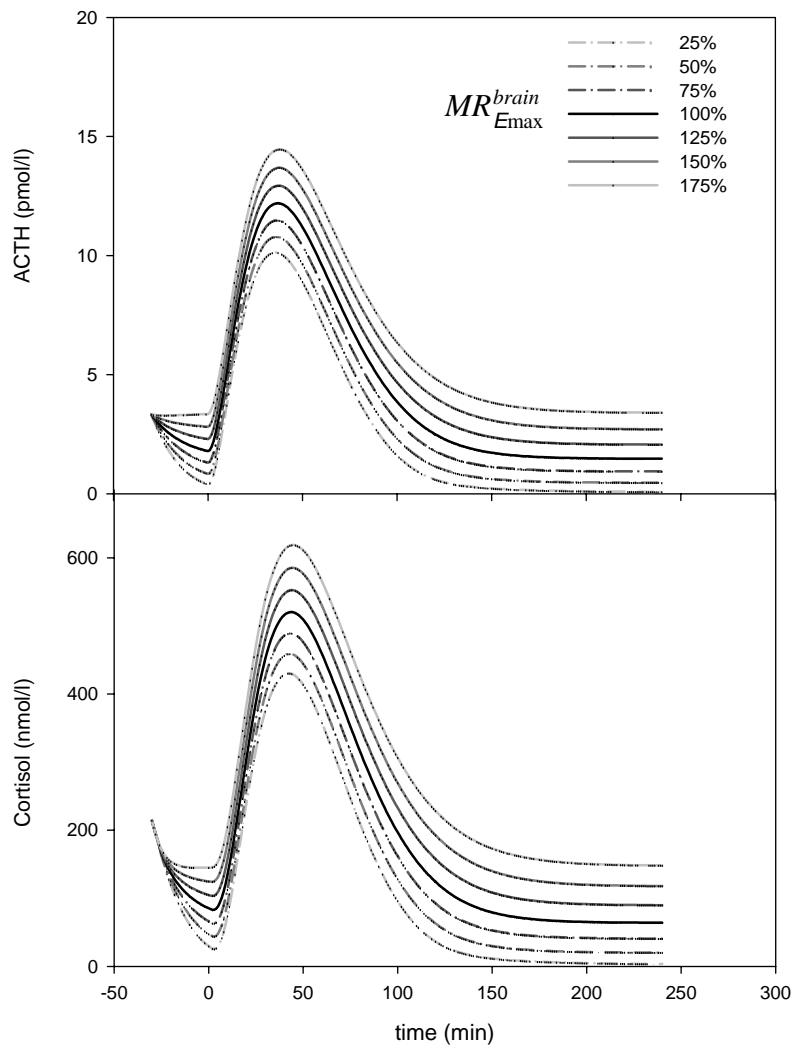




Figure 4c, d

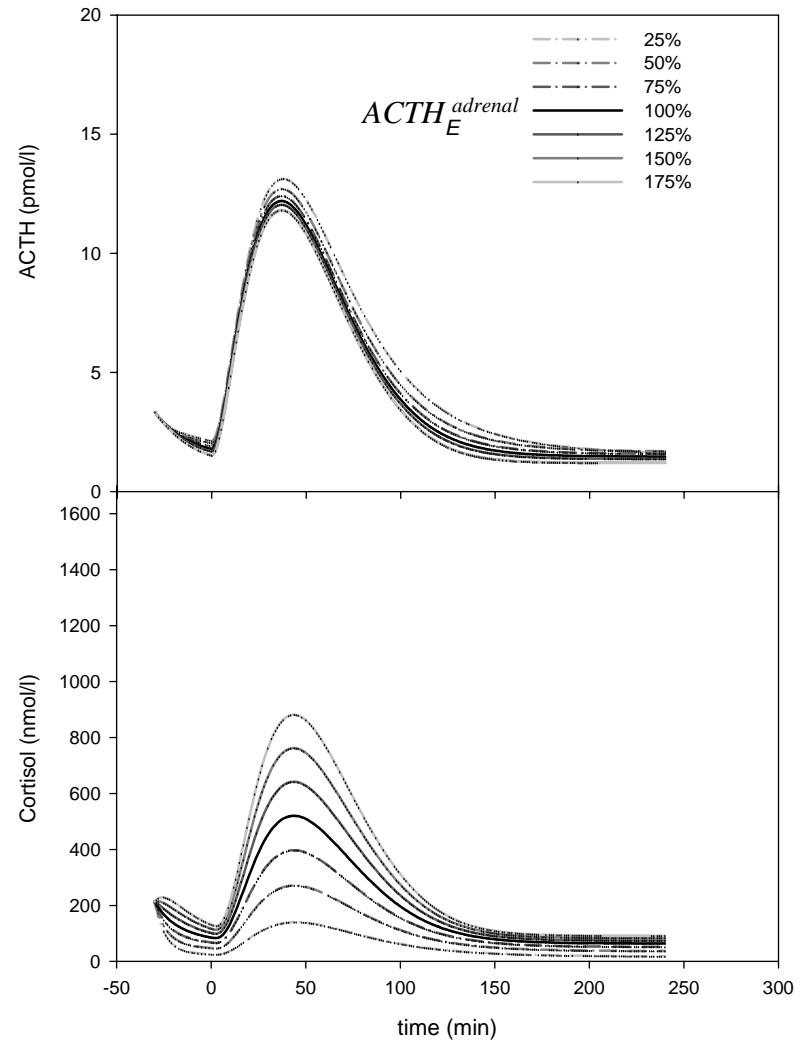
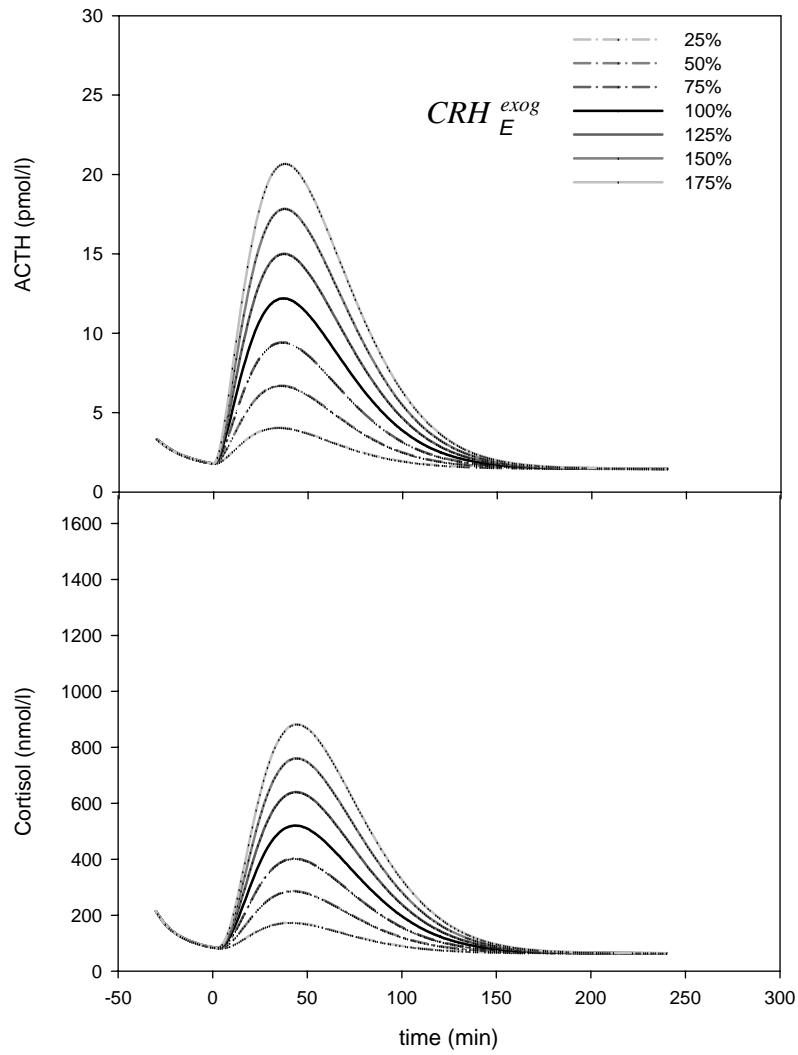


Figure 4e

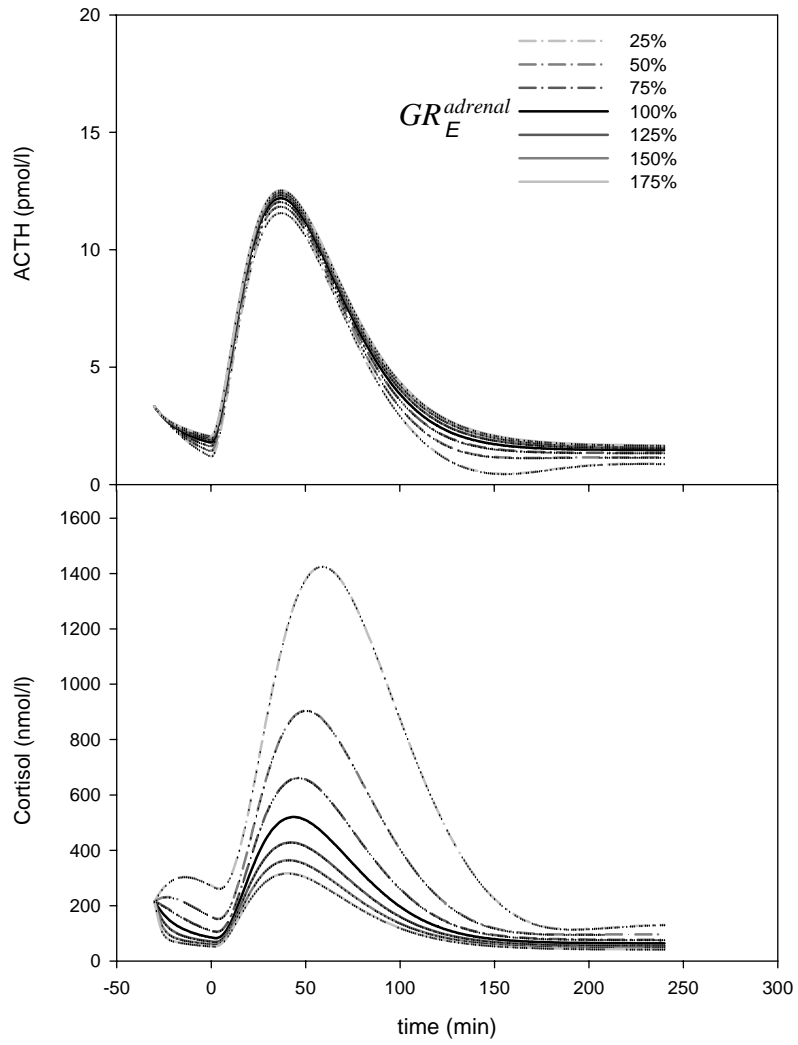


Figure 5

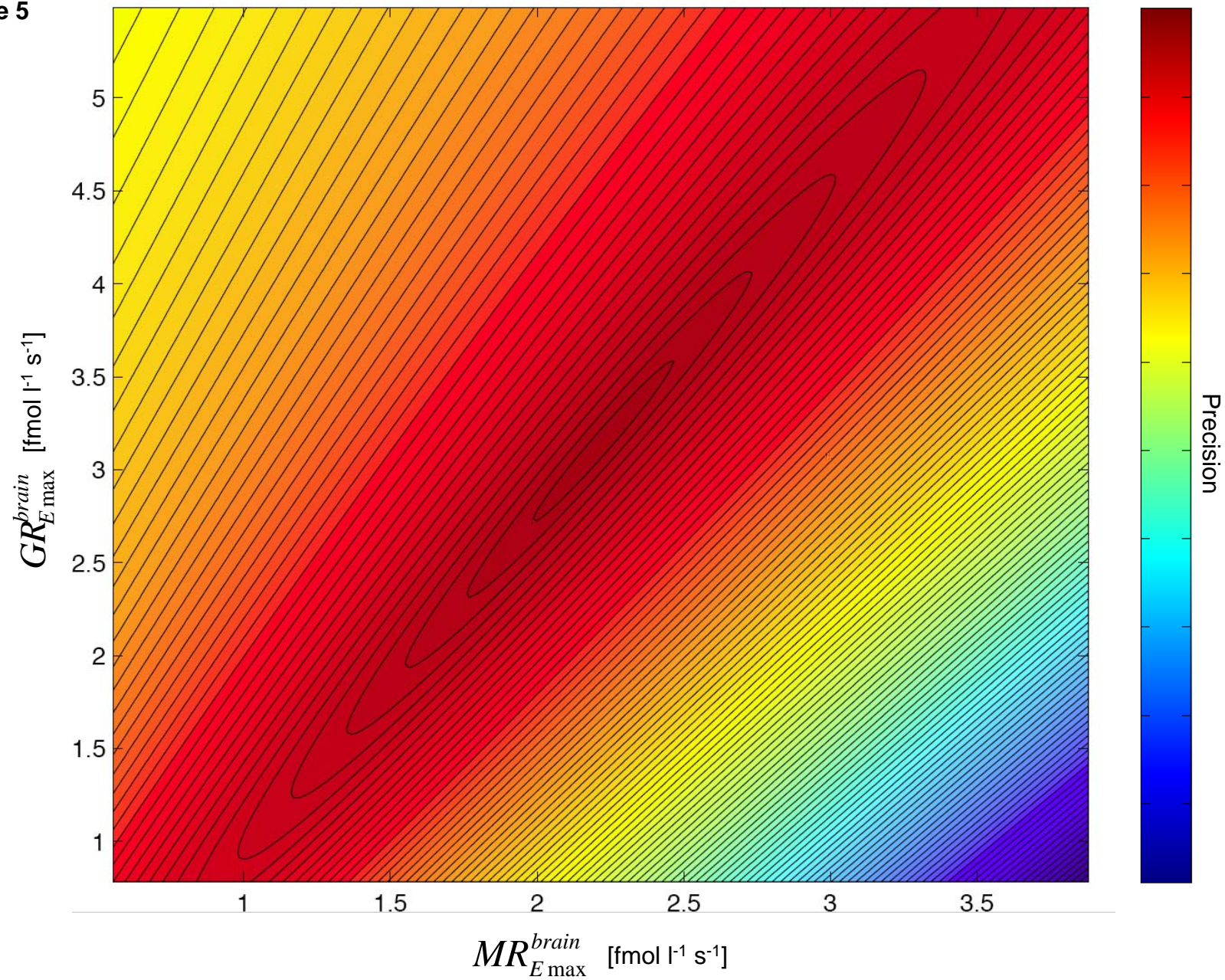


Figure 6

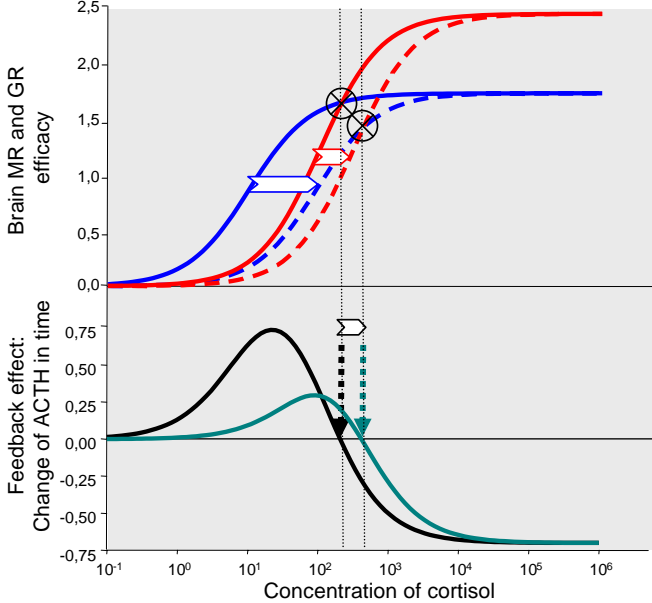


Figure S1

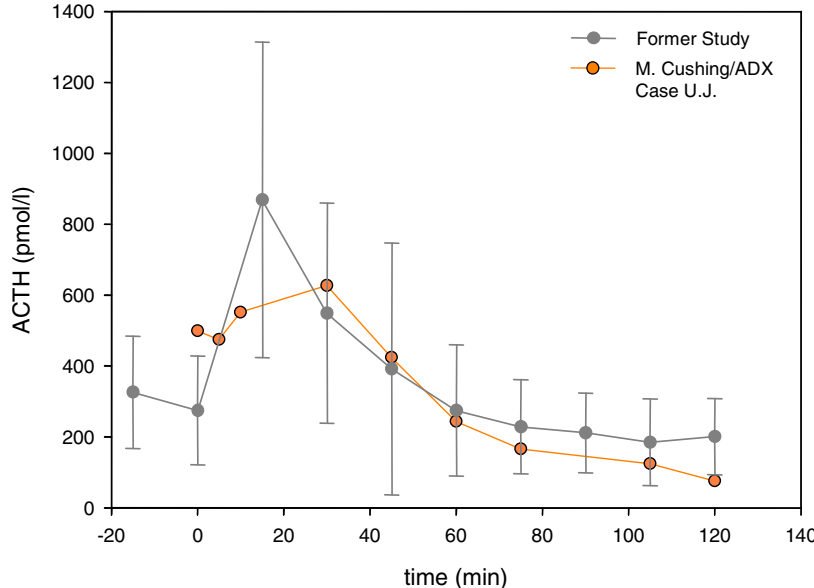


Figure S2

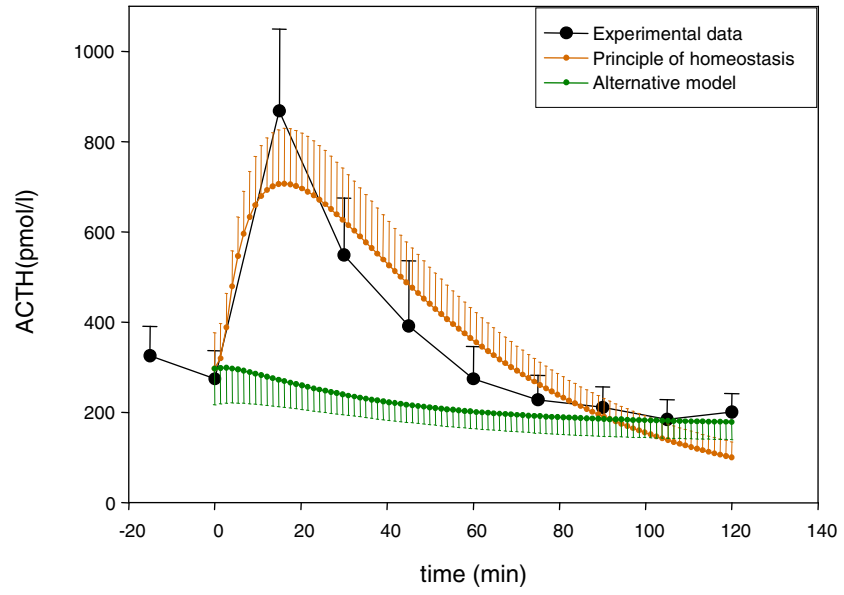


Figure S3

