Title

Hormonal dysregulation after prolonged HPA axis activation can be explained by changes of adrenal and corticotroph masses

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Abstract

Stress activates a complex network of hormones known as the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis regulates multiple metabolic, immune, and behavioral end-points. Dysregulation of the HPA axis is a hallmark of several psychiatric conditions, including depression and substance-abuse disorders. However, little is known about the origin of this dysregulation, and we currently lack mathematical models that can explain its dynamics on the timescale of weeks. Specifically, accumulated experimental evidence indicates that after prolonged activation of the HPA axis, ACTH responses become blunted, and this blunting persists for weeks even after normalization of cortisol levels and responses. Here, we use mathematical modelling to show that this dysregulation can be explained by changes in the functional masses of the glands (total mass of the cells) that secrete ACTH and cortisol. These mass changes occur because the hormones CRH and ACTH regulate the growth of corticotroph and adrenal cortex cells, respectively. Furthermore, we show that impaired glucocorticoid receptor (GR) feedback exacerbates this dysregulation, providing a rationale for the role of GR in depression. We propose that this dysregulation is a side-effect of a circuit with a physiological function, in which gland-mass changes provide dynamical compensation to physiological variation. These findings suggest that gland-mass dynamics may play a role in the pathophysiology of stress-related disorders.

Introduction

Physical and psychological challenges activate a network of endocrine interactions, known as the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1A, (1–4)). The HPA axis generates an adaptive stress response, and its dysregulation is implicated in a range of diseases (5−10). Activation of the HPA axis begins in the hypothalamus, which secretes corticotropin releasing hormone (CRH) in response to stressors. CRH stimulates the secretion of adrenocorticotrophic hormone (ACTH) from corticotroph cells in the anterior pituitary. ACTH is secreted into the circulation and causes cells in the adrenal cortex to secrete cortisol. Cortisol then inhibits HPA axis activity by feedback through high affinity receptors (Mineralocorticoid Receptor, MR) and low affinity receptors (Glucocorticoid Receptor, GR). This feedback affects the response to stressors, as well as circadian and ultradian rhythms of the HPA axis (6,11,12). The activity of the GR is affected by epigenetic regulation (13–15). Cortisol, the primary output of the HPA axis, affects multiple physiological endpoints, including behavioral, immune and metabolic systems.

Several psychiatric disorders and physiological situations involve dysregulation of the HPA axis which develops over months. In major depression, a condition associated with chronic stress, about 40%-60% of drug-free patients have hypercortisolemia (16). One way to test the HPA axis is the CRH test, in which CRH is administered and cortisol and ACTH dynamics are measured. Major depression is associated with a blunted (reduced) ACTH response (17–19) in the CRH test (Figure 1A). Elevated cortisol and blunted ACTH responses are also observed in other conditions that involve prolonged HPA axis activation, including anorexia nervosa (20) (Figure 1B), alcohol abuse disorder (21) (Figure 1C), and pregnancy (22) (Figure 1D). In all these conditions, the resolution of HPA over-activation (e.g. by weight normalization after anorexia, cessation of alcohol consumption, and childbirth respectively) leads to return to baseline of cortisol levels within a few weeks. In contrast, ACTH responses remain blunted for many weeks after cortisol levels normalize. This blunting may be causal for some of the clinical aspects in these conditions, since ACTH is co-regulated with other peptides that modulate pain and mood (23–28). In anorexia and postpartum, both ACTH and cortisol normalize many weeks after stress resolution (for alcohol abuse disorder this time-point was not measured in (21)) (Figure 1B,D).

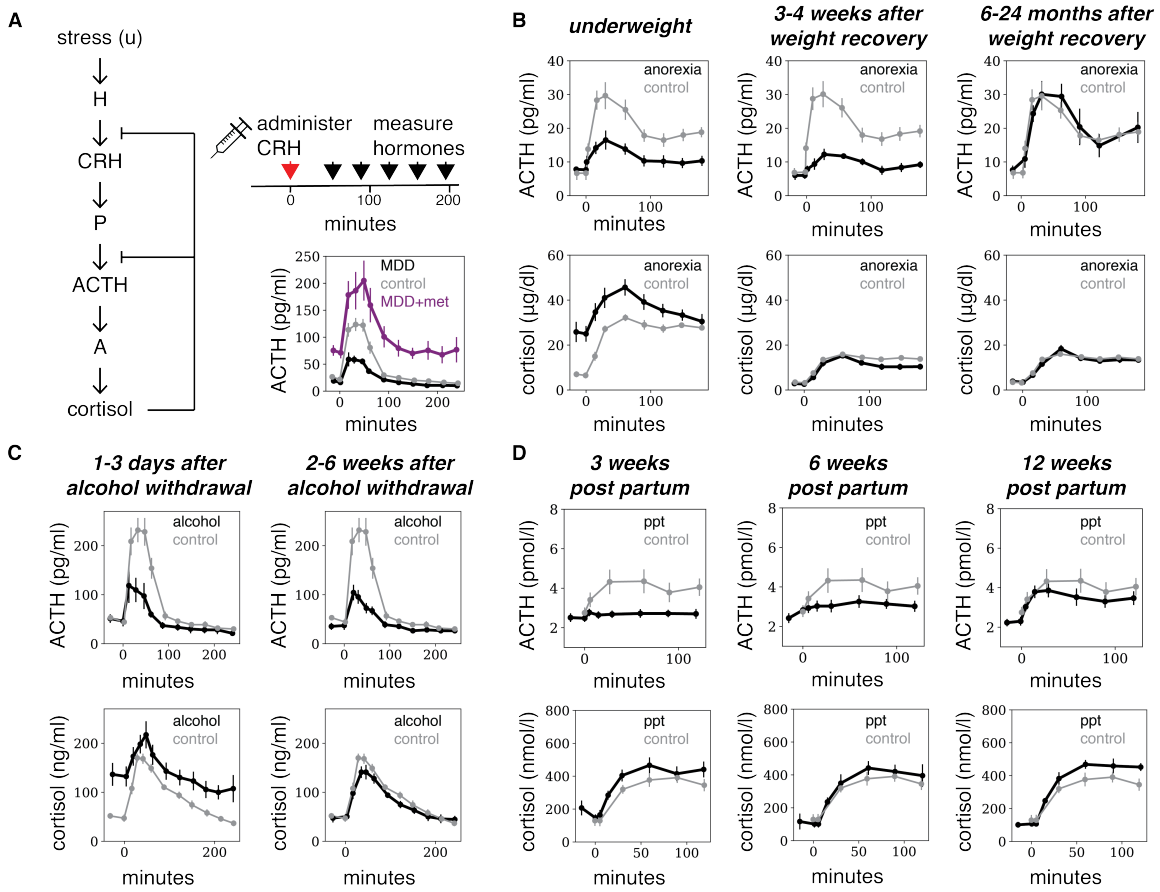

Figure 1. After prolonged stress, ACTH response is blunted for weeks after cortisol response normalizes. (A) Schema of the classic HPA axis. CRH causes the secretion of ACTH and cortisol. In the CRH test, the secretion of these hormones is measured after CRH administration. Patients suffering from major depressive disorder (MDD) show a blunted response to CRH (black line), compared with control (gray line) and with MDD patients treated with a blocker of cortisol synthesis (metyrapone, purple line)- data from (29). (B) Patients suffering from anorexia and admitted to treatment show a blunted ACTH response and hypercortisolemia, which resolves within 6-24 months after weight normalization- data from (20). However, 3-4 weeks after weight normalization, cortisol dynamics are normal whereas ACTH dynamics are blunted. (C) Individuals recovering from alcohol abuse show hypercortisolemia and blunted ACTH response after admission – data from (21). After 2-6 weeks, these individuals show normal cortisol dynamics, but blunted ACTH responses persist. (D) Pregnancy is associated with elevated cortisol levels due to CRH secretion by the placenta. 3-6 weeks after delivery, cortisol levels and dynamics return to normal, whereas ACTH dynamics are blunted- data from (22). After 12 weeks, ACTH dynamics normalize as well. In all panels, patient data are denoted by thin gray line, and controls by a thicker black line.

The persistent blunting of ACTH responses after the resolution of hyper-cortisolemia is surprising. The dynamics of ACTH in the classical HPA axis model is strongly associated with the dynamics of cortisol, and so once cortisol normalizes, so should ACTH. It is also unclear how a deficient ACTH response produces a normal cortisol response, given that ACTH is the main regulator of cortisol secretion. Explaining this dysregulation thus requires a process on the scale of weeks that decouples the dynamics of ACTH and cortisol. This timescale cannot be readily explained by existing models of the HPA axis, where the relevant timescale is the lifetime of hormones, which is minutes to hours (30). One important process with potentially a timescale of weeks is epigenetic regulation of GR sensitivity (31–33). This process, however,

does not break the association between ACTH and cortisol, and cannot explain, on its own, the dysregulation.

To address the dysregulation of ACTH and cortisol after prolonged HPA activation, we therefore turn to additional interactions in the HPA axis, which are experimentally characterized but have not been considered on the systems level. These are the interactions between the HPA hormones and the functional mass of the HPA glands, whereby 'functional mass of a gland' we mean the total capacity of the cells for the secretion of a hormone. A large body of research, starting from the pioneering research of Hans Selye in the 1930s, showed that the mass and number of adrenal cortisol-secreting cells increases under stress. Subsequent studies established the role of ACTH as the principle regulator of the functional mass of the adrenal cortex (34,35). Imaging and post-mortem studies also demonstrated that adrenal mass increases in humans suffering from major depression (36–42), and returns to its original size after remission (43).

Similarly, CRH causes the growth of pituitary corticotrophs: prolonged administration of CRH (or a CRH-secreting tumor) leads to dramatic increases in POMC content and ACTH output (44,45), as well as to growth of corticotroph cell mass (46–52). Adrenalectomy, which removes negative feedback inhibition from the HPA axis, shows similar effects in rodents (44,47,53,54), and leads to increased proliferation of corticotrophs (53) which is potentiated by CRH treatment.

Changes in functional masses can occur by hypertrophy (enlarged cells) and/or hyperplasia (more cells); the exact mechanism does not matter for the present analysis. The changes in functional mass take weeks, due to the slow turnover time of cell mass. We therefore asked whether the interplay of interactions between hormones and gland mass in the HPA axis can explain the observed dysregulation of the HPA axis on the time scale of weeks to months in the pathological and physiological situations mentioned above. We also tested other slow processes such as epigenetic regulation of GR (and more generally, GR resistance), slow changes in the input signal, or in the removal rate of cortisol.

To understand the dynamics of gland mass changes, we developed a mathematical model that incorporates both the hormonal interactions and the gland mass dynamics in the HPA axis. The model incorporates cortisol feedback through the high-affinity MR and low-affinity GR. We find that prolonged HPA activation enlarges the functional masses of the pituitary corticotrophs and adrenal cortex, and that the recovery of these functional masses takes weeks after stress is removed. The dynamics of this recovery explains the observed HPA dysregulation: ACTH responses remain blunted for weeks after cortisol has normalized. The

other slow processes we tested cannot explain this dysregulation because they do not break the strong association between ACTH and cortisol. We further show that the GR protects the HPA axis against this dysregulation after high levels of stress, providing a new explanation for the association between deficient GR feedback and depression. Finally, we demonstrate the physiological advantages conferred by the control of functional mass. Thus, functional mass changes provide an integrated explanation of HPA dysregulation and dynamics on the scale of weeks to months.

Results

Model for HPA axis dynamics that includes functional mass changes

We consider two timescales of HPA axis dynamics (Figure 2A). On the fast timescale, of minutes to hours, hormones are secreted and degraded. Physical, circadian or psychological stresses act as input signals to the HPA axis. Such inputs include low blood glucose, low blood pressure or inflammation signals, psychological stressors, or effects of drugs such as alcohol, cocaine, or amphetamines. We consider all inputs acting at a given time-point as a combined input signal which we denote as u. This input signal causes the secretion of CRH.

We modelled the classic HPA cascade and cortisol feedback loop based on previous modelling studies (6,55,56). The equations describe ACTH stimulation by CRH, cortisol stimulation by ACTH, and the feedback from cortisol on upstream hormones due to the MR and GR receptors. The equations are provided in the methods section. Hormone half-lives are on the order of minutes-hours (30), and therefore this model cannot explain dysregulation over the timescale of weeks presented in Figure 1. Most previous models of the HPA axis included only this fast timescale, and could follow phenomena such as changes during the circadian cycle (6), ultradian rhythms (57–59), and bistability (31,55,56).

To study the dysregulation of the HPA axis over the timescale of weeks and beyond, we incorporate interactions between the hormones and the total *functional mass* of the cells that secrete these hormones. We introduce two new variables, the functional mass of the corticotrophs, C(t), and of the cortisol-secreting cells in the adrenal cortex, A(t). To focus on the role of the mass, we separate the secretion of ACTH as follows: secretion rate of ACTH is $b_2 C\ CRH$, where C is the corticotroph mass, b_2 is the rate of ACTH secretion per unit corticotroph mass, and CRH is the concentration of CRH. The parameter b_2 thus includes the metabolic capacity of the corticotrophs, the number of CRH receptors, the effects of cytokines and neuronal inputs, and the total blood volume which dilutes out ACTH. A similar equation describes the production of cortisol, secretion rate of cortisol is $b_3 A\ ACTH$. To isolate the

effects of mass changes, we assume that the per-unit-biomass secretion rates b_2 and b_3 are constant.

In order to describe the dynamics of the functional masses, we model the known major factors that affect cell mass, namely the upstream hormones. CRH increases the growth of the functional mass of pituitary corticotrophs, and ACTH increases the growth of the functional mass of the adrenal cortex. After perturbations, the doubling time of the corticotrophs is on the order of days in rats (47), while that of the adrenal mass is on the order of weeks in rats and other animal models (34).

These hormone-driven mass changes are described by two additional equations, for the masses of the corticotrophs and the adrenal cortex (Figure 2A and Methods). The important parameters in these equations are the turnover times of the functional masses, which we estimate to be on the order of days-weeks in order to explain the observed hormone dynamics in Figure 1. Parameter values are given in Table 1. Note that the dynamics of the functional masses is thus much slower than the dynamics of the hormones which have half-lives of minutes to hours.

<u>Model shows HPA axis dysregulation after prolonged activation</u>

We simulated the response of the HPA model to a prolonged stress input. We model the effect of a prolonged stressor as a pulse of input u to the HPA axis which lasts for several weeks or longer.

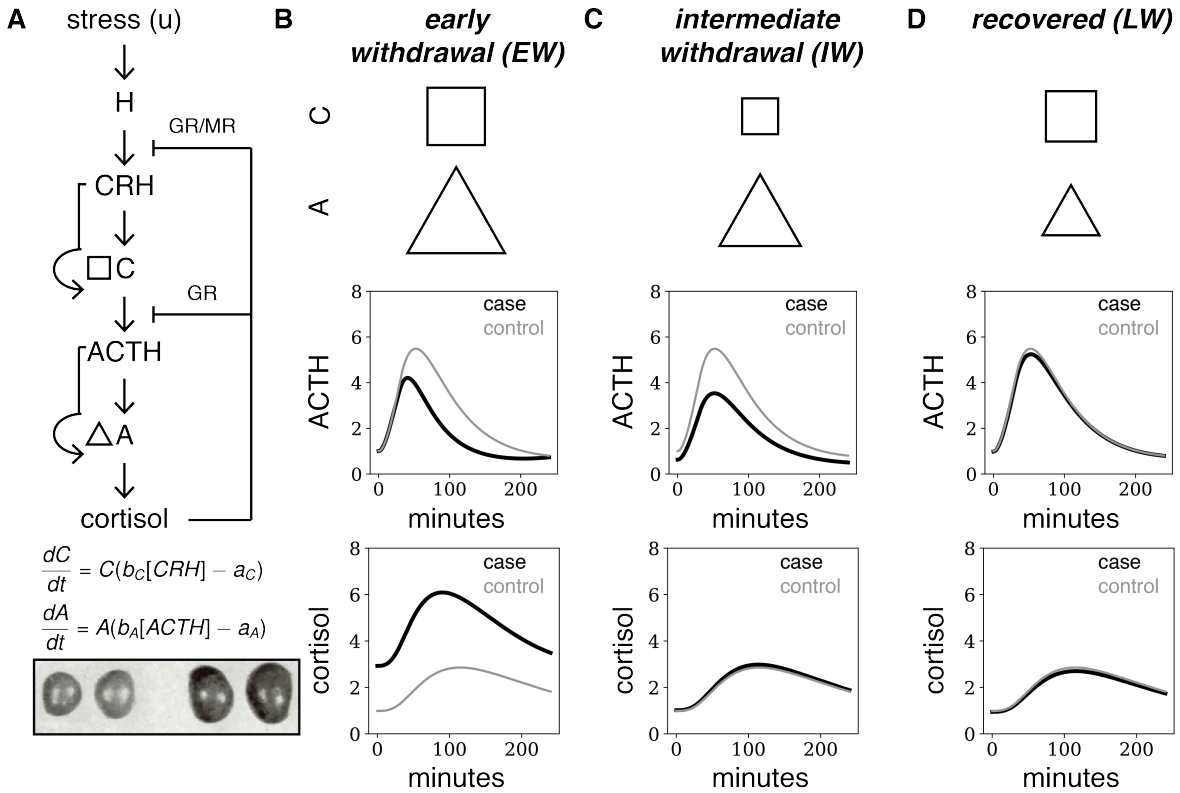

Figure 2. Model with functional mass dynamics shows ACTH blunting for weeks even after cortisol normalizes. (A) To account for the control of corticotroph growth by CRH and adrenal cortex growth by ACTH, we added to classical HPA models two equations that represent the dynamics of the functional mass of corticotrophs and the adrenal cortex, C and A. *(Inset)* Such dynamics explain, for example, the enlarged adrenals of stressed rats (inset, right) compared with control (inset, left), adapted from (60). (BCD) The model shows the three distinct phases of HPA axis dysregulation observed in experiments. At the end of a prolonged stress period, the adrenal mass is enlarged and the corticotroph mass is slightly enlarged, which results in hypercortisolemia and blunted ACTH responses in the CRH test (B). After a few weeks, corticotroph mass drops below baseline, while adrenal mass is slightly enlarged, causing normal cortisol dynamics with blunted ACTH responses to the CRH test (C). Finally, after a few months both tissue masses return to normal, leading to normalization of both cortisol and ACTH dynamics (D). In all panels, simulations are of a CRH test (Methods), where 'case' is after stress and 'control' is after basal HPA axis activation, as described in Figure 3.

We then simulated a CRH test at several time-points after the end of the input stress pulse. The CRH test is modelled by adding CRH and measuring ACTH and cortisol over 4 hours of simulated time (methods). We find three phases of recovery. In the first few weeks after the end of the input pulse, cortisol is high and ACTH responses are blunted (Figure 2B). Then, for a period of several months, cortisol has returned to its original baseline but ACTH is remains blunted (Figure 2C). Finally, after several months, both cortisol and ACTH return to their original baselines (Figure 2D). Thus, the model recapitulates the observed dynamics of Figure 1.

To understand these dynamics in detail, we plot in Figure 3 the full behavior of the functional masses and hormones during and after the pulse of input. Importantly, the qualitative conclusions are independent on the precise values of the model parameters. The HPA model

has several distinct phases of dysregulation during and after prolonged stress (Figure 3). These phases are caused by changes in the functional masses of the pituitary corticotrophs and adrenal cortex (Figure 3AB). These functional mass changes lead to changes in the level of the hormones (Figure 3C). We also simulated a CRH test at each timepoint and calculated the response as the peak hormone level after CRH administration relative to a control simulation without the prolonged stress input (Figure 3D). We plotted the CRH response as a function of time for ACTH and cortisol in Figure 3E. A blunted response corresponds a response below 1.0.

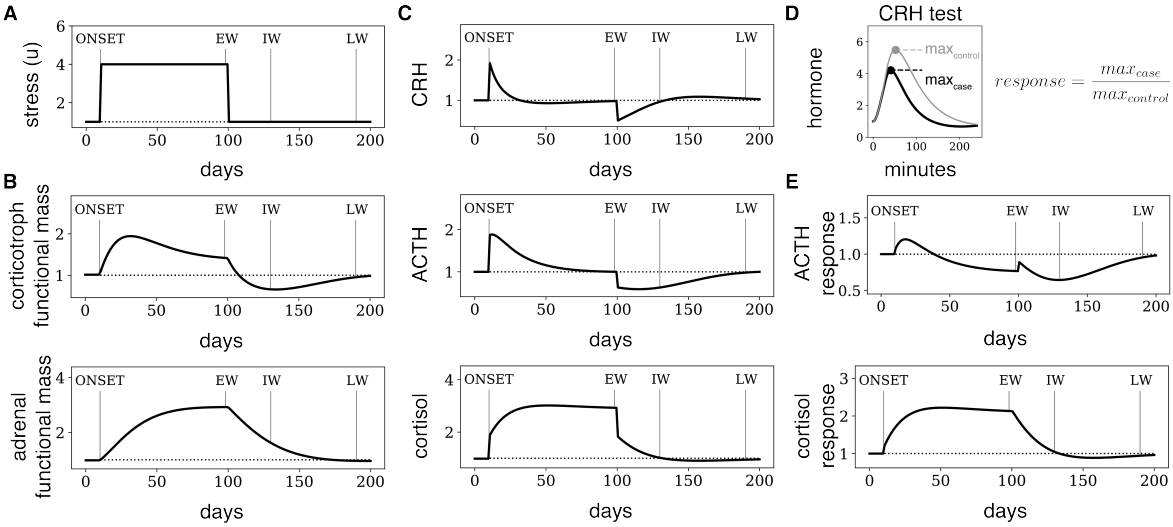

Figure 3. Model dynamics of HPA axis after prolonged stress. Numerical solution of the HPA model after a prolonged pulse of input (u=4) lasting 3 months, followed by recovery to baseline input u=1(A). During the pulse, gland masses (B) increase over weeks, leading to exact adaptation of ACTH and CRH levels after a few weeks (C) despite the increased input level. After stress ends, gland masses adjust back over weeks. During this adjustment period, the HPA axis is dysregulated. (D) To model a CRH test, we add CRH to the simulation and follow the hormones over several hours. The response is defined as the maximum response to a CRH test (case) relative to the maximum response to a CRH test in steady-state with basal input conditions (control). In E, the response to a CRH test given at time *t* is shown as a function of *t*. thus, this is the predicted response to CRH tests done at different days during and after the stressor. The model (black lines) shows blunted (reduced) responses to CRH tests after the stress similar to those observed in Figure 1, as well as a mismatch between cortisol and ACTH dynamics that develops a few weeks after cessation of stress.

The initial phase occurs after the onset of the stressor and before adaptation to the stressor (Marked ONSET in Figure 3). It lasts several weeks. In this phase, the increase in input *u* causes elevated levels and responses of hypothalamic CRH, ACTH and cortisol. However, over weeks of stress input, the corticotroph and adrenal masses grow. The gland masses thus effectively adjust to the stressor, as example of the more general phenomenon of *dynamical compensation* in physiological systems (61), discussed below. This functional mass growth causes a return to baseline of hypothalamic CRH and ACTH, due to negative feedback by cortisol. More precisely, a larger adrenal functional mass means that less ACTH is needed to produce a concentration of cortisol that drives ACTH down to baseline.

Such a return to baseline is called *exact adaptation*. Exact adaptation is a robust feature of this circuit due to a mathematical principle in the functional mass equations called integral feedback (61) (Methods). Exact adaptation does not occur in models without the effects of functional mass changes- the hormones do not adapt to the stressor (Figure S1, Supplementary Section 1).

The enlarged functional masses results in elevated cortisol levels during the stress period, but in adapted (that is, baseline) levels of CRH, ACTH, and blunted responses of CRH and ACTH to inputs (Figure 3DE). During prolonged stress there is thus a transition from an elevated to a blunted response of ACTH that occurs due to changes in functional masses, and results from cortisol negative feedback.

At the end of the prolonged stress pulse, which we term Early Withdrawal (or EW), the functional masses are abnormal, and take weeks to months to recover. This fundamental process is the reason for the hormonal dysregulation that is the subject of this study. In the first weeks after the stressor is removed, the adrenal and corticotroph functional masses shrink, accompanied by dropping cortisol levels. ACTH responses are blunted, and blunting may even worsen over time.

Then, cortisol and CRH levels and responses simultaneously normalize. This marks the beginning of the next phase which we term Intermediate Withdrawal (IW, Figure 3). In this phase, ACTH responses remain blunted, despite the fact that cortisol is back to baseline, because adrenal functional mass is enlarged, and corticotroph functional mass is deficient. Finally, over time, the entire dynamics of the HPA axis normalize (Late Withdrawal, LW in Figure 3), and the system has fully recovered.

These recovery phases are robust features of the HPA model with mass dynamics. After withdrawal of the stressor, cortisol and CRH levels and dynamics recover *together*, before the recovery of ACTH. This order occurs regardless of parameter values such as turnover times of the tissues (proof in Methods). The intuitive reason for this is that before CRH returns to baseline, the growth rate of the pituitary corticotrophs is negative, preventing ACTH from returning to baseline.

We conclude that the model is sufficient to explain the dynamics of recovery from chronic HPA activation in several conditions mentioned in the introduction – anorexia, alcohol addiction, and pregnancy (Figure 3). In order to explain the timescales of recovery, the only model parameters that matter are the tissue turnover times. Good agreement is found with turnover times on the scale of 1-3 weeks for the corticotrophs and adrenal cortex cells (see Figure S2 for comparison of different turnover times, Supplementary Section 2). The model

254 therefore explains how ACTH responses remain blunted despite normalization of cortisol
255 baseline and dynamics.
256 A model in which functional masses remain constant does not show these phenomena,
257 but instead shows an immediate normalization of hormone levels and CRH-test responses after
258 stress (Figure S1, red lines). We also tested several alternative mechanisms with a slow
259 timescale of weeks. We tested models with constant gland masses and the following processes
260 to which we assigned time constants on the order of one month: GR resistance following HPA
261 activation (Figure S1, purple lines), slow changes in input signal (Figure S1, blue lines), and
262 slow changes in cortisol removal rate (Figure S1, gray lines). None of these models shows the
263 dysregulation that we consider. The reason is that these slow processes do not cause a mismatch
264 between ACTH and cortisol needed to capture the blunting of ACTH despite normal cortisol
265 responses.
266 Deficient GR feedback exacerbates HPA dysregulation following prolonged stress
267 We next considered the role of glucocorticoid receptor (GR) in recovery from
268 prolonged HPA activation. GR mediates the negative feedback of cortisol on CRH and ACTH
269 secretion. Impaired feedback by GR is observed in many cases of depression. For example,
270 administration of dexamethasone, which binds the GR in the pituitary, fails to suppress cortisol
271 secretion in the majority of individuals suffering from depression (62). Reduced expression of
272 GR and impaired GR function in people with depression was also demonstrated in *post mortem*
273 brains (13,63–65) and in peripheral tissues (66). The feedback strength of the GR is regulated
274 epigenetically, and is affected by early-life adversity (13,67).
275 The relation between depression and impaired GR function seems paradoxical, since
276 GR signaling mediates many of the detrimental effects associated with high cortisol levels such
277 as hippocampal atrophy (68). One explanation for the association between impaired GR
278 feedback and depression is that impaired GR feedback leads to failure of the HPA axis to
279 terminate the stress response on the timescale of hours, leading to excessive cortisol levels
280 (7,14). GR feedback also plays a role in ultradian and circadian rhythms in the HPA axis
281 (12,57,69).
282 Here we present an additional explanation for the association between GR feedback
283 and depression, based on the functional mass dynamics model. Model simulations show that
284 negative feedback by GR protects the HPA axis against large changes in hormone levels and
285 responses on the timescale of weeks after the end of a period of prolonged stress input (Figure
286 4A). The extent of the impaired dynamics of cortisol and ACTH after prolonged stress is

reduced when GR affinity is high. In other words, dysregulation is more severe the weaker the feedback from GR.

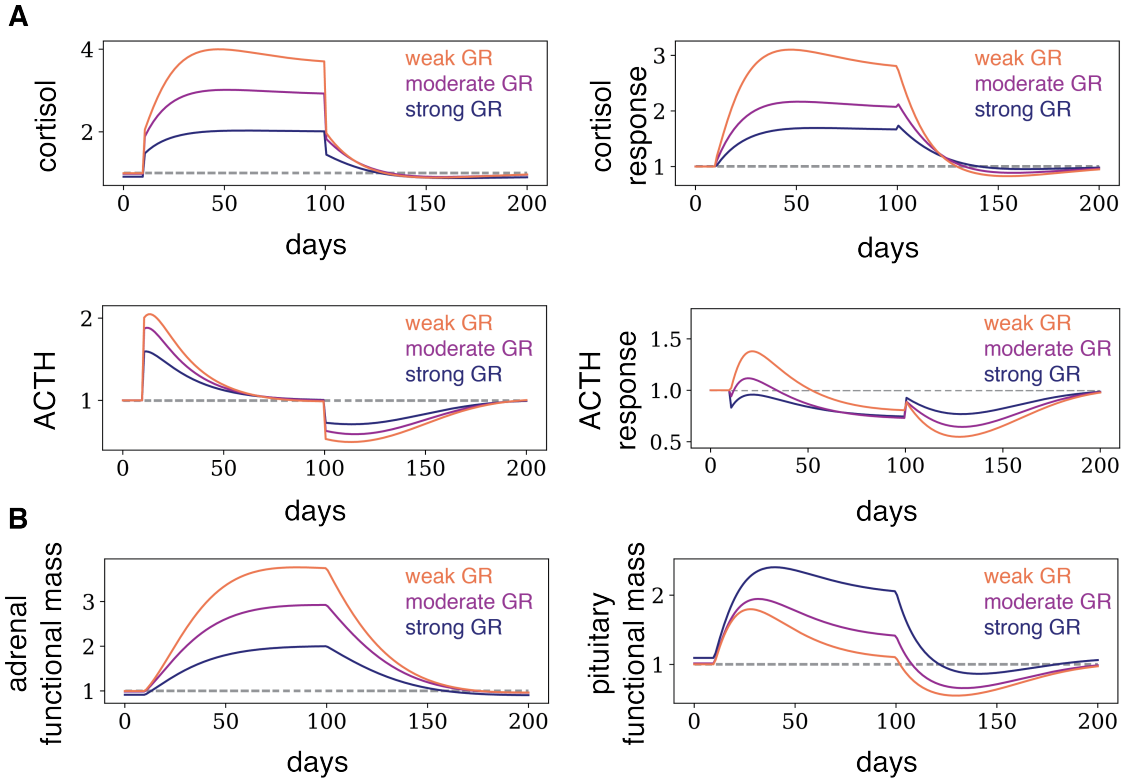

Figure 4. GR provides resilience to the HPA axis against prolonged stressors. Here we show the dynamics of the HPA axis during and after a prolonged pulse of stress, described in Figure 3, for K_{GR}=2 (strong feedback), K_{GR}=4 (moderate feedback), and K_{GR}=8 (weak feedback). Stronger feedback from the GR attenuates the dysregulation of all HPA axis hormones.

The reason for this effect is as follows. After an increase in stress levels ($u_1 \rightarrow u_2$), the adrenal gland mass increases (Figure 4B). When GR feedback is weak ($K_{GR} \gg u_2$), the adrenal increases by about a factor of u_2/u_1. However, if GR feedback is strong ($K_{GR} \ll u_2$), the adrenal increases to a smaller extent, because less cortisol is required to inhibit ACTH to the level required for precise adaptation. The smaller adrenal mass means a smaller dysregulation of cortisol and ACTH after stress. Strong GR feedback therefore provides resilience to the HPA axis against stress on the slow timescale.

Mass changes provide robustness and dynamic compensation to the HPA axis

Finally, we discuss several potential advantages provided by the functional mass changes in the HPA axis. The first advantage is a natural way for the cell populations to maintain their steady-state mass, by balancing growth and removal. Growth and removal must be precisely balanced in order to avoid exponential growth or decay of the tissue. Thus, although cells turn over, the feedback through the hormones couples with the hormone trophic effects to set the masses at a functional level.

308 The second beneficial feature is the robustness of the steady-state hormone levels with
309 respect to physiological parameters. The simple form of the equations for the masses C(t) and
310 A(t) (given in Figure 2) locks the hormones CRH and ACTH into a unique steady-state
311 determined only by the growth and removal parameters of the tissue. Thus $CRH_{st} = \frac{a_C}{b_C}$, and
312 $ACTH_{st} = \frac{a_A}{b_A}$. This is remarkable because all other model parameters do not affect these
313 steady-states. Similarly, the cortisol baseline level, $CORT_{st} \approx \left(\frac{b_1 b_C}{a_1 a_C} u\right)^{1/n_1}$, depends on very
314 few parameters. As appropriate for a stress-response hormone, cortisol steady state depends
315 monotonously on the input u to the hypothalamus (averaged over weeks), which corresponds
316 to physiological and psychological stresses. It is independent on almost all other parameters,
317 including production rate per cell (b_2, b_3) or removal rates (a_2, a_3) of CRH and ACTH, as
318 well as the rates of the proliferation and removal of the adrenal cortex cells b_A and a_A. This
319 robustness is due to the ability of the functional masses to grow or shrink to buffer changes in
320 these parameters.
321 This robustness also makes the steady-state level of cortisol and ACTH *independent of*
322 *total blood volume*. This is because blood volume only enters through the production
323 parameters b_2, b_3. These parameters describe the secretion rate per cell, and since hormone
324 concentration is distributed throughout the circulation, these parameters go as one over the
325 blood volume (b_1 relates to the hypophyseal portal vein and not to total blood volume (70)).
326 The functional masses therefore adjust to stay proportional to blood volume.
327 The third feature of the present model is dynamical compensation, in which masses C
328 and A change to make the fast-timescale response of the system invariant to changes in the
329 production rates b_2, b_3. A full analysis of dynamical compensation in the HPA axis is provided
330 in the SI (Supplementary Section 3).
331 **Discussion**
332 In this study we sought to understand the physiological mechanisms that underlies HPA
333 axis dysregulation after prolonged stress. In particular, we focused on a puzzling phenomenon
334 which recurs in several conditions: ACTH responses remain blunted for a few weeks after
335 cortisol dynamics return to baseline. This cannot be readily explained by existing models of
336 HPA axis activation. We show that a model which incorporates two known interactions that
337 govern functional masses – control of corticotroph growth by CRH and control of adrenal
338 growth by ACTH – is sufficient to explain this dysregulation. The dysregulation is a robust
339 property of this model, and explains clinical data with physiologically reasonable parameters.

This suggests that feedback interactions between masses and hormones may underlie the dysregulation of the HPA axis after prolonged stress.

There may be several implications of the blunted ACTH response that lasts for weeks after stress is removed. One set of psychopathological complications is due to the fact that ACTH secretion is tightly linked with the secretion of endogenous opioids such as β-endorphin. β-endorphin is secreted from the anterior pituitary, and also from POMC-neurons in the hypothalamus, in response to CRH. β-endorphin is cleaved from the same POMC polypeptide (24,71,72) that is the precursor for ACTH. Its secretion, like that of ACTH, is suppressed by cortisol (73). β-endorphin is the endogenous ligand of the mu-opioid receptor (MOR), the primary target of addictive drugs such as morphine and heroin. It mediates euphoria and analgesia in humans and animals (74–79). Dysregulation of β-endorphin occurs in depression (27), anorexia (28,80), and substance abuse disorders (26,81,82). Our results suggest that the dysregulation of β-endorphin, which we assume is similar to that of ACTH, persists for many weeks after cessation of stress. It can therefore contribute to pain and anhedonia in depression and addiction withdrawal.

The present mass model can further provide insight into a physiological hallmark of several psychiatric disorders: deficient GR feedback. Impaired GR feedback is implicated in depression (7), and several genetic and environmental factors have been associated with this impaired feedback (83,84). Deficient GR feedback has been suggested to affect ultradian (57) and circadian (69) HPA rhythms. We find that strong GR feedback (high GR affinity to cortisol) can also work via functional mass dynamics on the timescale of weeks: it protects the HPA axis from dysregulation after prolonged activation. Reduced GR feedback causes larger dysregulation after prolonged stress. This effect is directly mediated by changes in adrenal and corticotroph masses: strong feedback allows smaller changes of adrenal mass during the stress period, and hence to a smaller dysregulation after stress is removed.

Since chronic stress can by itself lead to reduced GR sensitivity ("glucocorticoid resistance") (31), the present findings suggest that prolonged stress makes the HPA less resilient to the next prolonged stress. Perhaps this reduced resilience is relevant to the progression of depressive episodes (85).

If the present model is correct, functional masses are potential targets to address HPA dysregulation. Measuring these masses and their dynamics using imaging may test this model and provide clinically relevant information. Interventions that seek to normalize functional masses can potentially reduce the extent of dysregulation during and after prolonged stresses. One class of interventions may use control-engineering approaches, by periodically measuring

masses or hormones and administering defined HPA agonists or antagonist doses in order to reach desired functional masses and hormone levels (86).

The interactions between functional masses and hormones which underlie the present HPA axis dysregulation also provide exact adaptation to CRH and ACTH (Figure 3) during stress. Exact adaption has been extensively studied in the context of biochemical circuits (87), but less is known about the role of exact adaptation in physiological circuits, and, in particular, about its relevance to psychopathologies. One exception is mass changes in the insulin-glucose system, in which exact adaptation can provide control of glucose levels and dynamics (61) despite changes in insulin resistance, a form of dynamical compensation. It would be fascinating to study what functional roles exact adaptation has in the HPA axis, in order to understand the trade-offs that impact its dysregulation. The present modelling approach may be able to address such questions.

Methods.

HPA axis model.

We model HPA axis using the following equations, where cortisol provides negative feedback on CRH secretion through MR, and negative feedback on both CRH and ACTH secretion through GR. The functional mass of corticotrophs in the pituitary is C, and that of the cortisol-secreting cells in the adrenal cortex is A. We assume a linear dependence of secretion on the upstream hormones (55).

$$\frac{d[CRH]}{dt} = b_1 u(t) \cdot MR([CORT]) \cdot GR([CORT]) - a_1[CRH] \quad [1]$$

$$\frac{d[ACTH]}{dt} = b_2 C[CRH] \cdot GR([CORT]) - a_2[ACTH] \quad [2]$$

$$\frac{d[CORT]}{dt} = b_3\, A[ACTH] - a_3[CORT] \quad [3]$$

$$MR([CORT]) = \frac{1}{[CORT]^{n_1}} \quad [4]$$

$$GR([CORT]) = \frac{1}{1 + \left(\frac{[CORT]}{K_{GR}}\right)^{n_2}} \quad [5]$$

We added to this classic description of the HPA axis the following equations for functional mass dynamics, C(t) and A(t). The equations describe functional mass growth due to the upstream hormones, and mass decline at turnover rates a_C and a_A, which are much smaller than the hormone turnover rates:

$$\frac{dC}{dt} = C(b_C[CRH] - a_C\,) \quad [6]$$

$$\frac{dA}{dt} = A(b_A[ACTH] - a_A) \quad [7]$$

In the simulations of Figure 2, and 3, we use dimensionless units such that the steady state levels of the hormones and functional masses are equal to 1. Thus,

$$\frac{d[CRH]}{dt} = \gamma_{CRH}(u(t) \cdot MR([CORT]) \cdot GR([CORT]) - [CRH]) \quad [8]$$

$$\frac{d[ACTH]}{dt} = \gamma_{ACTH}(C[CRH] \cdot GR([CORT]) - [ACTH]) \quad [9]$$

$$\frac{d[CORT]}{dt} = \gamma_{CORT}(A[ACTH] - [CORT]) \quad [10]$$

$$MR([CORT]) = \frac{1}{[CORT]^{n_1}} \quad [11]$$

$$GR([CORT]) = \frac{1}{1 + \left(\frac{[CORT]}{K_{GR}}\right)^{n_2}} \quad [12]$$

$$\frac{dC}{dt} = \gamma_C C([CRH] - 1) \quad [13]$$

$$\frac{dA}{dt} = \gamma_A A([ACTH] - 1) \quad [14]$$

Where the gamma parameters are the turnover rates. All parameters are provided in Table 1. Code to simulate the equations and generate the figures is provided in https://github.com/omerka-weizmann/hpa_dynamics.We note that one can solve the quasi-steady state of Eqs. [8-10] by setting time derivatives equal to zero. This yields at basal input:

$$[ACTH] \approx C^{\frac{1}{1+n_1}} A^{-\frac{n_1}{1+n_1}} \quad [15]$$

$$[CORTISOL] \approx (CA)^{\frac{n_1}{1+n_1}} \quad [16]$$

$$[CRH] \approx (CA)^{-\frac{n_1}{1+n_1}} \quad [17]$$

Alternative models.

The model without functional mass dynamics is provided by Eq. [8-12]. To this model, we added, instead of gland-mass dynamics, several alternative biological processes that have a slow timescale, potentially on the order of a month. The first process is glucocorticoid resistance, where chronically elevated cortisol levels cause weaker feedback from the GR (31,33). One possible mechanism involves epigenetic effects such as DNA methylation (15). To model GR resistance, we added a variable R that modifies the effective binding coefficient, into Eq. 5, yielding:

$$GR([CORT]) = \frac{1}{1 + \left(R\frac{[CORT]}{K_{GR}}\right)^{n_2}} \quad [18]$$

For R we use the following equation, based on a model of leptin resistance by Jacquier et al. (88). It describes the decline of R with CORT levels:

$$\frac{dR}{dt} = \gamma_R (f([CORT]) - g([CORT])R) \quad [19]$$

For high cortisol levels to induce strong resistance, we use the simple forms: *f(x)=1, g(x)=* $1 + \lambda x^2$.

The second alternative slow process is a slow decrease in input, *u*. We considered an exponentially decreasing input signal (Figure S1).

The third alternative process is a putative slow decrease in cortisol clearance rate. To model this, we added a term C_R to the removal term equation [3]:

$$\frac{d[CORT]}{dt} = \gamma_{CORT}(A \cdot [ACTH] - C_R^{-1}[CORT]) \quad [20]$$

Because there is no well-characterized biological process that governs removal on the scale of weeks, we use a putative description in which cortisol reduces its own removal rate by increasing C_R:

$$\frac{dC_R}{dt} = \gamma_{C_R}([CORT] - C_R) \quad [21]$$

All processes were provided with a month timescale, by setting $\gamma_{C_R} = \gamma_R = \frac{\log(2)}{30} day^{-1}$. Simulation results are shown in the SI.

CRH test.

We modeled the CRH test by adding the following equation for the concentration of externally administrated CRH, denoted $[CRH_E]$:

$$\frac{d[CRH_E]}{dt} = \gamma_{CRH_E}(\delta(t) - CRH_E) \quad [22]$$

Where $\delta(t)$ describes a dose D of external CRH injected at T_{inj} and lasting a time W:

$$\delta(t) = \begin{cases} D & T_{inj} < t \leq T_{inj} + W \\ 0 & otherwise \end{cases}$$

and the removal rate is γ_{CRH_E} as described in (89). Extrinsically administrated CRH causes the pituitary to secrete ACTH, as described by adding CRH_E to the intrinsic CRH (units of CRH_E are set to have equal biological effect to CRH). Thus, [Eq. 9] was modified to:

$$\frac{d[ACTH]}{dt} = \gamma_{ACTH}(C \cdot ([CRH] + [CRH_E]) \cdot GR([CORT]) - [ACTH]) \quad [23]$$

The dose D and pulse width W were calibrated to provide the observed mean CRH test results in non-stressed control subjects, providing D=20 and W=30 min.

Proof for dysregulation of ACTH after cortisol normalization.

We briefly show how the model Eq. 8-14 provide ACTH dysregulation even after cortisol normalizes. Consider a prolonged stressor, like the one presented in Figure 3 (that is, a pulse increase in the input *u*). Since ACTH and CRH are adapted to the stressor, within hours after withdrawal of the stressor, CRH and ACTH levels drop to below their pre-stressor baseline, whereas cortisol remains above its pre-stressor baseline. CRH and cortisol recover simultaneously over a few weeks because CRH dynamics depend only on cortisol and the input *u*. It therefore remains to be shown that ACTH does not recover before cortisol. Since ACTH recovers from below baseline ACTH=1, it recovers with a *positive temporal derivative*

$\frac{d[ACTH]}{dt} > 0$. If (by negation) this happens before CRH and cortisol recover, we will come to a contradiction: since CRH is below its baseline CRH=1, C has a negative derivative due to Eq. 13; the derivative of A is zero due to Eq 7. Because ACTH levels are approximately proportional to $C^{\frac{1}{1+n_1}}A^{-\frac{n_1}{1+n_1}}$ (Eq. 15) we find that ACTH has a *negative temporal derivative* when it crosses its baseline. We come to a contradiction. We conclude that CRH and cortisol return to baseline before ACTH does.

Cortisol shows normal fast timescale response after recovery despite ACTH blunting.

We now use the HPA model Eq. 8-14 to show that when CRH and cortisol levels return to baseline after stress (point IW in Figure 3), their entire dynamics in response to any fast-timescale input (such as a CRH test) normalize, even if ACTH responses have not yet normalized. The mass of the adrenal cortex and pituitary corticotrophs at baseline is denoted A_0, C_0, and their size at the timepoint IW where cortisol and CRH first normalize ($[CORT] = [CRH] = 1$) is $\lambda_A A_0, \lambda_P C_0$. Because cortisol and CRH are at baseline, and both are proportional to the product of gland masses AC (Eq. 15-16), as noted above, one obtains $\lambda_A \lambda_C = 1$. Replacing $[\widetilde{ACTH}] = \lambda_C [ACTH]$ in Eq. 8-10 yields revised equations for the fast timescale dynamics:

$$\frac{d[CRH]}{dt} = \gamma_{CRH}(u(t) \cdot MR([CORT]) \cdot GR([CORT]) - [CRH]) \quad [24]$$

$$\frac{d[\widetilde{ACTH}]}{dt} = \frac{d[ACTH]}{dt} \cdot \frac{1}{\lambda_C} = \frac{1}{\lambda_C}\gamma_{ACTH}\left(\lambda_C C_0 \cdot [CRH] \cdot GR([CORT]) - \lambda_C [\widetilde{ACTH}]\right)$$

$$= \gamma_{ACTH}\left(C_0 \cdot [CRH] \cdot GR([CORT]) - [\widetilde{ACTH}]\right) \quad [25]$$

$$\frac{d[CORT]}{dt} = \gamma_{CORT}(\lambda_A A_0 \cdot [ACTH] - [CORT]) = \gamma_{CORT}\left(A_0 \cdot [\widetilde{ACTH}] - [CORT]\right) \quad [26]$$

Note that Eq. [24-26] are the same as Eq. [8-10] when $A = A_0$ and $C = C_0$, that is, they are independent of λ_A, λ_C. In addition, the initial conditions for the hormones are also independent of λ_A, λ_C, because $[CORT] = [CRH] = 1$ are at baseline, while ACTH, which is proportional to $C^{\frac{1}{1+n_1}}A^{-\frac{n_1}{1+n_1}} = C_0^{\frac{1}{1+n_1}}A_0^{-\frac{n_1}{1+n_1}} \cdot \lambda_C$, scales with λ_C, so that $[\widetilde{ACTH_{ST}}]$ is also independent of λ_A, λ_C. We conclude that after cortisol and CRH return to baseline, the fast timescale dynamics of cortisol and CRH to any input (such as a CRH test) becomes normalized and equal to the dynamics before the stressor. In the simulations, after CRH and cortisol have normalized for the first time, they only deviate from baseline slightly (at most 9%), and so this consideration holds approximately for further times in the scenario of Figure 3.

Table 1. Parameter values.

Parameter	Value
γ_{CRH}	0.17/min (55)
γ_{ACTH}	0.035/min (55)
γ_{CORT}	0.0091/min (55)
γ_P	0.099/day
γ_A	0.049/day
K_{GR}	4
γ_{CRH_E}	0.016/min (89)
W	30 min
D	20
γ_{C_E}	0.023/day
λ	1
γ_R	0.023/day
n_1	1
n_2	3 (55)

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