**HIGHLIGHTS**

- Longitudinal cortisol over a year was measured using hair from 55 healthy individuals.
- A method was developed to adjust for decline along the length of the hair.
- Cortisol showed non-seasonal fluctuations with periods of months to a year.
- These fluctuations can be explained by turnover time of cortisol-related glands.

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**Cortisol rythms**

- **Known:**
  - Circadian cortisol
    - Peaks at 8am and 8pm
  - Seasonal cortisol
    - Increase in winter and decrease in summer

- **Unknown:**
  - Month-scale cortisol
    - Fluctuations in months 1 to 11

**Hair cortisol measurement and mathematical model of the HPA axis explain month-scale frequencies**

- 12 cm = 6 segments = 1 year
- Dominant frequency:
  - 1 year: 22%
  - 2 years: 0%
  - 3 years: 0%

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**References**

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SUMMARY
Cortisol is a major human stress hormone, secreted within minutes of acute stress. Cortisol also has slower patterns of variation: a strong circadian rhythm and a seasonal rhythm. However, longitudinal cortisol dynamics in healthy individuals over timescales of months has rarely been studied. Here, we measured longitudinal cortisol in 55 healthy participants using 12 cm of hair, which provides a retrospective measurement over one year. Individuals showed (non-seasonal) fluctuations averaging about 22% around their baseline. Fourier analysis reveals dominant slow frequencies with periods of months to a year. These frequencies can be explained by a mathematical model of the hormonal cascade that controls cortisol, the HPA axis, when including the slow timescales of tissue turnover of the glands. Measuring these dynamics is important for understanding disorders in which cortisol secretion is impaired over months, such as mood disorders, and to test models of cortisol feedback control.

INTRODUCTION
Cortisol is a major stress hormone in humans. It is secreted in response to psychological and physiological stress, under control of a cascade of hormones called the HPA (hypothalamus-pituitary-adrenal) axis. Cortisol has receptors in most cell types and exerts widespread actions that help the organism to prepare for stressors and to cope with them (Buckingham, 2009; McEwen, 1998; Sapolsky et al., 2000). Cortisol dysregulation is implicated in physiological and psychological pathologies, such as mood disorders, including depression (Daban et al., 2005; Ehlert et al., 2001; Nemeroff et al., 1992; Pearson Murphy, 1991; Sriram et al., 2012; Watson and Mackin, 2006; Wingenfeld and Wolf, 2015).

Cortisol has dynamics on several timescales (Figure 1A). It shows ultradian pulses throughout the day that last about 60–90 min (Young et al., 2004). Pulse amplitude is largest in the morning, forming a sizable circadian pattern (Walker et al., 2010). Cortisol also has a seasonal rhythm, which peaks in late winter (Hadlow et al., 2018; Miller et al., 2016; Tendler et al., 2020). The amplitude of this seasonal rhythm is a few percent in temperate clines and rises to tens of percents at high latitudes. Detecting this seasonal rhythm requires averaging over many individuals. On the timescale of decades, cortisol generally decreases at old age on average (Sharma et al., 1989).

Overlaid on top of these daily and seasonal patterns is the response of cortisol to the stressors that occur over time. Thus, one expects cortisol dynamics to fluctuate over weeks, months, and years in each individual. However, the nature of these slow fluctuations and their typical timescale have not been quantified.

Understanding fluctuations on a timescale of months is important to better understand the feedback loops in the HPA axis. Cortisol inhibits its upstream hormones (Figure 1B) in a classic feedback loop that acts on the timescale of minutes to hours (Andersen et al., 2013; Ronald De Kloet et al., 1998). This feedback can explain ultradian rhythms on the scale of hours (Walker et al., 2010). A recent model by Karin et al., 2020 points to an additional feedback loop, which acts over months. In this feedback loop, the functional mass of the glands in the HPA axis changes over time, under the control of the hormones that act as growth factors (Bicknell et al., 2001; Gertz et al., 1987; Horvath et al., 1999; Kataoka et al., 1996; Nolan et al., 1998; Westlund et al., 1985) (Figure 1C). This effectively forms a feedback loop between two glands in the HPA axis, the adrenal cortex cells and the pituitary corticotrophs (Figure 1D). This feedback loop is predicted to have a typical timescale of about a year, due to the slow timescale of tissue turnover (Figure 1E). Intuitively, this model predicts that stressors over time would stimulate the natural period of this feedback loop,
Understanding cortisol fluctuations in healthy individuals can also set a baseline to compare with the situation in mood disorders that play out over months, such as depression and bipolar disorder (Belvederi et al., 2016). Future studies of cortisol dynamics in individuals with mood disorders would benefit from a solid understanding of the control group, namely, cortisol fluctuations in healthy individuals.

Here we explore the dynamics of cortisol in healthy participants using longitudinal measurements over 1 year. We use hair cortisol, a measure that allows retrospective quantification (D’Anna-Hernandez et al., 2011; Davenport et al., 2006; Mayer et al., 2019). Cortisol passively lies in hair. Each centimeter of hair corresponds to about 1 month of growth, and thus analyzing hair allows measurement of cortisol levels averaged over long times (D’Anna-Hernandez et al., 2011; Smy et al., 2016). We developed a procedure to adjust for the decay of cortisol over 12-cm hair samples and use Fourier analysis to quantify the contribution leading to noisy fluctuations of cortisol with a timescale of about a year (added on top of the much smaller seasonal pattern).

Understanding cortisol fluctuations in healthy individuals can also set a baseline to compare with the situation in mood disorders that play out over months, such as depression and bipolar disorder (Belvederi et al., 2016). Future studies of cortisol dynamics in individuals with mood disorders would benefit from a solid understanding of the control group, namely, cortisol fluctuations in healthy individuals.
RESULTS
Longitudinal Cortisol Fluctuates around Its Baseline
We collected 12 cm of hair from healthy participants and assayed cortisol using ELISA in 2-cm segments. This provided a longitudinal time-series of six time points over about a year of growth (Figure 2A).

We corrected each time series for the decline of cortisol in distal segments by fitting an exponential decay model to each participant’s cortisol measurements (see Methods). The mean decay coefficient was $\approx 2.2 \pm 0.2$ (year$^{-1}$) (standard error of the mean, SEM). The exponential fit allowed estimate of the baseline cortisol for each individual. The baseline varied about 30-fold between individuals, as can be seen in Figure 2A.

After normalizing by the decline, the fluctuations around each individual baseline can be seen in Figure 2B.

Normalized cortisol showed fluctuations around the mean with an average coefficient of variance (CV) of 28%. When accounting for experimental noise with CV = 14% (see Methods), one obtains fluctuations with CV = 24%.

Longitudinal Hair Cortisol Shows a Dominant 1 Year$^{-1}$ Frequency Component
To explore the frequencies underlying these fluctuations, we used Fourier analysis, which quantifies the contributions of different frequencies to the signal. Six segments allow three frequencies to be detected: 1 (year$^{-1}$), 2 (year$^{-1}$), and 3 (year$^{-1}$), representing periods of a year, 6 months, and 4 months, respectively.

We averaged the Fourier amplitudes over the participants. The highest mean amplitude was obtained at the slowest frequency, 1 (year$^{-1}$) (Figure 3, black dots). This amplitude was about 1.45 $\pm$ 0.17 times higher than the amplitude at the highest frequency, 3 (year$^{-1}$).

To test the statistical significance of this observation, we compared the Fourier spectrum to a null model constructed by shuffled data and subjected to the same correction for cortisol decline (Methods, Figure 3, gray dots). The amplitude of the 1 (year$^{-1}$) frequency was significantly higher than shuffled control with a large effect size ($p = 0.004$, Cohens $d = 3.8$, non-parametric $p = 0.002$, Cliff’s delta = 0.99).

Note that the null model has lower amplitudes in the 1 (year$^{-1}$) frequency compared with the other frequencies. This decrease in the slowest frequency is due to correction for the cortisol decline along the hair: the subtraction (in log-transformed variables) of an exponential fit from the raw signals removes a major portion of the slowest frequency component from the normalized signals. This is generally true also for
random data, such as those generated from a normal distribution. The experimental data show high amplitude at low frequency (1 \( \text{year}^{-1} \)) despite this effect.

We also conducted a non-parametric correction for cortisol decline (see Methods Supplemental Information, section S4), which gave the same conclusion of a dominant 1 \( \text{year}^{-1} \) frequency component in cortisol spectrum.

We also corrected for seasonality—the effect of the month of the year on cortisol—by fitting the decline-corrected cortisol to a cosinor model. Cortisol showed a seasonality effect of about 15%. When corrected for seasonality (Supplemental Information, section S3), the CV of the decline-corrected fluctuations dropped from 24% to 22%. The \( \text{year}^{-1} \) frequency remains the dominant frequency. This indicates that the slow cortisol variations in each individual go beyond a seasonal effect.

Model of the HPA Axis with Gland Mass Dynamics can Explain the Year-scale Fluctuations

We asked what biological processes might underlie the observed frequency spectrum of hair cortisol, with large amplitude at \( \text{year}^{-1} \) frequency. One possibility is that stressor inputs to the HPA axis have dominant low-frequency components. For example, due to life events, some periods of several months may be more stressful than others (Dettenborn et al., 2010; Rothman, 1972; Staufenbiel et al., 2013), contributing to the observed fluctuations (beyond the variation with season).

Here we consider in detail an alternative explanation, in which the interactions within the HPA axis contribute to the low-frequency cortisol fluctuations. We thus ask whether stressor inputs randomly distributed over time can generate cortisol fluctuations with typical fluctuations of a year.

We simulated and compared two models of the HPA axis. First is the classic HPA model (Figure 1B), in which the only feedback mechanism is inhibition by cortisol to upstream hormones. This model has timescales given by the hormone half-lives, namely, hours. Simulating this model with white-noise stress input results in a Fourier spectrum without dominant \( \text{year}^{-1} \) frequency. Instead, all frequencies are found with approximately the same amplitude. Applying the decline correction to the simulated data, as done to the experimental data, results in a spectrum that is indistinguishable from the null model (Figure 4A).

In contrast, we simulated the model of Karin et al. (Figure 1C), which includes the changes of gland masses with typical timescale of months. Using the same input signals, this model provides a dominant \( \text{year}^{-1} \) frequency component (Figure 4B). The period of 1 year arises from the tissue turnover times, which are
on the order of a few weeks, due to the $2\pi$ term in the equation $period = \frac{2\pi}{frequency}$. The only model parameters that affect the timescales of months-years are the two tissue turnover times. All other model parameters are of the fast timescale of hours, such as hormone turnover times and secretion rates, and do not contribute to the dynamics on this slow timescale. This can be shown analytically using the Laplace transform on linearized model equations (Supplemental Information, section S6), as well as by a detailed sensitivity analysis using simulations (Supplemental Information, section S5).

We find that the turnover times of the two tissues in the model, corticotrophs and cortisol-secreting cells in the adrenal cortex, can range from days to months to obtain a similar shape to the observed Fourier spectrum. Thus, we conclude that the model is sufficient to explain the timescales of the measured cortisol fluctuations.

**DISCUSSION**

We analyzed longitudinal hair cortisol in healthy participants. Cortisol fluctuated by about 22% around the baseline, with variations that have a dominant low-frequency component with a period of 1 year. This variation goes beyond the effect of seasonality. The slow variations are consistent with a recent model of the HPA axis, which can explain low frequencies by the hormonal regulation of the functional mass of the cells that secrete adrenocorticotropic hormone and cortisol, namely, pituitary corticotroph cells and adrenal cortex cells. The timescale of months to years arises due to the slow turnover time of the tissues.

Hair has advantages for longitudinal cortisol profiling on the timescale of months, due to its low invasiveness, ease of acquisition, convenient storage, and the ability of hair to record cortisol from the past. A technical advance in this study was to correct for the decay in cortisol distally along the hair. This decay was reported in several previous studies and has led many researchers to use only the first few centimeters of hair closest to the scalp (Kirschbaum et al., 2009; Mayer et al., 2019; Schonblum et al., 2018). Here we corrected the decline by fitting an exponential decay to each time series and compared this to shuffled controls. This allowed us to estimate the extent and significance of the Fourier amplitudes over a year of dynamics.

One limitation of this study is that 12 cm of hair does not allow rhythms slower than 1 year to be detected. Indeed, the HPA model suggests that such slower rhythms are expected. As cortisol in most samples decayed close to background detection levels after 12 cm, a future longitudinal study with multiple hair samples is needed to estimate the amplitude of slower Fourier frequencies.

The present agreement with the Karin et al. HPA model adds to a picture in which the functional masses of the cells in the HPA axis are important variables. These masses are not considered in standard models of
the HPA axis. Karin et al. showed that such mass changes are sufficient to explain HPA dysregulation after prolonged stress. (Tendler et al., 2020) proposed that the same model can explain seasonal entrainment of hormones and their seasonal peaks and troughs. Here, we propose that the mass changes provide a “memory” to the HPA axis, which can integrate over the fast timescale fluctuations of stress inputs to generate fluctuations that last on the timescale of a year. Future work can further test this model by measuring gland masses over time and correlating them with hormonal measurements.

The HPA model studied here is not the only possible explanation for the slow fluctuations with periods of months to years. These fluctuations may also arise due to slow timescales in the distribution of stressors over time. Such slow timescales arise in many human activity patterns, which have long-tailed distributions of intervals between events (Vázquez et al., 2006). Investigating the role of the temporal stressor distribution requires following stressors over time and may be the focus of future work. Additional effects can introduce long timescales, including the effects of epigenetic regulation in the HPA axis.

The present study can serve as a baseline for future exploration of stress-related pathologies. For example, mood disorders display HPA dysregulation, but the precise dynamics of this dysregulation remains to be clarified in conditions such as bipolar disorder. Hair offers a rare opportunity to look retrospectively from the time of first diagnosis, showing the dynamical prodrome to disease onset. Cortisol dynamics can be used to test models of pathology, and to provide a detailed diagnostic for HPA axis function.

In the long term, one may envision using hair cortisol longitudinal dynamics as a basis for stabilizers of psychological diseases that involve dysregulation of the HPA axis on the scale of months (other HPA syndromes such as Addisonian crisis can have much faster timescales and cannot rely on hair measurements). In this paradigm, the goal is to return dysregulated HPA function back to baseline using a feedback-control approach. One measures cortisol, simulates a model of the axis, and determines the optimal dose of HPA agonists or antagonists to take in the next time period (e.g., a month) to return dysregulated HPA function back to baseline (Ben-Zvi et al., 2009). Following the interventions, hair measurements can be used to test if the desired state was reached.

Limitations of the Study
This study involved a sample of only 55 individuals from one country. Future work can enlarge sample size and sample additional populations, which is important given the large person-to-person variability in cortisol. Use of other methods to measure cortisol, such as mass spectrometry, can test the validity of the results. Use of multiple short hair samples from the same individual can extend the study period beyond 12 months and test the validity of the decline correction.

Resource Availability
Lead Contact
Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Uri Alon (uri.alon@weizmann.ac.il).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
The data and code generated during this study are available at https://github.com/tomermilo/hair-cortisol.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101501.
ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

L.M., T.M., and U.A. designed the study, L.M. recruited participants, L.M., R.S.M., T.D. and A.B performed the experiments, T.M., A.M. and L.M. analyzed the data, L.M., T.M. and U.A. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Timescales of Human Hair Cortisol Dynamics

Lior Maimon, Tomer Milo, Rina S. Moyal, Avi Mayo, Tamar Danon, Anat Bren, and Uri Alon
Supplemental Information for “Tim escales of human hair cortisol
dynamics over one year”

Transparent Methods

Ethics statement

The study protocol was approved by the Review Board of the Weizmann Institute of
Science (study code: 706-1). Written informed consent was obtained from all participants.

Participants

Healthy participants (N=59, females=45, average age=27±6) were recruited during
January-December of 2019 through social media. Inclusion criteria: participants older
than 18 years, with at least 12 centimeters of natural hair with no cosmetic treatment such
as dying or perming (Cooper, 2015). Exclusion criteria: diagnosis of a mental,
psychological or endocrine disorder, consumption of steroids, psychiatric drugs or other
drugs that might affect the endocrine system during the year prior to participation, and
pregnancy in the year before participating. Oral contraceptives were allowed as long as
there was no change in prescription during the year prior to participation. Each subject
was asked to complete a personal information questionnaire prior to the collection of a
hair sample. The study was anonymous; each hair sample received a serial number. Male
height was 179±6 cm, weight 73±11 kg and BMI (body mass index) 22±3; Female height
was 164±6 cm, weight 61±9 kg and BMI 23±3 (all values are mean±STD). 26% of the
females reported taking oral contraceptives.

Hair Cortisol Measurements

A lock of hair (a pencil-width group of about 100 hair strands) from the vertex posterior
area (Cooper, 2015; Sauvé et al., 2007) of the head was tied with a thread and cut with
fine scissors as close to the scalp as possible. We estimate that hair is cut at 0.8±0.1 cm
from the scalp, in agreement with Cooper et al. (2015). Cut samples were masking-taped
at their distal end to a piece of aluminum foil; the tied thread marked the proximal end.
Samples were kept in the laboratory at room temperature before analysis.

We adapted a protocol by Schonblum et al. (2018) for the extraction and measurement
of hair cortisol. The first 12 centimeters of each hair sample, starting from the proximal
end, were segmented to six 2 cm segments (segments of 1 cm dropped below the
detection threshold too often and thus 2 cm segments were used). The segments were
placed in vials (Fisherbrand, 21x70 mm) and washed twice with 5 ml isopropanol while
mixing on an orbital rotator for 3 minutes. Isopropanol was then decanted and the open vials were left in a chemical hood to dry overnight. Then, 2 ml of methanol was added to each vial, sonicated for 60 min, and incubated overnight (approximately 20 hours) at 50°C while shaking. The following day, all the methanol was transferred to 2 ml Eppendorf tubes and centrifuged for 10 minutes at 4°C. Methanol (1.5 ml) from each tube was transferred to a glass vial (Falcon, 12x75 mm) and evaporated under a stream of nitrogen at 45 °C. Samples were reconstituted in 10% methanol and 90% assay buffer provided by the kit manufacturer and cortisol was quantified using competitive Enzyme-Linked Immunosororbent Assays (ELISA; Salimetrics Europe, Newmarket, cat.no1-3002-5 for cortisol, UK). Reported antibody cross-reactivity was 19.2% with dexamethasone, and less than 1% with 15 other tested steroids. Linearity was observed between 30-70 mg of hair, hence we used 30-70 mg of hair to measure cortisol. The assay detection threshold was 112 pg as specified by the manufacturer. All 6 segments from the 12 cm sample of hair from each participant were analyzed in the same batch of washing, sonication, extraction and ELISA plate. To control for inter-assay variation, we generated a standard curve for each plate, consisting of 6 known concentrations of cortisol supplied by the kit manufacturer assayed in 6 wells. To estimate the inter-batch variation, we assayed multiple standard hair samples. Each standard sample was taken from a large, well-mixed, sample of hair collected from a single individual. The coefficient of variation (CV=STD/mean) of 13 standard samples measured on 2 different day was 14%.

We included in this study the 55 participants (32 females) with all 6 cortisol measurements above detection threshold. Mean cortisol levels did not show a significant correlation with age (spearman r=0.19, p=0.2), weight (spearman r=0.01, p=0.9), height (spearman r=0.06, p=0.7), and BMI (spearman r=0.03, p=0.8). Mean cortisol levels did not significantly differ between the sex groups (Mann Whitney U test, p=0.22) or by taking oral contraceptives (Mann Whitney U test, p=0.35).

Analysis of cortisol time series

Each hair sample provided six 2 cm segments. The average rate of scalp hair growth in humans is approximately 1 cm/month, with a reported range of 0.6 to 1.5 cm a month (Cooper, 2015); we thus assumed that each 2 cm segment represents two months of growth and contains cortisol that accumulated during that period. Due to factors including hair washing (Hamel et al., 2011), cortisol levels in hair decline as a function of distance from the scalp (Gao et al., 2010; Kirschbaum et al., 2009; Steudte et al., 2011). Studies on glycosylated proteins in hair showed a similar decline that was well-described as exponential with time (Nissimov et al., 2007). To account for the decline, we performed a linear regression on the log of the measurements, equivalent to assuming an exponential decline of $A_i \exp(-\alpha_i t_i)$ for individual $i$, where $t_i$ is the time corresponding to segment $i = 1 \ldots 6$, assuming hair growth rate of 1 cm/month. We constrained the slope to be negative
Thus, if \( \hat{c}_{ij} \) is the raw cortisol measurement, we define \( z_{ij} = \log(\hat{c}_{ij}) \), then use linear regression on \( z_{ij} \) to define the decline as \( d_{ij} = \log(A_j) - \alpha_j t_i \), and subtract this from \( z_{ij} \) to obtain the normalized log cortisol in segment \( i \) for person \( j \), \( y_{ij} = \log(c_{ij}) = z_{ij} - d_{ij} \). Fourier analysis was computed on \( c_{ij} = \exp(y_{ij}) \) using the dFFT function of python v3.7.4, numpy v1.16.5.

Fourier analysis is a widely-used method to decompose a time-varying signal into its constituent frequency components. It provides a breakdown of the signal into a sum of sine waves of different frequencies. Each sine wave has an amplitude and a phase. The higher the amplitude at a given frequency, the higher the contribution of that frequency to the signal.

The number of different frequencies provided by this analysis equals half of the number of time-points in the signal. For a signal with six time-points, three frequencies are available: the lowest frequency corresponds to a period equal to the total duration \( D \) of the 6 measurements, and the two other frequencies correspond to periods that are 1/2 and 1/3 of \( D \). In the present case, \( D=1 \) year, and the frequencies correspond to sine waves with periods of 1 year, 6 months and 4 months.

We also estimated the effect of the month of the year on cortisol measurements. For this purpose, we averaged the decline-corrected \( c_{ij} \) according to the calendar month corresponding to the middle of the segment, taking into account an offset of 3mm inside the scalp and 8mm outside the scalp at the point of hair cutting, and a growth rate of 1cm/month. We then fit the resulting average, denoted \( C(t) \) where \( t \) is the month of the year, to a cosinor model \( Acos(\omega t + \phi) \) with \( \omega = \frac{2\pi}{12 \text{ month}} \). To correct for month of the year, we then normalized \( c_{ij} \) by the best-fit cosinor model using the month of the year for each mid-segment (see SI, S3).

To estimate significance, we compared our results with the Fourier analysis of a shuffled control. We shuffled the segments \( i = 1 \ldots 6 \) within each participant's normalized data \( c_{ij} \). This yielded shuffled data, \( s_{ij} \). The shuffling keeps each participant's normalized cortisol distribution but breaks temporal correlations. We then added the best-fit decline of that participant, to get simulated raw log data, \( z'_{ij} = \log(s_{ij}) + d_{ij} \). We then repeated the analysis by fitting the decline with a new regression (which yields a slightly different decline, \( d'_{ij} \), due to the data shuffling), subtracted the decline \( d'_{ij} \) to obtain \( y'_{ij} = z'_{ij} - d'_{ij} \), and performed the same Fourier analysis on \( \exp(y'_{ij}) \). This controls for the fact that the decline correction affects the long-wavelength components of the data. We repeated this procedure 1,000 times in order to estimate statistical significance (See SI, S2 for details).
We also performed a second, non-parametric analysis. We assumed that the decline of cortisol is monotonic with distance from the proximal segment. We therefore performed a rank regression on $\hat{c}_{ij}$ versus segment number ($i = 1...6$). We then subtracted the rank regression from the rank of $\hat{c}_{ij}$, to obtain the rank residuals $r_{ij}$. Finally, we performed a Fourier analysis on $r_{ij}$. As a shuffled control, we shuffled $r_{ij}$ within each participant $j$, added the rank regression for that participant, rank-regressed again, and performed Fourier analysis on the residuals. The results are qualitatively similar to the parametric test and are shown in the SI, S4.

**HPA model**

We employ a recently developed model for the HPA axis, which incorporates the effects of the hormones on the total functional mass of hormone-secreting cells (Karin et al., 2020; Tendler et al., 2020). The concentrations of the hormones CRH, ACTH and cortisol are denoted $x_1, x_2$ and $x_3$. The input to the hypothalamus, which describes the combined impact on CRH secretion due to physiological, circadian, and psychological stressors is denoted $u$. The total functional mass of pituitary corticotroph cells that secrete ACTH is $C$, and that of the adrenal cortex cells that secrete cortisol is $A$. The secretion of CRH due to input $u$ is as follows:

$$\frac{dx_1}{dt} = b_1 u f(x_3) - a_1 x_1$$

Where $b_1$ is the secretion parameter of CRH, and $a_1$ is CRH removal rate. $f(x_3)$ describes the feedback by cortisol, due to the mineralocorticoid and glucocorticoid receptors (MR and GR, respectively) in the hypothalamus, $f(x_3) = MR(x_3) GR(x_3)$. Since the high-affinity receptor MR is usually bound by cortisol at physiological levels we use an approximation to the Michaelis–Menten binding kinetics; GR is a cooperative (n) receptor that binds cortisol with lower affinity, $K_{GR}$:

$$MR(x_3) = \frac{1}{x_3}$$

$$GR(x_3) = \frac{1}{1+(x_3/K_{GR})^n}$$

The dynamics of ACTH are as follows:

$$\frac{dx_2}{dt} = b_2 x_1 C g(x_3) - a_2 x_2$$

While $b_2$ is the secretion parameter per unit corticotroph functional mass. The parameter $b_2$ includes per-cell effects with signaling pathways such as CRH receptor numbers per cell, neuronal inputs and cytokine inputs that affect corticotrophs. $g(x_3)$ is the feedback from cortisol due to the GR receptors in the pituitary, $g(x_3) = GR(x_3)$. 

The dynamics of cortisol are as follows:

\[
\frac{dx_3}{dt} = b_3 x_2 A - a_3 x_3
\]

While \(b_3\) includes all per-cell effects on cortisol secretion rates, and \(a_3\) is cortisol removal rate. To this classical model, Karin et al. added the effects of the hormones on the total functional mass \(C(t)\) and \(A(t)\), which are important for the present study. The mass changes can be due to cell division (hyperplasia) or cell growth (hypertrophy); the precise mechanism is not important for the present analysis. The main growth factor for corticotrophs is CRH, so that proliferation rate is \(b_c x_1\) and removal rate is \(a_c\), resulting in the following:

\[
\frac{dC}{dt} = C(b_c x_1 - a_c)
\]

Note that \(C\) occurs in both proliferation and removal terms, because differentiated corticotrophs divide to produce new corticotrophs (Gulyás et al., 1991) (with additional supply, not considered here, from pituitary stem cells (Andy Levy, 2007; Nakane et al., 1977)). The main growth factor for cortisol-secreting cells in the adrenal cortex is ACTH and therefore:

\[
\frac{dA}{dt} = A(b_A x_2 - a_A)
\]

The timescales for the change in mass are governed by the removal rates \(a_A\) and \(a_C\), which are experimentally found to be on the scale of weeks in model organisms (A Levy, 2002; Nolan et al., 1998; Swann, 1940; WESTLUND et al., 1985). An analytical solution of the steady state stability found a spiral fixed point with a period on the order of a year (Tendler et al., 2020). The parameter values used in the present simulations are given in table 1 (SI, S1). In a version of the model without cell mass dynamics, we used constant \(A(t) = C(t) = 1\) and omitted equations 6 and 7. We numerically solved the model using python’s solver, ‘odeint’ of scipy v1.3.1 (Virtanen et al., 2020) The input was piecewise-constant \(u\) in every 2-month time period, with a value of \(u\) drawn from a lognormal distribution with a mean of 1 and a standard deviation (STD) of 0.65, determined to give cortisol STD similar to measurements. We simulated four years of dynamics, and took the last year for analysis, in order to avoid transients due to initial conditions. We simulated individual participant data by multiplying \(x_3\) by the exponential decline fit for that participant and averaging this over six consecutive 2-month periods. This generated a simulated dataset with the same number of participants as the experimental data. We then performed the same analysis as for the experimental cortisol measurements. To test the significance of the model results, we repeated this procedure 1,000 times with new simulations.
To complement the empirical analysis with simulations we performed an analytical spectral analysis on the linearized model equations, using Bode plots (SI, S6).

S1. Table of reference parameter values, related to Figure 4

| Parameter | Value       | Reference            |
|-----------|-------------|----------------------|
| $b_1$     | 0.17 [1/min]| (Andersen et al., 2013) |
| $b_2$     | 0.035 [1/min] | (Andersen et al., 2013) |
| $b_3$     | 0.0091 [1/min] | (Andersen et al., 2013) |
| $b_p$     | 1/30 [1/day] | (Nolan et al., 1998) |
| $b_A$     | 1/30 [1/day] | (Kataoka et al., 1996) |
| $K_{GR}$  | 4           | (Karin et al., 2020) |
| $n$       | 3           | (Andersen et al., 2013) |

S2. Statistical tests for significance of Fourier amplitudes

We tested the significance of the mean Fourier amplitude at $1[year^{-1}]$ compared to a shuffled control in which the 6 time-points of each individual are shuffled. For this purpose, we generated 1000 bootstrapped datasets in which we chose from the 55 participants with returns. For each bootstrapped dataset we computed the mean Fourier amplitude (A) at $1[year^{-1}]$. This results in a distribution $P_{bootstrap}(A)$. We then shuffled the time points of each participant and generated 1000 shuffled datasets. We computed the mean Fourier amplitude at $1[year^{-1}]$ for each shuffled dataset, to obtain $P_{shuffled}(A)$. We find that both distributions are very close to Normal, as expected for distributions of means. We then computed significance in two different ways, parametric and non-parametric. The parametric test used the normality of the distribution to calculate the weighted p value
and effect size (Cohens d) analytically. The non-parametric calculation asked how often $P_{shuffled}(A)$ exceeds $P_{bootstrap}(A)$. The effect size was calculated using Cliff's delta whose range is [-1,1]).

S3. Correction for seasonality shows a dominant $year^{-1}$ frequency

We also analyzed the data according to calendar months, using a cosinor analysis. Hair cortisol showed a seasonal amplitude of 15%±3 with a peak at May-June. We correct for seasonality by dividing the decline-corrected cortisol values for each segment by the cosinor model for the relevant months (Methods), and repeated the Fourier analysis.

Fourier analysis shows that the lowest frequency of 1 $year^{-1}$ remains the dominant frequency (Figure S1). It exceeds shuffled control significantly ($p=0.01$, Cohen’s D=3.2, non-parametric $p=0.01$, Cliff’s delta=0.98). We conclude that hair cortisol shows variations on the scale of a year that go beyond seasonality.

Figure S1. Hair cortisol shows fluctuations with a dominant period of 1 year, after correcting for seasonality, related to Figure 3. Fourier amplitudes averaged over participants quantify the contribution of each frequency component ($1[year^{-1}]$, $2[year^{-1}]$ and $3[year^{-1}]$) to the cortisol signal (black dots). Error bars (SEM) were calculated by bootstrapping the participants. Shuffled control is shown as gray dots (1,000 repeats), with 97.5% and 2.5% confidence intervals shown in dashed gray lines.
The present finding of a May-June peak of hair cortisol is not consistent with most previous cortisol seasonality studies that identify peak cortisol (acrophase) in winter (Hadlow et al., 2018; Persson et al., 2008; Tendler et al., 2020), including a study on hair cortisol on 3,507 British civil servants (Abell et al., 2016). There is one exception of a large study with a peak phase in summer - a study from Netherlands on 1,768 children (age 10-12) (Rosmalen et al., 2005). The reason for the discrepancy of the present peak season (acrophase) with most previous studies is not clear.

S4. Non-parametric correction for cortisol decline shows dominant \( \text{year}^{-1} \) frequency as well

We made a non-parametric correction for the cortisol decline along the hair by rank regression (see Methods). Despite the large loss of information due to using ranks, the \( 1 \text{ year}^{-1} \) frequency showed a trend of being higher than shuffled control \((p=0.06, \text{Cohen's } d=2.2; \text{nonparametric } p=0.06, \text{Cliff's delta}=0.88)\) (Figure S2). The highest mean amplitude was obtained at the slowest frequency, \( 1 \text{ year}^{-1} \), this amplitude was about \( 1.3 \pm 0.2 \) times higher than the amplitude of the highest frequency, \( 3 \text{ year}^{-1} \).

Figure S2. Rank-normalized hair cortisol shows fluctuations with a dominant \( \text{year}^{-1} \) frequency, related to figure 3. Mean Fourier amplitudes of hair cortisol data, which was normalized by rank regression (black dots with 1 STD error bars obtained by bootstrapping). Shuffled control is shown as gray dots (1,000 repeats), with 97.5% and 2.5% confidence interval shown in dashed gray lines.
S5. Sensitivity analysis of HPA model simulations

We tested the sensitivity of the main conclusion of the simulation analysis to the model parameters. We varied each of the five model parameters around its reference value (table S1) by a factor of up to $2^{10} = 1024$-fold. For each case, we repeated the simulation of Figure 4. We computed the ratio $R$ between the $1\text{ year}^{-1}$ and $3\text{ year}^{-1}$ amplitudes. Figure S3 shows the percent change in $R$ as a function of the fold-change in each parameter. The slow-timescale parameters for the tissue turnover processes, $b_A$ and $b_p$, match the observed ratio within its experimental error (gray region) over an approximately 8-fold range around their reference values. The fast timescale parameters, that describe hormone production and removal, have minor effects on $R$ across the entire 1024-fold range (inset). We conclude that the dominance of the $1\text{ year}^{-1}$ frequency is insensitive to the model parameters.

Figure S3. Sensitivity analysis of HPA model with mass dynamics suggests that the dominance of the low frequency is insensitive to all model parameter, related to Figure 4 and Table S1. Each of the five model parameters was varied around its reference range by a range of $2^{10} = 1024$. For each case, the simulation of Figure 4 was repeated, and the ratio between the $1\text{ year}^{-1}$ and $3\text{ year}^{-1}$ amplitudes was computed. The plot shows the percent change in the ratio as a function of the fold change in each parameter. The slow-timescale parameters for the tissue turnover...
processes, $b_A$ and $b_P$, match the observed ratio within its experimental error (gray region) for an approximately 8-fold range. The fast timescale parameters of hormone production and removal have minor effects on the ratio across the entire range (inset).

S6. Frequency response of the linearized HPA model

In order to put the findings in perspective, and to add analytical understanding, we calculate here the frequency response of the linearized HPA model. The frequency response allows one to calculate the magnitude and the phase of the system’s output as a function of frequency, for a given input. We analyze the system around its steady state. Although in reality the simulations are not strictly in the linear range, the linear approximation can be used to gain intuition. We thus assume $x_3 << K_{GR}$, therefore the GR is not activated and we can set $GR(x) = 1$. Substituting this in the model equations (1)-(7) (Methods). We used dimensionless variables for the hormones and glands. We do so by normalizing the steady-state of all variables to be 1 for an input $u = 1$. Scaling the variables gives:

$$\frac{dx_1}{dt} = b_1 \left( \frac{u}{x_3} - x_1 \right)$$

$$\frac{dx_2}{dt} = b_2 (C x_1 - x_2)$$

$$\frac{dx_3}{dt} = b_3 (A x_2 - x_3)$$

$$\frac{dC}{dt} = b_C C (x_1 - 1)$$

$$\frac{dA}{dt} = b_A A (x_2 - 1)$$

The linearization of these equations around their steady state and using a Laplace transform gives:

$$sX_1(s) = b_1 (U(s) - X_1(s) - X_3(s))$$

$$sX_2(s) = b_2 (X_1(s) - X_2(s) + C(s))$$

$$sX_3(s) = b_3 (X_2(s) - X_3(s) + A(s))$$

$$sC(s) = b_C X_1(s)$$

$$sA(s) = b_A X_2(s)$$
The capital letters denote the Laplace transform of each variable. Solving these equations we get:

\[
\begin{align*}
H_1(s) &= \frac{X_1(s)}{U(s)} = \frac{b_1(b_2 + s)(b_3 + s)s^2}{D(s)} \\
H_2(s) &= \frac{X_2(s)}{U(s)} = \frac{b_1b_2(b_3 + s)(b_C + s)}{D(s)} \\
H_3(s) &= \frac{X_3(s)}{U(s)} = \frac{b_1b_2b_3(b_C + s)(b_A + s)}{D(s)} \\
H_C(s) &= \frac{C(s)}{U(s)} = \frac{b_1b_C(b_2 + s)(b_3 + s)s}{D(s)} \\
H_A(s) &= \frac{A(s)}{U(s)} = \frac{b_1b_2b_A(b_3 + s)(b_C + s)}{D(s)}
\end{align*}
\]

Where \( D(s) = s^5 + (b_1 + b_2 + b_3)s^4 + (b_1b_2 + b_1b_3 + b_2b_3)s^3 + 2b_1b_2b_3s^2 + b_1b_2b_3(b_A + b_C)s + b_1b_2b_3b_Ab_C \)

\( H(s) \) are the transfer functions of each variable. By substituting \( s = j\omega \) (\( j = \sqrt{-1} \)), we obtain the frequency response of the system. The amplitude of each variable as function of frequency is obtained by calculating the magnitude of the transfer function, \( A_x = |H_x(j\omega)| \). Using the parameters listed in table S1 we plot the cortisol frequency response (Figure S4). Note that a white noise input corresponds to input with constant amplitude at each frequency. Thus, the frequency response curve corresponds to the Fourier amplitudes expected for a white noise input. At very high frequencies corresponding to periods of days or hours, the response decays. At frequencies of around 1 year\(^{-1}\) the frequency response shows the qualitative trend observed in the present study: the 1 year\(^{-1}\) frequency has higher amplitude than the 2 year\(^{-1}\) and 3 year\(^{-1}\) frequencies as found in the results section. Note that frequencies slower than 1 year\(^{-1}\) are also predicted to occur. Observing such variations with a period longer than a year require a longer longitudinal study.
Figure S4. Analytical linearized frequency response for cortisol shows dominant $\text{year}^{-1}$ frequency, related to Figure 3. Cortisol amplitude as function of the stressor input frequency in the HPA model with mass dynamics. Vertical dashed lines correspond to the frequencies in the hair cortisol experiment - 1, 1.2, and 3 $\text{year}^{-1}$.

S7. Analysis of the gland turnover times

Combining theoretical hypotheses and experimental evidence, we construct different independent constraints on the relationships between the parameters of the gland turnover rates ($a_c$ and $a_d$). Each constraint is projected onto the parameter space and is satisfied in some region of this space (Figure S5). The intersection (if it exists) between these different regions provides a range of consistent parameter values.

Using the linearized frequency response of the HPA system (SI, S6) and the experimental results of this study, one can constrain the turnover parameters to fulfill a condition on the ratio between the cortisol amplitudes $|H_3|$ at the slowest frequency ($1 \text{ year}^{-1}$), to the fastest ($3 \text{ year}^{-1}$). The experimentally observed ratio is in the range

$$1.3 < \frac{|H_3(w = 1 \text{ year}^{-1})|}{|H_3(w = 3 \text{ year}^{-1})|} < 1.65$$
We added this constraint to the ones discovered in a previous study (Tendler et al., 2020) on seasonal entrainment of the hormone circuit: 1) A resonance frequency of approximately 1 year (range 9-13 months) for the gland-mass negative feedback circuit; 2) Experimental observation that cortisol blood and urine tests peak at late winter.

These constraints are independent, and hence a-priori they do not have to converge in a specific intersection region. The existence of a region (green region) provides a consistent parameter range. Reassuringly, this range is consistent with gland turnover measurements in model organisms (GERTZ et al., 1987; Kataoka et al., 1996; Nolan et al., 1998; WESTLUND et al., 1985).
Figure S5. Constraint analysis for gland turnover parameters suggests a range consistent between several independent measurements, related to Figure 4 and Table S1. Each region corresponds to parameter values that satisfy a certain constraint. (i) ratio between 1 year$^{-1}$ and 3 year$^{-1}$ frequencies in response to white noise in the present experimental range (orange region). (ii) cortisol peak in winter for a seasonal input as measured and calculated in Tendler et al. (light green) (iii) resonance frequency of the linearized model in the range of 9-13 months (blue region). Intersection: dark green region.