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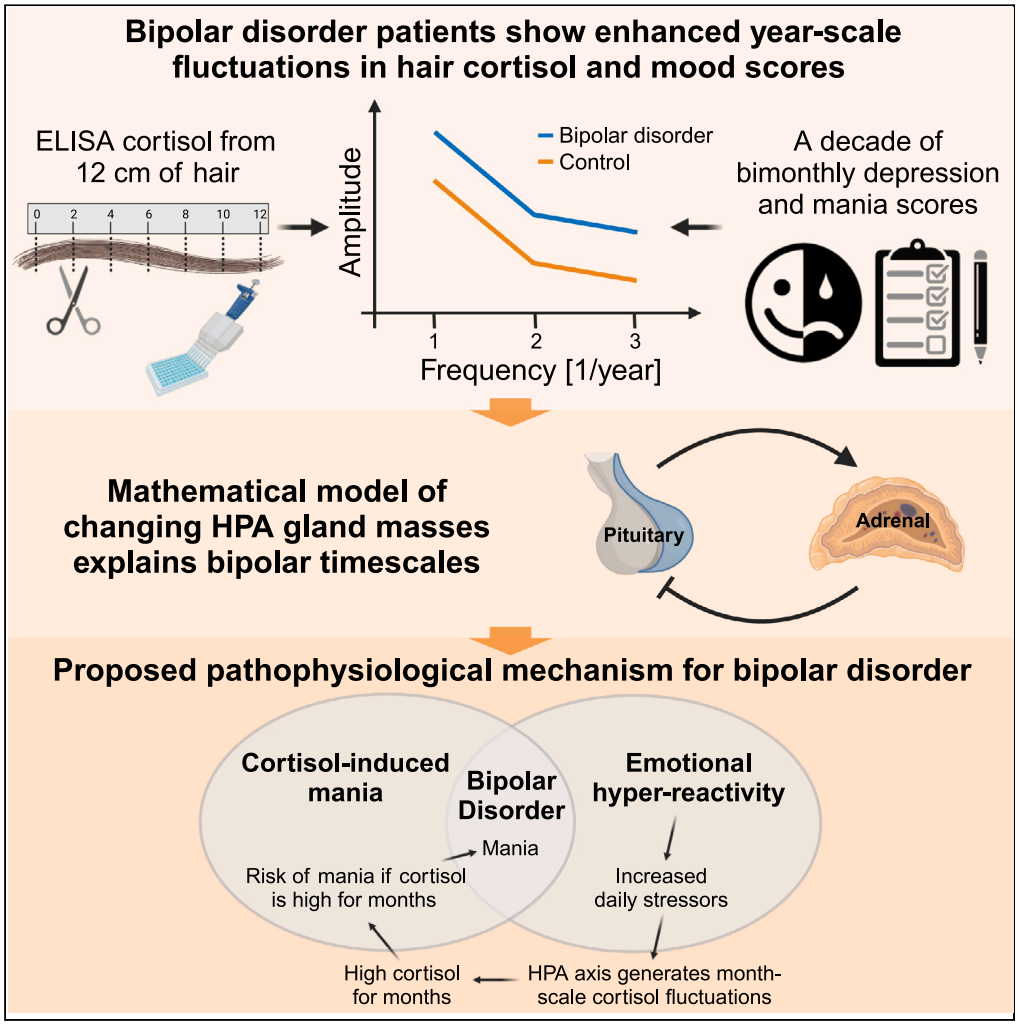

Tomer Milo, Lior Maimon, Ben Cohen, ..., Gadi Cohen Rappaport, Melvin McInnis, Uri Alon

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Highlights

Longitudinal hair cortisol from bipolar and control participants

Cortisol and mood scales show similar elevated year-scale fluctuations

Depression scales correlate with proximal hair cortisol

Proposed mechanism where cortisol fluctuations trigger mood episodes

Milo et al., iScience 27, 109234
March 15, 2024
https://doi.org/10.1016/j.isci.2024.109234

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Longitudinal hair cortisol in bipolar disorder and a mechanism based on HPA dynamics

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SUMMARY

Bipolar disorder (BD) is marked by fluctuating mood states over months to years, often with elevated cortisol levels. Elevated cortisol can also trigger mood episodes. Here, we combine longitudinal hair cortisol and mood measurements with mathematical modeling to provide a potential mechanistic link between cortisol and mood timescales in BD. Using 12 cm hair samples, representing a year of growth, we found enhanced year-scale cortisol fluctuations whose amplitude averaged 4-fold higher in BD (n = 26) participants than controls (n = 59). The proximal 2 cm of hair correlated with recent mood scores. Depression (n = 266) and mania (n = 273) scores from a longitudinal study of BD showed similar frequency spectra. These results suggest a mechanism for BD in which high emotional reactivity excites the slow timescales in the hypothalamic–pituitary–adrenal (HPA) axis to generate elevated months-scale cortisol fluctuations, triggering cortisol-induced mood episodes.

INTRODUCTION

Bipolar disorder (BD) is a mood disorder with temporal variation between mania, depression, mixed states, and euthymic states. The biological mechanisms underlying BD are heterogeneous and complex.[1,2] Advances in understanding the pathophysiological mechanisms of BD are needed in order to develop new avenues for treatment.[3,4]

A defining characteristic of the mood changes in BD is their timescale of months to years. Mania episodes typically last several weeks. Depression episodes typically last months or longer.[5] There does not appear to be a typical frequency for mood changes,[6] but rather a wide range of frequencies with fluctuations on the scale of months to years. Episode rate can range from four times or more per year in rapid-cycling BD to once every few years,[7–9] with some people experiencing euthymic conditions without aberrant mood swings most of the time. The origin of these long timescales and the underlying factors contributing to their temporal variability are not understood.

The purpose of this study is to identify potential mechanisms contributing to the observed timescales of BD. To do so, we focused on a physiological system implicated in BD—the hypothalamic–pituitary–adrenal (HPA) axis, which controls the stress hormone cortisol.[10,11] Cortisol is produced by the adrenal cortex, under control of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn is induced by corticotropin-releasing hormone (CRH) secreted from the hypothalamus in response to stressor inputs. Cortisol has wide-ranging effects on metabolism and cognition.[12–14] It has receptors in many brain areas, including the prefrontal cortex and hippocampus.[15] The effects of cortisol depend on whether the stressor is acute or chronic. Chronically high levels of cortisol are generally damaging to neurological and physiological systems.[16,17]

Recent advances in mathematical modeling show that the HPA glands, the pituitary corticotrophs and the adrenal cortex, can grow or shrink on the timescale of months, providing fluctuations in cortisol that can last months to years. These gland-mass changes arise from the fact that the HPA hormones CRH and ACTH are growth factors for the secretory cells in the pituitary and the adrenal cortex, respectively.[18–21] The timescale for mass changes of these glands is determined by the turnover time of their cells, on the order of 1–2 months.[18] These growth-factor interactions cause the pituitary and adrenal masses to act as a damped oscillator, which can be induced by stressor inputs to exhibit noisy oscillations of gland masses with a wide range of frequencies corresponding to periods of months to years.[22] Indeed, a control population without BD showed months-scale fluctuations of hair cortisol, with the strongest contributions from the lowest frequency measured, corresponding to a period of one year.[22]

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https://doi.org/10.1016/j.isci.2024.109234

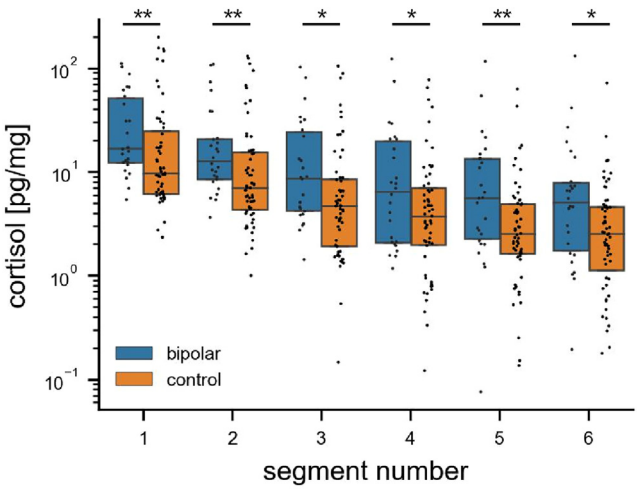

Figure 1. BD participants had higher hair cortisol than control participants

Cortisol levels of control (orange) and BD patients (blue) along six successive 2-cm hair segments of a 12 cm hair sample, where segment 1 is closest to the scalp. Effect sizes, calculated as the ratio between BD and control median cortisol, and Mann-Whitney U test p values for segment 1 to 6 are: 1.7, p = 0.008; 1.8, p = 0.003; 1.85, p = 0.01; 1.7, p = 0.04; 2.2, p = 0.005; 2, p = 0.01. Boxes show 25%, 50% (median) and 75% percentiles.

Further implicating the HPA axis in BD are studies showing that average serum, saliva and hair cortisol levels are about 2-fold higher in BD patients than in control populations.[23–28] BD patients also exhibit dysregulation of the HPA axis, measured as a reduced suppression of cortisol in the dexamethasone test[29–32] and altered pituitary size.[33–35]

Elevated cortisol is not only a feature of BD, but can also cause BD-like mood episodes. People treated with glucocorticoid steroids, which are cortisol analogues, are at high risk to develop manic or depressive symptoms.[36–38] The risk for developing these neuropsychiatric symptoms increases with glucocorticoid dose,[37] and a previous history of unstable mood patterns further increases the risk.[38]

Similar findings occur in Cushing's syndrome, in which cortisol levels are elevated due to hormone-secreting tumors.[39,40] About 30% of Cushing patients experience mania or hypomania, and 50–60% present with depression.[41] One study found that of 30 patients with Cushing's syndrome, 20 met criteria for depressive episode and 8 of these also reported an episode of mania or hypomania during their endocrine disturbance.[42] These findings suggest that a fraction of individuals are sensitive to chronically high cortisol levels in triggering mood episodes.

Several additional lines of evidence suggest that the HPA axis plays a role in the pathophysiology of BD. Early BD episodes are often triggered by stressful life events.[43,44] Unaffected relatives of patients with BD display abnormal HPA axis activity,[45,46] though this is not an endophenotype of BD, but rather seems related to environmental risk factors, such as childhood trauma.[28]

Despite the fact that BD is a dynamic disease with strong ties to cortisol, the dynamics of cortisol in BD over the timescales of months to a year are poorly characterized. Previous studies of BD typically measured cortisol at one or two time points.[23,26,27] There is thus a need for longitudinal cortisol measurements over months in a cohort of people with BD.

Here, we addressed this by using hair to measure longitudinal cortisol levels in a BD population and a control population over one year. We compared the cortisol dynamics to the dynamics of mood scales.[47] The results were analyzed by using the gland-mass mathematical model of the HPA axis. Informed by these findings, we propose a mechanism for the timescales of BD based on the HPA axis.

RESULTS

Longitudinal hair cortisol in participants with BD is higher than in controls

We compared hair cortisol from participants with BD (n = 26) to control participants (n = 59). We measured hair cortisol in 12 cm of hair, corresponding to about a year of growth. Cortisol was evaluated in 6 segments of 2 cm each. Cortisol levels declined along the length of hair as previously described.[22] In all six hair segments, measurements in participants with BD had about 2-fold higher median cortisol than control participants (Figure 1).

BD participants had enhanced year-scale fluctuations in cortisol levels

The finding of higher cortisol in BD agrees with previous studies which used a single time point measurement. The longitudinal nature of the present data allows asking also about the typical timescale for cortisol change.

We therefore studied the variation of cortisol over time in each individual. We accounted for the natural decline along the hair and evaluated the cortisol fluctuations in both control and BD participants using the methods developed in the study by Maimon et al.[22] This resulted in normalized cortisol readings for six segments (the length of each segment is 2 cm, representing 2 months of growth) from each 12 cm hair sample (Figure 2A).

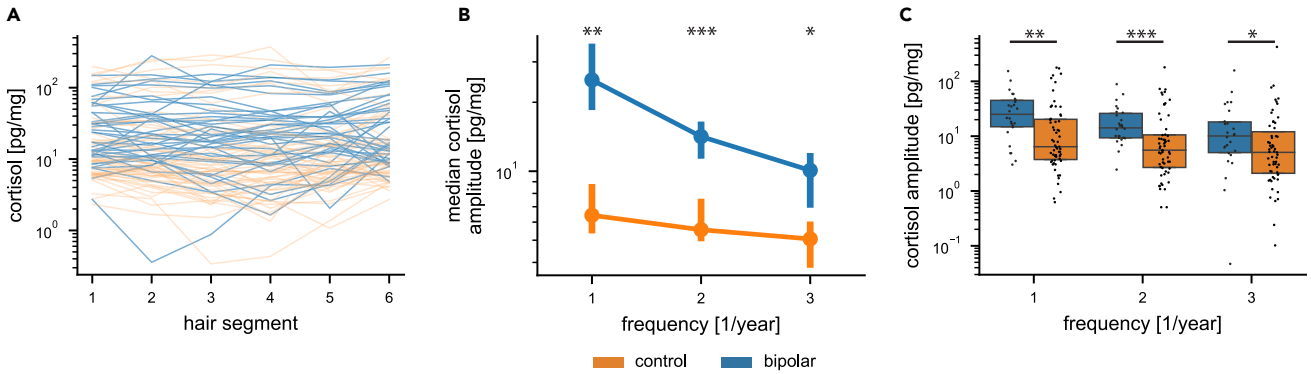

Figure 2. Participants with BD showed enhanced year-scale fluctuations of hair cortisol

(A) Individual cortisol time series of 6 hair segments after correcting for the cortisol decay along the hair.

(B) Median Fourier amplitudes of longitudinal hair cortisol. Error bars are 68% confidence intervals from bootstrapping. The ratios between BD and control median Fourier amplitudes were: 3.9 ($p = 0.001$) for the 1 $year^{-1}$ frequency; 2.55 ($p = 0.0003$) for the 2 $year^{-1}$ frequency, and 2 ($p = 0.02$) for the 3 $year^{-1}$ frequency. All p values from Mann-Whitney U test.

(C) Fourier amplitudes of all participants (dots). Boxes show 25%, 50% (median) and 75% percentiles.

To evaluate the contribution of different timescales to the cortisol dynamics we used Fourier analysis. Fourier analysis is a mathematical technique used to decompose a complex signal into a set of simpler sinusoidal components, revealing the frequency content of the original signal. It involves expressing a function or signal as a sum of sinusoidal functions, each with a specific frequency and amplitude called a Fourier amplitude. The resulting representation in the frequency domain specifies the underlying harmonics and their contributions to the overall signal. Fourier analysis is widely employed in fields such as signal processing, telecommunications, and physics to analyze and manipulate various types of signals and waveforms.

We used Fourier analysis to decompose the cortisol time course into a sum of oscillatory components at different frequencies, and quantify the contribution of each frequency component as Fourier amplitude. Six segments, spanning one year, 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.

In both groups the Fourier amplitudes declined with frequency (Figures 2B and 2C), in agreement with a previous study on a control population.[22] In controls, median amplitude at the lowest frequency (1 $year^{-1}$) was about 1.3 ($p = 0.02$, Mann-Whitney U test) times higher than the median amplitude at the highest frequency (3 $year^{-1}$). In the BD group, the median amplitude at the lowest frequency was higher by a factor of about 2.5 ($p = 0.006$, Mann-Whitney U test) than that of the highest frequency.

The Fourier amplitudes of the BD group were significantly higher than controls ($p = 0.001$, 0.0003, 0.02 for the three frequencies, Mann-Whitney U test). At the lowest frequency, the median amplitude was about 4 times larger in BD than in controls. This shows that BD participants exhibit year-scale peaks and troughs in cortisol levels that are much larger than those of the control group.

Hair cortisol of participants with BD correlated with depression and anxiety mood scores

We also tested whether hair cortisol was associated with mood scales. At the time the BD participants contributed the 12 cm hair sample, they completed four mood scales, the Young Mania Rating Scale (YMRS) for mania, Beck Depression Inventory (BDI), and Hamilton Depression Rating Scale (HDRS) for depression and HAM-A for anxiety (see STAR methods). Thirteen participants returned and donated a second hair sample more than two months after the first sample, and were evaluated using these mood scales a second time. One participant did not fill out the scales. In total, we had 38 hair samples with 38 corresponding sets of mood scores.

Since the mood scales are designed to estimate mood over the recent weeks, we compared the mood scale scores to the 2 cm segment of hair most proximal to the scalp, which corresponds to cortisol in the 2 months prior to hair collection.

Low YMRS mania scale scores were recorded for all participants (mean score = 2, std = 3.3), indicating that none of the participants showed significant manic symptoms in the weeks before donating hair. YMRS scores did not correlate with log cortisol ($r = 0.23, p = 0.13$, adjusted partial Pearson correlation).

A wide range of scores were recorded on the depression and anxiety scales, indicating a range of depression and anxiety symptoms in the BD group. The two depression scales BDI and HDRS both correlated positively with log hair cortisol ($r = 0.47$, $p = 0.008$ BDI, $r = 0.55$, $p = 0.002$ HDRS, adjusted partial Pearson correlation). The anxiety scale HAM-A also correlated positively with log cortisol ($r = 0.47, p = 0.007$, adjusted partial Pearson correlation).

We also repeated the analysis, excluding the 13 repeat measurements. The HDRS correlation remained significant ($r = 0.5, p = 0.04$). The other two scales show positive correlation trends (BDI $r = 0.34$, $p = 0.1$ and HAM-A $r = 0.3, p = 0.15$).

The BDI, HAM-A, and HDRS scale scores also correlated well with each other ($r = 0.88$, $p = 6 \cdot 10^{-13}$ BDI vs. HDRS, $r = 0.72$, $p = 5 \cdot 10^{-7}$ BDI vs. HAM-A, $r = 0.66$, $p = 7 \cdot 10^{-6}$ HDRS vs. HAM-A) indicating satisfactory reliability of the questionnaires.

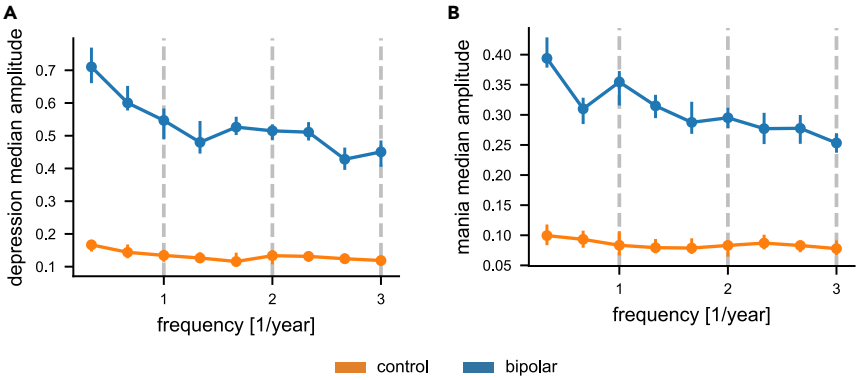

Figure 3. Frequency spectra of mood scales from a cohort of individuals with BD show year scale fluctuations

(A) Median Fourier amplitudes of PHQ-9 depression scale scores from 266 BD and 179 control participants measured every 2 months for at least two years. (B) Median Fourier amplitudes of ASRM mania scale scores from 273 BD and 178 control participants measured every 2 months for at least two years. Error bars are 68% confidence intervals from bootstrapping.

We conclude that hair cortisol in the 2 cm segment most proximal to the scalp correlates with depression and anxiety mood scales in this cohort.

Longitudinal BD mood scales show similar frequency spectra to hair cortisol

We next asked whether the low frequency components observed in the hair cortisol dynamics of BD participants resemble the frequencies found in a much larger study of longitudinal mood measurements (which did not measure cortisol). For this purpose we analyzed longitudinal mood scale scores data from the Prechter BD cohort, where participants completed depression (PHQ-9) and mania (ASRM) questionnaires every two months over multiple years[47] (see STAR methods).

The Prechter BD cohort includes 541 bipolar type-1 patients and 267 controls. We analyzed the BD participants with at least two years of consecutive mood measurements and took their longest consecutive time series for each mood scale. Thus, we included 266 BD and 179 control time series of depression scores (PHQ-9), and 273 BD and 178 control time series of mania scores (ASRM). In total we analyzed 24,627 mood measurements.

The PHQ-9 scores exhibited a Fourier spectrum displaying the highest amplitudes at low frequencies (Figure 3A). The 1 $year^{-1}$ median amplitude was 1.2-fold ($p = 0.01$, Mann-Whitney U test) higher than the 3 $year^{-1}$ median amplitude. The Fourier amplitudes were higher in BD participants than in controls. A similar feature was observed in the ASRM scores with declining frequency amplitudes along the Fourier spectrum, where the 1 $year^{-1}$ median amplitude was 1.4-fold ($p = 0.001$, Mann-Whitney U test) higher than the 3 $year^{-1}$ median amplitude (Figure 3B).

We conclude that hair cortisol and mood scales in BD show similar frequency distributions, with dominant low frequencies on the scale of a year that have higher amplitude than those on the scale of months.

Mathematical model of the HPA axis in BD predicts enhanced year-scale fluctuations

In one of our prior studies,[22] a Fourier spectrum constructed from hair cortisol levels measured from control participants was similar to the control data in Figures 2B and 2C, with dominant low frequencies on the scale of a year. These frequencies cannot be simply explained by the classical mechanism of the HPA axis (STAR methods and Figure S1). The classical mechanism works on the timescale of the hormone lifetime, about an hour, and thus does not intrinsically show timescales of months and years.

We then showed that a recent mathematical model of the HPA axis[18,20] that considers the temporal changes in the size of the HPA glands over months (Figure 4A) is able to reproduce the frequency spectrum of control participants. Stressor inputs that vary from day to day, modeled as white noise, cause the glands to grow and shrink on the timescale of many months, providing the observed Fourier spectrum. The HPA system modulates the typical flat Fourier spectrum of the noisy input (Figure 4C) and amplifies low frequencies (Figure 4E) due to gland changes.

Here, we extend this mathematical model to BD. We identify a possible reason why the BD group exhibits a 4-fold increase in low frequency amplitudes compared to controls. We propose that the daily stressor inputs to the hypothalamus in people with BD are higher than in the control population. We modeled this as a white noise input to the HPA equations that has a larger amplitude than in controls (Figures 4B and 4C).

We find, using simulations of the mathematical model, that the empiricial BD cortisol spectrum can be obtained by providing daily stressor noise that is 4-fold larger than the noise needed to obtain the Fourier spectrum of control participants (Figures 4D and 4E). We thus conclude that a possible explanation of the enhanced slow cortisol fluctuations in BD participants may be larger day-to-day fluctuations in their hypothalamic input to the HPA axis.

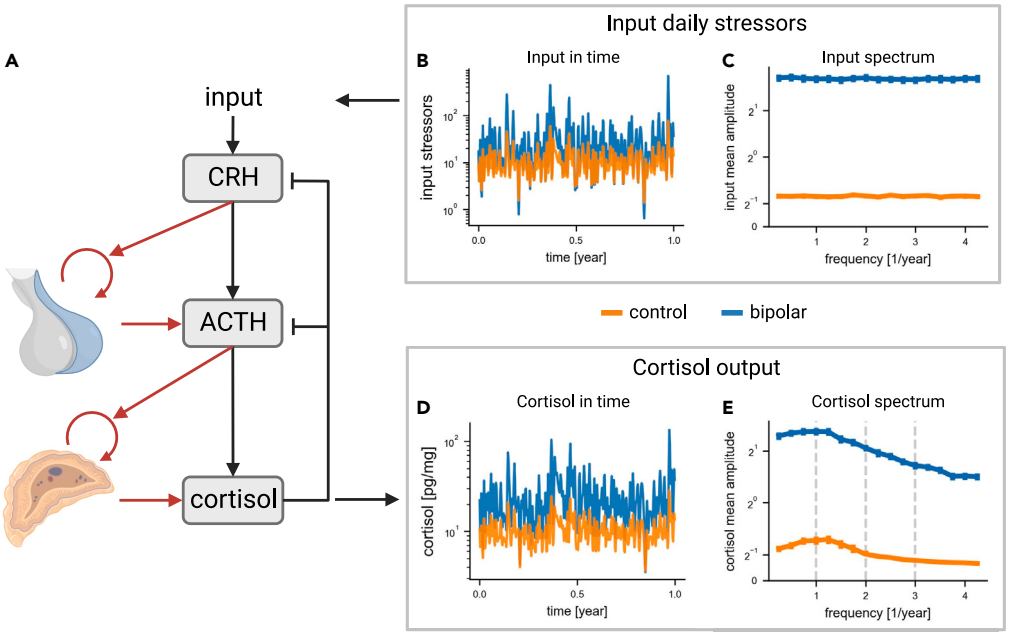

Figure 4. HPA model with gland mass changes predicts enhanced year-scale fluctuations in BD in agreement with the experimental measurements

(A) HPA axis circuit with gland mass dynamics. Red arrows represent growth-factor activities of hormones on the pituitary ACTH-secreting cells and the adrenal cortex.

(B) One realization of simulated noisy daily stressor inputs in time for BD (blue) and control (orange).

(C) A flat Fourier spectrum of the simulated white noise inputs.

(D) The simulated response of cortisol to the stressor inputs shown in (B) over a year, where a baseline cortisol level was set to be the mean of the first segment hair data (E) Fourier spectra of simulated cortisol shown in (D). The three gray dashed lines mark the frequencies measured in the present hair cortisol experiment. Data shown in (C) and (E) are simulation means +/− SEM.

Hypothesis for a pathophysiological mechanism for the timescales of BD

Taking the present findings together, we propose a mechanism for the timescales of mood episodes in BD based on the HPA axis. This mechanism builds on decades of research showing the connections between the HPA axis and BD.[28,48] The new aspect of the proposed mechanism is the ability of the HPA axis to generate fluctuations in cortisol on the timescale of many months[22] due to changes in the effective mass of the glands[18] (Figure 4A).

The basic premise of the proposed mechanism is that individuals susceptible to BD have two neurobiological traits (Figure 5A): a susceptibility in which high cortisol levels over weeks can trigger mood episodes (mania, in particular, as the primary psychiatric criterion for BD1), and emotional reactivity in which individuals generate larger hypothalamic inputs to the HPA axis in response to life events than the typical population. The HPA axis amplifies these enhanced inputs to generate large cortisol fluctuations over months that can trigger mania and depression.

These two neurobiological traits are supported by multiple lines of evidence. Susceptibility to cortisol-induced mania is found in studies on long-term use of glucocorticoid steroids, which are cortisol analogues. According to a large-scale study, people treated with glucocorticoids have a 2-fold higher risk of developing depression and a 4- to 5-fold higher risk of developing mania compared with people unexposed to glucocorticoids.[37] The effect is dose-dependent (Figure 5B), with an apparent threshold of about 40 mg prednisone, equivalent to about 8 times the normal level of cortisol.[49]

As described in the introduction, a similar effect is observed in Cushing's syndrome in which cortisol levels are elevated due to a hormone-secreting tumor. Mania or hypomania is reported in about 30% of Cushing's syndrome patients and depression in about 50–60% of the patients.[39] In both Cushing's syndrome and steroid treatment there is evidence that mania tends to occur before depression.[38,39]

Along with susceptibility to glucocorticoid-induced mood episodes, our proposed mechanism also requires special conditions which promote prolonged excess cortisol that can cross the mania and depression threshold. One such condition is a neuropsychological trait called emotional hyper-reactivity,[50] which is the generation of stronger-than-typical emotional responses to stimuli and is often reported in BD.[51,52] We operationalize emotional hyper-reactivity by defining it as greater hypothalamic sensitivity leading to more pronounced input signals from daily stressors than those of the typical population. A related variable is deregulation of circadian cycles as evident by dysregulated melatonin dynamics in BD. Circadian rhythm is an important input to the HPA axis. Both emotional reactivity and circadian dysregulation have fast-scale dynamics (hours-day) that can, in our model, excite slow timescale fluctuations (months) along the lines of Figure 4.

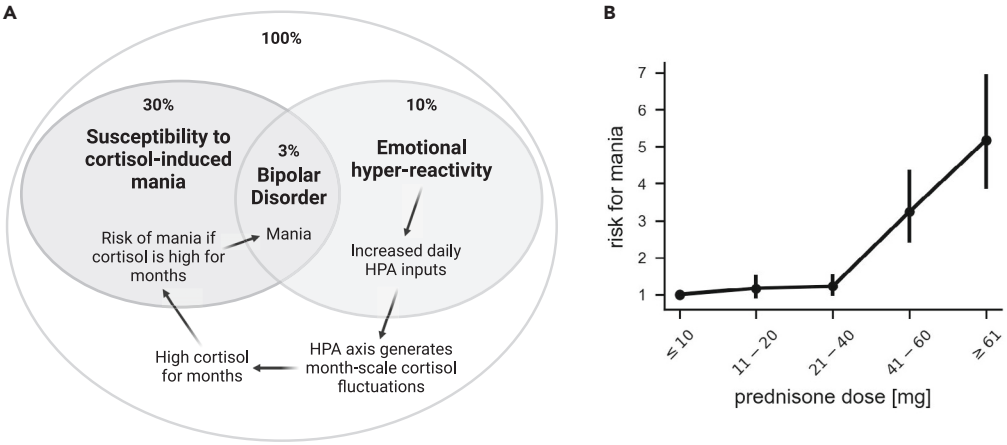

Figure 5. Hypothesis for a pathophysiological mechanism for the timescales of BD

(A) Overview of the proposed mechanism. BD can occur in individuals with both cortisol-induced mania (and depression) susceptibility and emotional hyper-reactivity. Emotional hyper-reactivity causes higher HPA inputs that fluctuate over hours and days. The HPA axis transforms these into months-scale fluctuations because of gland mass changes. High cortisol over months increases the probability of mania and depression in individuals with susceptibility to cortisol induced mood episodes. Percentages are estimates of the fraction of the population with each trait (STAR methods and Tables S3 and S4).

(B) Risk of mania in a population (n = 372,696) taking glucocorticoid steroids representing 89,298 years of glucocorticoid exposure compared to a control population. Normal cortisol baseline is equivalent to about 5 mg prednisone. Adapted from the data of[37] error bars are 95% CI.

The heart of the proposed mechanism is the ability of the HPA axis to generate months-scale fluctuations of cortisol. This timescale of months is provided by the growth and shrinkage of the pituitary and adrenal functional masses. The months-scale property of the HPA axis converts stressor inputs that fluctuate rapidly over hours and days to large months-scale fluctuations. We posit that these months-scale fluctuations are larger in those with emotional hyper-reactivity because of their enhanced hypothalamic inputs. This coincides with the hair cortisol fluctuations measured here. As a result of prolonged periods of high cortisol, there is an increased probability of triggering mood episodes.

The prevalence of BD in the general population is about 2%. This may correspond to the intersection of the two traits mentioned previously. Cortisol-induced mania appears in about 30% of Cushing's patients, so that susceptibility to cortisol-induced mania may characterize about 30% of the general population. Emotional hyper-reactivity appears in at least 10% of the general population (STAR methods and Tables S3 and S4). If these traits are independent, one may expect about 3% to have both (Figure 5A).

DISCUSSION

We present longitudinal hair cortisol measurements from participants with BD and combine this with mathematical modeling to propose a mechanism for mood fluctuations in BD. Participants with BD showed higher mean hair cortisol and enhanced cortisol fluctuations on the year-timescale as compared to a control population. Hair cortisol in the proximal 2 cm segment correlated with mood scales within the BD cohort. The frequency spectrum of cortisol is similar to that of mood scales from the Prechter longitudinal BD cohort. The enhanced year-scale fluctuations can be explained by using a model of the HPA axis based on fluctuations in gland masses on the timescale of months. We discuss a mechanism for the timescales of BD based on these results.

This study aligns with the view that longitudinal measurements are important for studying BD, a complex and dynamic disease.[53] Studies that explore a single time point can assess the differences in mean between populations, such as higher mean cortisol in BD,[24,27,28] which was also found here. Single time point studies, however, cannot address questions like the amplitude of 12-month or 6-month cortisol fluctuations. The present data indicate that such months-scale cortisol fluctuations are larger in BD than in control populations.

To measure cortisol we used hair, because it offers advantages over other assays, namely blood, saliva, and urine measurements.[54–57] Cortisol accumulates passively in the hair, and protocols exist to measure hair cortisol. A 2 cm hair sample represents about 2 months of growth, offering a window into the mean cortisol over the last two months.[54,58] For example, hair cortisol reports accurately on cortisol dynamics over months in patients with Cushing's syndrome.[59] Averaging cortisol over 2 months bypasses the sensitivity of blood or saliva cortisol tests to circadian phase, the menstrual cycle and acute stressors at the time of the test. Hair is also easy to collect and store.

We used 12 cm of hair, and each participant filled out mood scales when hair was collected. We could thus correlate mood with the proximal 2 cm of hair that represents the last 2 months of growth. Hair cortisol correlates well with depression and anxiety scales in the BD cohort.

Cortisol is a well-studied correlate and causal agent for major depressive disorder (MDD).[37,60] We recently applied the HPA gland-mass model to understand the timescale of weeks of MDD. To do so, we modeled a toggle-switch between the HPA axis and brain inhibition of HPA inputs.[61] The model portrays MDD as a bistability and explains why many depression treatments take weeks to have effect. It predicts a hysteresis phenomenon in which depression is more easily entered than exited, and explains HPA dysregulation in DEX and DEX-CRH tests in

MDD. Future work can connect between the present model of BD, based on oscillatory phenomena in HPA gland masses, and the MDD model based on a toggle switch between the HPA and its brain inhibition.

We could not deduce association between hair cortisol and mania scales since no participant in the hair cortisol study had high mania scale scores. Notably, we find that mania and depression scales showed similar Fourier spectra in the Prechter longtitudonal BD cohort (in which cortisol was not measured). Further study can collect shorter hair segments repeatedly along with mood scales, in order to test the temporal correlation of cortisol and mood in each individual over time.

The enhanced months-scale fluctuations in cortisol offer a possible origin, at least in some BD patients, for the timescales of mood swings. Enhanced month-scale fluctuations are relevant to BD when the following is true. First, the individual needs to be more sensitive to daily stressors than the typical population, due to their neuropsychological makeup and their life events. This sensitivity manifests as stronger hypothalamic input to the HPA axis. Such enhanced varying inputs causes the HPA glands to slowly fluctuate in mass, and hence in their hormone secretion, to produce enhanced month-scale cortisol fluctuations. Second, the individual needs to be susceptible to cortisol-induced mood episodes. Such susceptibility may be analogous to that seen in endogenous Cushing's syndrome and in individuals treated with glucocorticoids that respond with BD-like symptoms. For the BD patients that meet these two conditions, a strategy that reduces cortisol and its fluctuations might be therapeutic.

Other physiological mechanisms that can in principle underlie the months-timescale of BD might include epigenetic modifications, such as DNA methylation and histone acetylation, which can have timescales of months, and may conceivably affect mood episodes. Our present model used the assumption that the hypothalamic input in people with BD is higher than in the control population. An alternative explanation is that people with BD have different HPA parameters. There are claims for HPA related epigenetic changes such as DNA methylation in the GR gene,[62] encoding the glucocorticoid receptor, which is also implicated in MDD.[61,63] However, common genetic variants explain a very small percentage of the genetic risk for BD[64,65]; there are no HPA-related genetic variants cited in a recent literature review that explain significant portions of BD risk.[65] Other biological processes such as neurotransmitter function and gene expression are unlikely to accommodate the observed timescale because they typically work on the timescale of hours to days.

Depression is also observed in situations of reduced cortisol.[66] Several stress-associated neuropsychiatric disorders, notably posttraumatic stress disorder, chronic pain, and fatigue syndromes, paradoxically exhibit somewhat low plasma levels of cortisol in many studies. The effects appear greatest in those initially traumatized in early life, implying a degree of developmental programming.[67] The present study mainly concerns cases of high cortisol levels, and future work may connect the HPA mathematical model to conditions of low cortisol.

The HPA axis in BD has potential crosstalk with other endocrine systems. For example, thyroid hormones interact with both BD and the HPA axis. There is interest in thyroid hormone augmentation therapy in bipolar depression and other mood disorders.[68–70] Thyroid output is upregulated in individuals with borderline personality disorder and psychological trauma.[71] Hypothyroidism appears to reduce cortisol degradation rate causing higher levels of cortisol.[72,73] Interestingly, our model predicts that cortisol steady-state on the scale of months doesn't depend on its degradation rate (or its production rate per unit adrenal mass), due to compensation by gland mass changes. This suggests that high cortisol in hypothyroidism may result from stressor input rather than reduced degradation rate of cortisol.

The mathematical model for the HPA axis used here has been tested in several contexts,[21] such as hormone seasonality,[20] HPA function in addiction,[19] HPA recovery from prolonged stress,[18] and cortisol variation in healthy controls.[22] Therefore, it seems to be a reasonable description of the slow timescale of the HPA axis.

In summary, we present data on hair cortisol in BD participants and find dominant low frequencies of a year. We find a similar frequency spectrum in mood scales from a large cohort of people with BD. The frequency spectrum is explained by a mathematical model of the HPA axis due to the slow growth and shrinkage of the gland functional mass. Taken together, these data suggest a three way correspondence of cortisol, mood scales, and HPA physiology. It therefore suggests a mechanism for the timescales of BD based on the HPA axis amplification of enhanced daily stressor inputs in people where cortisol can induce mood episodes. We hope that these findings will lead to further exploration of the timescales of BD and their physiological origin, in order to better understand the etiology of this disorder.

Limitations of the study

This study involved a sample of only 59 control participants and 26 BD participants from one country, primarily female. 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 of hair samples, or blood urine or saliva cortisol assays, can test the validity of the results. Use of multiple hair samples from the same individual over time can extend the study period beyond 12 months.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109234.

ACKNOWLEDGMENTS

We thank the members of the Alon lab for discussions. This research was supported by Heinz C Prechter Bipolar Research Program at the University of Michigan Eisenberg Family Depression Center and The Richard Tam Foundation. With gratitude, we acknowledge the Bipolar Longitudinal Research participants and thank the research team lead for collection, stewardship, and sharing of the data used in this publication. Funding was provided by European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No. 856587). We thank Dr. Eric Simon of the Research Innovation Core at the University of Michigan's Eisenberg Family Depression Center for reviewing and editing this manuscript. Figures 4 and 5 were created with BioRender.com.

AUTHOR CONTRIBUTIONS

Conceptualization, T.M., L.M., and U.A.; methodology, T.M., L.M., T.D., A.B., and U.A.; software, T.M. and D.S.; formal analysis, T.M., D.H., D.S., and A.M.; investigation, T.M. and L.M.; resources, B.C., G.C.R., and M.M.; data curation, M.M.; writing—original draft, T.M. and U.A.; writing—review and editing, T.M., D.H., D.S., A.M., G.C.R., M.M., and U.A.; visualization, T.M.; supervision, B.C., A.B., G.C.R., M.M., and U.A.; project administration, T.D.; funding acquisition, M.M. and U.A.

DECLARATION OF INTERESTS

McInnis has received research support from Janssen Pharmaceuticals and has served as a consultant for them. This research has been filed as a patent to the international patent system, patent cooperation treaty (PCT).

Received: October 20, 2023
Revised: December 22, 2023
Accepted: February 9, 2024
Published: February 15, 2024

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Longitudinal human hair cortisol	This paper	https://github.com/tomermilo/hair-cortisol-in-bipolar-paper
Experimental models: Organisms/strains		
Healthy humans for collecting hair cortisol	This paper. Subjects were recruited using social media approved by the Weizmann Institute IRB (study code 1312-1)	https://github.com/tomermilo/hair-cortisol-in-bipolar-paper
Bipolar disorder participants for collecting hair cortisol	This paper. Subjects were recruited from Be'er Ya'akov mental hospital and via ads approved by the Weizmann Institute IRB (study code 1312-1)	https://github.com/tomermilo/hair-cortisol-in-bipolar-paper
Control and bipolar disorder participants for collecting mood data from the Heinz C. Prechter Bipolar Research Program	McInnis et al.[47]	https://medicine.umich.edu/dept/prechter-program/bipolar-research/data-repositories
Software and algorithms		
Python software for analyzing the data and for mathematical modeling	This paper	https://github.com/tomermilo/hair-cortisol-in-bipolar-paper

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents 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

- Python code needed to reconstruct the analysis and figures is provided in the GitHub repository: https://github.com/tomermilo/hair-cortisol-in-bipolar-paper.
- Longitudinal hair cortisol data is deposited in the GitHub repository: https://github.com/tomermilo/hair-cortisol-in-bipolar-paper.
- The Heinz C. Prechter longitudinal study of bipolar disorder data[47] is available through a data use agreement with the Heinz C. Prechter Bipolar Research Program at the University of Michigan. Information about the dataset, the data dictionary, and guidelines for access are on the Prechter Program website: https://medicine.umich.edu/dept/prechter-program/bipolar-research/data-repositories. In addition, summary statistics describing these data can be found in Table S2.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants

The study protocol was approved by the Helsinki committee of Be'er Ya'akov mental hospital (study code: 613) and by the Review Board of the Weizmann Institute of Science (study code: 1312-1). Written informed consent was obtained from all participants.

Control group

Healthy participants (N = 71, females = 55, average age = 28±5 years) were recruited using social media. Inclusion criteria were age 18–65 years, with at least 12 cm of natural hair with no cosmetic treatment such as dying or perming.[74] Exclusion criteria were 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 participant 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.

The 71 control participants include 59 participants from a previous study.[22] Of these, 21 participants returned to provide a new 12 cm hair sample for the present study, and these data was used. For the remaining 38 participants from the previous study, the previous data was used. The entire dataset can be found online on https://github.com/tomermilo/hair-cortisol-in-bipolar-paper.

Bipolar disorder group

Participants diagnosed with BD (N = 28, Table S1) were recruited from Be'er Ya'akov mental hospital and via ads approved by the Weizmann Institute IRB (study code 1312-1). Inclusion criteria were diagnosis of BD (DSM III-V), age 18–65, stable medical treatment in the last 2 months or without medical treatment, with at least 12 cm of natural hair with no cosmetic treatment such as dying or perming.[74] Exclusion criteria: substance use disorder, diagnosis of PTSD (due to reports of lower resting levels of circulating cortisol, and evidence of heightened HPA axis response to challenge tests in chronic PTSD[75,76] or schizophrenia), steroid treatment in the 6 months prior to participation, surgery or brain stimulation in the 6 months 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. The study was anonymous; each hair sample received a serial number, case report form (CRF) was coded with initials and a serial number. Incentive payment for their participation in the study was provided. The dataset can be found online in https://github.com/tomermilo/hair-cortisol-in-bipolar-paper.

Participants from Prechter cohort

Participants were enrolled in the Prechter Bipolar Longitudinal Cohort[47] (Table S2), an observational cohort study gathering phenotypic and biological data at the University of Michigan. Participants were recruited into the cohort through advertisements on the web, in the newspaper, in an outpatient specialty psychiatric clinic, community mental health centers, community outreach events and in an inpatient psychiatric unit from 2006 to 2018. At study entry, BD and healthy controls were evaluated using the Diagnostic Interview for Genetic Studies[77] and a best estimate process by at least two clinicians was used to confirm diagnosis using DSM-IV criteria. Participants were excluded if they had active/current substance abuse (per DSM-IV criteria) at enrollment or neurological disease. Healthy controls were included if they had no history of DSM-IV axis I psychiatric illness and no family history of psychiatric diagnosis. This study was approved by the University of Michigan (UM) IRB and written informed consent was obtained and incentive payment for their participation in the study was provided.

We analyzed data collected from bipolar I and control individuals followed prospectively for at least 4 years in the Prechter Longitudinal Study of Bipolar Disorder at the University of Michigan.[47,78] The Altman Self-Reported Mania (ASRM)[79] and the Patient Health Questionnaire for Depression (PHQ9)[80] scales were completed at 2 months intervals.

The UM IRB approved recruitment, assessment, and research procedures (HUM606).

METHOD DETAILS

Hair cortisol measurements

We used the method of Maimon et al.[22] A lock of hair (a pencil-width group of about 100 hair strands) from the vertex posterior area[57,74] of the head was tied with a thread and cut with fine scissors as close to the scalp as possible. We estimate that hair was cut at 0.8 $\pm$ 0.1 cm from the scalp, in agreement with Cooper.[74] Cut samples were kept in 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.[81] for the extraction and measurement of hair cortisol. The first 12 cm of each hair sample, starting from the proximal end, were segmented to six 2 cm segments (segments of 1 cm often dropped below the detection threshold in preliminary experiments and thus 2 cm segments were used). The segments were placed in vials (Fisherbrand, 21 × 70 mm) and washed twice with 5 mL isopropanol while mixing on an orbital rotator for 3 min. 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 h) at 50°C while shaking. The following day, all the methanol was transferred to 2 mL Eppendorf tubes and centrifuged for 10 min at 4°C. Methanol (1.5 mL) from each tube was transferred to a glass vial (Falcon, 12 × 75 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 Immunosorbent 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 and 70 mg of hair, hence we used 30–70 mg of hair to measure cortisol. The assay detection threshold specified by the manufacturer was 112 pg. 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 control individual. The coefficient of variation (CV = SD/mean) of 13 standard samples measured on 2 different days was 14%.

We included in this study the 91 participants (63 controls, 28 with bipolar disorder) that had all 6 cortisol measurements above detection threshold.

Psychological measurements

Interviews were conducted by a graduate student with training and supervision of a psychiatrist. Each participant was interviewed to collect medical history, medication treatment and mood evaluation. The interview included a clinical interview and supervised taking of the Hamilton Depression Rating Scale (HDRS),[82] together with self-report using the Beck Depression Inventory (BDI).[83] Mania was rated using the Young Mania Rating Scale (YMRS).[84] Anxiety was measured using the Hamilton Anxiety Rating Scale (HAM-A),[85] general functioning was assessed using the Clinical Global Impressions inventory (CGI)[86] and the Global Assessment of Functioning scale (GAF).[87]

HPA model

We used a mathematical model for the HPA axis that incorporates the pituitary and adrenal gland mass dynamics.[18,20] The Karin et al.[18] model includes the basic hormone cascade and feedback loops (black arrows in Figure 4A):

$$\frac{dx_1}{dt} = b_1 \cdot GR(x_3) \cdot MR(x_3)\, u - a_1 x_1 \quad \text{(Equation 1)}$$

$$\frac{dx_2}{dt} = b_2 x_1 \cdot GR(x_3)\, P - a_2 x_2 \quad \text{(Equation 2)}$$

$$\frac{dx_3}{dt} = b_3\, x_2\, A - a_3 x_3 \quad \text{(Equation 3)}$$

$$\frac{dP}{dt} = P(b_P\, x_1 - a_P) \quad \text{(Equation 4)}$$

$$\frac{dA}{dt} = A(b_A x_2 - a_A) \quad \text{(Equation 5)}$$

Physiological or psychological stressors (u) cause the hypothalamus to secrete CRH (x_1) at a b_1 rate. CRH induces ACTH (x_2) secretion at a b_2 rate by corticotrophs in the pituitary (P) which, in turn, stimulates the adrenal cortex (A) to secrete cortisol (x_3) at a b_3 rate. Cortisol exerts negative feedback on the secretion of the upstream hormones, CRH and ACTH, through the mineralocorticoid and glucocorticoid receptors, given by $MR(x) = \frac{1}{x}$; $GR(x) = \frac{1}{\left(\frac{x}{K_{GR}}\right)^n + 1}$. CRH, ACTH and cortisol are degraded in a_1, a_2 and a_3 rates, respectively. This classic part of the HPA axis, relevant on the timescale of hours, is based on the model of Ottesen et al.[88,89] The Karin et al.[18] model adds the effective masses of the pituitary corticotrophs and adrenal cortex as variables, allowing mass to change and thus to affect the secretion capability of the pituitary and adrenal glands. These interactions are the trophic (growth factor) effects of CRH on pituitary corticotrophs ($b_P x_1$) and of ACTH on the adrenal cortex cortisol secreting cells ($b_A x_2$) (red arrows in Figure 4A). These growth effects add long timescale dynamics of months to the HPA axis due to slow cell turnover (a_P and a_A).

To simulate noisy daily stressor input, we generated a signal that is a sum of 360 sine functions, each with different frequency (ranging from 1 per 4 years up to 1 per 4 days) and a random phase (Figures 4B and 4C). To analyze cortisol response to noisy stressors, we generated 1,000 simulations with such input signals. We used a burn-in period of one year and then computed the average Fourier amplitudes of cortisol over 4 additional years.

HPA model without gland mass changes does not show slow-timescale fluctuations

As an alternative model, we consider only the interactions between the HPA hormones without considering the dynamics of the endocrine gland masses (Figure S1A; Equations 1, 2, and 3). We ran simulations of the alternative model with the same inputs fed to our main model (Figures S1B and S1C). A model of the HPA axis without gland dynamics provides a flat cortisol spectrum, similar to the input spectrum (Figure S1E). This model cannot explain the shape of the hair cortisol spectrum (Figures 2B and 2C) with elevated amplitudes at low frequencies.

QUANTIFICATION AND STATISTICAL ANALYSIS

Hair cortisol time series analysis

We used the method of Maimon et al.[22] to correct for the decline along the hair and obtain normalized cortisol levels from the 6 hair segments. We calculated the mean, standard deviation (SD), coefficient of variation (CV = SD/mean) and the Fourier spectrum for each participant. We removed participants with average cortisol or cortisol CV that was higher by more than three SDs than the population mean. Specifically, we removed one control participant and two BD participants with cortisol exceeding 240 pg/mL, and 3 control participants with CV that exceeded 1.1. The analysis thus included 59 healthy controls and 26 BD patients. 13 BD participants had repeat measurements spaced by 2 months or more. When comparing raw cortisol, we averaged the first and second repeats per segment. When assessing cortisol temporal fluctuations we averaged the Fourier amplitudes of the first and second repeats for each of these 13 participants. To estimate the Fourier

frequency spectrum of the Prechter mood scale time series we used the Lomb-Scargle method[90–92] which is suitable for time series of differing lengths.

We used Mann-Whitney U test to compare cortisol concentrations and Fourier amplitudes between participants with bipolar disorder and controls.

Association of hair cortisol and mood scales

One participant out of the 26 BD participants didn't fill mood questionnaires. Thus, a total of 25 participants were analyzed. 13 participants returned for a second measurement. They were thus tested twice at intervals longer than 2 months, to provide a total of 38 hair samples with 38 corresponding sets of psychological scores. Sample size meets the requirements for detecting correlation of 0.45 with power = 0.8 and alpha = 0.05. We used log cortisol to adjust for the variation in cortisol between individuals.[22] We calculated partial Pearson correlations in order to adjust for participant's age, gender, family status, education and medication. The null hypothesis was that cortisol does not correlate positively with scale scores. Hence we used a one tailed statistical test to evaluate significance. Statistical analysis was conducted using Python Scipy and pingouin packages.[93,94]

Estimation of hyper-reactivity prevalence

We didn't find a previous estimate for the prevalence of hyper-reactivity in the population. To estimate this prevalence we summarized the prevalences of all the pathological conditions that are well-associated with hyper-reactivity, which total to 38% (Table S3). We then accounted for comorbidities (Table S4) to find an estimated hyper-reactivity prevalence of 11%. This is an underestimate in the sense that we don't take into account individuals without pathology.

ADDITIONAL RESOURCES

More information on the hair cortisol clinical trial (registry number MOH_2019-05-30_007171) can be found in: https://my.health.gov.il/CliniTrials/Pages/MOH_2019-05-30_007171.aspx

More information on the Heinz C. Prechter longitudinal study of bipolar disorder data can be found in: https://medicine.umich.edu/dept/prechter-program/bipolar-research/data-repositories.

iScience, Volume *27*

Supplemental information

Longitudinal hair cortisol in bipolar disorder and a mechanism based on HPA dynamics

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