# Circadian fluctuations in glucocorticoid level impact perceptual sensitivity

Jonas Obleser [1,3*],
Jens Kreitewolf [1,3],
Ricarda Vielhauer [1],
Fanny Lindner [1],
Carolin David [1],
Henrik Oster [2,3 §]
& Sarah Tune [1,3 §]

1 – Department of Psychology, University of Lübeck, 23562 Lübeck, Germany
2 – Institute of Neurobiology, University of Lübeck, 23562 Lübeck, Germany
3 – Center for Brain, Behavior, and Metabolism, University of Lübeck, 23562 Lübeck, Germany

§ shared senior authorship

* Lead contact: Jonas Obleser, Dept. of Psychology, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany, Telephone +49 451 3101 3620, jonas.obleser@uni-luebeck.de

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## Abstract

Slow neurobiological rhythms, such as the circadian expression of glucocorticoid (GC) hormones, modulate a wide variety of brain and body functions. Whether and how such endocrine fluctuations also exert an influence on perceptual abilities is largely uncharted. Here, we show that phasic, moderate increases in GC availability prove beneficial to auditory discrimination. In an age-varying sample of N = 68 healthy human participants, we characterise the covariation of saliva cortisol with perceptual sensitivity in an auditory pitch-discrimination task at five time points across the sleep--wake cycle. First, momentary saliva cortisol levels were captured well by the time relative to the wake-up cycle and overall sleep duration. Second, within individuals, higher cortisol levels just prior to behavioural testing improved participant's pitch discrimination abilities, expressed as a steepened psychometric curve. This effect of glucocorticoids on perceptual sensitivity held under a set of statistical control models. Our results pave the way for more in-depth studies on neuroendocrinological determinants of sensory encoding and perception.

## Introduction

Most physiological functions in humans exert circadian rhythmicity [1]. That is, bodily homeostatic functions oscillate with a period of about 24 hours and are vital in adapting the organism to its environment [2]. These functions are regulated through the endogenous circadian clock system with the suprachiasmatic nucleus (SCN) as pacemaker, synchronising subordinate tissue clocks located throughout the body [1].

While sensory, perceptual, and cognitive functions all have been shown to also be subject to—much faster—rhythmicity and to co-vary with brain states at the sub-second ("neural oscillations", e.g. [3]) or seconds-to-minutes scale (e.g., [4, 5]), a potential circadian role of the endocrine system in the regulation of sensation, and perception in particular, has received much less attention.

The endocrine system, with glucocorticoids as the main effector of the hypothalamic-pituitary-adrenal (HPA) axis, exhibits also prominent circadian rhythmicity [6]. Cortisol as the major human endogenous glucocorticoid is recognised in psychophysiological research primarily a stress hormone, reactive to physical and emotional stress [7]. More important to the current investigation, however, blood cortisol levels, approximated well by the saliva cortisol level lagging it [8], are lowest in the late afternoon up to midnight and begin to rise up again during the second half of the night to peak during the early morning [9] (Fig. 1a). It has been a long-standing hypothesis that glucocorticoids and their circadian dynamics are linked to cognitive function. There is evidence of a cortisol influence on different cognitive phenomena such as attention, memory formation [10], and executive functions in general [11].

Understanding better this potential association between central levels of glucocorticoid hormones and sensory–cognitive performance has implications for the notorious relation of stress-related and hearing disorders on the one hand [12], but also for a better understanding of how healthy variations in the central availability of stress hormones like cortisol might help regulate sensory and cognitive function on the other.

Generally, we do not know much on how strongly, and in which direction, glucocorticoid availability impacts cognitive function due to a large dynamic range and a variety of pathways by which GCs can act upon central nervous processes [13-15]. Previous results have been mixed: for instance, high baseline cortisol levels have been associated with impaired memory, executive functions, and visual perception [16], but also with improved attention and sensory performance in dichotic hearing [17]. Dijckmans and colleagues [18] reported better performance in high cognitive function tasks for participants exhibiting larger variation of cortisol levels throughout the day. An earlier peak and greater magnitude of the typical cortisol awakening response (CAR, a cortisol peak 30–45 min post wake-up) has been shown to be predictive of relatively better executive functions-related performance [19, 20].

More generally, it is assumed that a decrease in the dynamic range of circadian GC secretion, either due to an attenuated CAR or due to a slowed elimination of stress-induced cortisol increases are associated with cognitive impairment in elderly subjects [20-22]. Importantly, little is known on how and to what extent circadian changes in GC availability can influence perceptual processes directly. Visual sensitivity has been reported to fluctuate with time of day [16, 23, 24]. Clinically, in patients with GC insufficiency syndromes (e.g., Addison's disease; [25]) lowered perceptual thresholds (i.e., better detection) have been observed

along with a generally lowered dynamic range for audition. Not least, the systemic administration of synthetic corticosteroids has become a mainstay in treating various hearing disorders, assuming a protective effect of GCs in the inner ear [26].

Most directly pertaining to the present study, there is little evidence on how physiological endocrine fluctuations along the circadian cycle influence perception. First evidence with respect to a possible involvement of the circadian system in auditory function is given by the existence of a molecular circadian clock in the cochlea [27] as well as in the inferior colliculus [28]. In addition, Meltser and colleagues [27] reported higher auditory sensitivity, both on molecular and behavioural levels, at specific times of the day (for review see [29]).

Note that a direct impact of cortisol on auditory perception is physiologically plausible: first, experimental cortisol exposure stimulates the auditory system, but leads to damages in the long term [30]. Second, GC receptors are expressed in the inner ear, especially in the cochlea [31], as well as in brainstem nuclei [32]. Thus, GC availability might impact auditory function directly and at different levels of the auditory pathway.

In the current study, we focus on the impact of the circadian variation of GC availability on auditory perceptual sensitivity. We used a psychophysical method, a two-alternative forced-choice (2AFC) task, to describe individual sensitivity for pitch discrimination. The sparse literature available would predict a positive-sign association between momentary cortisol level (be it plasma or saliva) and perceptual discrimination sensitivity. If, as hypothesized, GCs drive GABAergic inhibition in cortical areas such as auditory cortex or, more generally, along the auditory pathway, this would effectively allow for an increased neural signal-to-noise ratio [15].

Our lead hypothesis here was that GC levels (as proxied by saliva cortisol) impact perceptual performance above and beyond expected drivers such as sex or chronological age: higher levels of saliva cortisol just prior to performing a challenging pitch-discrimination task should lead to a steeper psychometric curve; indicating a state of elevated perceptual sensitivity and, thus, better auditory discrimination abilities. As auxiliary hypotheses, we expected older participants to (i) show less perceptual sensitivity in auditory pitch-discrimination (e.g., [33]) and (ii) to present with lower levels of saliva cortisol [20]. The current design allowed us to control for potential confounds of cross-sectional age differences when studying GCs and auditory perception.

We tested a large, age-varying sample of participants to investigate the relationship of saliva cortisol and perceptual performance at the state (i.e., within individuals) and trait level (i.e., between individuals). In detail, we tested a cohort of healthy young adults and a cohort of middle-aged to older participants at five different measurements covering a time interval of approximately 18 hours (see Fig. 1a). We recorded individual sleep duration and aligned cortisol sampling and behavioural testing relative to the sleep–wake cycle to optimally capture the post-awakening rise and subsequent fall in GC levels [34, 35].

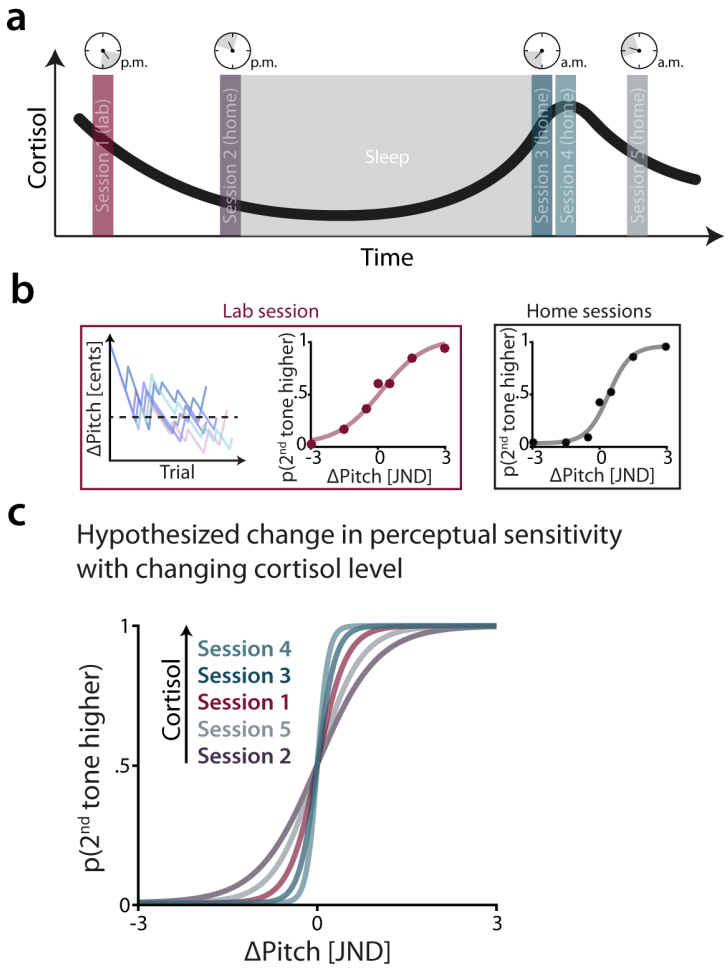


**Figure 1. Experimental design and hypothesis.**

**(a)** Design. In five sessions, participants were asked to take saliva samples, from which their cortisol levels were measured. After a first laboratory session (in the afternoon), participants were asked to perform the other four sessions at home. To capture circadian differences in cortisol levels (black curve), these 'home' sessions were timed to align with the individual participant's sleep-wake cycle such that sessions 2 and 3 had to be completed immediately before going to sleep and immediately after wake-up, respectively. Two further sessions (4 and 5) were performed 30 and 120 minutes after wake-up.

**(b)** Psychophysical testing. In addition to the collection of saliva samples, participants performed a pitch discrimination task in each session. In the lab session, we first assessed individual participants' pitch discrimination thresholds (just-noticeable difference; JND) using five separate staircases (see Methods for details). These individual JNDs were then used in an online experiment, which participants performed in all five sessions. Psychometric functions were fit to the data obtained in each session. The slope of the psychometric function served as a measure of perceptual sensitivity.

**(c)** Hypothesis. Increased levels of GC availability should result in steeper psychometric functions, reflecting higher perceptual sensitivity. Note that here, sessions are not ordered chronologically but by cortisol level.

## Results

We investigated the impact of circadian variation in cortisol levels on perceptual sensitivity in a challenging auditory pitch discrimination task. Task difficulty was titrated based on the individual just-noticeable difference (JND). We used separate linear mixed-effects models to (i) test how salivary cortisol secretion changes as a function of time relative to the sleep-wake cycle and age cohort, and (ii) to understand how the observed fluctuation in cortisol levels, in turn, impacts perceptual sensitivity, represented by the slope of the psychometric function. Each model tested for the impact of additional potentially confounding influences such as sex or sleep duration.

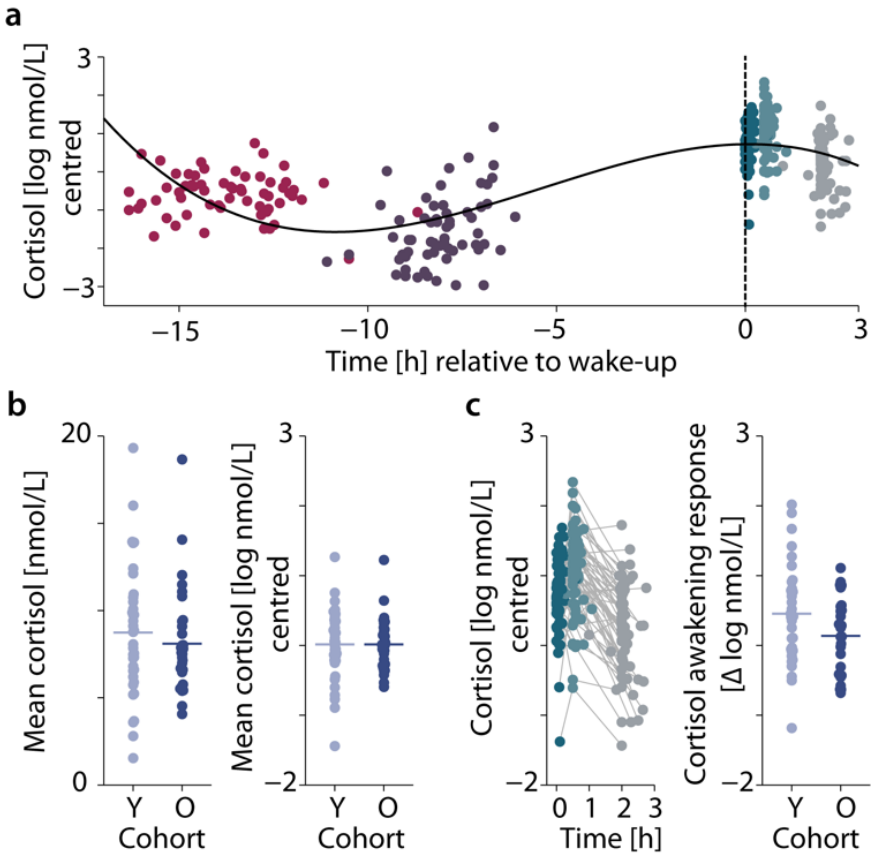


**Figure 2. Momentary states of glucocorticoid levels (salivary cortisol)**

**(a)** Changes in individual salivary cortisol concentration measured in log nmol/L across five experimental sessions. Cortisol levels are mean-centred across all N=68 participants. Sessions are grouped by colour and aligned by wake-up time (dashed vertical line). Black curve shows the cubic trend of time.

**(b)** Left panel: individual mean cortisol levels [nmol/L] across sessions shown separately for the younger (Y, light blue) and older (O, dark blue) age cohort. Coloured dots represent individual mean values (N=68), coloured horizontal lines show the respective group average. Right panel: individual mean cortisol levels per group after log-transformation and mean centring for statistical analysis.

**(c)** Left panel: trajectory of individual cortisol levels [log nmol/L] following wake-up. Time is expressed relative to wake-up time. Note the rise in cortisol levels 30 min after wake-up (session 4, light teal). Right panel: individual cortisol awakening response (CAR) expressed as the difference in cortisol levels [log nmol/L, centred] 30 min after wake-up relative to wake-up shown separately for the younger (Y, light blue) and older (O, dark blue) age cohort. Horizontal coloured lines indicate the group mean.

### *Explaining momentary states of saliva cortisol*

As revealed by model comparison, the momentary level of salivary cortisol was well accounted for by the daytime of measurement (expressed relative to the individual wake-up time) and total sleep duration in-between measurements (conditional $R^2$=.75; see Table S1 for full model details). Increased sleep duration led to overall lower levels of cortisol ($\beta$ = –.16, *standard error (SE)* = .04, p = .001, *log Bayes factor*$_{10}$ *(logBF)* = 3.2) while changes in cortisol over time were best described by a cubic trend ($\beta$ = –.15, *SE* = .07, *p* = .025, *logBF* = –.13). As shown in Fig. 2a, this cubic trend captures the decline in cortisol levels from afternoon to late evening and the characteristic CAR (see Fig. 2c). The considerable improvement of model fit by the inclusion of session-specific random intercepts further attests to the impact of daytime on cortisol level (likelihood ratio test; $\chi^2_1$ = 69.5, *p* < .001, *logBF* = 31.9). Overall levels of cortisol did not differ significantly between the younger and older cohort ($\chi^2_1$ = 2.05, *p* = .15, *logBF* = –1.9; see Fig. 2b). Neither did the cortisol awakening response exhibit a clear effect of age-cohort (Fig. 2c). The inclusion of participants' sex did not improve model fit, either ($\chi^2_1$ = 1.0, *p* = .31, *logBF* = –2.4).

### *Saliva cortisol predicts perceptual sensitivity*

As the main analysis (Fig. 3), we probed the influence of cortisol levels measured just prior to performing a challenging pitch-discrimination task on participants' perceptual sensitivity. As indicated by the best-fitting linear mixed-effects model, perceptual sensitivity, operationalized as the slope of the psychometric function, was significantly influenced by the momentary level of cortisol, age cohort and sex (conditional $R^2$ = .47; see Table S2 for full model details).

In line with our hypotheses, increased levels of cortisol led to heightened perceptual sensitivity ($\beta$ = .13, *SE* = .04, *p* = .004, *logBF* = 1.4). More specifically, as illustrated in Figure 3a (right panel), an increase in cortisol by one unit log(nmol/L) steepened the slope of the psychometric curve by one tenth of the just-noticeable difference (JND). Importantly, as illustrated by the inclusion of subject-specific random slopes in Figure 3a (left panel), this relationship was consistently observable across individual participants. An additional analysis including separate regressors for the state- (i.e., within-subjects) and trait-level (i.e., between-subjects) effect of cortisol on perceptual sensitivity provided additional support for cortisol-driven changes in perceptual sensitivity at the level of the individual participant (within-subject effect of cortisol: $\beta$ = .13, *SE* = .04, *p* = .004, *logBF* = 1.2; between-subject effect cortisol: $\beta$ = .13, *SE* = .23, *p* = .56, *logBF* = –2.7; see Methods and Table S3 for full model details).

As expected, we observed a significant decrease in perceptual sensitivity for the older compared to the younger cohort ($\beta$ = –.52, *SE* = .18, *p* = .005, *logBF* = 1.3; see Fig 3b). More precisely, we observed shallower slopes for older participants with an overall difference in the slopes of younger and older participant of nearly half a JND. Participants' sex proved to be an additional significant predictor with females showing overall lower perceptual sensitivity ($\beta$ = –.36, *SE* = .18, *p* = .049, *logBF* =–.84; see Figure S1). Participants' sleepiness or response bias [indicated by the point of subjective equality (PSE) on the psychometric function], however, did not influence behavioural performance. The inclusion of these predictors did not significantly improve model fit (likelihood ratio tests, all *p* > .067, all *logBFs* < –1.2).

Lastly, we investigated whether changes in cortisol would differentially impact perceptual sensitivity across the two age groups, despite overall comparable levels of cortisol observed for younger and older adults. However, the inclusion of the respective interaction term did not improve the model fit ($\chi^2_1 = .91$, $p = .34$, $logBF = -2.4$).

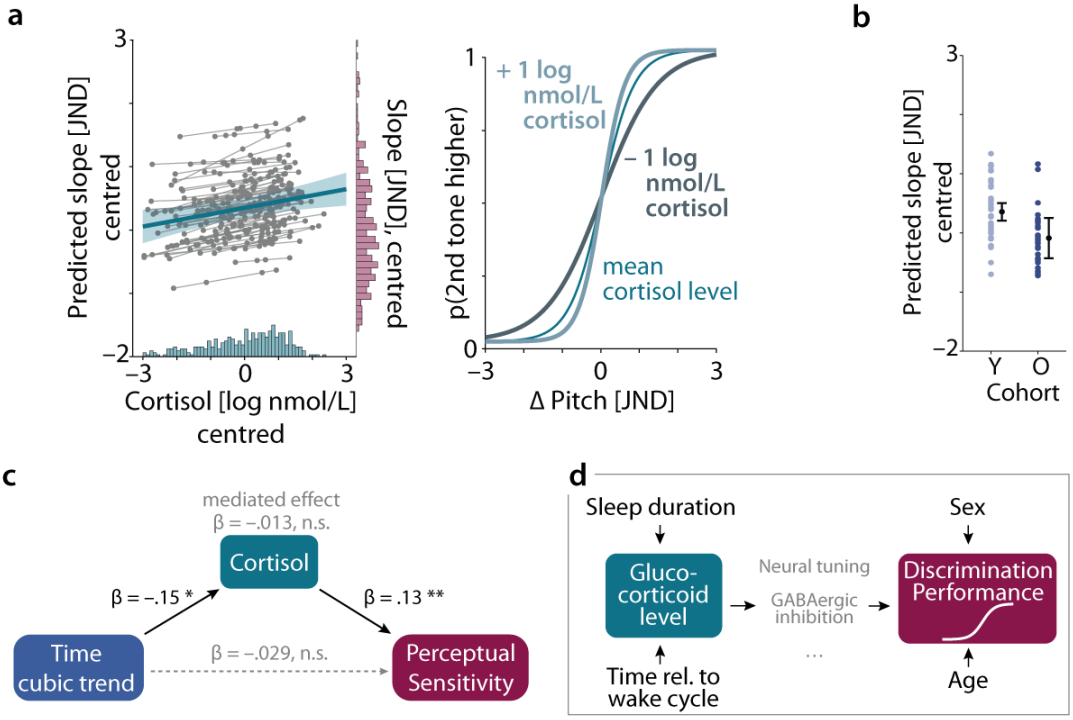


**Figure 3. Salivary cortisol impacts perceptual sensitivity**

**(a)** Left panel: change in perceptual sensitivity (operationalised by the slope of psychometric function) as predicted by cortisol. Predicted group-level fixed-effect (green slope) with 95% confidence interval (CI) error band is shown along with the estimated subject-specific random slopes (thin grey lines) and single-subject, single-session predictions (grey dots). Note that subject-specific random slopes did not improve the model fit and were added for illustrative purposes only. Histograms on the bottom and right side of the plot display the distribution of log-transformed cortisol and raw slope values, respectively. Right panel: illustration of how variation in cortisol level impacts the steepness of the psychometric curve.

**(b)** Change in perceptual sensitivity between age groups. Coloured dots (light blue, young (Y) cohort; dark blue, older (O) cohort) show single-subject predicted slope values based on the best-fitting linear mixed-effects model. Black dots represent the fixed-effect group-level prediction and 95% CI.

**(c)** Results of causal mediation analysis. Formally accounting for the potentially mediating role of cortisol does not lead to a significant change in the effect of the cubic trend of time on perceptual sensitivity.

**(d)** Summary of effects observed. The panel summarises observed (black solid) and statistically excluded (absence of arrows) effects. Intervening (i.e., mediating) effects of *how* GCs can act upon resulting perceptual outcomes must obviously exist, but remain subject to future experimentation. For illustration only, viable paths via a sharpening of neural tuning and/or increased levels of GABAergic inhibition are shown in grey.

### *Cortisol does not impact response bias*

To investigate whether the impact of momentary cortisol levels was specific to perceptual sensitivity, we ran a control model probing for their effect on response bias. We found a significant increase in PSE for older participants ($\beta$ = .44, *SE* = .2, *p* = .027, *logBF* = –.34). Importantly, however, circadian fluctuations in cortisol did not significantly predict changes in response bias ($\beta$ = .001, *SE* = .04, *p* = .98, *logBF* = –2.9, see supplemental Fig. S3 and Table S4).

### *Ruling out confounding effects of task proficiency*

One concern we aimed to target is the obvious repetition of the pitch discrimination task in close succession, especially in the morning of the second testing day (i.e., three times of testing within approximately two hours). Reassuringly, however, no training or time-of-day effects on our main outcome measure of pitch-discrimination perceptual sensitivity were evident (Fig. S2) as the inclusion of session ($\chi^2_1$ = 1.6, *p* = .21, *logBF = –2.1*) or time (linear, quadratic, cubic trend) did not improve model fit (all p > .4, *logBFs* < –2.5).

### *Cortisol is not simply mediating an effect of daytime on perceptual sensitivity*

An additional control analysis considered the possibility that the observed link between cortisol and perceptual sensitivity could reflect an indirect effect of daytime on perceptual sensitivity. While the absence of any systematic changes in the slope of the psychometric function with time (see above) rendered this scenario unlikely, we still formally tested this possibility using causal mediation analysis. As shown in Figure 3c, the comparison of the estimated total and direct effect of time (cubic trend) on perceptual sensitivity showed a comparably small and non-significant change (–.042, CI[–.13 – .04] vs. –.029, CI[–.13 – .07]; proportion mediated = .21, p=.74) when accounting for the indirect influence via cortisol (–.13, CI[–.07 – .04]). In other words, the observed increase in perceptual sensitivity with increasing levels of salivary cortisol does provide evidence for their potentially causal relationship.

## Discussion

Is the momentary availability of GCs affecting perceptual abilities, and if so, to what degree? The current study set out to gather decisive data on this seemingly simple question. In a mixed between- and within-participants design using multiple saliva cortisol samples and multiple associated behavioural assessments of perceptual sensitivity throughout the circadian cycle, we here have shown that this is indeed the case.

A first result lending overall credibility to our approach is the circadian modulation of saliva cortisol. A highly consistent pattern of relative cortisol level displacement dependent on daytime of measurement was observable (Fig. 2a), which in concert with the individual duration of sleep (taking place between measurements 2 and 3) could explain the observed GC variance to a large degree.

Second, as the main result of our study, saliva cortisol levels just prior to performing the pitch discrimination task were predictive of the perceptual sensitivity. Statistically dissecting the influence of trait-level (i.e., person to person) versus state-level (i.e., session to session) variation in cortisol showed that it was

the momentary cortisol level just prior to behavioural testing that covaried with perceptual sensitivity. The robustness and size of this effect is illustrated in Figure 3a: a change of one's own cortisol level by one unit log(nmol/L) steepens one's psychometric curve by approximately 1/10 of the just-noticeable difference in pitch. Essentially all participants showed this positive relationship of momentary saliva cortisol levels with steepness of the psychometric curve in pitch discrimination. Lastly, a series of control analyses underscores the directness and putative causality of the effect of cortisol on auditory discrimination performance.

This result fills various gaps in our knowledge on how the endocrine system impacts perception and behaviour. We will discuss potential mechanisms, limitations, and implications below.

### *Potential mechanisms: how could GC levels act upon perceptual sensitivity?*

The present results imply that, within normal levels of a healthy endocrine system, relative increases in centrally available GCs lead to an objective improvement in the ability to discriminate sounds. This is broadly in line with a view of stress hormones and activity of the hypothalamic-pituitary-adrenal (HPA) axis as preparing the body for action [36]. Enhanced discrimination abilities certainly fit into this view. What are mechanistic pathways by which glucocorticoids could bring such an improved discrimination about, and how specific to auditory discrimination might these pathways be?

Both peripheral effects of GC and central effects at various stages need to be considered and tested in detail in future studies. However, a two-alternative forced-choice (2AFC) discrimination task such as the present one requires the system to detect equally well two stimuli and to arbitrate between the two with respect to one task-relevant dimension (here, tone frequency). In signal detection theoretic terms, one stimulus (here, the one higher in tone frequency) is considered as "signal plus some noise" and should be chosen by the listener, while the other is considered "only noise". Thus, improving sensitivity in such a task requires a mechanism that is able to improve the "signal to noise" ratio—either at the level of neural encoding (inner ear, midbrain, auditory cortex), or at the level of decision-making (auditory cortex and beyond), or both.

A concept viable at all these levels is neural tuning, the degree to which a neuron or neuronal population is selectively responsive to a certain range along a given featural dimension, here, pitch or sound frequency. Neural tuning in auditory cortex is known to be highly adaptive to task demands in any given listening situation [37]. Additionally, improved discriminability of tones is a phenomenon with a clear auditory-cortical contribution [38]. Recent work in humans also underscores that ongoing neural population dynamics, which should be especially amendable to endocrine modulation, can flexibly (i.e., from trial to trial) affect behavioural sensitivity (e.g., [39, 40]).

It remains to be shown how GCs can mechanistically improve neural tuning and, thus, result in better discrimination abilities—a viable path here lies in improved GABAergic signalling, that is, inhibitory control. GCs are known to facilitate inhibitory GABAergic synaptic input (and to concomitantly suppress excitatory glutamatergic drive) at least in hypothalamic neurons, part of the HPA axis [15]. It remains speculative at this point whether such a combined effect of GCs might also tip other brain areas towards inhibition, with concomitant improvements of discrimination abilities both at the neural and behavioural level.

Not least, this conjecture receives support by the clinical observation that primary GC insufficiency as observed in Addison's disease is accompanied by paradoxically improved detection thresholds but a decrease in discrimination abilities [25, 41]. Most importantly, it poses a testable pathway: recent neurophysiology work using optogenetic stimulation of layer-6 cortical neurons in the rodent in fact provided compelling evidence for a dissociation and rapid switching of detection-optimal versus discrimination-optimal configurations, at a local neural and a resulting behavioural level [42, 43]. There thus lies great promise in better understanding the differential GC susceptibility of these detection and discrimination processes in the auditory system.

In sum, as a next step, new experiments should aim to manipulate GC availability directly. In healthy participants, a relatively unspecific but carefully titrated administration of synthetic GC analogues can easily be used to obtain experimental control over GC levels within the normal dynamic range of HPA axis activity [44]. In clinical patients with primary GC insufficiencies (e.g. M. Addison) or GC hyper-availability (M. Cushing), it will be fruitful to build on pioneering but technically limited work linking lowered thresholds (e.g., an over-sensibility) but lowered discrimination abilities in the auditory domain to cortisol states (e.g., [25]).

### *Potential confounders of a causal influence of cortisol on perceptual sensitivity*

As summarised in Figure 3d, the current work helps us rule out two potential (i.e., theoretically plausible) confounders. Namely, both participants' age and participants' sex were indeed predictive of perceptual performance (with younger and male participants outperforming older and female participants, respectively). However, they are both highly unlikely to confound the observed effect of GCs on this performance, as neither of the two could account for momentary cortisol levels (note the absence of arrows from sex or age into GC in Fig. 3d).

Not shown in Figure 3d, but reported in detail above, other more global "state" variables such as time of day (recall that most data here were acquired either just prior to bedtime or immediately after waking up) or total duration of sleep were good predictors of the momentary cortisol level. These, however, failed to account for any meaningful variance in the behavioural outcome (note the absence of arrows from *Time relative to wake cycle* and *Sleep duration* to *Discrimination performance*). Thus, it was not the case that, for example, participants who had slept more were overall providing higher perceptual sensitivity across all testing instances or vice versa. Neither did testing in the evening yield lower performance, all other things being equal.

Unsurprisingly, the mediation analysis we performed for the sake of completeness (see Results) also did not provide any evidence for a potential mediation (i.e., daytime → cortisol → performance). Instead, our results expose a more direct link from momentary salivary cortisol level to sensitivity in perceptual discrimination.

Note that we made a set of design choices (e.g., cortisol sampling always directly preceding the behavioural test) that help to rule out a conceivable, reverse causal relationship (i.e., worse performance in the behavioural task leading to perceived stress and, thus, to higher cortisol level). Such a hypothesis, however, is rendered unlikely on two grounds. First, a previous study has found no effect of task effort or hearing status on cortisol as a marker of stress [45]. Second, the present data themselves invalidate this notion, as higher cortisol levels just prior to testing were accompanied by better, not worse performance at test.

This leaves us with one potential unobserved confound, namely, arousal. Could elevated levels of arousal have led to higher cortisol levels and, hence, to better behavioural performance? Arousal is generally assumed to establish an inverted u-shaped impact on performance (the "Yerkes-Dodson law"; [39, 40, 46]). However, the fact that we did not observe such a pattern does not necessarily rule out a confounding influence of arousal as our paradigm might have captured only activity along the "rising" flank of such an inverted u.

Nevertheless, we deem a confounding influence of autonomic arousal on both, GC levels and performance, unlikely on various grounds. First, the relationship of stress on individual cortisol levels is not as clear-cut as often assumed [47]. Second, autonomic arousal markers, such as heart rate and blood pressure on the one hand and cortisol on the other, have been shown to exert a dissociable, that is, sufficiently independent impact on performance in a verbal memory task [48]. Third, even if we assume a mechanistic link between arousal and cortisol, it is hard to imagine why autonomic arousal should have covaried so consistently across our diverse cohorts of young and old adults and time of day in order to yield the consistent behavioural effects. The Karolinska Sleepiness Scale, assessed here as a control measure and arguably a proxy of arousal did not indicate any systematic covariation with behavioural outcome.

Nevertheless, a future study co-registering pupil dilation (as an established proxy of arousal) or physiological markers such as skin conductance and heart rate, ideally in a setting where GC levels are manipulated experimentally, should help to illuminate the causal links between arousal, cortisol, and discrimination performance.

### *Implications*

We here have shown that a main neurobiological circadian rhythm in the human body, the secretion of glucocorticoids (here, captured as saliva cortisol), covaries with the individual fluctuation of auditory perceptual abilities (here, captured as pitch-discrimination sensitivity immediately after taking the saliva sample). We have demonstrated that momentary GC levels show the expected circadian change, and that these within-individual fluctuation of GC levels exhibit a direct influence on perceptual sensitivity.

This result opens at least two new research avenues: first, experimental control and manipulation of endocrine modulators such as the GC system can help to constrain future research into the organisation of the auditory system. Second, our study opens new paths to improving or restoring discrimination abilities, a particularly vulnerable aspect of auditory function in both ageing generally and age-related hearing-loss specifically.

## Materials and methods

### *Participants*

Seventy-five participants took part in this study, acquired in two waves (younger participants in April–May 2018, older participants in April–May 2019). The participants were recruited through the database of the Department of Psychology at the University of Lübeck, using the online recruiting system ORSEE [49]. The cohort of younger participants consisted of 37 university students (24 females, mean age 22.6, SD = 2.58, age range 19–30 years). The cohort of middle-aged and older participants consisted of 38 persons (19 females, mean age 60.6, SD = 5.98, age range 50–70 years); 16 of them had already been retired.

All participants were screened to avoid any history of disorders that could have impacted their GC balance, such as neurological or psychiatric disorders as well as any known metabolic diseases. Furthermore, none showed a BMI over 30 $kg/m^2$ or had been working in shifts. None reported any known hearing disorders, severe current hearing loss, or a persistent tinnitus. Note, however, that participants with age-related mild hearing loss had not been excluded from the cohort of older participants due to its high prevalence in this age group.

In the cohort of younger participants, none took any medication that could have influenced their GC balance, including medication for asthma- or allergy treatment, systemic immunosuppressants or antihypertensives.

In the cohort of older participants, more lenient inclusion criteria with respect to medication applied (see Table S5). Here, participants who took any type of antihypertensives were still included to allow for a representative sample of older adults.

Written informed consent was collected from all participants according to procedures approved by the Research Ethics Committee of the University of Lübeck. Listeners were paid 25–30 € or received course credit for their participation in the experiment.

### *Experimental protocol*

On the first day, participants came to the laboratory between 4pm and 6pm for the first session, lasting about one and a half hours. A maximum of four participants conducted the first session on a given day. The session started with an adaptive tracking procedure that measured auditory pitch thresholds (see section Psychoacoustic testing for details). Participants were then asked to complete three questionnaires on their general medical history, their chronotype [50], and their momentary sleepiness (assessed using the Karolinska Sleepiness Scale; [51]) The scale consists of three items: (1) sleepiness during the last 10 minutes (nine steps on a Likert-Scale), (2) the current state with relaxation on one end and tension on the other end of a visual analogue scale and (3) the current fatigue (visual analogue scale).

Next, participants received detailed instructions for the subsequent measurements. Each session included taking a saliva sample and performing a challenging pitch discrimination task in a browser-based online study (Labvanced, Osnabrück), followed by the sleepiness questionnaire. According to their auditory pitch threshold, participants were assigned to an experimental group, designed to yield equivalent difficulties of the pitch discrimination task (see *Assessment of pitch discrimination thresholds* below), and provided with an individual link, which gave them exactly five times access to the online task.

Finally, participants completed the first session: taking a saliva sample first (see Saliva cortisol collection for details) and performing the online pitch discrimination task secondly before they were sent home. Throughout all sessions, participants in the younger cohort used their own technical devices (laptop and headphones) whereas participants in the older cohort used their own headphones for all experimental sessions but were provided with computers for the first session due to their lack of portable computers. Usage of participants' own equipment ensured that the acoustic properties of the pitch discrimination task remained constant across sessions and, whenever possible, that the experiment could be adequately performed with the participants' personal equipment.

All other measurements were conducted at home, scheduled at certain times of day relative to the participants' sleep–wake cycle: Session 2 had to be performed just before going to sleep, Session 3 immediately after waking up (participants were instructed to place the equipment, or at least the Salivette tube for the saliva sample, next to their bed), Session 4 30 minutes, and Session 5 about 120 minutes after awakening. To assess compliance and to gather information about the time of events, participants recorded the starting time of each session as well as the activities that they were engaged in between two consecutive sessions in a time protocol. Additionally, they were asked to maintain their typical sleeping and wake-up times, which they had recorded for the last two weeks.

### *Saliva cortisol collection*

Salivary cortisol level was measured to deduce the amount of unbound cortisol in blood [52]. To capture a comprehensive cycle of cortisol secretion as used in former studies [18, 20, 53], including the characteristic morning rise, a saliva sample was collected at each single experimental session. As described above, sessions were scheduled according to the individual participant's wake-up time. Following instructions and the collection of a first saliva sampling in the lab session, participants were provided with a saliva self-collection pack containing four Salivette Cortisol tubes (Sarstedt, Nümbrecht, Germany), pre-labelled with participant code and number of session, and written instructions. For a correct usage, the Salivette dental swab from the correctly labelled Salivette had to be chewed until fully saturated and then be put back into the tube. Saliva samples were then stored in the participants' own freezer until they were brought back or picked up after one to seven days, together with the time protocol and stored in the freezer of the Department of Psychology.

To avoid bias, participants were asked not to smoke, eat, drink (except of water) or brush their teeth 30 minutes before sampling.

All saliva samples (180 from the younger cohort and 185 from the older cohort) were analysed at the Biochemical Laboratory of the Technical University Dresden. The fraction of free cortisol in saliva (salivary cortisol) was determined using a time-resolved immunoassay with fluorometric detection (for detailed method see [54]) and reported back to the authors in the unit of measurement, nmol/l, to 1-decimal precision.

### *Psychoacoustic testing*

*Assessment of pitch discrimination thresholds.* In the first session, we assessed individual participants' pitch discrimination thresholds (i.e., their so-called just-noticeable differences; JNDs) using a weighted one-up, one-down method [55].

On each trial, participants heard two pure tones. Each tone had a duration of 100 ms with a silence period of 25 ms between tones. The first tone always had a frequency of 1 kHz; the frequency of the second tone differed from that of the first tone by delta f. The participants were asked to indicate via button press which of the two tones had the higher frequency. The next trial started 750 ms after the participants' response. Responses were self-paced. No feedback was given.

The assessment of pitch discrimination thresholds comprised five staircases per participant. Each staircase started with a delta f of 100 cents (i.e., one semitone). In the first phase, delta f was increased by a factor of 2.25 following an incorrect response and was decreased by the cube root of 2.25 following a correct response. Hence, the magnitude of upward steps was three times larger than the magnitude of downward steps, estimating approximately 75%-correct on the psychometric function. In the second phase, we used a factor of 1.5 and cube root of 1.5 for up- and down-steps, respectively. Each staircase was terminated after the twelfth reversal; there were four reversals in the first phase and eight reversals in the second phase. The threshold in each staircase was defined as the arithmetic mean of delta fs visited on all second-phase reversal trials. Finally, individual JNDs were defined as the average of thresholds across all five staircases per participant.

*Assessment of psychometric curves.* In each of the five sessions, participants performed a pitch discrimination task in a browser-based online study (Labvanced, Osnabrück). This task was similar to the assessment of pitch discrimination thresholds, which was completed in the first session only (see above): on each trial, participants heard two pure tones which differed in frequency and were asked to indicate which tone had the higher pitch. Here, however, we used a method of constant stimuli to assess participants' individual pitch sensitivity. In each session, participants completed 148 trials, comprising seven stimulus levels relative to their individual pitch discrimination threshold (JND). This means that participants were assigned to different groups based on their individual thresholds to ensure similar difficulty levels across participants. We considered five different groups: 5ct, 10ct, 15ct, 20ct, and 25ct. Participants were assigned to the group closest to their individual JND (e.g., a participant with a JND of 7.5ct would be assigned to the 10ct group, while a participant with a JND of 7.4ct would be assigned to the 5ct group).

The stimulus levels were approximately -3, -1.5, -0.5, 0, 0.5 1.5, and 3 JNDs. This choice of stimulus levels allowed us to sample the linear part of the logistic function (slope), while also capturing its asymptotes [39, 56]. Note that a stimulus level of zero JND means that the two tones on a given trial had the same frequency of 1 kHz. Hence, there was no correct response for this stimulus level. Each stimulus level was presented 21 times per session. We additionally included one dummy trial at the beginning of each session. The response in this trial was excluded from the analysis; however, inclusion of this dummy trial allowed us to present the stimulus levels using a type-1 index-1 sequence [57]. Type-1 index-1 sequences control for potential carry-over effects by first-order counterbalancing. This means that each stimulus level has the same probability to occur after each other stimulus level, including itself.

In each session, we calculated the proportion of 'second tone higher' responses per stimulus level and fitted a logistic function to the data using the Palamedes toolbox (version 1.7.0; [58]) in MATLAB (MathWorks, Natick, Massachusetts, USA; R2017b). We fitted three parameters: The point of subjective equality (PSE; i.e., the point where subjects reported 'second tone

higher' in 50% of trials), the slope at the PSE (i.e., our measure of perceptual sensitivity), and the lapse rate (i.e., the lower asymptote). The guess rate (i.e., the higher asymptote) was fixed at 1 minus the guess rate, which resulted in symmetric asymptotes of the psychometric fit.

Data sets from eight individual sessions did not follow a psychometric curve and no fit was possible. Additionally, we excluded fits with extreme slopes (i.e., larger than 5) as well as flat psychometric curves. Based on these criteria, six participants produced less than two usable fits. All data from these participants were therefore excluded from further analyses.

The data from one participant in the younger cohort who reported to follow an unusually shifted sleep-wake cycle were excluded prior to analysis. The data of six participants in the older cohort were excluded from analysis because they either dropped out of the study after the first session (N=3), or because of missing or unusable data in more than three sessions (N=3; see details on psychoacoustic testing below).

The final sample consisted of N=68 individuals and, in sum, we used 318 of a possible maximum of 340 observations in the statistical analyses.

### *Statistical analysis*

We used linear mixed-effect models to investigate how circadian fluctuations in salivary cortisol level influence perceptual sensitivity. To this end, we first modelled how cortisol expression levels change throughout the day and as a function of sleep duration, age cohort (young/old), and sex (male/female). In the main analysis, we then modelled the influence of momentary cortisol levels on auditory perceptual sensitivity, expressed as the slope of the psychometric function. We also tested for the impact of time (expressed relative to the individual wake-up time), age cohort, sex, sleep duration, pitch group, sleepiness (assessed using the Karolinska Sleepiness Scale), and response bias (expressed as the point of subjective equality on the psychometric curve, PSE).

Estimation and selection of linear mixed-effect models (Gaussian distribution, identity link function) followed an iterative model fitting procedure (e.g., [59, 60]). We started with an intercept-only null model including subject-specific random intercepts and added fixed-effects terms in a stepwise procedure following their conceptual importance. Main effects were added prior to higher-order interaction terms. Lastly, we tested whether the inclusion of a session-specific random intercept or subject-specific random slopes for time-varying within-subject effects would improve model fit. Change in model fit was assessed via likelihood ratio tests on models (re-fit with maximum-likelihood estimation for comparison of fixed effects).

We used deviation coding for categorical predictors. Single-subject observations with unusually high cortisol levels of above 60 nmol/L were discarded. To model circadian fluctuations of cortisol expression or perceptual sensitivity throughout the day, we tested for linear, quadratic and cubic time trends using polynomial regression. Cortisol levels were log-transformed and as all other continuous variables z-scored prior to modelling. To facilitate interpretation, in the visual presentation of model results, we transformed the continuous variables back to their original units.

An additional control analysis included two separate predictors for the influence of cortisol on perceptual sensitivity to tease apart within- and between-subject effects of cortisol on behaviour. Mean cortisol levels per subject captured the trait-

like, between-subject effect while the state-like, within-subject effect was modelled by the session-by-session deviation from this subject-level mean [61].

In a second control analysis, we performed a causal mediation analysis [62] to formally test the possibility of cortisol only mediating a daytime effect on perceptual sensitivity. We estimated the direct, indirect (mediated) and total effect of the cubic trend of time on perceptual sensitivity using the same set of covariate regressors in the mediation and outcome model. We calculated 95 % quasi-Bayesian confidence intervals using 5,000 replications.

We report p-values for individual model terms that were derived using the Kenward-Roger approximation for degrees of freedom [63]**.** As goodness-of-fit measures, we report $R^2$ (marginal and conditional $R^2$; taking into account only fixed or fixed and random effects, respectively) along with the Akaike information criterion (AIC) [64]. To facilitate interpretation of (non-)significant effects, we also calculated the Bayes factor (BF) based on the comparison of Bayesian information criterion (BIC) values as proposed by Wagenmakers [65]. Throughout we report log Bayes Factors, with a log BF of 0 representing equal evidence for and against the null hypothesis; log BFs with a positive sign indicating relatively more evidence for the alternative hypothesis than the null hypothesis, and vice versa. Magnitudes in the $|\log BF| > 1$ are taken as moderate, $|\log BF| > 2.3$ strong evidence for either of the alternative or null hypotheses, respectively. All analyses were performed in R (version 3.6.1) using the lme4 [66], mediation [67]**,** and sjPlot [68] packages.

### *Data availability*

All data and scripts for data analysis will be made available at Open Science framework (OSF; https://osf.io/ns26m/) upon publication.

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