Ultradean rhythmicity of plasma cortisol is necessary for normal emotional and cognitive responses in man


*Henry Wellcome Laboratories of Integrative Neurosciences and Endocrinology, School of Clinical Sciences, University of Bristol, BS1 3NY, United Kingdom; †Joint Clinical Research Unit, Bristol Royal Infirmary, University Hospitals Bristol NHS Foundation Trust, BS2 8HW, Bristol, United Kingdom; †Clinical Research and Imaging Centre, University of Bristol and University Hospitals Bristol NHS Foundation Trust, BS2 8DX, Bristol, United Kingdom; †Deparment of Psychiatry, Oxford University and Oxford Health NHS Foundation Trust, OX3 7JX, Oxford, United Kingdom; and †School of Experimental Psychology, University of Bristol, BS8 1TU, Bristol, United Kingdom

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Glucocorticoids (GCs) are secreted in an ultradian, pulsatile pattern that emerges from delays in the feedforward-feedback interaction between the anterior pituitary and adrenal glands. Dynamic oscillations of GCs are critical for normal cognitive and metabolic function in the rat and have been shown to modulate the pattern of GC-sensitive gene expression, modify synaptic activity, and maintain stress responsiveness. In man, current cortisol replacement therapy does not reproduce physiological hormone pulses and is associated with psychopathological symptoms, especially apathy and attenuated motivation in engaging with daily activities. In this work, we tested the hypothesis that the pattern of GC dynamics in the brain is of crucial importance for regulating cognitive and behavioral processes. We provide evidence that exactly the same dose of cortisol administered in different patterns alters the neural processing underlying the response to emotional stimulation, the accuracy in recognition and attentional bias toward/away from emotional faces, the quality of sleep, and the working memory performance of healthy male volunteers. These data indicate that the pattern of the GC rhythm differentially impacts human cognition and behavior under physiological, nonstressful conditions and has major implications for the improvement of cortisol replacement therapy.

glucocorticoid rhythmicity | human brain | emotional processing | fMRI study

**Significance**

The hypothalamic-pituitary-adrenal axis is a critical neurohormonal network regulating homeostasis and coordinating stress responses. Here we demonstrate that an oscillating pattern of plasma cortisol is important for maintenance of healthy brain responses as measured by functional neuroimaging and behavioral testing. Our data highlight the crucial role of glucocorticoid rhythmicity in (i) modulating sleep behavior and working memory performance, and (ii) regulating the human brain’s responses under emotional stimulation. Current optimal cortisol replacement therapies for patients with primary or secondary adrenal insufficiency are associated with poor psychological status, and these results suggest that closer attention to aspects of chronotherapy will benefit these patients and may also have major implications for improved glucocorticoid dynamics in stress and psychiatric disease.


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**1**K.K. and G.M.R. contributed equally to this work.

**2**To whom correspondence should be addressed. Email: Stafford.Lightman@bristol.ac.uk.

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also in peripheral tissues, such as the brain (55). Furthermore, the brain responds dynamically to these oscillations (56) with differing genomic (57) and rapid nongenomic responses, including the accumulation of glutamatergic receptors into synapses and induction of long-term potentiation (58). Even the behavioral responses of rodents to a mild stressor are dependent on endogenous pulses (59).

Motivated by the clinical need for improved GC-based therapeutics and the preclinical evidence, we hypothesized that under normal, nonstressful, nonpathological conditions, different ultradian GC rhythms might be translated differently in relevant GC-responsive human brain regions, and that this differential processing should be detectable using well-designed experimental protocols. We have developed a "block and replace" protocol (i.e., combined metyrapone administration with hydrocortisone infusion) in which we can reliably impose definitive patterns of plasma hydrocortisone (60). This has allowed us to provide three predetermined patterns of cortisol replacement therapy: (i) normal circadian rhythmicity lacking any physiological ultradian rhythm [s.c.-continuous hydrocortisone infusion (SCC)], (ii) normal circadian and ultradian rhythmicity [i.e., pulsatile hydrocortisone infusion (SCP)], and (iii) current optimal oral replacement therapy (PO), characterized by suboptimal circadian and ultradian rhythms. We have used these three treatment regimens in a double-blind, placebo-controlled, crossover study in healthy male volunteers to assess the importance of cortisol rhythmicity for normal brain activity in man, using a combination of functional magnetic resonance imaging (fMRI) and psychological tasks (Fig. 1), based on stimulation with emotionally valenced cues [implicit facial expression processing task (IFEPT) and parts of the P1vital emotional test battery, respectively], that recruit GC responsive brain regions previously shown to be sensitive to changes in GC infusion patterns in our preclinical studies (58, 59). We also gathered dynamic measurements of affective state throughout these interventions.

**Results**

Ecological momentary assessment (EMA) data obtained throughout the study showed no difference in either positive or negative affect between the SCC and SCP groups [$F(1.587, 22.221) = 0.196; P = 0.773$ and $F(1.321, 18.493) = 2.303; P = 0.141$, respectively]. However, individuals in the PO group showed higher negative mood ratings compared with the SCP group (mean difference of $3.154; 95\% CI, 0.754–5.554; P = 0.009$) (Fig. 24).

**The Nonpulsatile GC Rhythm Is Associated with Poorer Quality of Sleep.** An interaction of cortisol dynamics was found in one of the four domains of sleeping behavior assessed by the Leeds sleep evaluation questionnaire (LSEQ) [$F(1.914, 26.801) = 4.137; P = 0.029$; $\omega^2 = 0.12$]. Based on the volunteers’ responses on a visual analog scale (VAS), the quality of sleep is poorer (i.e., more and/or longer periods of restlessness and wakefulness) when undergoing the s.c.-continuous hydrocortisone infusion compared with the other two modes of hydrocortisone replacement (Fig. 2B and SI Appendix, Fig. S1). Post hoc analysis with a Bonferroni adjustment did not reveal any pairwise differences between any combinations of groups, however.

**The N-Back Task Reveals an Effect of Optimal Pulsatile GC Replacement in Retention of Working Memory Capacity Under Increased Cognitive Demands.** The n-back task is considered to reflect working memory processes, when n equals 2 or more; in the latter cases, the working memory buffer must be updated continuously to keep track of what the current stimulus must be compared with, necessitating maintenance and manipulation of information in working memory. Zero- and one-back tasks are used as control

![Fig. 1. Study design. Each healthy male volunteer took part in three 5-d, randomized-order, block and replace studies. In each arm of the study, endogenous hydrocortisone was suppressed with metyrapone, and hydrocortisone was replaced at a dose of 20 mg/d via (i) s.c. with a normal circadian rhythm provided by continuous s.c. infusion with an Animas Vibe pump (SCC), (ii) pulsatile s.c. infusion providing both circadian and ultradian rhythms with a Canè CRONO P pump (SCP), or (iii) oral treatment (PO), using a three times daily regimen, resulting in just three pulses during the day and a prolonged low level at night. During each pharmacologic intervention, participants were given hydrocortisone/placebo tablets and were connected to one of the pumps (infusing placebo/ hydrocortisone). Between pharmacologic interventions, there was a washout period of at least 2 wk. In the morning of the fifth day of each pharmacologic intervention, participants were attending the clinical facility and undergoing time-controlled functional (fMRI) and perfusion (ASL) magnetic resonance imaging experiments, completing an LSEQ and engaging with various computerized behavioral and cognitive tests. VS, visual stimulation task (flashing checkerboard). The mean timing (and the corresponding SD) per outcome measure per group is displayed.](image-url)
The Face Expression Recognition Task.

Volunteers in the SCP group showed greater accuracy in recognizing positive emotions compared with negative emotions, in agreement with earlier research (61, 62). While the accuracy in recognizing positive emotions did not change between treatments, this was not the case with negative emotions [F(1, 1480, 20.714) = 6.492; P = 0.011; ω² = 0.20]. The perception of negative facial expressions was reduced in the SCP group compared with the SCC group (mean difference of 4.9%; 95% CI, 0.2–9.6%; P = 0.039) and the PO group (mean difference of 4%; 95% CI, –0.2 to 8.2%; P = 0.067).

The SCP Group Shows Reduced Accuracy in Recognizing Negatively Valenced Emotional Input, as Assessed by PtVital Face Expression Recognition Task. The Face Expression Recognition Task (FERT) measures individuals' accuracy and speed in interpreting facial expressions. After each brief exposure to a human face, subjects need to make one of seven choices, indicating that they encountered a neutral expression, a positive expression (i.e., happy, surprise), or a negative expression (i.e., angry, fear, disgust, sad). The %accuracy scores for recognizing neutral faces did not differ across the treatment groups [F(1, 780, 24.914) = 0.463; P = 0.612] and were higher than those for recognizing faces with an emotional valence. ANOVA elicited a two-way interaction of [valence] × [cortisol dynamics] on the %accuracy [F(1,629, 22,809) = 3.747; P = 0.047]; across the treatment groups, participants showed greater accuracy in recognizing positive emotions compared with negative emotions, in agreement with earlier research (61, 62). While the accuracy in recognizing positive emotions did not change between treatments, this was not the case with negative emotions [F(1, 1480, 20.714) = 6.492; P = 0.011; ω² = 0.20]. The perception of negative facial expressions was reduced in the SCP group compared with the SCC group (mean difference of 4.9%; 95% CI, 0.2–9.6%; P = 0.039) and the PO group (mean difference of 4%; 95% CI, –0.2 to 8.2%; P = 0.067).

sessions. Two subjects performed poorly (i.e., significantly low %accuracy scores, with values of studentized residuals below −3) in these control sessions in at least one of the study arms, which indicates a systematic bias; therefore, these subjects were excluded from further analysis for this task (n = 13 per study group).

ANOVA elicited a two-way interaction of [cognitive load] × [cortisol dynamics] on participants' performance in the n-back task [F(1,525, 18,304) = 4.437; P = 0.035]. Volunteers in the SCP group retained the same performance across the two- and three-back sessions, in contrast to the performance in the other two treatment modes, where performance in three-back sessions was poorer compared with that in the two-back sessions, especially in the SCC group (mean drop in %accuracy score from two- to three-back sessions, 7.9%; 95% CI, 2.7–13%; P = 0.006; ω² = 0.28; mean difference in the %accuracy in three-back sessions between the SCP and SCC groups, 6.6%; 95% CI, –0.3 to 13.5%; P = 0.063; ω² = 0.10) (Fig. 3).

ANOVA did not demonstrate any two-way interaction of [cognitive load] × [cortisol dynamics] on participants' reaction times for correct responses [F(1,403, 16,835) = 0.663; P = 0.477], or any significant interactions for the main effects of each factor. Therefore, the differences in %accuracy reported above have not been confounded by differences in reaction times.

The SCP Group Shows Reduced Accuracy in Recognizing Negatively Valenced Emotional Input, as Assessed by PtVital Face Expression Recognition Task. The Face Expression Recognition Task (FERT) measures individuals' accuracy and speed in interpreting facial expressions. After each brief exposure to a human face, subjects need to make one of seven choices, indicating that they encountered a neutral expression, a positive expression (i.e., happy, surprise), or a negative expression (i.e., angry, fear, disgust, sad). The %accuracy scores for recognizing neutral faces did not differ across the treatment groups [F(1,780, 24.914) = 0.463; P = 0.612] and were higher than those for recognizing faces with an emotional valence. ANOVA elicited a two-way interaction of [valence] × [cortisol dynamics] on the %accuracy [F(1,629, 22,809) = 3.747; P = 0.047]; across the treatment groups, participants showed greater accuracy in recognizing positive emotions compared with negative emotions, in agreement with earlier research (61, 62). While the accuracy in recognizing positive emotions did not change between treatments, this was not the case with negative emotions [F(1, 1480, 20.714) = 6.492; P = 0.011; ω² = 0.20]. The perception of negative facial expressions was reduced in the SCP group compared with the SCC group (mean difference of 4.9%; 95% CI, 0.2–9.6%; P = 0.039) and the PO group (mean difference of 4%; 95% CI, –0.2 to 8.2%; P = 0.067).
These treatment-related perceptual variations were confirmed by ANOVA on the %origin of misclassifications \(F(1.637, 22.916) = 4.120; P = 0.036; \omega^2 = 0.12\), since subjects receiving the optimal pulsatile hydrocortisone replacement are more likely to misclassify negative emotional faces compared with those receiving the other two modes of hydrocortisone replacement, as well as by the main effect of treatment on the %destination of misclassifications \(F(1.665, 23.312) = 6.522; P = 0.008; \omega^2 = 0.20\), since the SCP group misclassified toward emotional faces with a significantly higher frequency compared with the other two groups (SI Appendix, Fig. S2). Overall, the optimal pulsatile treatment resulted in lower accuracy for recognizing negatively valenced faces and, consequently, more misclassifications of negatively valenced faces.

There was no two-way interaction of [valence] \times [cortisol dynamics] by ANOVA on the reaction time of subjects’ responses \(F(1.927, 26.971) = 1.959; P = 0.162\). There was also no main effect of treatment on reaction time \(F(1.668, 23.351) = 1.026; P = 0.361\). However, emotional valence showed a main effect \(F(1, 14) = 48.857; P < 0.001\), similar to that found in earlier studies (61, 62); independent of treatment group and on average, participants tended to respond faster to faces with a positive emotional valence compared with faces with a negative emotional valence (mean difference, 211 ms; 95% CI, 164–257 ms; \(P < 0.001\)).

The Pivital Emotional Face-Related Attentional Bias Task Indicated That the SCP Group Shows Facilitation of Attentional Deployment Toward Positive Stimuli. The Facial Dot-Probe Task (FDOT) assesses attention to positive and negative stimuli using a reaction time measure, the vigilance score. While subjects are asked to maintain their attention to the middle of a screen, emotionally valenced and neutral stimuli are presented above and below this central point. After presentation, one of the stimuli is replaced by two dots, vertically or horizontally oriented. The subjects are instructed to guide their attention and correctly identify the orientation of the two dots as quickly as possible. The facial stimuli are presented for either 100 ms (unmasked) or 16 ms and then replaced for the remaining 84 ms with a jumbled face (masked) (63). In the three treatment groups, the mean %accuracy scores for identifying the orientation of the two dots were very high and very similar (97.5%, 97.1%, and 96.7%). ANOVA elicited a three-way interaction of [masking] \times [valence] \times [cortisol dynamics] on the vigilance score of the subjects’ responses \(F(1.771, 24.778) = 4.039; P = 0.035\), driven by the differential, treatment-dependent deployment of attention toward or away from emotional faces when moving from the subliminal level (masked faces; Fig. 4B) to the brief presentation level (unmasked faces; Fig. 4C).

Across treatments and emotional valences (happy and fearful faces), we can observe a pattern in which subjects showed a negative vigilance score (i.e., attentional bias away from emotional faces) at a subliminal level, transformed to a positive one (i.e., attentional bias toward emotional faces) at a brief presentation level. This pattern not only was reversed for fearful faces in subjects receiving oral hydrocortisone replacement, but...
the difference in the vigilance scores between the two perceptual levels was notable \( F(1.879, 26.301) = 3.456; P = 0.049; \omega^2 = 0.08 \); mean difference, 25.1; 95% CI, −2 to 52.3; \( P = 0.067 \), especially due to the deployment of these subjects’ attention away from unmasked fearful faces [absolute attentional bias: \( t(14) = 2.363; P = 0.033; d = 0.31 \)]. At a preconscious level, subjects receiving the optimal pulsatile treatment tended to be strongly attracted by happy faces, not only per se [absolute attentional bias: \( t(14) = 3.872; P = 0.002; d = 1.00 \)], but also in comparison with fearful faces \( F(1, 14) = 5.874; P = 0.030; \omega^2 = 0.14 \); mean difference in vigilance score, 23.1; 95% CI, 5.5–40.7; \( P = 0.014 \). Overall, GC rhythmicity seems to interact with the fast-acting neural pathways that coordinate the deployment of attention in the presence of emotional stimuli.

**Different GC Rhythms Change the Neural Processing of Emotional Input.** Our fMRI protocol assessing the processing of emotional faces (i.e., IFEPT) provoked significant activations from most of the predefined regions of interest (ROIs; SI Appendix, Table S3) and the combined evaluation of the fMRI experiments (on emotional face processing and nonemotional visual stimulation) with the ROI analysis on the regional perfusion data revealed the presence of GC-susceptible brain areas in which the underlying neural processing of emotional input is rhythm-sensitive. These rhythm-dependent neural responses specifically relate to facial expression processing rather than to nonspecific differences due to neural reactivity or neural coupling or due to differences in the resting perfusion of these brain areas between the groups.

Whole-brain analysis of the fMRI data acquired during the IFEPT identified differential patterns of activation in brain regions (familywise error-corrected; Z-threshold = 2.3; \( P < 0.05 \)) in which blood oxygen level-dependent (BOLD) signal activity patterns corresponding to emotional face discrimination showed notable variations as a function of emotional expression and group. These included some of our predefined ROIs and were observed in the differential processing of happy and sad faces, and fearful and sad faces, between the SCC and SCP groups (SI Appendix, Fig. S4) and in the differential processing of happy and sad faces between the SCC and PO groups (SI Appendix, Fig. S5).

The five ROIs specified were parts of the right amygdala, right striatum, right orbitofrontal cortex, and right and left insula; the corresponding data are presented in Figs. 5 and 6 and SI Appendix, Fig. S6.

During our control task, visual stimulation (i.e., flashing checkerboard) whole-brain analysis did not reveal any notable BOLD signal variations between the treatment groups in areas responsible for visual processing (occipital and temporal lobes) or any of the ROIs predefined for this study (SI Appendix, Fig. S7). Thus, the variations detected in those five ROIs in the IFEPT fMRI experiment should reflect differences in the processing of facial traits and emotion rather than nonspecific differences due to neural reactivity or neural coupling.

At another level, resting perfusion has been shown to have an inverse relationship with the BOLD responses and thus is an important confounding factor in IMRI studies (64). Our arterial spin labeling (ASL) data showed comparable regional resting perfusion (pairwise comparisons presented in SI Appendix, Fig. S6 and Table S8) across the five GC-sensitive ROIs responding differentially to emotional faces in the IFEPT fMRI experiment, further strengthening the concept that these variations reflect...
existing differences in the underlying neural processing of facial traits and emotion.

Different GC Rhythms Change the Functional Connectivity of GC-Sensitive Brain Regions and Their Functional Roles in Processing of Emotional Input. Pursuant to our fMRI data assessing the neural processing of happy and sad faces, and our behavioral data from FERT on the recognition of happy and sad faces, we investigated whether there is a functional link between these GC rhythm-sensitive neural and psychological processes, and how different GC rhythms might alter that link. We conducted a multiple post hoc correlation analysis between the absolute values of the effect estimates (absolute %BOLD signal changes) of the brain regions, which were differentially responsive to viewing happy and sad faces across the treatment groups, an index of ambiguity in recognizing emotion corresponding to happy and sad faces, derived from the FERT data, and the EMA data illustrating the positive and negative affective state of individuals presented earlier (Fig. 7).

This post hoc analysis was decided in the light of the existing strong evidence about the role of the amygdala in the detection of ambiguous, emotion-relevant cues (65, 66) and the insular cortex in the integration of internal cues, like affective state (67) and empathic processing (68, 69), to the cognitive evaluation of emotional input, as well as the involvement of the orbitofrontal cortex and striatum in the neural systems mediating emotional processing (70, 71).

To define the ambiguity in recognizing emotional faces, we created an index derived from the data of the FERT. We divided the number of misclassifications from or toward each valence (sad and happy faces) by the number of emotional faces of that valence correctly recognized for each mode of hydrocortisone replacement such that the higher the index, the greater the degree of ambiguity or difficulty in specifically recognizing each emotion. This index was chosen because of its concurrent very high correlation coefficient with both, the %accuracy scores (for correctly identifying), and the number of misclassifications involving the corresponding emotional valence (SI Appendix, Fig. S9).

In the SCC and SCP groups, only in the latter were the %BOLD signal changes of the right amygdala and right insula highly correlated with the monitoring of ambiguity in recognizing happy and sad faces [r(30) = 0.430; P = 0.018] and the affective state [r(30) = 0.521; P = 0.003], respectively. Moreover, in the SCP group, these two brain structures showed an increased in-between functional connectivity compared with the SCC group (Fig. 7A). Furthermore, subjects on the oral replacement showed an increased functional connectivity between the right orbitofrontal cortex with bilateral insulae [rFROC(30) = 0.501; P = 0.005 for the right insula and rPO(30) = 0.428; P = 0.018, for the left insula] during the neural processing of the happy and sad faces, not present in the SCC group (Fig. 7B).

Relationship of Sleep Quality with Outcomes of the Study. Since cortisol impacts sleep physiology, and the latter influences emotional and memory processing in man (72–75), we plotted our LSEQ-derived sleep evaluations against the rest of our data (SI Appendix, Fig. S6 and Table S10). Quality of sleep had a moderate positive correlation with the %BOLD signal changes in the pulsatile and continuous infusion groups reported in the right insula in the context of discriminating between fearful and sad faces [rFROC(30) = 0.344; P = 0.063, accounting for the 11.8% of the statistical variance of these BOLD signal responses], and a moderate negative correlation with the number of misclassifications originating from negatively valenced faces [rFROC(46) = −0.386; P = 0.014, accounting for the 13.4% of the statistical variance on the number of misclassifications]. In all other cases, no significant correlations were specified.
Correlation analysis between the absolute values of the effect estimates of the brain regions, which were differentially responsive to viewing happy and sad faces across the treatment groups, an index of ambiguity in recognizing emotion corresponding to happy and sad faces, and the positive and negative affective states of individuals. The values of each outcome measure are illustrated on a white to dark-blue scale (with white indicating the lowest value and dark-blue indicating the highest value). Spearman's rank-order correlation test was used; positive correlations (between the corresponding datasets) are indicated by a green arrow, and negative correlations are indicated with a red arrow. The scales of the negative and positive effects are (as expected) strongly anticorrelated with each other. A positive functional connectivity between the right insula and the right striatum (during emotional processing) is observed. (A) The differences in the neural processing of emotional input observed in the right amygdala and the right insula of the SCP group compared with the SCC group seem to (at least partially) reflect changes in their functional role (like monitoring ambiguity in recognizing facial expressions or the affective state) during the processing of emotional input. (B) The differences in neural processing of emotional input observed in the bilateral insula and the right orbitofrontal cortex of the PO group compared with the SCC group seem to (at least partially) reflect changes in their in-between functional connectivity during the processing of emotional input. %BOLD, absolute %BOLD signal change (compared with baseline) during exposure to the corresponding emotional face (happy and sad); Li/Lins, left insula; NEG.Aff., negative affect score; POS.Aff., positive affect score; RA/Ramy, right amygdala; Recg. Amb., index of recognition ambiguity when engaging with the corresponding emotional faces (happy and sad); Ri/Rins, right insula; ROFC, right orbitofrontal cortex; RS, right striatum.

**Discussion**

We have investigated the neural processing and behavioral responses related to emotional perception and cognitive performance in healthy male volunteers on three patterns of the same total dose hydrocortisone replacement therapy. The SCP group was characterized by physiological circadian and ultradian GC rhythms (SI Appendix, Fig. S11), the SCC group was characterized by a normal circadian but no ultradian GC variation, and the PO group (in which we used current optimal GC replacement recommendations) was characterized by a delayed circadian peak of cortisol, two further pulses during the day, and very low levels at awakening. It is the low quality of life, reduced activity, low motivation, and mental fatigue reported in patients on oral replacement therapy or continuous infusion therapy that motivated the present study.

The pattern of GC replacement differentially modulates processes related to working memory and sleep physiology. In particular, a lack of physiological cortisol pulsatility is associated with poorer working memory performance at times of increased cognitive demands. Moreover, the absence of ultradian rhythmicity correlates with a poorer self-perceived quality of sleep. Our data demonstrate that different patterns of plasma cortisol oscillations have a differential impact on the GC-sensitive brain regions underlying emotional processing, with distinct consequences for (i) the accuracy of recognizing emotional faces (particularly the negatively valenced ones), (ii) the direction of this perceptual bias, and (iii) the attentional bias toward or away from emotional faces. These GC rhythm-dependent changes could reflect functional modifications among corticolimbic brain regions underlying the differential recognition of negatively valenced faces as observed in the FERT, or the differential deployment of attention across perceptual levels, in the presence of emotional stimuli, as observed in the FDOT.

While the %BOLD signal change in the right amygdala of the SCP group in response to emotional faces relates to the difficulty of the individuals in correctly recognizing emotions, the same brain region becomes dissociated from monitoring emotional ambiguity in the SCC group. In this context, it is worth noting that amygdala %BOLD signal changes in the SCP group were significantly higher when subjects were viewing sad (negatively valenced) faces compared with happy (positively valenced) faces. This is in line with the FERT data, supporting the notion that the decreased accuracy (i.e., increased uncertainty) of subjects on the optimal pulsatile infusion in recognizing emotional faces is negative valence-specific. Similarly, while in the SCP group the right insula seems to integrate internal cues, like the affective state, into the process of encoding facial expressions, it becomes dissociated from that process in the SCC group. Finally, the functional connectivity between the right orbitofrontal cortex and insular cortices during emotional face presentation appears to be very strong in the PO group, which is not the case in the SCC group.

Previous studies using the FERT and FDOT for assessing neuropsychological processes involved in depression and anxiety (76) have shown similar responses of healthy subjects receiving antidepressant and anxiolytic regimens to the responses that we find in our patients on the pulsatile SCP regimen. This implies an involvement of GC rhythmicity in the psychophysiological mechanisms regulating mood and anxiety.

Previous neuroimaging experiments have provided evidence that high levels of exogenous GCs, mimicking stress-associated states, alter both the neural processing in response to emotional

Fig. 7. Correlation analysis between the absolute values of the effect estimates of the brain regions, which were differentially responsive to viewing happy and sad faces across the treatment groups, an index of ambiguity in recognizing emotion corresponding to happy and sad faces, and the positive and negative affective states of individuals. The values of each outcome measure are illustrated on a white to dark-blue scale (with white indicating the lowest value and dark-blue indicating the highest value). Spearman’s rank-order correlation test was used; positive correlations (between the corresponding datasets) are indicated by a green arrow, and negative correlations are indicated with a red arrow. The scales of the negative and positive effects are (as expected) strongly anticorrelated with each other. A positive functional connectivity between the right insula and the right striatum (during emotional processing) is observed. (A) The differences in the neural processing of emotional input observed in the right amygdala and the right insula of the SCP group compared with the SCC group seem to (at least partially) reflect changes in their functional role (like monitoring ambiguity in recognizing facial expressions or the affective state) during the processing of emotional input. (B) The differences in neural processing of emotional input observed in the bilateral insula and the right orbitofrontal cortex of the PO group compared with the SCC group seem to (at least partially) reflect changes in their in-between functional connectivity during the processing of emotional input. %BOLD, absolute %BOLD signal change (compared with baseline) during exposure to the corresponding emotional face (happy and sad); Li/Lins, left insula; NEG.Aff., negative affect score; POS.Aff., positive affect score; RA/Ramy, right amygdala; Recg. Amb., index of recognition ambiguity when engaging with the corresponding emotional faces (happy and sad); Ri/Rins, right insula; ROFC, right orbitofrontal cortex; RS, right striatum.

**Table 1.**

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*P < 0.1; **P < 0.01; ***P < 0.001*
or cognitive stimulation and the resting state functional connectivity (7–14). These studies suggest a specific role for MRs in some of these stress-related changes in both neural processing and functional connectivity (15, 16), while MR or GC receptor antagonism impacts the amygdala-dependent processing of emotional faces (77). Our study findings now provide evidence that even in the absence of a stressful stimulus, the ultradian GC rhythm is critical in regulating neural dynamics and, consequently, behavioral and cognitive phenotypes. Future studies in patients with adrenocortical insufficiency are now needed, not only to help reduce the morbidity of current replacement regimens, but also to provide evidence from longer-term modification of replacement cortisol rhythmicity for improved brain function and a more personalized approach to GC therapeutics.

Materials and Methods

Study Design. This randomized, double-blind, placebo-controlled crossover study of three different modes of hydrocortisone replacement in healthy subjects was registered with the United Kingdom Clinical Research Network (IRAS reference 106181; UKCRN-ID-15236; October 23, 2013). The study followed the CONSORT guidelines for randomized controlled trials (SI Appendix, Fig. S12).

Participants. Fifteen right-handed, healthy male volunteers age 20–33 y were included in the study (SI Appendix, Table S13). The subjects had no history of neuropsychiatric disease as confirmed by clinical assessment and were excluded if they had a family history of a psychiatric disorder. The Ethics Committee of the University of Bristol approved the study, and all participants provided informed written consent. Each volunteers passed a detailed screening session, part of which was the acquisition of a high-resolution anatomic MRI scan.

Pharmacologic Interventions. The cortisol biosynthesis blocking agent (metyrapone) was taken orally in all three arms of the study, together with the maintenance treatment of their chosen GC replacement regimen. All the participants received a fixed daily dose of 20 mg of hydrocortisone: (i) s.c. via a pump, delivering normal circadian but no ultradian rhythm (SCP group), (ii) normal circadian and ultradian rhythm (SCC group), or (iii) per os, three times daily (after waking up, during lunch and dinner) (PO group) (60).

Randomization and Blinding Procedures. Block randomization schedules were generated by staff members not directly involved in data collection for this study. Each subject was randomly assigned to one of the six possible orders of treatment, with the limitation that the difference in the final number of subjects between any order of treatments should not exceed 1, when the total number of participants reaches 15. Dispensing and processing of all medication/placebo was managed by a third party (Bristol Royal Infirmary University Hospital Pharmacy). In all treatment arms, subjects were required to wear a face mask when handling test stimuli and body contact with any other participants. Anonymized data from all outcome measures were stored in the University of Bristol central servers, were cleaned at an individual level without knowledge of which session corresponds to which subject, and, consequently, further postprocessed and compared statistically at a group level, without knowledge of which group corresponds to which mode of hydrocortisone substitution.

EMA. Throughout each 5-d treatment period, subjects had to reply (via a provided android phone) to a fixed set of nine questions about self-perceived reactivity and feelings of well-being at multiple time points during each day (SI Appendix, Fig. S14). A total of 1,018 assessments were collected. Principal component analysis was used to reduce the nine items of the questionnaire and identified two factors: positive and negative affect.

Neuroimaging Study. Prior to the fMRI experiments, streaming along the longitudinal axis of the main magnetic field was performed to reduce the impact of geometric distortions and improve signal acquisition from the areas of the orbitofrontal cortices. Moreover, field maps were acquired and used during the preprocessing stage of the functional image analysis, improving the coregistration of the functional images to the corresponding high-resolution anatomic images (SI Appendix, Table S15). This study follows the guidelines for reporting fMRI data suggested by Poldrack et al. (78). The ROIs for the fMRI studies have been defined as a priori (60). Functional and perfusion neuroimaging data analyses were carried out using FMRIb soft-

ware library 5.0 (79) and Statistical Parametric Mapping version 8, and involved a number of preprocessing and postprocessing steps (SI Appendix, Figs. S16 and S17) (80–83).

LSEO. Ten self-rating questions examined four domains of sleeping behavior: (i) getting to sleep, (ii) quality of sleep, (iii) awakening from sleep, and (iv) behavior following waking. Rating was done on a 100-mm VAS, with a higher score indicating poorer sleep. The score for each of the four domains (per study arm and participant) was derived from the average of the scores for the corresponding questions.

FERT. Two hundred and fifty images of artificially created human faces were displayed (one at a time) on the computer screen for 500 ms and each time replaced by a blank screen until the subject responded. The facial characteristics of each image were developed based on the Pictures of Affect Series (84) but morphed between each prototype emotion and neutral in such a way as to produce images depicting the full emotion (100%) and images presenting the emotion with gradually increasing degrees of ambiguity. For each emotion, 40 images were displayed (equally weighted for other non-emotional features, such as sex, skin color, eye color, etc.). In addition, 10 faces with a neutral expression were included. Images were presented in a random order. More details on the dependent variables constructed are provided in SI Appendix, Fig. S18.

FDOT. One hundred and ninety-two pairs of images depicting facial expressions were displayed on the screen (one pair at a time) for 100 ms. The images used in this task were taken from the JACFEE/JACNeuF sets of facial expressions (85). Each pair of faces comprised one emotional expression and one neutral expression of the same individual (in 128 of the trials) or two neutral expressions of the same individual (in 64 of the trials). One-half of the emotional faces were fearful, and the other half were happy. In the fearful-neutral and happy-neutral face trials, the emotional faces appeared above and below the central fixation position with equal frequency. The task design involved congruent trials (dots replace an emotional face) and incongruent trials (dots replace a neutral face while an emotional face is present). Incorrect trials were excluded from the data analysis. Attentional vigilance scores were calculated for each participant by subtracting the mean reaction time of congruent trials from incongruent trials.

N-Back. This test comprised 16 sessions of letter presentation (one letter at a time) for 100 ms. The images used in this task were taken from the JACFEE/JACNeuF sets of facial expressions (85). Each pair of faces comprised one emotional expression and one neutral expression of the same individual (in 128 of the trials) or two neutral expressions of the same individual (in 64 of the trials). One-half of the emotional faces were fearful, and the other half were happy. In the fearful-neutral and happy-neutral face trials, the emotional faces appeared above and below the central fixation position with equal frequency. The task design involved congruent trials (dots replace an emotional face) and incongruent trials (dots replace a neutral face while an emotional face is present). Incorrect trials were excluded from the data analysis. Attentional vigilance scores were calculated for each participant by subtracting the mean reaction time of congruent trials from incongruent trials.

Statistical Analysis of the Behavioral Data. Statistical analysis was performed using SPSS version 23, and corresponding graphs were created in GraphPad Prism version 5.03. The influence of the different cortisol rhythms on subjects' responses as assessed by the EMA, LSEQ, FERT, FDOT, and n-back was evaluated with repeated-measures mixed-model ANOVA, either three-way (FDOT), two-way (FERT, n-back), or one-way (EMA, LSEQ), depending on the psychological test. In all cases, one of the within-subject factors was the treatment group (three levels: SCC, SCP, and PO). In the case of FDOT, one-sample t tests were also used to compare attentional bias scores to zero within each group to clarify where an absolute bias was present. Tests for detecting distribution of the data (Shapiro-Wilk test), and sphericity have been used and taken into consideration for the data analysis. Two genuinely unusual values were detected in the FERT dataset referring to reaction time; in this case, repeated-measures ANOVA was

All study-related procedures not directly available in the main text are provided in SI Appendix. Additional information is available in Kalafatakis et al. (60).

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