Progress in Neurobiology 205 (2021) 102127

Contents lists available at ScienceDirect

Progress in Neurobiology

journal homepage: www.elsevier.com/locate/pneurobio

Original Research Article

Brain preparedness: The proactive role of the cortisol awakening response in hippocampal-prefrontal functional interactions

Bingsen Xiong [a], Changming Chen [b,1], Yanqiu Tian [a,1], Shouwen Zhang [c,1], Chao Liu [a,1], Tanya M. Evans [d], Guillén Fernández [e], Jianhui Wu [f], Shaozheng Qin [a,g,*]

[a] State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing, 100875, China
[b] School of Education, Chongqing Normal University, Chongqing, 401331, China
[c] West Essence Clinic, Beijing Institute of Functional Neurosurgery & Xuanwu Hospital, Capital Medical University, Beijing, 100053, China
[d] School of Education and Human Development, University of Virginia, Charlottesville, VA, 22904, USA
[e] Donders Institute for Brain, Cognition and Behaviour & Department for Cognitive Neuroscience, Radboud University Medical Centre, Nijmegen, 6525 EN, the Netherlands
[f] Shenzhen Key Laboratory of Affective and Social Cognitive Science, Shenzhen University, Shenzhen, 518060, China
[g] Chinese Institute for Brain Research, Beijing, 100069, China

ARTICLE INFO

Keywords:
Cortisol awakening response
Prefrontal cortex
Hippocampus
fMRI
Working memory

ABSTRACT

Upon awakening from nighttime sleep, the stress hormone cortisol in humans exhibits a robust rise within thirty to forty-five minutes. This cortisol awakening response (CAR), a crucial point of reference within the healthy cortisol circadian rhythm, has been linked to various psychological, psychiatric and health-related conditions. The CAR is thought to prepare the brain for anticipated challenges of the upcoming day to maintain one's homeostasis and promote adaptive responses. Using brain imaging with a prospective design and pharmacological manipulation, we investigate the neurobiological mechanisms underlying this preparation function of the CAR across two studies. In Study 1, a robust CAR is predictive of less hippocampal and prefrontal activity, though enhanced functional coupling between those regions during a demanding task hours later in the afternoon. Reduced prefrontal activity is in turn linked to better working memory performance, implicating that the CAR proactively promotes brain preparedness based on improved neurocognitive efficiency. In Study 2, pharmacologically suppressed CAR using Dexamethasone mirrors this proactive effect, which further causes a selective reduction of prefrontal top-down functional modulation over hippocampal activity. These findings establish a causal link between the CAR and its proactive role in optimizing functional brain networks involved in neuroendocrine control, executive function and memory.

1. Introduction

Upon awakening from night sleep, cortisol, the major glucocorticoid stress hormone in humans, exhibits a burst typically by 50–160 % within thirty to forty-five minutes – that is known as the cortisol awakening response (CAR) (Clow et al., 2010; Pruessner et al., 1997). Since its first discovery, the CAR, a hallmark of the hypothalamus-pituitary-adrenal (HPA) axis activity as well as a crucial point of reference within the healthy cortisol circadian rhythm, is thought to prepare the body for anticipated challenges of the upcoming day (Adam et al., 2006; Elder et al., 2014; Fries et al., 2009; Law et al., 2013). In support of this "preparation" hypothesis, an individual's CAR predicts anticipated workload and cognitive demands of the upcoming day (Kunz-Ebrecht et al., 2004; Law et al., 2015, 2020; Schlotz et al., 2004; Stalder et al., 2010a, b), while atypical CAR including either a blunted or elevated pattern is often linked to stress-related psychopathology such as anxiety, depression and higher-order cognitive impairments (Chida and Steptoe, 2009; Clow et al., 2004; Fries et al., 2009; Kudielka and Wust, 2010). However, our understanding of the CAR's neurobiological mechanisms is still in its infancy.

Cortisol acts as one of the key modulators of the human brain and cognition. It is released mainly by the zona fasciculata of the adrenal

* Corresponding author at: State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing, 100875, China.
E-mail address: szqin@bnu.edu.cn (S. Qin).
[1] Equal contributions.

https://doi.org/10.1016/j.pneurobio.2021.102127
Received 4 March 2021; Received in revised form 23 June 2021; Accepted 29 July 2021
Available online 31 July 2021

cortex (Spencer and Deak, 2017) and can cross the blood-brain barrier to affect neuronal excitability and functional organization of brain networks, thereby fostering adaptation to cognitive and environmental challenges (McEwen, 1998). The neurobiological models posit that glucocorticoids exert both rapid nongenomic and slow genomic actions on the limbic-frontal networks especially the hippocampus and prefrontal cortex (PFC), via high-affinity mineralocorticoid receptors (MRs) and low-affinity glucocorticoid receptors (GRs) that are co-expressed abundantly in these brain regions (de Kloet et al., 2019; McEwen et al., 2015). The MR initiates rapid changes in the assembly of neural circuits allowing a quick and adequate response to an ongoing stressful event (Vogel et al., 2016). As this process is energetically costly and may have deleterious consequences when over-engaged, MR-mediated rapid actions are complemented by slower actions via GRs on preventing these initial defence reactions from overshooting and becoming damaging. The GR-mediated slow genomic effect on neuronal activity is not expected to start earlier than approximately 90-min after cortisol administration, and often lasts for hours (Henckens et al., 2011; Joels and de Kloet, 1992). This process can promote contextualization, rationalization and memory storage of experiences, thereby priming brain circuits to be prepared for upcoming challenges in similar contexts (de Kloet et al., 2019; Herman et al., 2003). Thus, it is conceivable that the CAR, with a burst of the cortisol concentration in response to awakening in the morning, may proactively affect the brain and cognition via similar MR/GR-mediated actions of cortisol.

Beyond the conventional cortisol responses, the CAR exhibits unique features which may involve fundamentally distinct mechanisms (Stalder et al., 2016). Specifically, the CAR consists of a superimposed response (reflected by a burst effect of cortisol increase) to awakening, which is not a mere continuation of pre-awakening cortisol increase (Wilhelm et al., 2007). It is regulated by multiple neuroendocrine and psychological processes, including i) rapid attainment of consciousness followed by slow re-establishment of one's full alertness (Clow et al., 2010), ii) activation of hippocampal-dependent prospective memory representations for upcoming stress (Fries et al., 2009), and iii) an interplay with concurrent catecholaminergic activation when facing demanding tasks (Arnsten, 2009). Moreover, findings from previous studies point to a critical role of hippocampal and/or prefrontal involvement in regulating CAR. Patients with lesions to the hippocampus (Buchanan et al., 2004) or retrograde amnesia (Wolf et al., 2005), for instance, do not exhibit a reliable CAR. The magnitude of CAR also negatively correlates with prefrontal cortical thickness (Kremen et al., 2010), suggesting prefrontal involvement in the CAR. Additionally, functional organization of hippocampal-prefrontal networks is crucial for regulating information exchange and flexible reallocation of neural resources in support of executive function and memory (Egan et al., 2003; Liu et al., 2016). Little, however, is known regarding the neurobiological mechanisms of whether and how the CAR proactively modulates the human brain for executive function. Based on the aforementioned unique features of the CAR and empirical observations, we hypothesized that the CAR would prepare the brain for upcoming demands of the day ahead via optimizing the functional organization of hippocampal and prefrontal systems.

We tested this hypothesis across two studies using blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD-fMRI) with a prospective design and pharmacological manipulations dedicated to CAR (Figs. 1A and 3 A). We opted for a well-established numerical N-back working memory (WM) paradigm to probe task-invoked activation and deactivation in the dorsolateral prefrontal cortex (dlPFC) and the hippocampus, respectively (Cousijn et al., 2012; Owen et al., 2005). Such antagonistic organization is known to enable a flexible reallocation of neural resources to support higher-order executive function while inhibiting task-irrelevant interference (Cousijn et al., 2012; Pomarol-Clotet et al., 2008; Qin et al., 2009), making this domain an ideal model for studying human prefrontal-hippocampal interactions.

In Study 1, sixty participants underwent fMRI while performing the WM task with low and high cognitive demands after 6 hours relative to awakening in the afternoon. Six salivary samples were obtained to assess the CAR in the morning and cortisol levels before and after fMRI scanning. To further test whether there is a causal link between an individual's CAR and its proactive effects on task-related prefrontal and hippocampal activity, we conducted a pharmacological fMRI experiment (Study 2) by implementing a randomized, double-blind, placebo-controlled design. Sixty-three participants received either 0.5-mg *dexamethasone* (DXM) or placebo at 20:00 on Day 1 to suppress their CAR on Day 2. DXM, a synthetic glucocorticoid, can temporally suppress CAR via imitating negative feedback from circulating cortisol to adrenocorticotropic hormone-secreting cells of the pituitary (Cole et al., 2000; Ebrecht et al., 2000). Saliva samples were collected at 15 time points spanning over three consecutive days. Other procedures were similar to Study 1. These two studies allowed us to investigate the potential causal link between CAR and its proactive role in preparing hippocampal-prefrontal networks involved in higher-order cognitive processing.

2. Methods and materials

2.1. Participants

A total of 123 young, healthy, male college students participated in two separate studies, with 60 (mean age: 21.6 ± 0.76 years old; range: 20–24 years old) in Study 1 and 63 (mean age: 22.9 ± 1.9; range: 18 - 27 years old) in Study 2. Only men were included because of hormonal fluctuations across the menstrual cycle and the impact of hormonal contraceptives in young adult females (Cousijn et al., 2010; Kirschbaum et al., 1999). Participants reported no history of neurological, psychiatric or endocrinal disorders. Exclusion criteria included current medication treatment that affects central nervous or endocrine systems, daily tobacco or alcohol use, irregular sleep/wake rhythm, intense daily physical exercise, abnormal hearing or (uncorrected) vision, predominant left-handedness, current periodontitis (Mathew et al., 2019), stressful experience or major life events (see Supplementary Materials for more details).

Data from 12 participants were excluded from the analyses due to excessive (beyond 2 mm/degree) head movement during scanning (5 and 3 participants for Study 1 and 2, respectively) or incomplete salivary samples (3 and 1 participants for Study 1 and 2, respectively). Thus, a final sample of 52 participants (28 and 24 participants for robust- and lower-CAR groups, respectively) were included in Study 1, and another sample of 59 participants (26 and 33 for placebo and DXM groups, respectively) were included in Study 2 (Table S1). Informed written consent was obtained from all participants before the experiment, and the study protocol was approved by the Institutional Review Board for Human Subjects at Beijing Normal University. The protocol with pharmacological manipulation was registered as a clinical trial before the experiment (https://register.clinicaltrials.gov/; Protocol ID: ICBIR_A_0098_002).

2.2. General experimental procedure

In Study 1, we explored the relationship between individual differences in CAR and the neurocognitive correlates of WM in a natural setting. Salivary samples were obtained at 10 time points to assess CAR and diurnal rhythms of cortisol levels. The brain imaging data were acquired while participants performed a numerical N-back task with two loading conditions (i.e., 0- and 2-back) in the afternoon of the same day (Fig. 1A).

In Study 2, we implemented a randomized, double-blind, placebo-controlled design to investigate the causal link of CAR with brain activity during a WM task. Participants orally received either a dose of 0.5-mg Dexamethasone (i.e., DXM group) or an equal amount of Vitamin C (i.e., placebo group) pill at 20:00 on Day 1. Participants completed a

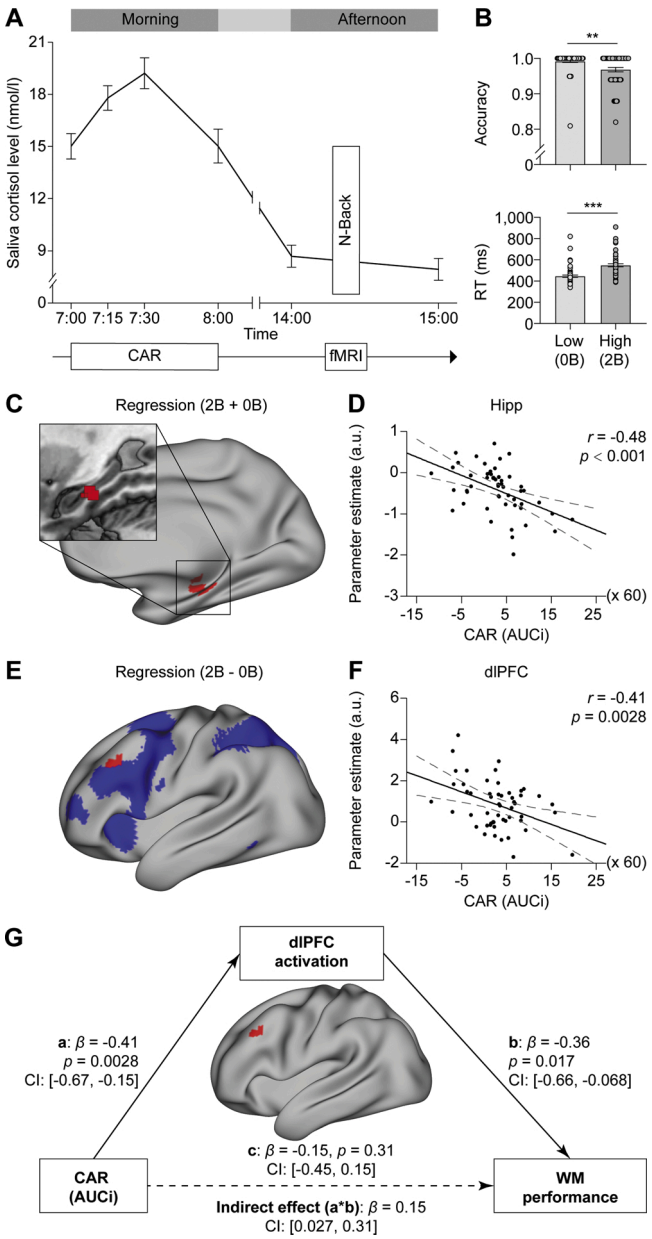

Fig. 1. Experimental design, cortisol awakening response (CAR), and CAR-related proactive effect on brain systems from Study 1. **A**. Salivary cortisol levels at 4 time points after awakening in the morning and 2 time points right before and after fMRI scanning during working-memory (WM) task about 6 hours later in the afternoon. **B**. Behavioural performance on accuracy and response time (RT). **C-D**. Significant cluster in the hippocampus with a negative correlation between an individual's CAR and hippocampal activity in general. **E-F**. Significant cluster in the dorsolateral prefrontal (dlPFC, in red) overlapping with the main effect of WM-loads (in blue). Scatter plot depicts a negative correlation between an individual's CAR and WM-related dlPFC activity. **G**. The mediating effect of the dlPFC activity on the association between higher CAR and better WM performance. Paths are marked with standardized coefficients. Notes: Hipp, hippocampus; 0B, 0-back; 2B, 2-back; AUCi, area under the curve with respect to the cortisol increase; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

similar numerical N-back task with three conditions (0-, 1- and 2-back) during fMRI scanning in the afternoon on Day 2. A total of 15 saliva samples were collected through 3 consecutive days, while participant's subjective mood was monitored concurrently by the positive and negative affection scale (PANAS) (Watson et al., 1988). The other experimental settings were identical to those of Study 1 (Fig. 3A).

2.3. Physiological and psychological measures

2.3.1. Salivary cortisol measure

Cortisol levels were measured from saliva samples. In Study 1, we collected morning cortisol samples within 1 hour immediately post-wakening from two consecutive days. A total of 10 saliva samples were collected, with 4 time points spanning within 1 hour immediately after awakening (i.e., 0, 15, 30 and 60 min) on both Day 2 (i.e., fMRI scanning day) and Day 3 respectively, and extra 2 time points right before and after fMRI scanning on Day 2 (Fig. 1A). In Study 2, participants orally took a pill of 0.5-mg DXM (or placebo) at 20:00 on Day 1, then a total of 15 saliva samples (i.e., S0 to S14) were collected to cover cortisol's diurnal rhythms across 3 consecutive days at the following time points: on Day 1 at 22:00; on Day 2 at 0 min (7:00), 15 min (7:15), 30 min (7:30) and 60 min (8:00) after awakening; 11:00 before lunch, right before and after the fMRI scanning in the afternoon from 14:00 to 17:00, and 22:00 in the evening; on Day 3 at 0 min (7:00), 15 min (7:15),

30 min (7:30) and 60 min (8:00) after awakening, 11:00 and 16:00 respectively (Fig. 3A). Saliva was collected using Salivette collection device (Sarstedt, Germany). Participants were asked not to brush their teeth, drink or eat within 1 hour before sampling in order to avoid saliva contamination. They were also required to refrain from any alcohol, coffee, nicotine consumption as well as excessive exercise at least one day before the experiment. To ensure participant's compliance and the quality of saliva collection for the CAR assessment, we adopted four strategies derived from previous studies (Stalder et al., 2016; Tian et al., 2021; Wu et al., 2015; Zhu et al., 2019), including: **i)** enhancing participants' compliance by one-on-one instructions one day prior to the experiment; **ii)** setting up individualized wake-up alarm; **iii)** using time-stamped photo-taking of saliva collection, **iv)** using electronic monitoring devices to obtain the exact time of awakening and sampling. The first three strategies were used in both Studies 1 and 2. The fourth strategy was used only in Study 2. The detailed procedures are provided in the Supplementary Materials.

Salivary samples were returned back to the laboratory and kept frozen (−20 °C) until the assay. After thawing and centrifuging at 3000 rpm for 5 min, the samples were analyzed using an electrochemiluminescence immunoassay (ECLIA, Cobas e601, Roche Diagnostics, Mannheim, Germany) with sensitivity of 0.500 nmol/L (lower limit) and a standard range in assay of 0.5–1750 nmol/L. Intra and inter-assay variations were below 10 %. The CAR was computed by the area under the curve with respect to increase (AUCi) by the following equation: AUCi = (S1 + S2) × 15 min/2 + (S2 + S3) × 15 min/2 + (S3 + S4) × 30 min/2 − S1 × (15 min + 15 min + 30 min). S1 to S4 represent the measurements of 4 samples collected within 1 hour immediately after awakening. The AUCi reflects the dynamics of the cortisol awakening response and emphasizes diurnal changes over time (Clow et al., 2010; Pruessner et al., 2003).

Given our prior hypothesis and experimental questions on the proactive role of the CAR on prefrontal-hippocampal functional organization 6 hours later on the same day, we focused on our fMRI data analyses for the CAR assessment from the same day (Day 2) in both Studies. We have also conducted additional control analyses to explore intra-subject variability of the CAR assessment across 2 experimental days for Studies 1 and 2 separately (see Supplemental Materials for more details).

2.3.2. Cognitive task

A blocked-design N-back task was used in both studies. In Study 1, the entire task included 10 blocks of alternating 0- and 2-back conditions. In Study 2, the task consisted of 12 blocks of alternating 0-, 1- and 2-back conditions. Each block started with a 2-s cue indicating the experimental condition, followed by a pseudo-randomized sequence consisting of 15 digits. Each digit was presented for 400 ms, followed by an inter-stimulus-interval of 1400 ms. The blocks were interleaved by a jittered fixation ranging from 8 to 12 s, resulting in a mean inter-block duration of 38 s. During the 0-back condition, participants were instructed to detect whether the current digit was '1'. During the 1-back condition, participants were instructed to detect whether the current digit had appeared 1 position back in the sequence. During the 2-back condition, participants were instructed to detect whether the current digit had appeared 2 positions back in the sequence. Each sequence contained either 2 or 3 targets, and participants were asked to make a button press with their right index finger as fast as possible when detecting a target.

We adopted a simplified design with only two WM loads (i.e., 0- and 2-back) in Study 1 in order to localize task-invoked prefrontal activation and hippocampal deactivation in the contrast of 2- with 0-back conditions. This design has been proved effective and robust by previous studies (Egan et al., 2003; Hur et al., 2017; Owen et al., 2005; Qin et al., 2012, 2009), which allows us to further test the proactive effects of the CAR on prefrontal and hippocampal functional organization during WM task. We decided to incorporate an additional 1-back into Study 2 because of two considerations: **i)** to examine the robustness of our observations in Study 1 and the causal link to CAR via pharmacological manipulation (see Supplementary Materials), **ii)** to further explore whether the proactive effect of the CAR on task-invoked brain activity patterns during three WM loads exhibits a linear or non-linear pattern (see Supplementary Materials). Finally, we reported a comparison between (1- minus 0-back) vs (2- minus 0-back) in the main text, treating 0-back condition as the baseline condition of other ones, which could be a more serious way for the fMRI-based N-back task.

2.3.3. Questionnaires

When participants arrived at the laboratory in both Studies 1 and 2, they received two questionnaires: the State-Trait Anxiety Inventory (STATI) (Spielberger, 1985) measuring the participants' state and trait anxiety, and the Perceived Stress Scale (PSS) (Cohen, 1988) measuring their long term psychological stress levels. A sleep questionnaire was also used to log participants' sleeping quality across the experimental days (see Supplementary Materials). In Study 2, subjective mood state was also assessed using the PANAS at time points coinciding with collection of saliva samples.

2.4. Brain imaging data acquisition

Whole-brain images in both studies were acquired on a Siemens 3.0 T TRIO MRI scanner (Erlangen, Germany) in the National Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research at Beijing Normal University. Functional brain images were collected during the N-back task using a gradient-recalled echo planar imaging (GR-EPI) sequence (axial slices = 33, volume repetition time = 2.0 s, echo time = 30 ms, flip angle = 90 °, slice thickness = 4 mm, gap = 0.6 mm, field of view = 200 × 200 mm, and voxel size = 3.1 × 3.1 × 4.6 mm). High-resolution anatomical images were acquired in the sagittal orientation using a T1-weighted 3D magnetization-prepared rapid gradient echo sequence (slices = 192, volume repetition time = 2530 ms, echo time = 3.45 ms, flip angle = 7 °, slice thickness = 1 mm, field of view = 256 × 256 mm, and voxel size = 1 × 1 × 1 mm^3).

2.5. Brain imaging data analysis

2.5.1. Preprocessing

Image preprocessing and statistical analysis of fMRI data were performed using Statistical Parametric Mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm). The first four volumes of functional images were discarded for signal equilibrium and participant's adaptation to scanning noise. Remaining images were corrected for slice acquisition timing, realigned for head motion correction, co-registered to the gray matter image segmented from the anatomical T1-weighted images, and subsequently spatially normalized into a common stereotactic Montreal Neurological Institute (MNI) space. Images were then resampled into 2-mm isotropic voxels, and finally smoothed by an isotropic three-dimensional Gaussian kernel with 6 mm full-width at half-maximum. The data were then statistically analyzed under the framework of general linear models (GLM).

2.5.2. Univariate GLM analysis

To assess neural activity associated with the experimental conditions, each condition was modeled separately as boxcar regressor and convolved with the canonical hemodynamic response function (HRF) built in SPM12. The 6 parameters for head movement were also included

in the model as covariates to account for movement-related variability. A high-pass filtering cutoff of 1/128 Hz and a serial correlation correction by a first-order autoregressive model (AR) were also applied. Contrast images for each condition, generated at the individual level fixed-effects analyses, were submitted to a second-level group analysis treating participants as a random factor.

In Study 1, we first conducted a paired *t*-test to identify brain regions associated with WM by contrasting the 2- with 0-back condition and vice versa (collapsing across groups) ($P < 0.05$ familywise error rate (FWE) correction with Gaussian random field theory in SPM12; Fig. S1).

To examine how individual differences in CAR modulate WM-related brain activity, we then conducted whole-brain multiple regression analysis on the contrast of 2-back plus 0-back condition (i.e., 2B + 0B) and 2-back minus 0-back condition (i.e., 2B-0B), with CAR as the covariate of interest, while sleep duration, perceived stress and state-trait anxiety as covariates of no interest. Significant clusters were determined using a height threshold of $P < 0.001$ and an extent threshold of $P < 0.05$ with cluster-based FWE correction. Regions of interests (ROIs) were defined by overlapping these clusters with a template derived from automated meta-analysis of the most recent 1,091 fMRI studies with 'working memory' as a search term in Neurosynth (http://www.neurosynth.org). Correlation analyses for data extracted from these clusters were conducted and the patterns of correlation were illustrated on scatterplots.

To further characterize the interaction effect between individual differences in CAR and task-invoked brain activity, we conducted a complementary analysis by splitting participants into two groups of individuals with robust- and lower-CAR. According to the criterion outlined by previous studies (Clow et al., 2004; Wust et al., 2000), individuals whose cortisol level raised more than 50 % at 30 min after awakening were grouped into the robust-CAR group, whereas individuals with less than 50 % increase were assigned into the lower-CAR group. We first conducted independent-sample *t*-test to confirm the group difference of the CAR. We then conducted repeated-measure analysis of variance (ANOVA) on the whole-brain level, with WM (i.e., 0- and 2-back) as within-subject factor and Group (i.e., robust- and lower-CAR) as between-subject factor. Significant clusters were determined using a height threshold of $P < 0.001$ and an extent threshold of $P < 0.05$ with cluster-based FWE correction. The definition of ROIs was the same as above. ANOVAs for data extracted from these clusters were conducted and the main effect of Group and Group-by-Load interaction effect were plotted on bar graphs.

In Study 2, to test whether brain activity following suppressed CAR resembles that in Study 1, we conducted similar repeated-measure ANOVA on the whole-brain level, with WM-load (i.e., 1- and 2-back, relative to 0-back baseline) as the within-subject factor and pharmacological treatment (i.e., DXM and placebo groups) as the between-subject factor. Significant clusters were determined in the same way as in Study 1. ANOVAs were conducted for data extracted from these clusters to characterize the main effect of Group and Group-by-Load interaction effect.

2.5.3. Structural equation modeling

Structural equation models (SEMs) were constructed to examine the hypothesized mediating effects of prefrontal activation on the associations between the CAR and WM performance using Mplus 7.0 (https://www.statmodel.com/). Bias corrected bootstrap was conducted (5000 samples) to test the mediating effect (Shrout and Bolger, 2002). Both direct and indirect effects of prefrontal activation on the association between individual differences in CAR and WM performance were estimated, which generated percentile based on confidence intervals (CI). All reported *P* values are two-tailed.

2.5.4. Task-dependent functional connectivity analysis

To examine whether the hyper-activation caused by lower- (or DXM-suppressed) CAR was related to dlPFC coupling with brain regions, we conducted generalized psychophysiological interaction (gPPI) analysis (McLaren et al., 2012). The dlPFC seeds were defined as a cluster that showed Group-by-Load interaction from activation analysis in Studies 1 and 2, respectively. The mean time series from the seed ROIs were then deconvolved to uncover neuronal activity (i.e., physiological variable) and multiplied with the task design vector contrasting WM-load (i.e., 0-, 1- and 2-back) (i.e., psychological variable) to form a psychophysiological interaction vector. This interaction vector was convolved with a canonical HRF to form the gPPI regressor of interest. Task-related activations were also included in this GLM to remove out the effects of common driving inputs on brain connectivity.

Contrast images corresponding to PPI effects at the individual level were then submitted to group analysis. We conducted repeated-measure ANOVA on the whole-brain level, with WM-load as the within-subject factor and group as the between-subject factor. Significant clusters were determined using a height threshold of $P < 0.001$ and an extent threshold of $P < 0.05$ with cluster-based FWE correction. ANOVAs were conducted for data extracted from these clusters. The patterns of Group-by-Load interactions effect were plotted on bar graphs.

2.5.5. Dynamic causal modeling

To further investigate how suppressed CAR modulates functional interactions between the dlPFC and the hippocampus (ROIs identified from the above activation analysis) during WM, we estimated the effective connectivity between these two brain regions using dynamic causal modeling (DCM) (Friston et al., 2003). DCM explains regional effects in terms of dynamically changing patterns of connectivity during experimentally induced contextual changes. Importantly, this method allows inferences about the direction of causal interactions, i.e., whether the CAR modulates the 'top-down' connectivity from dlPFC to hippocampus or the reverse 'bottom-up' connectivity. We defined a standard model including both regions as nodes with bidirectional, intrinsic connectivity. This model was then modified to yield 36 models varied in the connectivity that could be modulated and in the locations of driving inputs during different WM-loads respectively. Fig. S8 illustrated the structures of 12 models including only 2-back as modulatory.

The models were estimated separately for each participant. To this end, we extracted the regional time series of the BOLD signal for each participant. First, two ROIs were defined as clusters in the hippocampus (showing main effect of Group in activation analysis in Study 2) and the dlPFC (showing Group-by-Load interaction from activation analysis in Study 2). The first eigenvariate from each ROI adjusted for effects of interest (i.e. 0-, 1-, and 2-back) constitutes its regional activity. Model fitting was based on these data and was achieved by adjusting the model parameters to maximize the (negnative) free-energy estimate of the model evidence (Friston et al., 2003).

Separated Bayesian model selection (BMS) for both DXM and placebo groups was then used to identify the model that could account best for the data (Penny et al., 2010). A random-effects approach was implemented, since it does not assume that the optimal model will be the best for each individual (Stephan et al., 2010). This analysis yielded the exceedance probability (EP), i.e., the probability to which a given model is more likely to have generated the data from a randomly selected participant than any other competing model. The group-level DCM analysis was also conducted using Bayesian model averaging (BMA) (Penny et al., 2010), which is less dependent on assumptions about model structure. BMA is a Bayesian approach that averages each parameter across models (and across participants) such that the contribution of each model (of each participant) for that parameter is

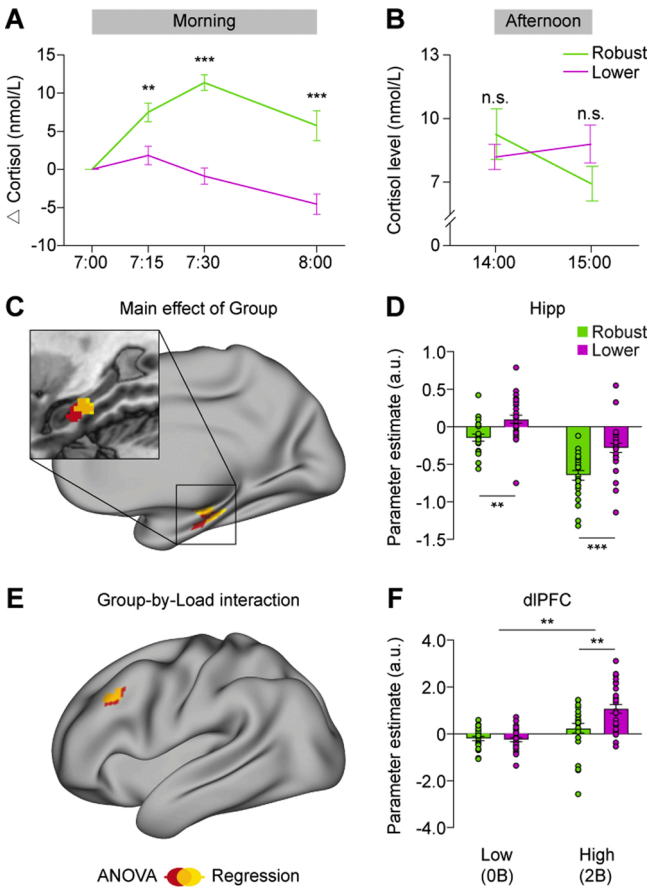

Fig. 2. Brain systems showing higher activation in individuals with lower- than robust-CAR from Study 1. **A-B.** Cortisol levels in individuals with robust- and lower-CAR in the morning, before and after fMRI scanning in the afternoon. **C.** Significant clusters showing a main effect of Group in the hippocampus (in red) and overlapping (in orange) with the one (in yellow) from the regression analysis. **D.** Bar graphs depict hippocampal hyper-activation regardless of WM-loads in individuals with lower- than robust-CAR. **E.** Significant cluster in the dlPFC (in red) showing an interaction effect between WM-loads and Group, and overlapping (in orange) with the one (in yellow) from the regression analysis. **F.** Bar graphs depict hyper-activation in the dlPFC in individuals with lower- than robust-CAR only in high (2-back) but not low (0-back) task demand. Notes are the same as Fig. 1.

weighted by the model's posterior probability. Independent-sample *t*-tests were conducted between groups for intrinsic coupling, modulatory, modulatory plus intrinsic effect separately.

3. Results

3.1. A robust CAR proactively predicts less hippocampal and prefrontal activity during WM in Study 1

We first assessed the overall CAR profile and diurnal cortisol levels for participants from Study 1. Cortisol levels peaked 30-minutes after awakening, followed by a decline at 60-minutes, and remained relatively low yet stable in the afternoon ($F_{5,\ 306} = 36.93$, $P < 0.001$; Fig. 1A). To verify the effectiveness of WM-load manipulation, we conducted separate paired *t*-tests on accuracy and RTs. This analysis revealed lower accuracy and slower reaction times (RTs) in the high than the low task demand condition (both $t_{51} > 3.43$, $P < 0.001$; Fig. 1B). To identify brain systems involved in WM processing, we conducted whole-brain analyses by contrasting 2- with 0-back condition and vice versa. These analyses replicated robust WM-related activation and deactivation in widespread regions in the frontoparietal network (FPN) and default mode network (DMN) respectively (Cousijn et al., 2012; Owen et al., 2005). Regions in the FPN include the dorsolateral prefrontal cortex (dlPFC) and intraparietal sulcus (IPS), and regions in the DMN include the posterior cingulate cortex (PCC), the medial prefrontal cortex and the hippocampus (Fig. S1).

Next, we examined via whole-brain regression analyses how an individual's CAR modulates brain functional activity involved in upcoming WM processing in the afternoon, while controlling for sleep duration, perceived stress, state and trait anxiety. The area under the curve with respect to the cortisol increase (AUCi) within 1 hour after awakening was computed to quantify the overall CAR and used as the predictor of interest. We observed a hippocampal cluster (Cluster $P <$ 0.05 FWE corrected; Fig. 1C; Table S2), with lower-CAR predictive of higher hippocampal activation (or less deactivation) regardless of task demands (Fig. 1D). Critically, we also identified clusters in the dlPFC and the intra-parietal sulcus (Fig. 1**E**; Fig. S2A; Table S2) with lower CAR predictive of more task-invoked prefrontal activation in the high (vs. low) demanding condition (Fig. 1F; Fig. S2B). Furthermore, we found a mediating effect of the dlPFC activity that could statistically account for an indirect association between the CAR and WM performance (Indirect Est. = 0.15, 95 % CI = [0.026, 0.31]), indicating that a robust CAR proactively promotes better WM performance via less dlPFC activation (Fig. 1G).

3.2. Interaction between CAR and task demands on hippocampal and prefrontal activity in Study 1

To further characterize the interaction effect between CAR and task-invoked brain activity, we conducted a set of complementary analyses by splitting participants into a robust- or lower-CAR group (see **Methods**) according to the criterion by previous studies (Clow et al.,

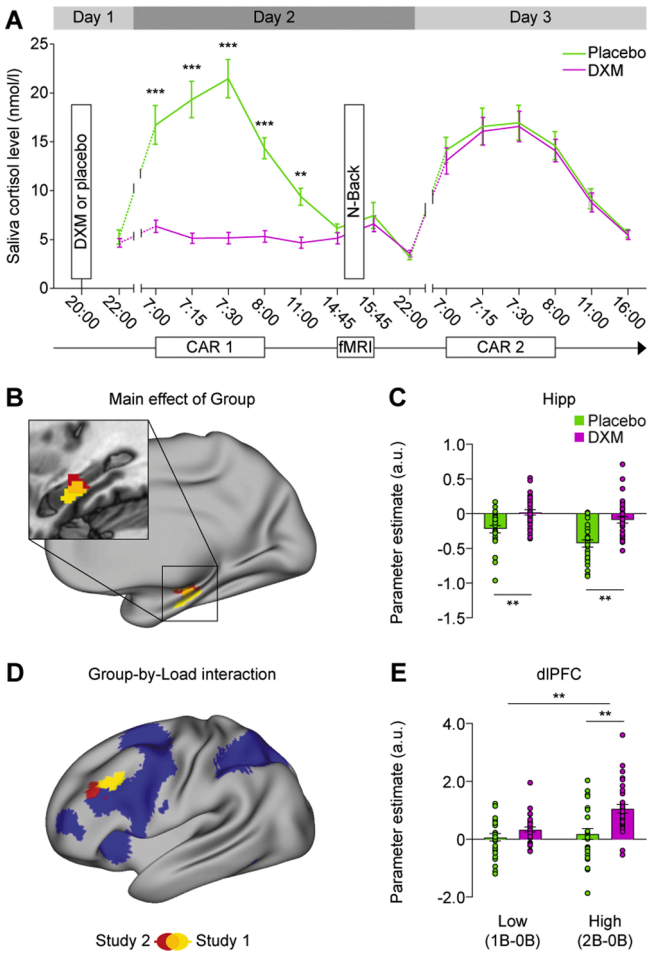

Fig. 3. Experimental design, pharmacological suppression of CAR and its effects on brain systems from Study 2. **A**. Salivary cortisol levels at 15 time points through three consecutive days. Participants received 0.5-mg either Dexamethasone (DXM) or placebo at 22:00 before sleep in the evening on Day 1. The CAR measured on Day 2 and Day 3, and fMRI data were acquired while performing a WM task with 0-, 1- and 2-back conditions in the afternoon on Day 2. **B-C**. Significant cluster in the hippocampus (in red) showing general hyper-activation in DXM (vs. placebo) group which is overlapped (in orange) with the one observed in individuals with lower- vs. robust-CAR from Study 1 (in yellow). **D-E**. Significant cluster in the dlPFC (in red) showing hyper-activation in the left dlPFC in DXM (vs. placebo) group only during high (but not low) task demand. Clusters in blue represent WM-related brain activation, and the cluster in yellow shows Group-by-Load interaction from Study 1. Notes are the same as in Fig. 1.

2004; Wust et al., 2000). Indeed, an independent-sample *t*-test confirmed a significant rise of cortisol level after awakening in the robust- relative to lower-CAR group ($t_{50} = 8.31$, $P < 0.001$; Fig. 2A), but no difference in cortisol levels either before or after fMRI scanning in the afternoon (all $P > 0.14$; Fig. 2B). There was no group difference in other behavioural and affective measures (all $P > 0.66$; Fig. S3; Table S1). A whole-brain 2 (Group: robust- vs. lower-CAR)-by-2 (Load: low vs. high) repeated-measure analysis of variance (ANOVA) revealed a main effect of Group in the hippocampus ($F_{1,50} = 21.54$, $P < 0.001$, $\eta^2 = 0.30$; Fig. 2C and D) and an interaction effect in the dlPFC ($F_{1,50} = 9.037$, $P = 0.004$, $\eta^2 = 0.15$; Fig. 2E and F) and the intraparietal sulcus (Fig. S4; Table S3). Remarkably, these regions overlap (Fig. 2C and E) with those from above-described regression analyses, highlighting the robustness of our observations. These results indicate that individuals with lower-CAR show higher hippocampal activation regardless of task demands, and higher dlPFC activation specific to a high task demand.

3.3. *Effectiveness of pharmacological suppression of the CAR and related control measures in Study 2*

To examine whether there is a causal link between an individual's CAR and its proactive effects on task-related prefrontal and hippocampal activity, we conducted Study 2 by suppression of the CAR using DXM. As expected, DXM administration on Day 1 suppressed participants' CAR in the morning on Day 2, as indicated by the main effect of Group ($F_{1,57} = 16.78$, $P < 0.001$, $\eta^2 = 0.23$) from a 2 (Group: DXM and placebo)-by-15 (Time: 15-samples) ANOVA. We also observed Group-by-Time interaction effect ($F_{14,798} = 19.91$, $P < 0.001$, $\eta^2 = 0.26$). Post-hoc tests revealed a flattened CAR at 0-, 15-, 30- and 60-minutes after morning awakening in DXM group (All $P < 0.001$), but no significant group differences in cortisol levels before and after fMRI scanning nor in the CAR on Day 3 when compared to placebo (All $P > 0.18$) (Fig. 3A). There was no significant group difference either in subjective mood across the 15 time points over three consecutive days (Fig. 5SA), behavioural performance, sleep duration, perceived stress or anxiety (All $P > 0.18$; Fig. S5B and C;Table S1). The effectiveness of the WM-load manipulation was evidenced by separate repeated-measure ANOVA for both accuracy ($F_{2,114} = 7.58$, $P < 0.001$) and RTs ($F_{2,114} = 48.67$, $P < 0.001$) during WM. Thus, as intended, the DXM administration selectively suppressed the CAR of the experimental day, but it did not alter cortisol levels before and after fMRI scanning nor affective measures over three days.

3.4. *Suppressed CAR proactively leads to increases in hippocampal and prefrontal activity in Study 2*

We then investigated whether CAR suppression using DXM in Study

2 could resemble our observed prefrontal and hippocampal hyper-activation above in individuals with lower-CAR from Study 1. We conducted a whole-brain 2 (Group: DXM vs. placebo)-by-2 (Load: Low vs. High) ANOVA. This analysis revealed a main effect of Group in the hippocampus (Cluster $P < 0.05$ FWE corrected, Fig. 3B; *Table S4*) and a Group-by-Load interaction effect in the dlPFC (Cluster $P < 0.05$ FWE corrected, Fig. 3D; *Table S4*). As shown in Fig. 3B and D, these two regions overlapped closely with the findings of Study 1, with a general hippocampal hyper-activation regardless of cognitive load ($F_{1,57} = 26.95$, $P < 0.001$, $\eta^2 = 0.32$; Fig. 3C) and a prefrontal hyper-activation specific to high (vs. low) WM-load in the DXM as compared to the placebo group ($F_{1,57} = 12.95$, $P < 0.001$ $\eta^2 = 0.19$; Fig. 3E). Other clusters are shown in Fig. S6 (Table S4). Thus, results from Studies 1 and 2 converge into a causal link between the CAR and its proactive effects on task-invoked activity in the dlPFC and hippocampus about 6 hours later in the afternoon of the same day.

3.5. Suppressed CAR reduces prefrontal-hippocampal functional coupling during WM in Studies 1 and 2

The above localization of brain activation linked to the CAR provides limited insight into how cortisol six hours later affects nuanced coordination of brain networks to support human WM. To test for the CAR-mediated effects on prefrontal network properties, we implemented a generalized form of psychophysiological interaction (gPPI) analysis (McLaren et al., 2012) to assess task-dependent functional connectivity of a specific seed (the dlPFC here; Fig. 4A) to the rest of the brain in Study 1 and 2. The dlPFC-seeded connectivity maps were then submitted to a 2 (Group)-by-2 (Load) ANOVA for statistical testing. This analysis revealed a Group-by-Load interaction in the hippocampus in Studies 1 and 2 independently (Fig. 4B; Table S5), with weaker dlPFC-hippocampal connectivity in individuals with lower- (or DXM-suppressed) CAR as compared to those with robust-CAR (or placebo), under high but not low task demands (Study 1: $F_{1,55} = 6.64$, $P = 0.013$, $\eta^2 = 0.12$; Study 2: $F_{1,57} = 23.77$, $P < 0.001$, $\eta^2 = 0.29$; Fig. 4C and D; Fig. S7). Notably, analyses of dlPFC-hippocampal intrinsic functional connectivity at resting state showed no group difference in the two studies (All $P > 0.47$). These results indicate that lower-/DXM-suppressed CAR proactively reduces prefrontal-hippocampal coupling at a cognitively demanding state.

3.6. Suppressed CAR reduces prefrontal top-down modulation over the hippocampus in Study 2

To further test the directionality of prefrontal-hippocampal connectivity, we modeled dynamic functional interactions between these two regions described above, by implementing Dynamic Causal Modeling to assess neural dynamics exerting from one region to another (Friston et al., 2003). Bayesian model selection was used to identify the optimal model structure of 36 variants (Figs. S8 and 9) that accounts best for the data in each group. For the placebo group, model evidence based on exceedance probabilities (EP) favored a model (10th variant, EP = 0.68; Fig. 4E) where inputs to the dlPFC drive the network, and high cognitive demand (i.e., 2-back) modulates the effective connectivity between dlPFC and hippocampus bidirectionally. Model evidence for DXM, however, favored a model (4th variant, EP = 0.89; Fig. 4F) in which input to the dlPFC also drives the network, but high demand only modulates the network coupling from the hippocampus to the dlPFC. Dynamic coupling parameters from the dlPFC to the hippocampus during high task demand were obtained using Bayesian model averaging across all models. Independent-sample *t*-tests revealed a reduction in positive modulation of effective connectivity (i.e., the modulatory; $t_{57} = -2.07$, $P < 0.05$) as well as absolute effective connectivity (i.e., the modulatory plus intrinsic effect; $t_{57} = -2.01$, $P < 0.05$), but not intrinsic coupling alone, in the DXM relative to the placebo group (Fig. 4G). Together, the placebo group exhibited dynamic influences between the dlPFC and the hippocampus bidirectionally in high task demand, whereas DXM-suppressed CAR selectively reduced the top-down modulation from dlPFC to the hippocampus during WM processing.

4. Discussion

By leveraging cognitive neuroimaging and pharmacological manipulations across two studies, we investigated the neurobiological mechanisms underlying the proactive effects of human CAR on hippocampal-prefrontal functioning. In Study 1, we found that a robust CAR was predictive of less hippocampal activation regardless of task demands and less dlPFC activation selectively in a high task demand, as well as enhanced functional coupling between those regions and better working memory performance, about 6 hours later in the afternoon of the same day. These results implicate the CAR in proactively promoting brain preparedness based on improved neural efficiency. Critically, pharmacological suppression of CAR (Study 2) resembled this proactive effect from Study 1, indicating the robustness of our findings. Further, dynamic causal modeling revealed a reduction in prefrontal top-down modulation over the hippocampus. Our findings establish a causal link between the CAR and optimized hippocampal-prefrontal functional organization, suggesting a proactive mechanism of the CAR in promoting human brain preparedness.

4.1. CAR promotes brain preparedness via improved prefrontal and hippocampal efficiency

Our observed proactive effects of the CAR on task-invoked activity in the dlPFC and hippocampus concur with the CAR-mediated "preparation" hypothesis (Adam et al., 2006; Elder et al., 2014; Fries et al., 2009; Law et al., 2013) and extend the theoretical framework of glucocorticoids (de Kloet et al., 2019; Herman et al., 2003; McEwen et al., 2015). Specifically, our observed less dlPFC activation in individuals with robust-CAR may implicate improved neural efficiency during WM processing, given the comparable behavioural performance between robust- and lower-CAR groups. This interpretation is further supported by the mediating effect of less dlPFC activity on the association between a robust CAR and higher WM accuracy. Indeed, an increase in neuronal efficiency has been linked to relatively weaker and focal activation in certain brain region(s) (Barulli and Stern, 2013; Haier et al., 1988), likely by utilizing fewer neural resources (Elman et al., 2014; Heinzel et al., 2014).

The dlPFC and hippocampus are known to play antagonistic roles in WM processing, with prominent activation in the dlPFC and deactivation in the hippocampus (Cousijn et al., 2012; Meyer-Lindenberg et al., 2005; Owen et al., 2005; Stretton et al., 2012). Such activation/deactivation enables a flexible reallocation of neural resources between hippocampal and prefrontal systems to support executive functions (Cousijn et al., 2012; Pomarol-Clotet et al., 2008; Qin et al., 2009). The hippocampal deactivation, likely involving a GABAergic inhibition mechanism (Schmitz et al., 2017), is to suppress task-irrelevant thoughts and/or mind-wondering in favor of information maintenance and updating in WM (Cousijn et al., 2012; Meyer-Lindenberg et al., 2005; Stretton et al., 2012). Thus, more hippocampal deactivation (or less activation) here may reflect more effective suppression of task-irrelevant thoughts in individuals with robust- than lower-CAR.

Our observation on prefrontal-hippocampal systems differs from previous findings of increased local activity in the dlPFC 4 hours after

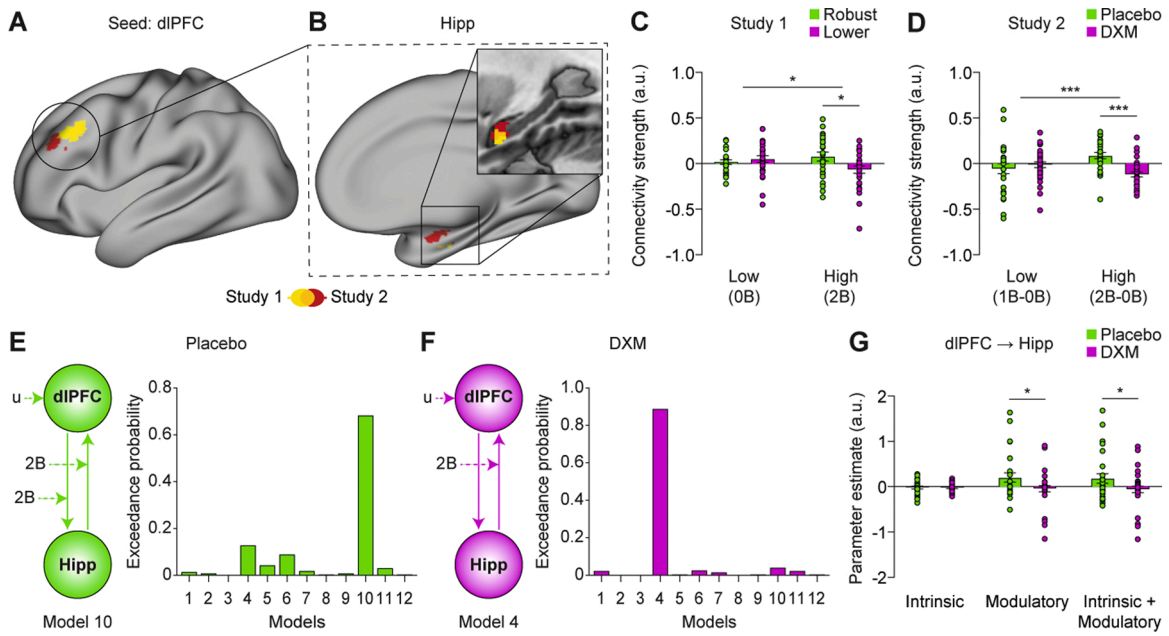

Fig. 4. Proactive effects of the CAR on prefrontal-hippocampal dynamic functional interactions. **A**. The dlPFC serving as the seed for task-dependent functional connectivity analysis. **B**. Significant clusters in the hippocampus showing Group-by-Load interaction effect in Study 1 and 2. **C-D**. Bar graphs depict weaker dlPFC functional coupling with the hippocampus in lower-CAR (or DXM) than robust- (or placebo) group during high but low task demand. **E**. Model evidence in placebo group from dynamic causal modeling analysis favored the 10th model: input to the dlPFC drives the network, and high task demand (i.e., 2-back) modulates dynamic influences between the dlPFC and hippocampus bidirectionally. **F**. Model evidence in DXM group favored the 4th model: inputs to dlPFC drives the network, while high task demand only modulates dynamic influence from the hippocampus to dlPFC. **G**. Bar graphs depict greater dynamic modulation as well as greater intrinsic plus modulatory dynamic influence from the dlPFC to hippocampus in placebo than DXM group. Notes: u, driving input; Others are the same as Fig. 1.

administration of exogenous corticosteroid to mimic a cortisol rise (Henckens et al., 2011). Given the CAR's unique features in morning awakening, it takes us from the general GR-mediated slow effect to a CAR-specific "preparation". The CAR is believed to be accompanied by activation of prospective memory representations of upcoming challenges for the day ahead (Fries et al., 2009). Such mnemonic aspects of the CAR could be important determinants of its proactive effects on brain networks. According to the neurobiological models of glucocordicoids, the brain can generate memory-dependent inhibitory traces to control cortisol responses and prime specific neural circuits to be prepared for future threats in similar contexts (de Kloet et al., 2019; Herman et al., 2003), through the MR/GR-mediated actions on initiating rapid reactions, contextualizing and regulating subsequent neuroendocrinal and behavioural adaptation to stress. Mnemonic-related brain circuits, for instance, can diminish responsiveness to repeatedly exposed stimuli to save engergy consumption (Herman et al., 2003). Thus, we speculate that the CAR, via similar MR/GR-mediated actions, may proactively set up a tonic tone with memory-dependent inhibitory traces to promote neuroendocrine control and mnenomic-related brain functions, thereby improving prefrontal-hippocampal efficiency during WM processsing. Suppressed-CAR implicates a decrease in such tonic inhibitory tone, which may account for more activity in those brain circuits that were also found in individuals with lower-CAR. To take it one step further, more dlPFC activity observed only under high but not low task demands in individuals with lower-/suppressed-CAR may result from an interplay between reduced tonic inhibition in the background (de Kloet et al., 2019) and task-induced phasic catecholaminergic actions on prefrontal networks during WM processing (Arnsten, 2009). Most likely, this proactive effect of the CAR on improved prefrontal-hippocampal efficiency can in turn optimize a flexible reallocation of neurocognitive resources among these systems to meet ever-changing cognitive demands. Our findings below from connectivity and dynamic causal modeling further support this interpretation.

4.2. CAR promotes brain preparedness via optimizing prefrontal-hippocampal network coupling and dynamic interactions

Beyond regional activation, a robust CAR proactively enhances functional coupling between the dlPFC and the hippocampus during WM, with higher connectivity in individuals with robust- than lower-CAR. Pharmacological suppression of CAR in Study 2 resembles this observation again. Prefrontal-hippocampal functional organization is recognized to play a critical role in various cognitive tasks including WM (Colgin, 2011; Harris and Shepherd, 2015; Spellman et al., 2015), through both direct and indirect neuronal connections (Hoover and Vertes, 2007; Rajasethupathy et al., 2015). Higher dlPFC coupling with the hippocampus in individuals with robust-CAR may reflect more efficient functional communication to support a flexible reallocation of neurocognitive resources to meet cognitive demands. Notably, weaker prefrontal-hippocampal coupling in individuals with lower-CAR came along with stronger dlPFC activation during high task demands. A stronger activation in the dlPFC may implicate compensation for suboptimal prefrontal-hippocampal functional organization (Barulli and Stern, 2013; Elman et al., 2014).

Our dynamic causal modeling further revealed that pharmacological suppression of CAR reduces the effective connectivity from the dlPFC to the hippocampus during WM about 6 hours later. Such metrics have been linked to the directionality of neural dynamics that one neuronal system exerts over another (Friston et al., 2003). Thus, our observation is most likely to reflect a reduction in prefrontal down-regulation over hippocampal activity during WM. Findings from previous studies have suggested that similar down-regulation involves a goal-directed signal that originates in the dlPFC and spreads downstream via polysynaptic pathways to the hippocampus, thus integrating these regions in a task-dependent manner (Anderson and Hanslmayr, 2014; Benoit and Anderson, 2012). If the CAR is responsible to promote the route of goal-directed input from the dlPFC to suppress hippocampal processing of task-irrelevant thoughts during WM, DXM-suppressed CAR in the

morning would mute the dlPFC influence on this network dynamics. Indeed, two aspects of our results support this assumption. First, the placebo group favored a model with inputs to the dlPFC driving the network and high task demands modulating connectivity between the hippocampus and the dlPFC bidirectionally, whereas the DXM-suppressed CAR group favored a model with the same inputs to the dlPFC driving the network, but reduced top-down modulation of network dynamics from the dlPFC to the hippocampus during high task demands. Second, this top-down modulation showed a strong trend to be positive, i.e., according to dynamic causal modeling reduced dlPFC recruitment actually caused less hippocampal activation (or more deactivation) during WM processing.

Taken together, our findings from activation, connectivity and dynamic causal modeling converge into a model of how the CAR prepares brain networks for the upcoming challenges: the CAR-mediated tonic inhibitory tone may work in concert with task-induced phasic catecholaminergic actions to support neuroendocrinal control, executive function and memory, through proactively improving neural efficiency in hippocampal-prefrontal networks and optimizing the flexible reallocation of neurocognitive resources in these networks. Indeed, many accounts have emphasize the interplay of glucocorticoid and catecholaminergic actions on modulating not just neural activities of different systems but also the dynamic organization of large-scale brain networks (Arnsten, 2009; Hermans et al., 2014). Future studies are required to address the complex interplay of the CAR and other neuromodulatory systems.

4.3. Limitations

Our findings should be considered in light of several limitations. First, our study focused on male participants in order to avoid potential confounds on the CAR by menstrual cycles. Whether our findings could be generalized into a female population remains open for future work. Second, we did not use electronic monitoring devices to obtain the exact time of awakening and sampling in Study 1, though findings from Study 2 provide evidence to verify the effectiveness of our CAR assessment. Finally, while sleep duration, state-trait anxiety, and perceived stress were controlled in our CAR assessment, other variables such as stress anticipation should also be taken into account in future studies.

4.4. Conclusion

Our findings establish a causal link between the CAR and its proactive role in the functional coordination of prefrontal-hippocampal networks involved in executive functioning. Combining cognitive neuroimaging with pharmacological manipulation advances our understanding of the CAR-mediated neuromodulatory pathways for upcoming cognitive and environmental challenges. Our study also highlights the proactive role of CAR on brain preparedness for the day ahead after awakening and could lead to the development of useful CAR-inspired biomarkers in both healthy and clinical populations.

Funding

This work was supported by the National Natural Science Foundation of China (32130045, 31522028, 81571056, 82021004, 31530031, 31871110, 31771246), the Open Research Fund of the State Key Laboratory of Cognitive Neuroscience and Learning (CNLZD1503), the Major Project of National Social Science Foundation (19ZDA363, 20&ZD153) and the Fundamental Research Funds for the Central Universities.

Author contributions

S.Q. conceived the experiments. B.X., C.C., Y.T., S.Z., C.L. & J.W. performed the study and data analysis. B.X., C.C., T.E., G.F. & S.Q. wrote the manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Code used to generate the analyses are available from https://github.com/QinBrainLab/2020_CAR_preparedness.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

The authors would like to thank Wanjun Lin, Wanqing Li, Haiyang Geng, Lei Hao and Liping Zhuang for their assistance in data collection and data analysis.

Appendix A. The Peer Review Overview and Supplementary data

The Peer Review Overview and Supplementary data associated with this article can be found in the online version, at doi:https://doi.org/10.1016/j.pneurobio.2021.102127.

References

Adam, E.K., Hawkley, L.C., Kudielka, B.M., Cacioppo, J.T., 2006. Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. Proc. Natl. Acad. Sci. U. S. A. 103, 17058–17063.
Anderson, M.C., Hanslmayr, S., 2014. Neural mechanisms of motivated forgetting. Trends Cogn. Sci. 18, 279–292.
Arnsten, A.F., 2009. Stress signalling pathways that impair prefrontal cortex structure and function. Nat. Rev. Neurosci. 10, 410–422.
Barulli, D., Stern, Y., 2013. Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts in cognitive reserve. Trends Cogn. Sci. 17, 502–509.
Benoit, R.G., Anderson, M.C., 2012. Opposing mechanisms support the voluntary forgetting of unwanted memories. Neuron 76, 450–460.
Buchanan, T.W., Kern, S., Allen, J.S., Tranel, D., Kirschbaum, C., 2004. Circadian regulation of cortisol after hippocampal damage in humans. Biol. Psychiatry 56, 651–656.
Chida, Y., Steptoe, A., 2009. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol. Psychol. 80, 265–278.
Clow, A., Thorn, L., Evans, P., Hucklebridge, F., 2004. The awakening cortisol response: methodological issues and significance. Stress 7, 29–37.
Clow, A., Hucklebridge, F., Stalder, T., Evans, P., Thorn, L., 2010. The cortisol awakening response: more than a measure of HPA axis function. Neurosci. Biobehav. Rev. 35, 97–103.
Cohen, S., 1988. Perceived stress in a probability sample of the United States. The Social Psychology of Health. Sage Publications, Inc, Thousand Oaks, CA, US, pp. 31–67.
Cole, M.A., Kim, P.J., Kalman, B.A., Spencer, R.L., 2000. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 25, 151–167.
Colgin, L.L., 2011. Oscillations and hippocampal-prefrontal synchrony. Curr. Opin. Neurobiol. 21, 467–474.
Cousijn, H., Rijpkema, M., Qin, S., van Marle, H.J., Franke, B., Hermans, E.J., van Wingen, G., Fernandez, G., 2010. Acute stress modulates genotype effects on amygdala processing in humans. Proc. Natl. Acad. Sci. U. S. A 107, 9867–9872.
Cousijn, H., Rijpkema, M., Qin, S., van Wingen, G.A., Fernandez, G., 2012. Phasic deactivation of the medial temporal lobe enables working memory processing under stress. Neuroimage 59, 1161–1167.
de Kloet, E.R., de Kloet, S.F., de Kloet, C.S., de Kloet, A.D., 2019. Top-down and bottom-up control of stress-coping. J. Neuroendocrinol. 31, e12675.
Ebrecht, M., Buske-Kirschbaum, A., Hellhammer, D., Kern, S., Rohleder, N., Walker, B., Kirschbaum, C., 2000. Tissue specificity of glucocorticoid sensitivity in healthy adults. J. Clin. Endocrinol. Metab. 85, 3733–3739.
Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.
Elder, G.J., Wetherell, M.A., Barclay, N.L., Ellis, J.G., 2014. The cortisol awakening response–applications and implications for sleep medicine. Sleep Med. Rev. 18, 215–224.

Elman, J.A., Oh, H., Madison, C.M., Baker, S.L., Vogel, J.W., Marks, S.M., Crowley, S., O'Neil, J.P., Jagust, W.J., 2014. Neural compensation in older people with brain amyloid-beta deposition. Nat. Neurosci. 17, 1316–1318.
Fries, E., Dettenborn, L., Kirschbaum, C., 2009. The cortisol awakening response (CAR): facts and future directions. Int. J. Psychophysiol. 72, 67–73.
Friston, K.J., Harrison, L., Penny, W., 2003. Dynamic causal modelling. Neuroimage 19, 1273–1302.
Haier, R.J., Siegel, B.V., Nuechterlein, K.H., Hazlett, E., Wu, J.C., Paek, J., Browning, H. L., Buchsbaum, M.S., 1988. Cortical glucose metabolic-rate correlates of abstract reasoning and attention studied with positron emission tomography. Intelligence 12, 199–217.
Harris, K.D., Shepherd, G.M., 2015. The neocortical circuit: themes and variations. Nat. Neurosci. 18, 170–181.
Heinzel, S., Lorenz, R.C., Brockhaus, W.R., Wustenberg, T., Kathmann, N., Heinz, A., Rapp, M.A., 2014. Working memory load-dependent brain response predicts behavioral training gains in older adults. J. Neurosci. 34, 1224–1233.
Henckens, M.J., van Wingen, G.A., Joels, M., Fernandez, G., 2011. Time-dependent corticosteroid modulation of prefrontal working memory processing. Proc. Natl. Acad. Sci. U. S. A 108, 5801–5806.
Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front. Neuroendocrinol. 24, 151–180.
Hermans, E.J., Henckens, M.J., Joels, M., Fernandez, G., 2014. Dynamic adaptation of large-scale brain networks in response to acute stressors. Trends Neurosci. 37, 304–314.
Hoover, W.B., Vertes, R.P., 2007. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct. Funct. 212, 149–179.
Hur, J., Iordan, A.D., Dolcos, F., Berenbaum, H., 2017. Emotional influences on perception and working memory. Cogn. Emot. 31, 1294–1302.
Joels, M., de Kloet, E.R., 1992. Control of neuronal excitability by corticosteroid hormones. Trends Neurosci. 15, 25–30.
Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom. Med. 61, 154–162.
Kremen, W.S., O'Brien, R.C., Panizzon, M.S., Prom-Wormley, E., Eaves, L.J., Eisen, S.A., Eyler, L.T., Hauger, R.L., Fennema-Notestine, C., Fischl, B., Grant, M.D., Hellhammer, D.H., Jak, A.J., Jacobson, K.C., Jernigan, T.L., Lupien, S.J., Lyons, M.J., Mendoza, S.P., Neale, M.C., Seidman, L.J., Thermenos, H.W., Tsuang, M.T., Dale, A. M., Franz, C.E., 2010. Salivary cortisol and prefrontal cortical thickness in middle-aged men: a twin study. Neuroimage 53, 1093–1102.
Kudielka, B.M., Wust, S., 2010. Human models in acute and chronic stress: assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. Stress 13, 1–14.
Kunz-Ebrecht, S.R., Kirschbaum, C., Marmot, M., Steptoe, A., 2004. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psychoneuroendocrinology 29, 516–528.
Law, R., Hucklebridge, F., Thorn, L., Evans, P., Clow, A., 2013. State variation in the cortisol awakening response. Stress 16, 483–492.
Law, R., Evans, P., Thorn, L., Hucklebridge, F., Clow, A., 2015. The cortisol awakening response predicts same morning executive function: results from a 50-day case study. Stress 18, 616–621.
Law, R., Evans, P., Thorn, L., Hucklebridge, F., Loveday, C., Clow, A., 2020. The cortisol awakening response predicts a same-day index of executive function in healthy young adults. Int. J. Psychophysiol.
Liu, Y., Lin, W., Liu, C., Wu, J., Bayley, P.J., Qin, S., 2016. Memory consolidation reconfigures neural pathways invoveld in the suppression of emotional memories. Nat. Commun. 7, 13375.
Mathew, A., Mn, P., Menon, P.K., Radeideh, A., Varma, S., Thomas, S., Varughese, N., Hamed, G.M., 2019. A clinical study on the circadian rhythm of salivary cortisol on aggressive periodontitis and its correlation with clinical parameters using electrochemiluminescence immunoassay method. J. Contemp. Dent. Pract. 20, 482–488.
McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179.
McEwen, B.S., Bowles, N.P., Gray, J.D., Hill, M.N., Hunter, R.G., Karatsoreos, I.N., Nasca, C., 2015. Mechanisms of stress in the brain. Nat. Neurosci. 18, 1353–1363.
McLaren, D.G., Ries, M.L., Xu, G., Johnson, S.C., 2012. A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. Neuroimage 61, 1277–1286.
Meyer-Lindenberg, A.S., Olsen, R.K., Kohn, P.D., Brown, T., Egan, M.F., Weinberger, D. R., Berman, K.F., 2005. Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. Arch. Gen. Psychiatry 62, 379–386.
Owen, A.M., McMillan, K.M., Laird, A.R., Bullmore, E., 2005. N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. Hum. Brain Mapp. 25, 46–59.
Penny, W.D., Stephan, K.E., Daunizeau, J., Rosa, M.J., Friston, K.J., Schofield, T.M., Leff, A.P., 2010. Comparing families of dynamic causal models. PLoS Comput. Biol. 6, e1000709.
Pomarol-Clotet, E., Salvador, R., Sarro, S., Gomar, J., Vila, F., Martinez, A., Guerrero, A., Ortiz-Gil, J., Sans-Sansa, B., Capdevila, A., Cebamanos, J.M., McKenna, P.J., 2008. Failure to deactivate in the prefrontal cortex in schizophrenia: dysfunction of the default mode network? Psychol. Med. 38, 1185–1193.
Pruessner, J.C., Wolf, O.T., Hellhammer, D.H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., Kirschbaum, C., 1997. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 61, 2539–2549.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.
Qin, S., Hermans, E.J., van Marle, H.J., Luo, J., Fernandez, G., 2009. Acute psychological stress reduces working memory-related activity in the dorsolateral prefrontal cortex. Biol. Psychiatry 66, 25–32.
Qin, S., Cousijn, H., Rijpkema, M., Luo, J., Franke, B., Hermans, E.J., Fernandez, G., 2012. The effect of moderate acute psychological stress on working memory-related neural activity is modulated by a genetic variation in catecholaminergic function in humans. Front. Integr. Neurosci. 6, 16.
Rajasethupathy, P., Sankaran, S., Marshel, J.H., Kim, C.K., Ferenczi, E., Lee, S.Y., Berndt, A., Ramakrishnan, C., Jaffe, A., Lo, M., Liston, C., Deisseroth, K., 2015. Projections from neocortex mediate top-down control of memory retrieval. Nature 526, 653–659.
Schlotz, W., Hellhammer, J., Schulz, P., Stone, A.A., 2004. Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response. Psychosom. Med. 66, 207–214.
Schmitz, T.W., Correia, M.M., Ferreira, C.S., Prescot, A.P., Anderson, M.C., 2017. Hippocampal GABA enables inhibitory control over unwanted thoughts. Nat. Commun. 8, 1311.
Shrout, P.E., Bolger, N., 2002. Mediation in experimental and nonexperimental studies: new procedures and recommendations. Psychol. Methods 7, 422–445.
Spellman, T., Rigotti, M., Ahmari, S.E., Fusi, S., Gogos, J.A., Gordon, J.A., 2015. Hippocampal-prefrontal input supports spatial encoding in working memory. Nature 522, 309–314.
Spencer, R.L., Deak, T., 2017. A users guide to HPA axis research. Physiol. Behav. 178, 43–65.
Spielberger, C.D., 1985. Assessment of state and trait anxiety: conceptual and methodological issues. Southern Psychol. 2, 6–16.
Stalder, T., Evans, P., Hucklebridge, F., Clow, A., 2010a. Associations between psychosocial state variables and the cortisol awakening response in a single case study. Psychoneuroendocrinology 35, 209–214.
Stalder, T., Evans, P., Hucklebridge, F., Clow, A., 2010b. State associations with the cortisol awakening response in healthy females. Psychoneuroendocrinology 35, 1245–1252.
Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wust, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., Miller, R., Wetherell, M.A., Lupien, S.J., Clow, A., 2016. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432.
Stephan, K.E., Penny, W.D., Moran, R.J., den Ouden, H.E., Daunizeau, J., Friston, K.J., 2010. Ten simple rules for dynamic causal modeling. Neuroimage 49, 3099–3109.
Stretton, J., Winston, G., Sidhu, M., Centeno, M., Vollmar, C., Bonelli, S., Symms, M., Koepp, M., Duncan, J.S., Thompson, P.J., 2012. Neural correlates of working memory in Temporal Lobe Epilepsy–an fMRI study. Neuroimage 60, 1696–1703.
Tian, T., Young, C.B., Zhu, Y., Xu, J., He, Y., Chen, M., Hao, L., Jiang, M., Qiu, J., Chen, X., Qin, S., 2021. Socioeconomic disparities affect children's amygdala-prefrontal circuitry via stress hormone response. Biol. Psychiatry.
Vogel, S., Fernandez, G., Joels, M., Schwabe, L., 2016. Cognitive adaptation under stress: a case for the mineralocorticoid receptor. Trends Cogn. Sci. (Regul. Ed.) 20, 192–203.
Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J. Pers. Soc. Psychol. 54, 1063–1070.
Wilhelm, I., Born, J., Kudielka, B.M., Schlotz, W., Wust, S., 2007. Is the cortisol awakening rise a response to awakening? Psychoneuroendocrinology 32, 358–366.
Wolf, O.T., Fujiwara, E., Luwinski, G., Kirschbaum, C., Markowitsch, H.J., 2005. No morning cortisol response in patients with severe global amnesia. Psychoneuroendocrinology 30, 101–105.
Wu, J., Zhang, S., Li, W., Qin, S., He, Y., Yang, Z., Buchanan, T.W., Liu, C., Zhang, K., 2015. Cortisol awakening response predicts intrinsic functional connectivity of the medial prefrontal cortex in the afternoon of the same day. Neuroimage 122, 158–165.
Wust, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., 2000. The cortisol awakening response - normal values and confounds. Noise Health 2, 79–88.
Zhu, Y., Chen, X., Zhao, H., Chen, M., Tian, Y., Liu, C., Han, Z.R., Lin, X., Qiu, J., Xue, G., Shu, H., Qin, S., 2019. Socioeconomic status disparities affect children's anxiety and stress-sensitive cortisol awakening response through parental anxiety. Psychoneuroendocrinology 103, 96–103.