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Simon BN Thompson[1,2,4*], Souhir Daly[3,4], Alain Le Blanche[3,5], Malek Abidi[3], Chama Belkhiria[3], Tarak Driss[3,4] and Giovanni de Marco[3,4]

1 Faculty of Science & Technology, Bournemouth University, BH12 5BB, UK
2 Dementia Institute, Bournemouth University, BH1 3LT, UK
3 Laboratoire CeRSM, EA2931, Université Paris Ouest Nanterre La Défense, 92000 Nanterre, France
4 International Scientific Council for Research, Université Paris Ouest Nanterre La Défense, 92000 Nanterre, France
5 Hôpital René-Dubos de Pontoise and Université de Versailles-Saint-Quentin, Simone Veil UFR des Sciences de la Santé, France

Corresponding author:
Simon BN Thompson

simont@bournemouth.ac.uk

Faculty of Science & Technology, Bournemouth University, BH12 5BB, UK

Tel: +44 1202 961558

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Abstract

Cortisol is an important hormone in the protective stress response system, the Hypothalamus-Pituitary-Adrenal (HPA)-axis. It becomes especially salient in immune suppression syndromes such as multiple sclerosis and Cushing's disease. Fatigue is a common symptom; mental and motor tasks are difficult and laboured. The role of cortisol in mental and motor tasks and the recruitment of key brain regions in completion of these tasks is explored together with functional magnetic resonance imaging in healthy participants. Cortisol levels were found to be higher and had greater reduction in levels during mental versus motor tasks. Recruitment of brainstem and hypothalamus regions, important in cortisol activity, was affected differently. At low cortisol levels, mental task participants had less activity in the regions than their physical task counterparts. When cortisol levels were higher, wider spread recruitment of these brain regions was observed in the mental task participants, and for the physical task participants, the spread was at comparative low levels of cortisol. It is concluded that cortisol is implicated in these brain regions and that brain region recruitment is likely to be dependent upon factors including cortisol levels as well as perception of stress in the task. It is suggested that mental tasks are perceived more stressful than physical and therefore require higher cortisol levels to promote wider spread brain region activity. Implication for neurological disease includes the use of cortisol in the proposed development of a potential new diagnostic biomarker for early detection of neurological sequelae.

Keywords: Brainstem; Cortisol; fMRI; Hypothalamus; Mental/Motor tasks

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Introduction

The association between cortisol and fatigue is complicated depending on whether fatigue is acute or chronic in nature. Cognitive fatigue has been defined as arising from the prolonged performance of cognitively demanding tasks requiring sustained mental efficiency [1]. Cortisol has been linked to fatigue in studies showing reduced cortisol levels in chronic fatigue syndrome [2].

Fatigue manipulation is usually achieved using neuropsychological tasks to generate cognitive exhaustion or by introducing either motor (physical) or mental (psychological) stimuli to cause stressful conditions [3]. Cortisol is regulated by the Hypothalamus-Pituitary-Adrenal (HPA)-axis where it is linked to specific receptors throughout the limbic system: hippocampus, amygdala, and prefrontal cortex [4]. Levels of cortisol secretion and the activity of brain regions are possibly dependent upon the stressor factors being of either physical or mental.

The amygdala is a crucial part of the limbic system and known as an important regulator of the stress-related glucocorticoid secretion [5] and boosts the activation of the HPA axis when the body is exposed to either a physical or psychological stressor [6].

Prefrontal cortex and its specific components (orbitofrontal PFC, ventrolateral PFC and medial PFC) have an important role in the processing of the stress response and cortisol regulation with decreased activity in orbitofrontal PFC associated with increased cortisol secretion in response to psychological stress [7].

Cortisol regulation is the domain of the central nervous system where binding occurs with limbic system receptors, hippocampus (HC), amygdala (AG), and prefrontal cortex (PFC) [4,8] **(Figure 1)**.

Levels of cortisol secretion and the brain regions activated are dependent upon the stressor factors being of either "motor" (physical) or "mental" (psychological). Diverse neuroimaging and animal studies on brain activity changes in response to stressors suggest contribution of the brainstem in physical stress, while psychological stressors tend to engage limbic system regions such as the HC, the AG, and the PFC in regulating the HPA axis.

Dedovic and colleagues [9] found significant interaction between cortisol release and fatigue in the right hippocampus with significantly decreased activation over time. This region is responsible for short-term changes in cortisol in association with levels of fatigue [10].

The amygdala is important in regulating glucocorticoid secretion during the stress response [5]; and adjusts vigilance levels whether positive or negative in nature [6]. Prefrontal cortex and its specific components (orbitofrontal PFC, ventrolateral PFC and medial PFC) emerge as candidates for the processing of the stress response and cortisol regulation. Decreased activity in orbitofrontal PFC has been demonstrated with increased cortisol secretion in response to a psychological stress task [7,11].

Similarly, increased activity in medial PFC regions correlates with decreased cortisol secretion [12], because projections emanate from the ventrolateral PFC towards the HC. This mechanism could allow ventrolateral PFC to decrease activity in orbital and medial PFC areas related to stress processing since this inappropriate control level could be associated with sustained cortisol secretion.

Temperature regulation and circadian rhythm is the responsibility of the hypothalamus which is intimately linked to the pituitary gland, which regulates oxytocin for social bonding activity [13], and the adrenal glands. Typically, the HPA-axis produces sufficient hormones to protect against stress and prepares us for physical activity [14,15].

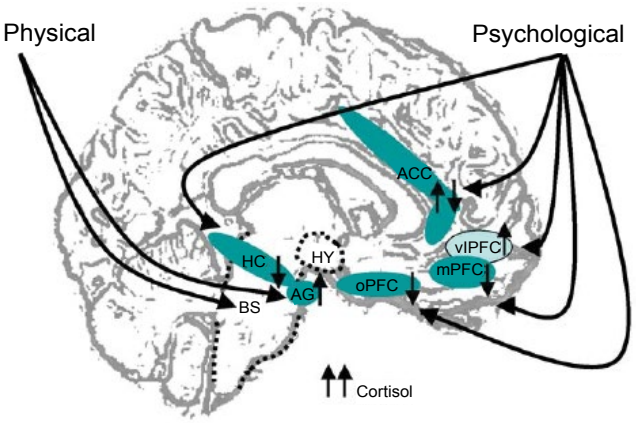


Figure 1 Brain activation by physical (motor) and psychological (mental) tasks.

The link between excessive yawning and neurological disease has been noted [16,17] as the first evidence-based report to link cortisol with yawning, demonstrating that cortisol rises when yawning. It is probable that the critical threshold level of cortisol is reached due to fatigue to elicit the yawning response. Feedback via the HPA-axis regulates cortisol and adrenaline production within the closed loop [18].

Hippocrates, the famous philosopher, writing in 400 BC in his book, De Flatibus Liber (A Treatise on Wind), wrote that large quantities of air are exposed during yawning like steam escaping from hot cauldrons as temperature rises dramatically [19]. His theory was interesting since we need to protect against critical rises in brain temperature, particularly when we are fatigued [16,20]. In multiple sclerosis (MS), fatigue is a common symptom [21-23] and this may be related to yawning excessively with high rises in brain temperature [24-26].

It is known that in the pituitary gland oxytocin regulates social bonding, and circadian rhythm and temperature regulation is the responsibility of the hypothalamus [13]. Together with the adrenal glands, they control the maintenance of hormones within the HPA-axis to prepare the body for exercise and to protect the body from stressors [14,15,27].

Thompson [16] has found a link between yawning excessively and neurological diseases; Lano-Peixoto, and colleagues [28] have also noted excessive yawning in their five patients who had neuromyelitis optica spectrum disorder (NMOSD). From MRI brain scans, their patients showed lesions in the brainstem and hypothalamus, with the conclusion that yawning may be a neglected (but not a rare symptom) of NMOSD. Similarly adrenal insufficiency and Parkinson's disease is associated with excessive yawning [29]; possibly, due to an irregularity in the level of hormones within the HPA-axis.

The first evidence based announcement of the link between yawning and cortisol was made by Thompson [17] who describes the rise in cortisol, produced by the adrenal cortex zona fasciculate, and yawning, controlling brain temperature.

The British neurologist, Sir Francis Walshe, reported on his stroke patients in 1923. He noted that those with brainstem lesions, had the capacity to raise their paralyzed arm when yawning [30]. This has since been evidenced by a number of other researchers [31-32].

It is probable that there is a critical threshold for the level of cortisol before yawning occurs and is dependent upon fatigue, level of perception, and sleep deprivation. Communicative yawning may involve several brain regions – frontal lobes, parietal lobes, insula and amygdala [33,34]. In addition to brain fMRI studies, others have been implicated the mirror-neuron system [35]; and endogenous levels of cortisol have implicated in pathological gamblers where striatal sensitivity fluctuates [36].

In this study, the role of cortisol in mental and motor tasks and the recruitment of key brain regions in completion of these tasks are explored together using fMRI in healthy participants. We show 1) recruitment of brainstem and hypothalamus regions, important in cortisol activity, was affected differently 2) mental tasks are perceived more stressful than physical and therefore require higher cortisol levels to promote wider spread brain region activity.

Materials and Methods

Participants

13 healthy participants (6 males, 7 females) aged between 21-35 years (SD = 4.50) with no known history of neurological, psychiatric or sensorimotor disorders gave their prior, written, informed consent to participation in the study. All participants were recruited among STAPS (Sciences et Techniques des Activitiés Physiques et Sportives) students from Paris Ouest University and were granted 50 euros to optimize motivation and concentration. Participants were assessed using the Edinburgh inventory [37] for right-handedness, and with consent, were recruited at Pontoise Hospital Centre and University of Versailles-Saint-Quentin, France.

MR data acquisition

Neuroimaging data were acquired with 1.5-Tesla, whole-body MRI system equipped with Sigma head volume coil (General Electric Medical System, Milwaukee, WI). Functional images with Blood-Oxygen-Level Dependent (BOLD) contrast enhancement were acquired for each participant. Single-shot echo-planar images (EPIs) were acquired using a typical T2*-weighted gradient-echo sequence. Total of 13640 images were obtained for the experimental run, using forty four, 3.8-mm thick axial slices and 310 EPI volumes with no gap (TR/TE = 3000/44 ms; flip angle = 90 degrees; matrix = 64*64; FOV= 240x240 mm^2; isotropic voxel volume = 52.7 mm^3). Conventional 3D imaging used an FSPGR BRAVO sequence (matrix: 256x256; flip angle: 12; TR/TE: 8.6/3.3 ms; FOV: 240x240 mm^2; 168 slices, 1 mm thick).

Saliva samples

Saliva samples were collected at the start and again at the end of the run from each participant. Six participants started the run with the mental task and 7 participants started the run with the physical task. Each sample was analysed and destroyed after analysis. Data was held securely and coded to ensure anonymity of participants. Cortisol levels are easily and reliably measured in saliva and it is far less invasive than intravenous collection methods. Presence of cortisol in saliva is highly correlated with blood assay and cheaper to analyse in the specialised laboratory.

fMRI paradigm

The paradigm is a modified version of the paradigm published in Périn B, et al. 2010 [38]. A short training session was performed prior to scanning. Participants were asked to perform a continuous attention task for 15 mins when they were asked to squeeze with their left hand a handgrip located on their lap. They were asked to focus attention on a fixation cross in the Centre of the screen (5 degrees visual field) and squeeze the handle as quickly as possible with different levels of force (low, medium, high) when a square appeared. Force levels were pseudo-randomized to prevent possible order-effect. After performing the physical condition, participants were asked to attend to the stimulus in the same way (15 mins) but this time "imagining" squeezing the handle (without actually squeezing it) to the same level of force, i.e. the "mental" condition. The order of the two conditions (physical and mental) was randomized across participants.

Saliva samples were collected at the start and again at the end of the condition from each participant. Each sample was analysed and destroyed after analysis. Data was held securely and coded to ensure anonymity of participants. Cortisol levels are easily and reliably measured in saliva and it is far less intrusive than intravenous collection methods. Presence of cortisol in saliva is highly correlated with blood assay and cheaper to analyse in the specialised laboratory.

Data collected from analyzing the saliva cortisol samples was performed using SPSS software. Normative data for saliva cortisol lies within the ranges: (i) Morning collection: 3.7-9.5 nanograms per millilitre of saliva; (ii) Noon collection: 1.2-3.0 nanograms per millilitre; and (iii) Evening collection: 0.6-1.9 nanograms per millilitre. Since evidence of statistically significant differences were shown in the mental (*versus* physical task), we decided to present imaging data using two putative brain regions of interest: midbrain and hypothalamus.

So that fatigue set in gradually and fairly throughout the paradigm, a pseudo-random sequence was implemented for the 3 levels of strength and for each task. The participant lay in the fMRI watching slides that appear in front of him/her. The participant was required to press the handgrip with the left hand each time the white square appears respecting the required levels of strength. The participant was instructed not to move his/her head for the duration of the acquisition and should always be focused. Before entering the fMRI, the participant was familiarized with the protocol.

Data Processing

SPM analysis

Image processing was performed using Statistical Parametric Mapping (SPM12) software (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm). EPI volumes were corrected to adjust for within-volume time differences and realigned with the last volume to correct for head movements. Functional scans were spatially normalized against the standard, stereotactic space of the MNI. Spatial smoothing was performed with 8mm t Gaussian kernel. Haemodynamic responses were modelled as a box-car function convolved with a synthetic haemodynamic response function.

Group and individual analyses

Fixed-effect model was created for each individual subject in order to perform the based-conditions random-effect analysis

(one sample *t* test for group analysis). To assess fatigue effects induced by both tasks, two contrasts were defined to compare the last quartile with the first quartile of the whole task - approximately the first and the last 4 mins of the task, i.e. "Motor_last Vs Motor_first" and "Mental_last Vs Mental_first". In each single-subject analysis, a significance level of p = .05 was used to detect activated voxels for this contrast and corresponding mask.

ROI analysis

From a priori hypotheses reported in the literature, analysis of regions of interest (ROI) was conducted to compare activation changes between the beginning and end of the task. Two masks were applied using WFU PickAtlas software (http://fmri.wfubmc.edu/software/pickatlas) for the hypothalamus and the midbrain. WFU PickAtlas toolbox automatically generated segmented atlas ROI templates in Montreal Neurological Institute (MNI) space [39].

Results

Saliva samples

Mean age of participants was 26.4 years (SD=4.70). In saliva cortisol sample 1, the means for participants in the mental condition was 7.0 (SD=7.21), and for the physical condition was 2.6 (SD=.74). In sample 2, the means were 5.6 (SD=5.56) for those in the mental condition, and 2.5 (SD=.83) for those in the physical condition. Hence, those in the mental condition had higher levels of resting and post-experiment saliva cortisol levels than those in the physical condition **(Table 1)**.

Using paired samples correlations, there were significant correlations between saliva cortisol sample 1 and sample 2 (P=.0001) **(Table 2)** but not when comparing means (P=.247) **(Table 3)**. There were significant correlations between samples in the mental condition (P=.002) **(Table 4)** but not for those in the physical condition (P=.469) **(Table 5)**. Using paired samples test, means testing did not reveal differences between samples in either condition **(Tables 6** and **7)**.

fMRI analysis

Comparison of participants' brain scans between the two conditions (mental *versus* physical), as an average across participants, revealed more spread of activity across the brainstem and hypothalamus regions in the mental condition **(Figure 2)**. Brain scans for each participant were individually reviewed with the following results.

For participant with the lowest level of cortisol in the mental condition (P2), there was less activity and less spread of activity in the brainstem region compared with the corresponding participant in the physical condition (P4) **(Figure 3)**. Similar results were shown for these participants on comparing hypothalamus activity **(Figure 4)**. However, when comparing participants with the highest levels of cortisol in both conditions, P9 (mental) had a wider spread of activity in the brainstem region corresponding physical condition participant P10 **(Figure 5)**.

Discussion

Findings of this study are consistent with the action of cortisol on specific brain regions including the hypothalamus. In the physical condition, participants showed lower changes in cortisol and fewer changes than compared with the mental condition. This might be due to the effort involved in the mental task as compared with the physical task.

Participants with lower levels of cortisol and with smaller (or no) changes in level of cortisol, were found in the physical condition. In contrast, greater changes, often in reduction of levels, were found in the mental condition, signifying greater demands of the mental task as compared with the physical task. Hence, if the mental task was perceived by the participants as being stressful, this might explain the higher levels of cortisol; although the actual exertion is lower than in the physical condition and is seen by a reduction in levels after completion of the mental task.

In terms of cortical activity, the brainstem and hypothalamus regions appear to be more active during the physical condition at low levels of cortisol but the activity is more widespread in the brainstem region in the mental condition at higher levels of cortisol in participants.

Therefore, it would seem that participants in the mental condition have the greatest reductions in their cortisol levels during the mental task but when their levels are particularly high (e.g. P9) then there is greater spread of cortical brainstem activity. In the physical condition, the level of cortisol activity is greater during the task in the brainstem region and hypothalamus. However, this activity seems to be less spread when the cortisol levels were highest (e.g. P10).

These findings suggest that the mental task is more initially demanding on cortisol levels which reduce during the task; and

Table 1 Cortisol level of all participants.

P	M/F	AGE	S1	S2	PERIOD	MentPhys
P1	M	34	2.3	2.3	Morning	P
P2	F	30	2.6	2.3	Noon	M
P3	M	35	4.5	4	Morning	M
P4	M	27	1.9	1.9	Noon	P
P5	F	23	3.6	6.9	Noon	M
P6	F	21	2.8	3.1	Noon	P
P7	F	21	5.9	3.6	Morning	M
P8	F	29	4	1.6	Noon	P
P9	M	23	22.7	17.7	Morning	M
P10	F	27	2.2	3.8	Morning	P
P11	M	25	1.8	2	Noon	M
P12	F	27	2.5	2.1	Evening	P
P13	M	21	7.6	3	Morning	M

Key: S1, S2 = cortisol saliva sample 1, 2; MentPhys = Mental (M), Physical (P)

Table 2 All participants – correlation of S1 compared with S2.

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	Sample1 & Sample2	13	.925	.000

Table 3 All participants – means of S1 compared with S2.

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Sample1 - Sample2	.77692	2.29933	.63772	-.61255	2.16639	1.218	12	.247

Table 4 Mental condition – correlation of S1 compared with S2.

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	Sample1 & Sample2	7	.929	.002

Table 5 Physical condition - correlation of S1 compared with S2.

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	Sample1 & Sample2	6	-.371	.469

Table 6 Mental condition – means of S1 compared with S2.

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Sample1 - Sample2	1.31429	2.90484	1.09793	-1.37224	4.00081	1.197	6	.276

Table 7 Physical condition - means of S1 compared with S2.

Paired Samples Test									
		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Sample1 - Sample2	.15000	1.29885	.53025	-1.21305	1.51305	.283	5	.789

when cortisol levels have reached a higher point (e.g. 22.7 for P9) then they activate more widely the brainstem region as compared with the physical task which requires less demand on cortisol but more cortical brainstem and hypothalamus activity.

The results of this study are intriguing because they might explain the role of cortisol as a hormone that protects against stressful situations. It is known that cortisol is enlisted to cope with the demands of a perceived stressful task, as in the mental condition. This is demonstrated by the high levels of cortisol found in the mental condition participants. At the highest levels of cortisol, the brainstem region has a wider spread of activity; and in the less demanding physical condition, less cortisol is enlisted but there is a wider spread of cortical brainstem activity.

It would seem that cortisol works in two ways: for mental tasks, the demand for cortisol is high but recruitment of brain regions is lower than for physical tasks where the demands on cortisol levels are lower, consistent with elite athletes where cortisol activity is lowered with more training and possibly, more brain regions are recruited with an increase in skills set [40].

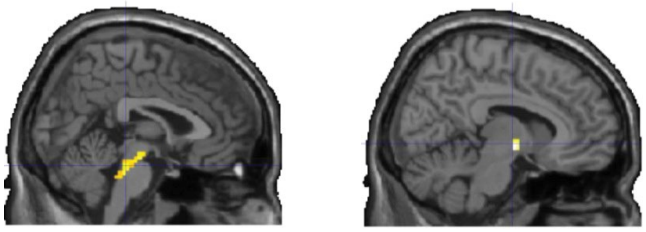
It is possible that cortisol levels fluctuate with atrophy and the demand for cortisol may alter according to structural changes in brain regions. Therefore, there is scope for investigating further the regional versus global changes in grey matter atrophy seen in multiple sclerosis [41,42] with a view to also considering how cortisol may impact these changes.

Averaging brain scan results across participants revealed that overall, recruitment of brain region activity is slightly greater than in the physical condition. Since the greater level of cortisol was found in the mental condition, it is likely that this contributed to the resultant average. However, it supports further the case that greater recruitment of brain regions is seen in the mental condition where cortisol levels diminish and in participants who have the greatest reduction in levels during the task.

Potential application of these findings is in the diagnosis of neurological diseases such as immune suppression syndromes where cortisol is important for good health maintenance. Diffusion Tensor Imaging (DTI) and Voxel-Based Morphometry (VBM) [43,44] is a useful tool for identifying atrophy in brain regions in neurological disease.

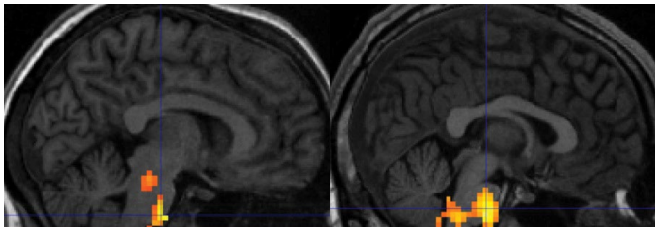
For example, in multiple sclerosis, grey matter atrophy occurs as a regional versus global process [41,42]. This study shows that the recruitment of brain regions changes with cortisol level and the type of task being carried out. It is possible that atrophy causes cortisol levels to change in these brain regions.

Evidence of cortisol changes associated with yawning [14,15,17,26], a common symptom of multiple sclerosis, may



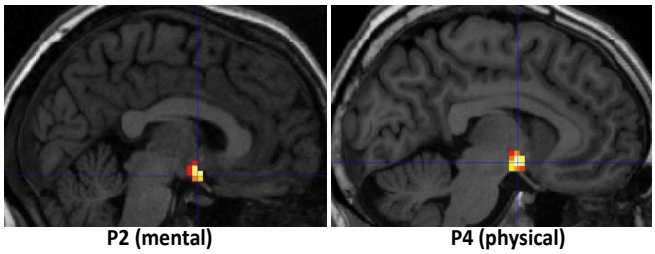
brainstem hypothalamus

Figure 2 Brain scans comparing brainstem and hypothalamus activity as an average across "Mental *versus* Physical" participants.



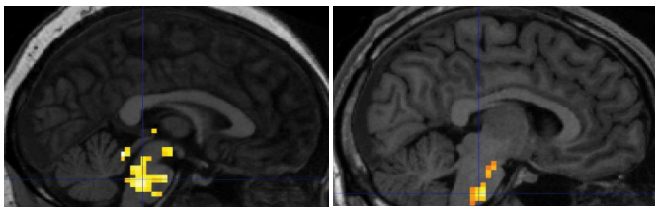
P2 (mental) P4 (physical)

Figure 3 Brain scans comparing brainstem activity in P2 (mental) and P4 (physical) for lowest level of cortisol.



P2 (mental) P4 (physical)

Figure 4 Brain scans comparing hypothalamus activity in P2 (mental) and P4 (physical) for lowest level of cortisol.



P9 (mental) P10 (physical)

Figure 5 Brain scans comparing brainstem activity in P9 (mental) and P10 (physical) for highest levels of cortisol.

help identify brain region recruitment in phases of the disease. It is hoped that the use of cortisol and yawning as a potential new biomarker for the detection of neurological disease may be a possibility in the near future.

Conclusions

These findings are encouraging because the establishment of threshold levels of cortisol across conditions might become indicative of poor performance and of impairment in brainstem and hypothalamic regions, important to vital and healthy functioning, and when impaired, indicative of HPA-axis malfunctioning. This has implications for the detection of cortisol insufficiency syndromes such as Cushing's disease, and other neurological sequelae.

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