

fMRI Randomized Study of Mental and Motor Task Performance and Cortisol Levels to Potentiate Cortisol as a New Diagnostic Biomarker

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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 and mental and motor tasks are difficult and labored. The role of cortisol is 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 brain-stem 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 seen 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 supporting the Thompson Cortisol Hypothesis 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 but demand 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: Brain-stem; Cortisol; fMRI; Hypothalamus; Mental/Motor task

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Introduction

Cognitive fatigue arises from prolonged exposure to the demands of a task that require continued mental efficiency [1]. Such tasks often give rise to the cortisol stress-response within the hypothalamic-pituitary-adrenal (HPA) axis.

Cortisol and fatigue

The link between fatigue and cortisol is dependent upon chronic or acute stress; with chronic fatigue syndrome being linked with cortisol level fluctuations [1].

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.

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) [2,3] (**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

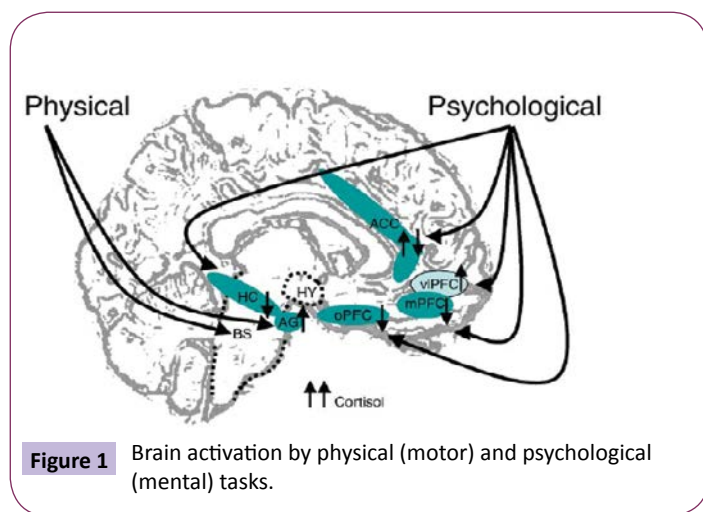


Figure 1 Brain activation by physical (motor) and psychological (mental) tasks.

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 [4] 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.

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 [7,8].

Similarly, increased activity in medial PFC regions correlates with decreased cortisol secretion [9], 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.

Yawning and cortisol

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 [10]. His theory was interesting since we need to protect against critical rises in brain temperature, particularly when we are fatigued [11,12]. In multiple sclerosis (MS), fatigue is a common symptom [13-15] and this may be related to yawning excessively with high rises in brain temperature [16-18].

It is known that the pituitary gland oxytocin regulates social bonding, and circadian rhythm and temperature regulation is the responsibility of the hypothalamus [19]. 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 [20-22].

Thompson [12] has found a link between yawning excessively and neurological diseases; Lano-Peixoto, and colleagues [23] have also noted excessive yawning in their five patients who has neuromyelitis optica spectrum disorder (NMOSD). From MRI brain scans, their patients showed lesions in the brain-stem 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 [24]; 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 [25] in his Thompson Cortisol Hypothesis and describes the rise in cortisol, produced by the adrenal cortex zona fasciculate yawning to control brain temperature.

The British neurologist, Sir Francis Walshe, reported to his stroke patients in 1923. He noted that those with brain-stem lesions, had the capacity to raise their paralyzed arm when yawning [26]. This has since been evidenced by a number of other researchers (Walusinski, et al., Kang & Dhand) [27,28].

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 [29,30]. In addition to brain fMRI studies, others have implicated the mirror-neuron system [31]; and endogenous levels of cortisol have implicated in pathological gamblers where striatal sensitivity fluctuates [32].

Materials and Methods

Participants

13 healthy participants (6 male, 7 female) aged between 21-35 years with no known history of neurological, psychiatric or sensorimotor disorders gave their prior, written, informed consent to participation in the study. Participants were assessed using the Edinburgh inventory [33] for right-handedness, and with consent, were recruited at 'Hôpital Universitaire Amiens and Jules Verne Université de Picardie, France.

Paradigm

Saliva samples were collected at the start and again at the end of the condition from each participant. Each sample was analyzed 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 analyze in the specialized laboratory.

Participants are randomly allocated to one of two conditions: mental task (intrinsic or extrinsic) or motor (physical) task. Inside

the fMRI scanner, the participant was presented with a slide that states "The experience will begin. Do not move your head, and pay attention to all instructions." The protocol was programmed so that the transition from the first slide to the second is performed manually by pressing the keyboard space bar in synchronism with the start of fMRI.

The second slide lasted 12s with the instruction: "Read all instructions carefully and practice fast and accurate pressures without moving your head." Apart from the first slide, subsequent slides were programmed to automatically follow for each task.

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 (Table 1).

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.

The protocol included 4 repetitions of 3 blocs: T1: Motor Task; T2: Intrinsic Task without Warning signal; T3: Extrinsic Task with warning signal. In Task 1 (motor), the instruction lasted 6s and specifies the level of force to execute: "Press with HIGH STRENGTH, quietly and fixing the flashing square". Alternation between the image of a white square and the image of a black screen, followed. The duration of each of these two images was 100 ms (Table 2).

In Task 2 (intrinsic without warning signal), the task began with a

statement that lasted 6s: "Press as soon as possible as soon as the square appears and release quickly." A cross and a white square, followed (Figure 2 and Table 3).

In Task 3 (extrinsic with warning signal), the task began with an instruction that lasted 6s: "The cross will turn off, Concentrate. Press as soon as possible as soon as the square appears and release quickly." A cross, a black screen, and a white square, followed (Figure 3 and Table 4). Summary of sequences is shown in Figure 4 and Table 5.

Results

Mean age of participants was 26.4 years (sd = 4.70); standard range from cortisol is (i) morning collection: 3.7 – 9.5 Nano grams (one billionth of a gram or 10⁻⁹) per milliliter of saliva; (ii) noon collection: 1.2 – 3.0 Nano grams per milliliter; (iii) evening collection: 1.9 Nano grams per milliliter.






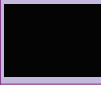
In saliva cortisol sample 1, the means for participants in the mental condition was 7.0 (sd = 7.21), and for the physical condition was 2.6 (sd = 0.74). In sample 2, the means were 5.6 (sd = 5.56) for those in the mental condition, and 2.5 (sd = 0.83) for those in the physical condition. Hence, those in the mental condition had higher levels of resting and post-experiment saliva cortisol levels than those in the physical condition (Table 6).

Using Paired Samples Test, there were significant correlations between saliva cortisol sample 1 and sample 2 (p=0.000) (Table 7) but not when comparing means (p=0.247) (Table 8). There were significant correlations between samples in the mental condition (p=0.002) (Table 9) but not for those in the physical condition (P=0.469) (Table 10). Means testing did not reveal differences between samples in either condition (Tables 11 and 12).

Table 1 Sequence of tasks.

Bloc N°1	T3			T1			T2		
	Low	Medium	High	High	Medium	Low	Low	High	Medium
Bloc N°2	T2			T1			T3		
	High	Low	Medium	High	Medium	Low	High	Medium	Low
Bloc N°3	T3			T1			T2		
	Medium	High	Low	Low	Medium	High	Medium	Low	High
Bloc N°4	T2			T1			T3		
	Low	Medium	High	Low	Medium	High	Low	High	Medium

Table 2 Tasks.

T1 High	Press with HIGH STRENGTH, quietly and fixing the flashing square		
T1 Medium	Press with MEDIUM STRENGTH, quietly and fixing the flashing square		
T1 Mild	Press with MILD STRENGTH, quietly and fixing the flashing square		
Duration (ms)	6000	100	100

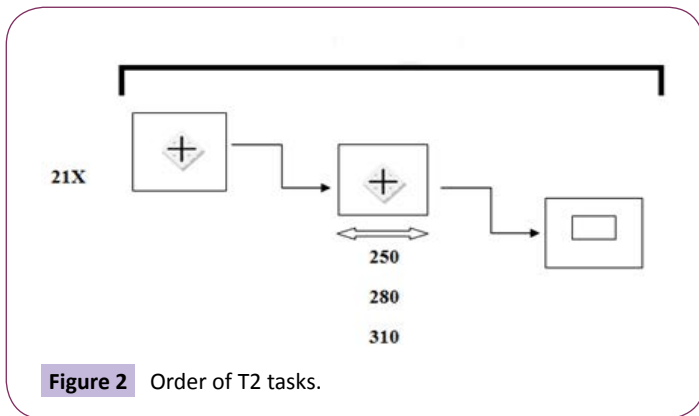


Figure 2 Order of T2 tasks.

Table 3 Order of T2 tasks.

	High strength	+	+	■	Duration
	T2 High	1500	1750	280	1000
		1750	310	1000	3060
		1690	250	1000	2940
		1720	250	1000	2970
		1720	310	1000	3030
		1690	280	1000	2970
		1720	280	1000	3000
T2 Medium		Medium strength	+	+	■
	1500	1720	250	1000	2970
		1690	310	1000	3000
		1750	280	1000	3030
		1750	310	1000	3060
		1720	280	1000	3000
		1690	250	1000	2940
		1690	310	1000	3000
T2 Mild	Mild strength	+	+	■	Duration
	1500	1720	250	1000	2970
		1690	280	1000	2970
		1750	250	1000	3000
		1750	310	1000	3060
		1690	250	1000	2940
		1720	310	1000	3030
		1750	280	1000	3030

Brain scans for each participant were reviewed with the following results. For participants with the lowest level of cortisol in the mental condition (P2), there was less activity and less spread of activity in the brain-stem region compared with the corresponding

participant in the physical condition (P4) (Figure 5). Similar results were shown for these participants on comparing hypothalamus activity (Figure 6). However, when comparing participants with the highest levels of cortisol in both conditions, P9 (mental) had a wider spread of activity in the brain-stem region corresponding physical condition participant P10 (Figure 7).

Comparison of participants' brain scans between the two conditions, as an average across participants, revealed more spread of activity across the brain-stem and hypothalamus regions in the mental condition; however, this might be skewed in favor of the high cortisol levels found in the mental condition participants, particularly P9 (22.7) (Figure 8).

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 brain-stem 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 brain-stem 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 brain-stem activity. In the physical condition, the level of cortisol activity is greater during the task in the brain-stem region and hypothalamus. However,

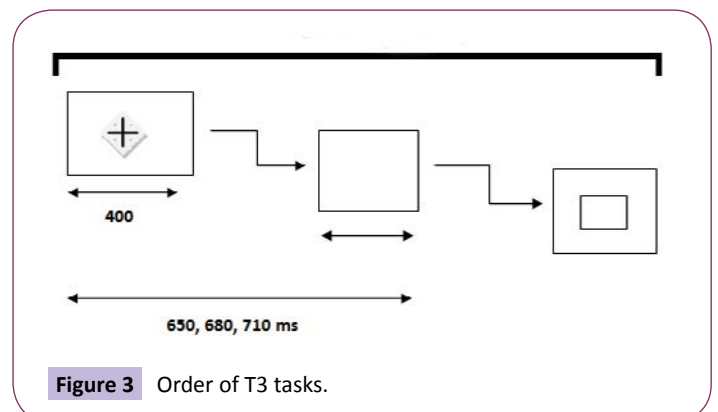


Figure 3 Order of T3 tasks.

Table 4 Order of T3 tasks

T3 High	High strength	+	+		■	Duration
	1500	1350	400	310	1000	3060
		1350	400	280	1000	3030
		1290	400	250	1000	2940
		1320	400	250	1000	2970
		1320	400	310	1000	3030
		1290	400	280	1000	2970
		1320	400	280	1000	3000
T3 Medium	Medium strength	+	+		■	Duration
	1500	1350	400	280	1000	3030
		1290	400	310	1000	3000
		1320	400	250	1000	2970
		1350	400	310	1000	3060
		1290	400	310	1000	3000
		1290	400	250	1000	2940
		1320	400	280	1000	3000
T3 Mild	Mild strength	+	+		■	Duration
	1500	1350	400	280	1000	3030
		1350	400	250	1000	3000
		1290	400	280	1000	2970
		1290	400	250	1000	2940
		1350	400	310	1000	3060
		1320	400	310	1000	3030
		1320	400	250	1000	2970

Table 5 Summary of timings

Total Duration of the protocol: 15min 17s 76ms
Boot set = 12s
Sets T3/T2 = 6s * 8 = 48s
Sets T1 = 6s * 12 = 72s
Images T3/T2 = 1.5s * 24 = 36s (High, Medium, Low strength)
Blocs T1 = 21 * 12 = 245.76s
Blocs T2 = 21 * 12 = 252s
Blocs T3 = 21 * 12 = 252s

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

Table 6 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	4.0	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	4.0	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	2.0	Noon	M
P12	F	27	2.5	2.1	Evening	P
P13	M	21	7.6	3.0	Morning	M

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

Table 7 All participants – correlation of S1 compared with S2.

Paired Samples Correlations				
Pair 1	Sample 1 & Sample 2	N	Correlation	Sig.
Pair 1	Sample 1 & Sample 2	13	0.925	0

Table 8 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	Sample 1 – Sample 2	0.77692	2.29933	0.63772	-0.61255	2.16639	1.218	12	0.247

Table 9 Mental condition – correlation of S1 compared with S2.

Paired Samples Correlations				
Pair 1	Sample 1 & Sample 2	N	Correlation	Sig.
Pair 1	Sample 1 & Sample 2	7	0.929	0.002

Table 10 Physical condition - correlation of S1 compared with S2.

Paired Samples Correlations				
Pair 1	Sample 1 & Sample 2	N	Correlation	Sig.
Pair 1	Sample 1 & Sample 2	6	-0.371	0.469

Table 11 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	Sample 1 – Sample 2	1.31429	2.90484	1.09793	-1.3722	4.00081	1.197	6	0.276

Table 12 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	Sample 1 – Sample 2	0.15	1.29885	0.53025	-1.2131	1.51305	0.283	5	0.789

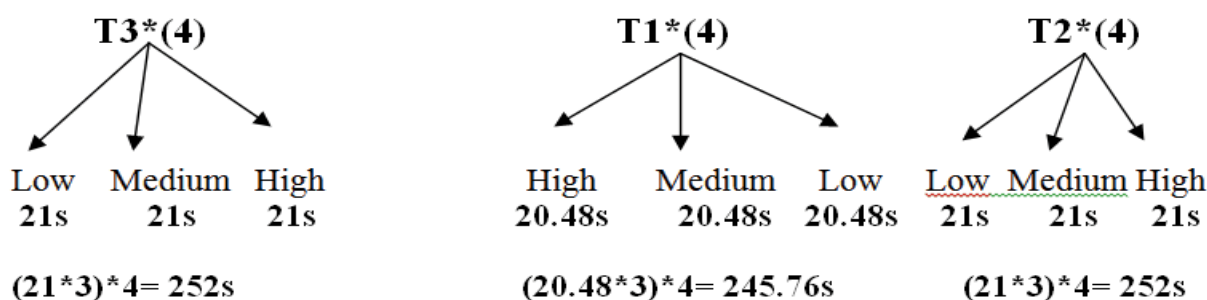


Figure 4 Summary of schema.

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 brain-stem 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 brain-stem activity.

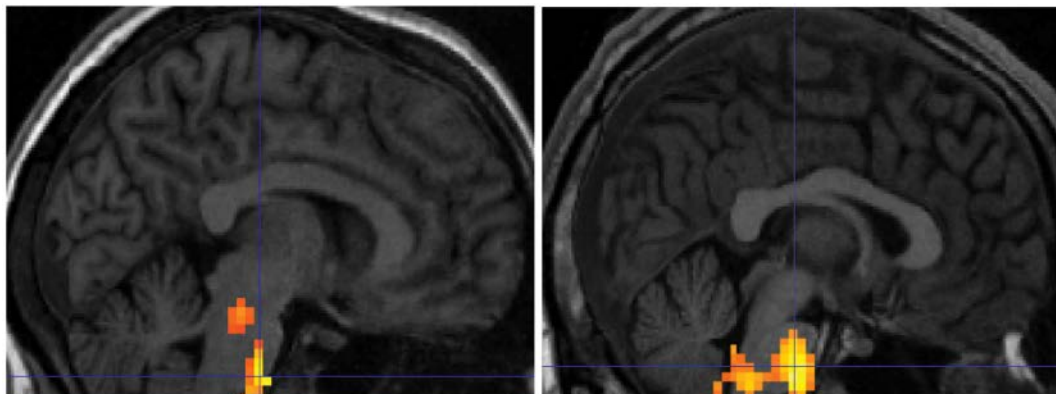


Figure 5 Brain scans comparing brain-stem activity in P2 (mental) and P4 (physical).

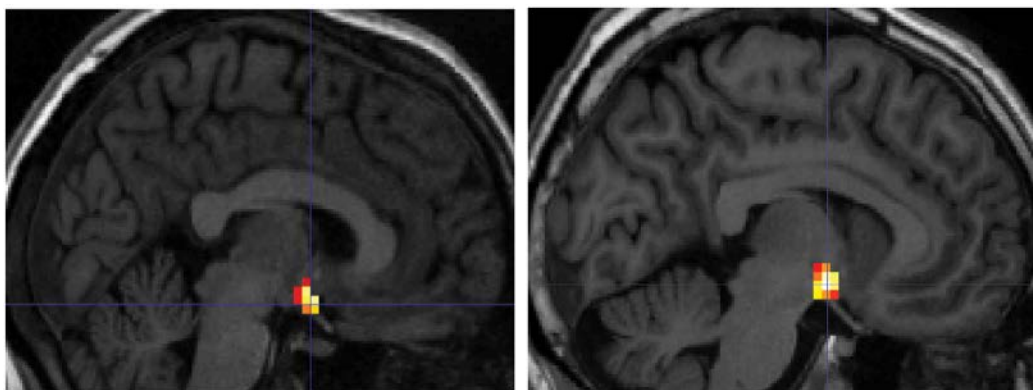


Figure 6 Brain scans comparing hypothalamus activity in P2 (mental) and P4 (physical).

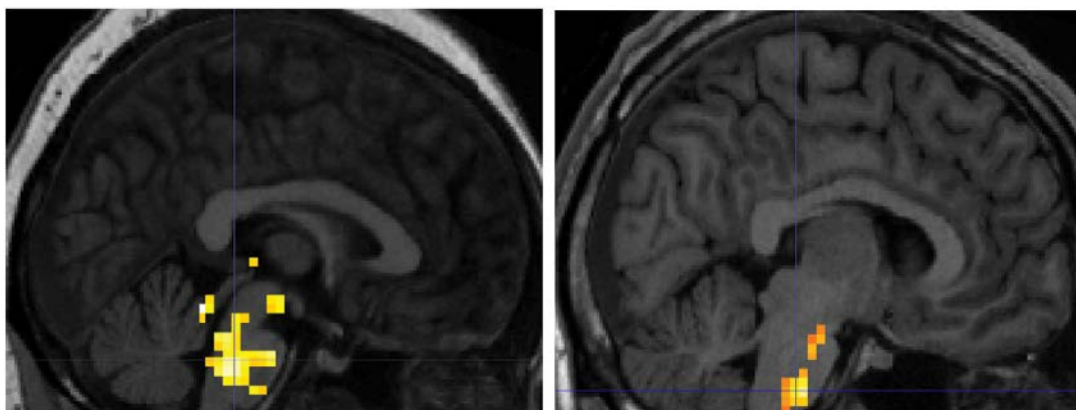


Figure 7 Brain scans comparing brain-stem activity in P9 (mental) and P10 (physical).

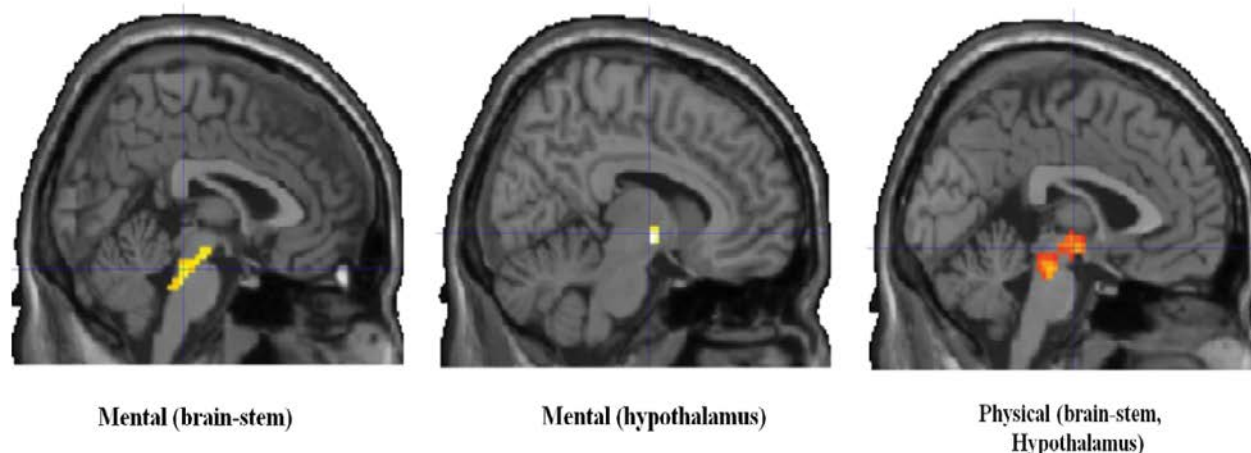


Figure 8 Brain scans comparing brain-stem and hypothalamus activity as an average across Mental versus Physical participants.

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 [34-36].

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 great 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) [37,38] 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 [39,40]. 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.

Conclusions

Clinical scientists are particularly interested in cortisol because of its role in the HPA-axis. The implications of cortisol in tasks and in association with yawning, makes it intriguing and important to neurologists, neuroscientists and practitioners because of the potential benefits of findings to neurological patients. Identifying threshold levels of cortisol and the recruitment of brain region activity may be important in determining future functioning deficits and neuronal damage.

Implications of these findings are potentially far reaching. For example, if known parameters of cortisol levels can be established across varying conditions, then they can become indicative of poor performance. Perhaps more interesting is the possibility that these findings might point towards a new biomarker of neurological disease where cortisol is particularly salient, such as in multiple sclerosis or Cushing's disease.

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