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THE ART OF CAPTURING A YAWN USING THE SCIENCE OF NERVE IMPULSES AND CORTISOL LEVELS IN A RANDOMIZED CONTROLLED TRIAL. THOMPSON CORTISOL HYPOTHESIS AS A POTENTIAL PREDICTOR OF NEUROLOGICAL IMPAIRMENT

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Background: Thompson Cortisol Hypothesis proposed yawning correlates with rises in cortisol levels. Cortisol is fundamental to immune system regulation. Pathological yawning is a symptom of MS. Electro-myographical activity (EMG) in the jaw muscles rises when stretched; and is likely to be correlated with yawning, and potentially correlated with cortisol levels in healthy people and in MS. Objectives: Investigate possible link between EMG in jaw muscles with rises in saliva cortisol levels during yawning. Method: Randomized controlled trial: 11 male and 15 female volunteers aged 18-53 years exposed to conditions that provoked a yawning response. Saliva samples were collected at start and after yawning or at the end of stimuli presentations if the participant failed to yawn, and EMG data was collected during rest and yawning phases and is novel. Yawning susceptibility scale, Hospital Anxiety and Depression Scale, General Health Questionnaire, demographic, health details were collected for yawners and non-yawners, between rest and yawning phases. Exclusion criteria: chronic fatigue, diabetes, fibromyalgia, heart condition, high blood pressure, hormone replacement therapy, multiple sclerosis, stroke. Results: Significant differences between saliva cortisol samples. Yawners, $t(11) = -3.115$, $p = 0.010$; $F(1, 11) = 13.680$ $p < 0.025$, and non-yawners, $t(14) = -2.658$, $p = 0.019$; $F(1, 14) = 4.758$ $p = 0.047$. Moderate, though not significant, correlation between cortisol change (from sample 1 to 2) and EMG score: $r(7) = 0.440$, $p = 0.071$. Significant difference in EMG readings between yawners and non-yawners, $t(7) = -2.959$, $p = 0.021$. Conclusions: Thompson Cortisol Hypothesis is supported, and EMG is correlated with yawning and elevated cortisol levels**.** Longitudinal study is planned with MS patients to develop an early diagnostic tool.

Keywords: Cortisol, Electro-myography, EMG, Neurological disorder, Yawning.

Introduction

Researchers are largely in agreement on the localisation of the process of yawning in the paraventricular nucleus of the hypothalamus and the brainstem. However, agreement is yet to be reached on which neuro-chemicals are fundamental to the yawning episode, albeit a number of neurotransmitters have been identified as being implicated [1].

Recently, the Thompson Cortisol Hypothesis [2] has proposed that the incidence of yawning is associated with rise in saliva cortisol levels. It suggests that either cortisol is a trigger for the yawn reflex or is an artefact that may protect the yawner. It is unclear how this mechanism may work within the

known Hypothalamus-Pituitary-Adrenal (HPA) axis and may be connected with hypothalamus temperature regulation theories such as those prosed by Gallup [3]. Stress and fatigue are known to cause elevations in cortisol, and yawning incidence has been shown to be increased at these times [4 - 7]. Cortisol is a fundamental component of the stress response and immune system regulation and consequently is sensitive to the physiological impact of neurological disorders such as Multiple Sclerosis (MS), an inflammatory disease of the central nervous system. Some evidence suggests that excessive and pathological yawning is a symptom of MS.

There is evidence for a potential relationship between yawning and cortisol in MS. Empirical evidence for excessive yawning in MS is limited though there is some indication of an association. Despite the absence of irrefutable evidence, yawning and MS, cortisol dysregulation and its association with stress, depression, fatigue and dysfunctional thermoregulation and the complexity of the interactions between these factors, indicates cortisol and yawning is tied in a certain relationship. The establishment of a connection between cortisol and yawning may shed further light on MS, and on the function of yawning in other neurological disorders.

Cortisol is often described as the stress hormone. The amygdala detects situations involving uncertainty or fear, sending messages to the hypothalamus. Increased stimulation of the hypothalamic-pituitary-adrenal axis (HPA) by physiological or psychological stressors stimulates an increase in production of cortisol from the adrenal gland [8]. The HPA axis is the regulatory neuro-endocrine mechanism connecting the central nervous system (CNS) with the hormonal and immune systems and maintains homeostasis by facilitating the body's adaptation to stress.

Physiological or psychological stressors prompt the secretion of corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus [9]. CRH triggers the pituitary gland to release adrenocorticotropic hormone (ACTH), the secretion of which is also influenced by the suprachiasmatic nucleus in the hypothalamus [10]. Stimulation of the adrenal cortex by ACTH induces the release of glucocorticoids such as cortisol [11].

An important homeostatic role of the hypothalamus is thermoregulation. Temperature is monitored by thermo-receptors located in the hypothalamus, which measure the temperature of blood passing through the brain to establish the core temperature of the body, and skin receptors track the ambient temperature. Deviations in core temperature are detected by the hypothalamus and, taking into account the external temperature, the autonomic nervous system (ANS) responds to correct the discrepancy through the involuntary mechanisms of conduction, convection, radiation and evaporation.

Excessive heat prompts sweating and vaso-dilation to promote heat loss and reduce core body temperature. Fall in temperature induces shivering and an increase in metabolic rate in an attempt to minimise further heat loss and promote warming.

Physiological adjustments are made constantly by the hypothalamus, and combine with voluntary changes in our behaviour, such as fanning when hot, or adding layers of clothing when cold, to maintain the body at its optimum temperature of 36.5-37.5˚C when at rest.

Attempts to understand the significance of yawning have challenged and eluded philosophers and scientists alike for centuries, and theories referring to yawning as a mechanism essential to maintenance of optimal oxygen levels has been contended for over 200 years [12].

Most people are aware of increased yawning when fatigued and there is evidence to suggest that yawning follows a circadian rhythm correlating with individual preferences for early waking or late retiring. Yawning may be evidenced more frequently in some in the morning, or alternatively, in the evening by other people [13]. Yawning at those times when the individual prefers to be asleep adds weight to the function of yawning as an arousal mechanism.

Gallup [14] is a leading proponent of the thermoregulation hypothesis which argues that yawning acts as a compensatory cooling mechanism when other homeostatic mechanisms have failed to respond to mild central hyperthermia. With homeostatic temperature control located in the hypothalamus, Gallup and Gallup Jr [15] propose that the intrinsic involvement of yawning in thermoregulation means that control must also be located in the hypothalamus. Like Corey and colleagues [16], they argue that the mechanism by which this occurs is a convective cooling, or radiator effect, whereby deep inhalation of cooled air

alters the temperature of the blood in the brain by cooling the arterial blood supply. They further propose two other mechanisms of cooling aside from counter-current heat exchange (enhanced cerebral blood flow and evaporation of sinus mucosa) [17]. Recent reports show the evidence in favour of the thermoregulatory theory to be tenable [18].

The most recent addition to theories of yawning is the Thompson [7] cortisol hypothesis which suggests that the incidence and frequency of yawning may be connected with cortisol levels. The variation in yawning incidence and cortisol levels in fatigue provides support for a correlation between the two as fatigue, which has implicit connections with yawning, has been identified as a consequence of hypocortisolism in a recent longitudinal study which accounted for multiple confounding variables [19].

Activation of the hypothalamic-pituitary-adrenal (HPA) axis by mental and physical stress is known to increase cortisol secretion to facilitate the body's management of the impact of stress and is cited as further support for the Thompson [7] cortisol hypothesis. Thompson and Zisa [6] propose that the stress imposed on the body by neurological conditions may impact upon cortisol levels and explain the incidence of pathological and excessive yawning during the onset of strokes [20] and acute relapse in MS [21].

Cortisol and multiple sclerosis

Increased cortisol levels, and the absence of cytokines, within the cerebrospinal fluid of deceased MS patients compared to healthy controls, also provides evidence of characteristic and sustained hyperactivity of the HPA axis [22]. As elevations in cortisol could not be attributed to the presence of current inflammation, they proposed that it may be part of the body's defence mechanism against MS and to promote recovery or to prevent relapse. However, the authors do not state whether they established symptom severity at the time of death, and it seems unlikely that all the participants passed away at times of remission or reduced inflammation. Thus, the absence of variations in cytokine levels between MS patients and controls appears unusual. People with MS appear to experience greater fluctuations in their plasma cortisol levels [23] and sustained hyperactivity within the HPA axis.

Insensitivity to glucocorticoids resulting from the excessive production of cortisol may facilitate increased inflammation due to an unregulated immune system response to infection relapses in MS inflammation [26]. Bergh [25] speculates this may increase autoimmunity which could cause a switch to a progressive form of the disease; hence, HPA hyperactivity is not merely an indicator but possibly the cause (or a predictor), of the onset of a progressive course of MS.

Michelson and colleagues [26] and suggest that MS differs from other disorders in which hypercortisolism is a symptom, such as Cushing's syndrome, because of the action of the corticotrophin-releasing hormone (CRH). This is supported by Erkut and colleagues [27] who found an increase in the number of cells that produce CRH whose action, in turn, stimulates production of adreno-corticotropic hormone (ACTH). They also found significant increase in arginine vasopressin (AVP) co-expressing CRH neurons. Vasopressin is a peptide hormone that controls the re-absorption of molecules in the tubules of the kidneys by affecting the tissue's permeability. It also increases peripheral vascular resistance, which in turn increases arterial blood pressure. It plays a key role in homeostasis, by the regulation of water, glucose, and salts in the blood [28]. As AVP potentiates CRH in its action upon the secretion of ACTH, this suggests that CRH neurons are hyperactive in MS.

Curiously, glycyrrhizic acid, found in liquorice, increases the activity of cortisol on the kidney functions [29]. Inhibition of the enzyme 11 -hydroxysteroid dehydrogenase type 2, which normally inactivates cortisol in the kidney, results in an increase of cortisol levels. One participant asked to eat liquorice after providing a saliva sample, was observed to have an increase in cortisol levels [7].

Fatigue and cortisol in multiple sclerosis

Fatigue is not only one of the most frequently reported symptoms of MS but has also been described as the most disabling aspect of the condition [29]. Mills and Young's [30] study reported that fatigue had consequences for both physical and cognitive activity causing such symptoms as muscle aches, poor

coordination, tremor, inability to sustain attention, and poor memory. MS patients tended to view energy as a fixed quota which once used results in fatigue and cannot be restored without rest, which is the predominant way in which MS patients attempt to manage fatigue. Just under fifty per cent of the respondents to the authors' survey were in agreement that excessive yawning was a feature of their fatigue.

Iriarte, Subira and De Castro [31] propose that fatigue in MS falls into three categories: fatigue at rest (asthenia), fatigability, and a generalised worsening of symptoms that can be attributed to other mechanisms. Their research identified a correlation between the severity of damage to the pyramidal tract, and fatigability as a consequence of exertion, whilst asthenia seems connected to immuno-activation. Of the participants with fatigue, had damage to the pyramidal tract; however, this was not predictive, as 51% with similar abnormalities reported no fatigue, which reduces the strength of their conclusion. The study benefited from a large sample size and appears to be comprehensive in its measurement and analysis of immuno-activation and Magnetic Resonance Imaging (MRI) scans.

Morelli, Ravera and Panfoli [32] suggest that the myelin sheath might itself have a part to play in energy during wakefulness. The sheath may act as a proton buffer capacitor as the constituents of myelin have been shown to be excellent at retaining energy, thus allowing it to provide energy during waking hours. Once this charge has been used, sleep would be induced, and the myelin restored with energy. The authors argue that increased sleep during infancy may be indicative of incomplete myelino-genesis which makes the myelin slower to accumulate energy.

Yawning in multiple sclerosis

Postert and colleagues [21] report on a patient with excessive pathological yawning, four times per minute, despite sufficient sleep, as a symptom of MS. An MRI scan showed that the patient had multiple demyelinated plaques within the brainstem. After three days of treatment with high dose steroids, yawning had completely remitted. However, demyelination of the brainstem is common in MS and so cannot be the source of pathological yawning. The authors speculate that inflammation in this area causes yawning by irritating the ascending activating reticular system.

Gallup and Gallup Jr [15] and Gallup and colleagues [33] cite the thermoregulatory dysfunction characteristic of MS as evidence for the pathological occurrence in the disorder. Evidence indicates that cooling improves and alleviates debilitating symptoms of MS with pre-cooling enabling increased participation in activity and exercise [34]. Gallup and Eldakar [35] suggest yawning rates are increased with initial rises in temperature, but then diminish as temperature approach or exceed body temperature. Gallup and colleagues [36] showed that decreases in ambient temperature (34 to 24 degrees Celsius) do not increase yawning frequency.

In healthy people, frequent and repetitive yawning is most commonly the consequence of sleep debt [37, 38]. Romeijn and colleagues [39] report that skin warming induces neuronal firing patterns in the brain similar to those occurring in sleep, and also inhibits patterns that indicate wakefulness in the hypothalamus. As low skin temperatures are correlated with greater efficiency in tasks and an improved ability to resist sleep [40] mild skin warming may therefore promote or induce sleep. The suprachiasmatic nucleus may become more sensitive to skin temperature if communication between its neurons is impaired, making sleep-wake rhythms more susceptible to the influence of skin temperature.

This is conceivable in MS as such connections could be damaged by demyelination. However, this does not provide unequivocal support for Gallup's [14] thermoregulatory hypothesis, as yawning in response to warming may be a consequence of increased feelings of tiredness in response to the change in brain activity, and not a homeostatic need to regulate temperature.

Pathological yawning in MS may also be the result of impaired hypothalamic function. Using MRI investigations, Zellini and colleagues [41] determined that despite similarities in hypothalamic volume in comparison with healthy controls, MS patients had longer T1 values, which could be a sign of demyelination or axonal damage, and that these values were positively correlated with fatigue. Impairment of the hypothalamus could also be the source of other MS symptoms such as sleep

disturbance, autonomic and metabolic dysfunctions, sexual disturbances and depression. Therefore, the hypothalamus may also be fundamental in thermoregulation as this may be the source of dysfunction.

Nahab's [42] neuroimaging research suggests involvement of the ventromedial prefrontal cortex (vmPFC) in yawning as this area seems to be activated only by yawns. The vmPFC could be a cortical releasing mechanism allowing yawning, when appropriate. Demyelination may prevent normal functioning of the vmPFC, thus preventing inhibition of inappropriate yawns and leading to the pathological and excessive yawning that has been described in people with MS.

Cortisol and yawning in multiple sclerosis

Thompson [2] is the first to propose a connection between yawning and cortisol in humans. The Thompson Cortisol Hypothesis suggests the incidence and frequency of yawning may be dependent upon fluctuations in cortisol. Animal studies are indicative of a link between yawning and cortisol. Removal of rats' adrenal glands almost completely eliminated yawning [43]. Miller and colleagues [44] also suggests a connection between yawning and cortisol in animals. Instead of measuring corticosterone, yawns may provide a non-invasive method to qualitatively measure stress. Others measures might include plasma CORT which can then potentially be correlated with yawning episodes. Despite the difficulty in relating animal research to humans, animal studies may give a preliminary indication of a connection.

Methods

11 male and 15 female volunteers aged between 18-53 years were recruited from students at Bournemouth University using the computerised recruitment system (SONA), and Facebook. All participants were properly consented according to code of conduct and research guidelines, and exposed, under randomised controlled trials guidelines, to three conditions intended to provoke a yawning response – 9 photos of people yawning; boring text about yawning; short 3:02 minutes video of person yawning (13 yawns during video clip). Comparisons were made with people exposed to the same conditions but who did not yawn.

Saliva samples were collected at start and again after first yawning response, together with electro-myographical (EMG) data of the jaw muscles to determine rest (5 minutes prior to yawn at post-session analysis) and yawning phases of neural activity. If there was no yawning response, then a second saliva sample was taken at the end of the experimental paradigm. A yawning susceptibility scale (questionnaire designed for this study), Hospital Anxiety and Depression Scale (HADS) [28, 45],

General Health Questionnaire GHQ28 [46 - 48], and demographic and health details were also collected from each participant.

To avoid disruption or inhibition of the participant's performance, the researcher sat just outside the experimental booth but in sight of the participant. In this way, the researcher could observe when a yawn occurred but did not hinder the participant's reaction to yawn or hinder concentration on viewing the presentation stimuli. Exclusion criteria were**:** chronic fatigue, diabetes, fibromyalgia, heart condition, high blood pressure, hormone replacement therapy, multiple sclerosis, and stroke. (People with multiple sclerosis were excluded for this study so that observation of people from a healthy population could be studied in terms of cortisol levels). Saliva sample is collected at start and again after yawning response (if produced). Electro-myographical data of the jaw muscles was collected via surface-placed electrodes to determine rest and yawning phases. Between- and within-subjects comparisons were made using t-tests and correlations using the SPSS package [version 19]. This enabled a comparison to be made between yawner and non-yawner participants as well as between rest status and yawning episodes.

Ethics

Bournemouth University Research & Ethics approval granted: BU-PS29/6/12; BU-PS18/10/12; BU-JC28/1/13; BU-KS6/9/13. Professional code of conduct, confidentiality, and safety issues were addressed

and approved in the Ethics submission. Data collected was made anonymous, coded, securely stored and all coding linking identification to individual participants was destroyed after completion of the study analysis. Protective measures were put in place for collection and analysis of saliva samples and the right to withdraw was made clear to all participants. Trials ID: ISRCTN61942768.

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Competing interests

None.

Results

Normative data for saliva cortisol is known, and lies within the following ranges: (i) Morning collection is 3.7 – 9.5 nanograms (one billionth of a gram or 10^{-9}) per millilitre of saliva; (ii) Noon collection is 1.2 – 3.0 nanograms per millilitre; (iii) Evening collection is 0.6 – 1.9 nanograms per millilitre.

There were differences between sample 1 (saliva cortisol) and sample 2 (saliva cortisol) for those who yawned, and for those who did not yawn, during the experiment (Tables 1 & 2).

Table 1. Descriptive data for overall group.

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Age	27	35.00	18.00	53.00	29.2593	11.24754	126.507
Sample1	27	6.20	.40	6.60	2.6111	1.73257	3.002
Sample2	27	8.80	.50	9.30	3.3481	2.25292	5.076
Yawn	27	1.00	.00	1.00	.4444	.50637	.256
EMG1LO	27	25.00	-15.00	10.00	-6.5148	5.38321	28.979
EMG1HI	27	78.00	2.00	80.00	20.1704	22.08018	487.534
EMG2LO	27	275.00	-225.00	50.00	-21.2963	45.90087	2106.890
EMG2HI	27	172.30	2.70	175.00	40.6481	43.24251	1869.915
Valid N (listwise)	27						

Table 2. Descriptive data for yawners and non-yawners.

Descriptive Statistics[a]

Yawn		N	Range	Minimum	Maximum	Mean	Std. Deviation
Non-yawn	Sample1	15	5.00	.50	5.50	2.3800	1.58619
	Sample2	15	8.70	.60	9.30	2.9133	2.19801
	Valid N (listwise)	15					
Yawn	Sample1	12	6.20	.40	6.60	2.9000	1.93109
	Sample2	12	6.90	.50	7.40	3.8917	2.29524
	Valid N (listwise)	12					

a. No statistics are computed for one or more split files because there are no valid cases.

For the yawners, repeated-measures t-test: t (11) = -3.115, p = 0.010 (Table 3); for the non-yawners, repeated-measures t-test: t(14) = -2.658, p = 0.019 (Table 4).

Table 3. Paired comparisons for yawners.

		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	.90000	1.00091	.28894	-1.53595	-.26405	3.115	11	.010

Table 4. Paired comparisons for non-yawners.

		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	1.13333	1.65169	.42647	-2.04801	-.21866	-2.658	14	.019

Using analysis for repeated measures, there was high significance for the groups overall but non-significant interaction, F (1, 26) = 1.588 p = 0.219 (Table 5).

Table 5. Within-subjects effects overall.

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
sample	Sphericity Assumed	7.752	1	7.752	17.584	.000
	Greenhouse-Geisser	7.752	1.000	7.752	17.584	.000
	Huynh-Feldt	7.752	1.000	7.752	17.584	.000
	Lower-bound	7.752	1.000	7.752	17.584	.000
sample * Yawn	Sphericity Assumed	.700	1	.700	1.588	.219
	Greenhouse-Geisser	.700	1.000	.700	1.588	.219
	Huynh-Feldt	.700	1.000	.700	1.588	.219
	Lower-bound	.700	1.000	.700	1.588	.219
Error(sample)	Sphericity Assumed	11.021	25	.441		
	Greenhouse-Geisser	11.021	25.000	.441		
	Huynh-Feldt	11.021	25.000	.441		
	Lower-bound	11.021	25.000	.441		

Electro-myographical data (EMG) was normally distributed: W (7) = 0.877, p = 0.212. Cortisol change scores were not normally distributed: W (27) = 0.743, p < 0.000, necessitating the use of Spearman's rho to enable correlation analysis. There was a moderate, though not significant, correlation between cortisol change (from sample 1 to 2) and EMG score: rho (7) = 0.440, p = 0.071 (Table 6).

Table 6. EMG correlations.

			Change	EMGhi
Spearman's rho	Change	Correlation Coefficient	1.000	.071
		Sig. (1-tailed)	.	.440
		N	7	7
	EMGhi	Correlation Coefficient	.071	1.000
		Sig. (1-tailed)	.440	.
		N	7	7

For the yawners, at rest, the EMG range was -15 to 70 millionth of a volt (mean of 29.7) as compared with -225 to 175 (mean of 114.1) after yawning. For non-yawners, the range was -4 to 80 (mean of 25.6) and -12 to 81 (mean of 26.2) after the stimuli presentation. Therefore, the yawners tended to show a larger peak following the yawn as compared with after the stimuli presentation for the non-yawners. Absolute means were also higher for yawners (Tables 7).

Table 7. EMG data for yawners and non-yawners.

	YAWNERS	YAWNERS	NON-YAWNERS	NON-YAWNERS
	Range	**Absolute Mean**	**Range**	**Absolute Mean**
REST	-15 to 70	29.7	-4 to 80	25.6
POST-STIMULI	-225 to 175	114.1	-12 to 81	26.2

There was a difference in EMG readings between the yawners and non-yawners, using t-test: t (7) = -2.959, p = 0.021 (Table 8). Using analysis of variance (ANOVA), there was a difference between measures at rest (p = 0.036) and after yawn (or after presentation) (p = 0.006) (Table 9).

Table 8. Analysis of EMG data.

		Levene's Test for equality of variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff	Std. Error Diff	Lower	Upper
EMGhi	Equal variances assumed	20.941	.003	2.959	7	.021	-183.86667	62.14239	-330.81006	-36.92327
	Equal variances not assumed			1.991	2.057	.181	-183.86667	92.36344	-570.86136	203.12803

Table 9. Differences between EMG values at rest and second time point

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Sample1	1.803[a]	1	1.803	.591	.449
	Sample2	6.381[b]	1	6.381	1.270	.270
	EMG1LO	100.018[c]	1	100.018	3.827	.062
	EMG1HI	15.134[d]	1	15.134	.030	.864
	EMG2LO	8962.963[e]	1	8962.963	4.891	.036
	EMG2HI	12721.557[f]	1	12721.557	8.860	.006
Intercept	Sample1	185.856	1	185.856	60.941	.000
	Sample2	308.720	1	308.720	61.456	.000
	EMG1LO	1207.811	1	1207.811	46.210	.000
	EMG1HI	10759.311	1	10759.311	21.245	.000
	EMG2LO	14518.519	1	14518.519	7.922	.009
	EMG2HI	49478.817	1	49478.817	34.460	.000
Yawn	Sample1	1.803	1	1.803	.591	.449
	Sample2	6.381	1	6.381	1.270	.270
	EMG1LO	100.018	1	100.018	3.827	.062
	EMG1HI	15.134	1	15.134	.030	.864
	EMG2LO	8962.963	1	8962.963	4.891	.036
	EMG2HI	12721.557	1	12721.557	8.860	.006
Error	Sample1	76.244	25	3.050		
	Sample2	125.587	25	5.023		
	EMG1LO	653.436	25	26.137		
	EMG1HI	12660.763	25	506.431		
	EMG2LO	45816.167	25	1832.647		
	EMG2HI	35896.230	25	1435.849		
Total	Sample1	262.130	27			
	Sample2	434.640	27			
	EMG1LO	1899.410	27			
	EMG1HI	23660.680	27			
	EMG2LO	67024.500	27			
	EMG2HI	93229.130	27			
Corrected Total	Sample1	78.047	26			
	Sample2	131.967	26			
	EMG1LO	753.454	26			
	EMG1HI	12675.896	26			
	EMG2LO	54779.130	26			
	EMG2HI	48617.787	26			

a. R Squared = .023 (Adjusted R Squared = -.016)
b. R Squared = .048 (Adjusted R Squared = .010)
c. R Squared = .133 (Adjusted R Squared = .098)
d. R Squared = .001 (Adjusted R Squared = -.039)
e. R Squared = .164 (Adjusted R Squared = .130)
f. R Squared = .262 (Adjusted R Squared = .232)

Yawning susceptibility scores, W (27) = 0.790, $p < 0.000$, and cortisol change scores (Tables 3 & 4) were not normally distributed. Using Mann-Whitney U test, there were no significant differences between yawners and non-yawners: U = 83.5, p = 0.755 (Table 10).

Table 10. Normality correction.

	Susceptibility
Mann-Whitney U	83.500
Wilcoxon W	203.500
Z	-.322
Asymp. Sig. (2-tailed)	.747
1-tailed Sig. (uncorrected for ties); grouping variable: yawn	.755

Differences between cortisol absolute values for yawners and non-yawners were found (p = 0.002 at Table 11; p = 0.034 at Table 12).

Table 11. Cortisol values between groups – analysis 1.

Tests of Between-Subjects Effects

Dependent Variable:Sample2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	123.492[a]	17	7.264	7.714	.002
Intercept	300.964	1	300.964	319.608	.000
Sample1	123.492	17	7.264	7.714	.002
Error	8.475	9	.942		
Total	434.640	27			
Corrected Total	131.967	26			

a. R Squared = .936 (Adjusted R Squared = .814)

Table 12. Cortisol values between groups – analysis 2

Tests of Between-Subjects Effects

Dependent Variable:Sample1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	77.822[a]	24	3.243	28.823	.034
Intercept	184.538	1	184.538	1640.334	.001
Sample2	77.822	24	3.243	28.823	.034
Error	.225	2	.113		
Total	262.130	27			
Corrected Total	78.047	26			

a. R Squared = .997 (Adjusted R Squared = .963)

Using Mixed 2 x 2 ANOVA, for yawners, there was a significant difference between cortisol samples, $F(1, 11) = 13.680$ $p < 0.01$. To account for multiple comparisons, the cut-off point for significance is reduced to $p < 0.025$. Hence, this result remains significant (Table 13). For non-yawners, samples were also different though not significant, $F(1, 14) = 4.758$ $p = 0.047$ (Table 13).

Table 13. Within-subjects effects: comparisons between yawners and non-yawners.

Tests of Within-Subjects Effects

Measure:MEASURE_1

Yawn	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Non-yawn	sample	Sphericity Assumed	2.133	1	2.133	4.758	.047
		Greenhouse-Geisser	2.133	1.000	2.133	4.758	.047
		Huynh-Feldt	2.133	1.000	2.133	4.758	.047
		Lower-bound	2.133	1.000	2.133	4.758	.047
	Error(sample)	Sphericity Assumed	6.277	14	.448		
		Greenhouse-Geisser	6.277	14.000	.448		
		Huynh-Feldt	6.277	14.000	.448		
		Lower-bound	6.277	14.000	.448		
Yawn	sample	Sphericity Assumed	5.900	1	5.900	13.680	.004
		Greenhouse-Geisser	5.900	1.000	5.900	13.680	.004
		Huynh-Feldt	5.900	1.000	5.900	13.680	.004
		Lower-bound	5.900	1.000	5.900	13.680	.004
	Error(sample)	Sphericity Assumed	4.745	11	.431		
		Greenhouse-Geisser	4.745	11.000	.431		
		Huynh-Feldt	4.745	11.000	.431		
		Lower-bound	4.745	11.000	.431		

Power and Effect Size

Power and effect sizes were computed based on repeated measures t-tests for both the yawning and non-yawning group. There was a medium effect size for the non-yawners group ($r = 0.467$) but a low power size (0.359). Results were similar for the yawners group: medium effect size ($r = 0.440$), and a low power size (0.331).

Discussion

The elevations in cortisol levels following yawning that are found in this study are consistent with previous work [7] which tends to lend support towards the Thompson Cortisol Hypothesis [2, 4, 6]. However, it is noted that there were also small rises in cortisol levels in the non-yawners which may be explained in terms of the experimental procedure. Since two time points of saliva cortisol sampling were

taken for both groups, it is possible to note that cortisol levels rose for both groups in the presence of yawn-stimuli; however, it is suggested that for the yawners, the cortisol levels did not reach the threshold for yawning, hence no yawn is elicited. This presumes that cortisol levels elicit the yawn response. If elevated cortisol levels are an artefact of the yawn, then these levels are lower than with the yawners because no yawn was elicited.

In addition, data arising from measuring jaw muscle neural activity has shown significant correlation with elevated cortisol levels following the episode of yawning. These data tend to support the notion that yawning is not just a mechanism for increasing air to the lungs; rather, it is a complex set of underlying sub-mechanisms.

Indeed, past research has commented on several important roles of neurotransmitters and amino acids involved during yawning [1]. It is possible that cortisol plays a much larger role in regulating our body's chemistry than has been previously thought; and the role of heightened electrical activity in muscles may also have effects upon body chemistry regulation. Simply put, when we yawn, the neurons regulating our jaw muscles are fired and the blood cortisol levels rise (Figure 1). However, it is unclear which happens first: perhaps cortisol levels rise during stress, fatigue, or when the brain temperature rises dramatically and triggers a yawn response. For non-yawners, although there is also a rise in cortisol, it is proposed that the elevation is insufficient to elicit a yawn, if cortisol is trigger. Alternatively, cortisol levels are associated with a yawn but rise after a yawn (ie non-elicitatory), then they may be inconsistent with the levels associated with a yawn episode.

Cortisol does seem to act, in many situations, in protection of our body. This is in terms of regulating the hormones released within the Hypothalamus-Pituitary-Adrenal axis. A yawn reflex may give rise to an increase in cortisol levels in order to afford relief in symptoms of stress; or alternatively, elevated cortisol levels are produced by a yawn reflex in order to provide symptom relief. Indeed, Gallup [33] reports how multiple sclerosis patients report symptom relief following yawns. It is possible that the temperature regulatory function of the hypothalamus is also governed by the levels of cortisol produced so as to maintain homeostasis of both temperature and to prevent fatigue as a result of stress.

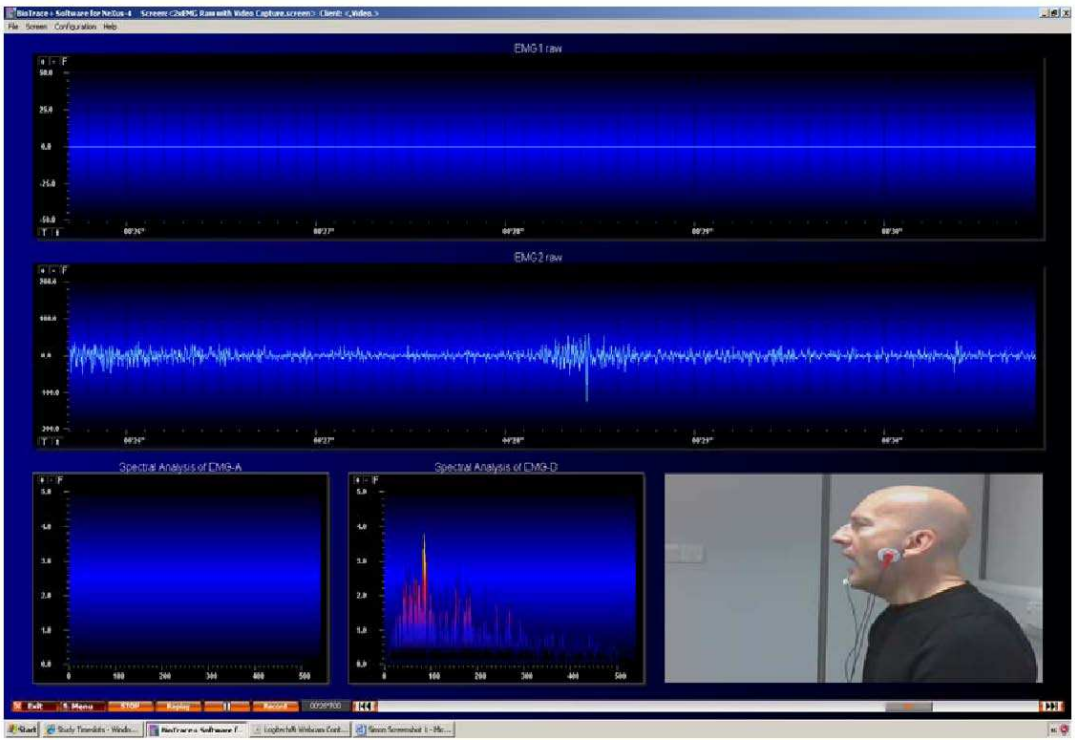


Figure 1. Episode of yawning and EMG trace.

Like many studies of neurological disorders, there is often a complexity in the interaction of neurotransmitters involved, just like that seen in Parkinson's disease or in the treatment management of Alzheimer's disease [49].

Age, recent anxiety and depression levels, and general health were measured. These are all potential factors that can affect the change data seen in EMG and cortisol levels. However, on analysis of these data, there were no contributory interference effects to change data.

Close inspection of data suggests that the differences between groups in terms of cortisol change between sample one and two is not huge in size. Greater numbers of participants will allow random allocation to each group of larger numbers; for example, using G*Power calculation, for a power size of 0.80, 27 participants will be required for each group (healthy yawners and healthy non-yawners). Aiming for 100 participants initially in the healthy group, this should enable random allocation of 50 participants per yawners and non-yawners. Additional numbers will be required for neurological groups, yawners and non-yawners.

In terms of EMG data, the range of values after a yawn was higher than that for the non-yawners after the stimuli presentation. Absolute means for yawners were higher than for the non-yawners at both rest and after the yawn (compared with after the stimuli presentation for non-yawners). It is suggested that the threshold for yawn is not reached in the non-yawners and that EMG data reflects the rises in cortisol levels at each of these time points.

The clinical research team led by the first author at Bournemouth University is working towards developing a diagnostic tool for the early identification of neurological sequelae. Along the way, this scientific pursuit may help shed light on the fascinating and complex entity that is cortisol and help us to understand better the ancient phenomenon of the yawning response.

Conclusions

Cortisol production appears to have many roles, including protection and regulation of other chemicals. Of particular interest is the role of cortisol in neurological disorders such as multiple sclerosis and stroke. Yawning seems to be a fundamental mechanism that occurs in all of us, yet malfunction, or increase and decrease in frequency may be associated with changes in cortisol levels.

Tapping into this interesting phenomenon has led the clinical research team at Bournemouth University, led by the first author, to conclude that cortisol affects yawning in some way. Neural activity is also closely associated with rises in cortisol levels during the yawning phase. It is uncertain whether or not elevated cortisol levels are an artefact of the yawn or trigger the yawn reflex. There also seems to be a threshold at which a yawn is produced and related to the level of cortisol produced. It is possible that the temperature regulation of the hypothalamus is also linked with these cortisol elevation in turn regulating the production of hormones associated with the Hypothalamus-Pituitary-Adrenal axis feedback mechanism.

These findings are of interest because they have potentially important implications for many neurological disorders where there is dysregulation of cortisol or where yawning frequency is altered. Diagnosis of neurological disorders is often a complex process with associated timelines. If we are able to understand the association of cortisol, neural activity and yawning, then we may have some direction in understanding how, and importantly, when neurological disorders are occurring in the individuals who present at our clinics.

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