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Chewing gum alleviates negative mood and reduces cortisol during acute laboratory psychological stress

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Running title: Chewing gum alleviates acute laboratory stress

ABSTRACT

The notion that chewing gum may relieve stress was investigated in a controlled setting. A multi-tasking framework which reliably evokes stress and also includes performance measures was used to induce acute stress in the laboratory. Using a randomised crossover design forty participants (mean age 21.98 years) performed on the multitasking framework at two intensities (on separate days) both while chewing and not chewing. Order of workload intensity and chewing conditions were counterbalanced. Before and after undergoing the platform participants completed the state portion of the State-trait anxiety inventory, Bond-Lader visual analogue mood scales, a single stress visual analogue scale and provided saliva samples for cortisol measurement. Baseline measures showed that both levels of the multi-tasking framework were effective in significantly reducing self-rated alertness, calmness and contentment while increasing self-rated stress and state anxiety. Cortisol levels fell during both levels of the stressor during the morning, reflecting the predominance of a.m. diurnal changes, but this effect was reversed in the afternoon which may reflect a measurable stress response. Pre-post stressor changes (□) for each measure at baseline were subtracted from □ scores under chewing and no chewing conditions. During both levels of stress the chewing gum condition was associated with significantly better alertness and reduced state anxiety, stress and salivary cortisol. Overall performance on the framework was also significantly better in the chewing condition. The mechanisms underlying these effects are unknown but may involve improved cerebral blood flow and/or effects secondary to performance improvement during gum chewing.

Key words: gum; chewing; stress; stressor; anxiety; multi-tasking; cortisol

1. Introduction

People chew gum for a variety of reasons including to modulate psychological states, for example to aid concentration and to help relieve stress. This last possibility was first addressed in the 1930s by Hollingworth [1] who described a series of studies into the mood and performance effects of chewing gum, in the form of candy-coated chicle (the sap from the sapodilla tree) on aspects of stress and performance. Typically subjects were studied under three conditions: not chewing; chewing gum; sucking a candy. In one set of experiments subjects were asked to rate how they felt on a 20-point linear rating scale ranging from extreme strain to extreme relaxation. In all three experiments the subjects rated themselves as between 10% and 15% more relaxed in the chewing conditions compared with the other conditions.

Since these classic studies the impact of chewing on psychological function received scant attention until the last decade. Several controlled laboratory studies have identified that chewing gum can improve memory [2-4]. An effect which may be related to insulin-mediated glucose uptake [3] or the re-instatement of learning context [2]. The latter possibility has not gone unchallenged [5-7], and one study has reported effects on attention but not memory [8]. While there is evidence of cortical activation during chewing gum [9] any relationship to cognitive processing is not clear at present. Certainly the exact mechanisms underpinning any cognition enhancing effects have been the subject of speculation but remain to be elucidated [10-13].

Measures of subjective state and aspects of performance have been included in studies into the effects of gum chewing during sleep deprivation. Subjects who were allowed to chew gum during an extended period without sleep were less sleepy than those who did not chew gum as measured using the Stanford Sleepiness Scale [14]. This effect may not be solely related to mastication since chewing an unflavoured, odourless substance (a strip of paraffin wax) had little effect on mood or performance during a night of sleep deprivation [15].

Thus while there are reports of beneficial effects of chewing gum on aspects of cognition and performance, there has been little research specifically addressing its effects on mood. This is despite the fact that chewing a flavoured gum may produce an electroencephalograph (EEG) pattern consistent with a state of relaxation [16]. A recent online study of 280 'heavy' gum chewers (> 10 pieces per week) assessed the effects of abstaining from chewing in a two-way crossover study. Three-day chewing abstention resulted in significantly higher scores on the state portion of the Speilberger

State-trait questionnaire. At the end of the study period 54% of the sample reported that chewing gum reduced stress (Zibell, personal communication). It was recently reported that chewing a neutral medium (paraffin wax) reduced salivary cortisol following an acute psychological stressor [17]. Such findings raise the possibility that chewing gum may have anti-stress properties which should be tested in more controlled conditions.

The aim of the proposed study was to identify if chewing gum can ameliorate both selfrated affective states and hormonal changes associated with acute, laboratory induced psychological stress. Mild but measurable stress can be induced in the laboratory in a variety of ways including via participants performing 'multi-tasking' activities. One such multi-tasking platform is the Multi-tasking Framework, also known as the Defined Intensity Stress Simulator (DISS), which has been developed as a platform for eliciting acute psychological stress via increases in cognitive workload. We have previously used a version of this framework in several randomised, doubleblind, placebocontrolled studies examining anti-stress effects of herbal extracts [1820]. The Multitasking Framework was chosen over other laboratory stressors (such as simulated public speaking), as it enables repeated testing of the same participant allowing a crossover design to be utilised. Additionally, unlike most other psychosocial stressors, performance measures are inherent in the task. Since the difficulty of the modules (cognitive tasks) making up the multi-tasking framework can be adjusted, the effects of chewing can be assessed at different workload intensities or stress levels. As elevated cortisol is associated with increased stress, salivary cortisol was measured as a physiological marker of stress. Given that chewing has been found to improve aspects of cognitive performance, and since each module produces a performance score, any positive or negative effects on psychomotor, memory or attentional performance were also examined. The present study therefore aimed to test the hypotheses that compared with a control, nonchewing condition, chewing gum would 1) reduce self-rated stress, 2) result in reduced cortisol levels, and 3) improve task performance.

2. Materials and Methods

2.1 Design

The study followed a controlled, randomised, balanced crossover design. Due to the nature of the intervention neither double blinding nor placebo control was possible. The study followed a 2 (mild stress, moderate stress) x 2 (chewing, not chewing)

design. In this way the effects of chewing gum on mild and moderate laboratory stress were assessed.

2.2 Participants

Forty healthy, non-smoking, young adults (8 male), mean age 21.98 years (\pm SD 4.79); BMI 23.80 (\pm 4.32) took part in the study. The participant information sheet stated that the study examined the effects of chewing gum on mood and cognitive performance, during mentally demanding tasks. Volunteers were paid £90 for three visits to the laboratory.

Inclusion criteria included having chewed gum at least once in the previous week. Subjects had to show a willingness to use the assigned products according to instructions, be available for appointments, and be likely to complete the study. Other inclusion criteria included the presence of at least 18 natural teeth in a good state of repair with the ability to chew gum for at least 30 minutes at a time. Any subjects using concurrent medication (including over-the-counter medication) or a medical history which may have affected any experimental outcomes were not entered into the study. Screening also ensured that no subjects were admitted who had any type of negative opinion or feeling related to chewing gum – (e.g. it's a bad habit, it's unhealthy, it's impolite, it's rude, etc.). No volunteers were excluded based on these pre-determined criteria.

Ethical permission was granted from the Northumbria University School of Psychology and Sport Sciences Ethics committee and all procedures were carried out in accordance with the Declaration of Helsinki.

2.3 Multi-tasking Framework

Participants were required to simultaneously perform four tasks presented via computer. The multi-tasking interface (Purple Research Solutions Ltd, UK) was presented on Dell Latitude laptops with high definition screens. The modules appeared as presented in Figure 1. In the present study the participant performed the four computerised tasks simultaneously for twenty minutes. Participants were instructed to pay equal attention to all four modules and to perform as quickly and accurately as possible. Participants performed the tasks on two separate study visits. On one visit

settings of 'low' intensity were used, on the other 'medium' intensity settings were used (with intensity defined according to the developers' definitions). The higher workload at medium intensity aims to produce higher levels of stress. The tasks are described in the following section.

[Figure 1 around here]

2.3.1 Mental Arithmetic

A series of arithmetic problems (additions) are presented (Figure 1, top left quadrant). Using a number pad to the right of the sum, participants use the mouse to enter the answer. They are instructed that in the case of any error they should click on the digit that they wished to change and then use the number pad to select a new answer. When the volunteer is satisfied with the answer they click 'Done' whereupon a new sum appears. 10 points are awarded for each correct answer and 10 points subtracted for an incorrect answer. At the low intensity level the sums involve addition of two two-digit numbers and at medium intensity the addition of two three-digit numbers.

2.3.2 *Stroop*

The Stroop task [21] is a classic psychological test of selective attention and response inhibition. In the current form, four colour blocks (Blue, Yellow, Red and Green) appear on the right hand side of the task (Figure 1, top right quadrant). At a given time interval, a colour name appears to the left of the colour blocks. The task is to click the colour block on the right related to the font colour, regardless of the colour it describes (e.g. the correct response would be the red block to the colour name 'blue' appearing in red font). 10 points are added for every colour word correctly identified, and 10 points subtracted for each incorrect answer, or for not making a response in the allotted time period (a 'timeout'). The frequency of stimulus presentation is higher in the medium than low intensity with 'timeouts' of 20 and 30 seconds respectively.

2.3.3 Memory Search

This working memory task is adapted from Sternberg [22]. An array of letters appears for the participants to remember. After 4 seconds, the letters disappear but can be viewed again by clicking on "retrieve list" button (Figure 1, bottom right quadrant).

Approximately every 10 seconds, a single target letter appears. Participants indicate whether the target letter had appeared in the original list of four letters by clicking on the "yes" or "no" buttons. Ten points are awarded for a correct answer, 10 points deducted for an incorrect answer or no response within 15 sec per stimulus. Five points are deducted every time the list is retrieved. There are two and four letters in the arrays displayed in the low and medium intensities respectively.

2.3.4 Visual monitoring

This task assesses psychomotor ability. A small dot drifts outwards from the centre of a target comprising five concentric circles (Figure 1, bottom left quadrant). The participant is instructed to allow the dot to travel as far out of the centre as possible, without letting it hit the edge of the target, before clicking on the "reset" button. Two points are added to the running total for every line that the dot passes (with a maximum of 10 points), with a penalty of 10 points for every half second that passed between the dot hitting the outer edge and the participant clicking on the "reset" button. The speed that the dot travelled was faster at the medium than the low intensity setting (approximately 5 and 2.5 pixels per second respectively).

In addition to the scores for each of the four modules, a total score was computed by adding the scores from individual modules.

2.4 Mood scales and other pencil- and-paper measures

2.4.1 State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) [23] comprises of two scales. The 'State' subscale is a widely used instrument for measuring fluctuating levels of anxiety. The subscale contains 20 statements (e.g. 'I am calm'). Participants rate how much they feel like each statement at the time of making the response by marking a 4-point scale ranging from 'not at all' to 'very much so'. Scores range from 20 to 80, with higher scores indicating more anxiety.

2.4.2 Bond-Lader Visual Analogue Scales

The Bond-Lader mood scale is made up of 16 x 100 mm visual analogue scales with the end points anchored by antonyms: alert-drowsy, calm-excited, strong-feeble, muzzy-clearheaded, well coordinated-clumsy, lethargic-energetic, contented discontented, troubled-tranquil, mentally slow-quick witted, tense relaxed, attentive dreamy, incompetent-proficient, happy-sad, antagonistic-friendly, interested-bored, withdrawn-sociable. Participants are presented with a sheet of paper containing all the scales and instructed to mark their current mood state on each line. These are combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'contented' [24], with scores on each ranging from 0 to 100.

2.4.3 Stress Visual Analogue Scale

The Stress Visual Analogue Scale consisted of a single unmarked 100 mm line with end-points labelled 'Not at all' and 'Very much so'. The scale was presented on a single sheet of paper with the words "Indicate on the line below how stressed you feel at this moment" written above it. The scale therefore gives a single subjective stress score between 0 and 100.

2.4.4 Chewing Gum Questionnaire

A questionnaire was given to each participant. It asked them first to write down why they chewed gum, secondly to indicate (yes or no) whether they ever chewed gum to relieve stress, and finally if they had any other comments.

2.5 Cortisol measurement

The participants provided salivary samples using salivettes (Sarstedt, UK) as a noninvasive measure of free, bio-available cortisol levels [25, 26]. Participants were required to place a cotton dental roll in their mouth for approximately 30 sec, these were immediately frozen at –20 °C. Samples were defrosted and centrifuged prior to testing for cortisol levels by luminescence immunoassay according to the instructions of the manufacturers (IBL Hamburg, Flughafenstrasse 52a, D-22335 Hamburg, Germany).

2.6 Procedure

All testing took place in a dedicated suite of testing facilities and testing sessions took place at least 24 hours apart. Participants visited the laboratory on three occasions. The first was a practice day during which the procedure was similar to the other two study days, with the exception that no treatment was given and that subjects completed both sections of the State Trait Anxiety Inventory (STAI). These data were not analysed other than to ensure that each participant's performance lay within an acceptable range of scores for this population.

During the practice day visit volunteers provided signed consent and information regarding their chewing habits was recorded. As the study was aimed at assessing the effects of chewing gum per se, rather then the impact of any particular brand, it was felt that providing a single brand of gum may have confounded this aim. Therefore each participant selected the brand of gum which they would use for the study days (from a selection of the ten top-selling brands in the UK).

On each of the two study days participants refrained from drinking anything other than decaffeinated products on the morning of the day of the study. They were asked to eat a light meal at least 2 hours before the study, the nature of the meal was recorded and participants were requested to eat the same meal on subsequent visits. In order to control for diurnal changes in cortisol, testing always began at the same time for each volunteer. Thus on study days individuals' sessions commenced at either 1100 or 1430 for the morning or afternoon sessions (respectively).

Participants performed the 4-module version of the Multi-tasking framework for twenty minutes. At each session, both before and after undergoing the framework, participants completed the Bond-Lader visual analogue mood scale, filled out the state portion only of the STAI and marked the Stress VAS. Up to five minutes was allowed for completion of the pencil-and-paper measures. They then produced a saliva sample (by salivette) for the measurement of cortisol. In the post testing condition, the saliva was sampled at a fixed 5 min from the cessation of chewing.

On each study day participants underwent the framework three times (i.e. three 'sessions') at either the low or medium intensity. The first session established baseline performance and stress reactivity on that day. It also served to confirm the effectiveness of the multi-tasking framework at eliciting psychophysiological stress responses at the selected stress intensities. The other two 'experimental' sessions were

performed under a chewing and a no chewing condition. In the chewing condition participants chewed throughout the 20 min stressor platform during the post-baseline session. Rest periods of twenty minutes, during which the participants sat quietly, occurred between all sessions. On the second study visit the procedure was repeated at the other level of stress and was followed by the chewing gum questionnaire and debriefing session.

The order of chewing/no chewing across experimental sessions and low/medium intensities across study days was counterbalanced across participants and visits.

2.7 Statistics

2.7.1 Effects of multi-tasking framework at baseline

As a manipulation check for the effectiveness of the multi-tasking framework as a stressor, the effects of the framework on mood and cortisol levels during baseline performance were analysed as follows. Pre- and post-framework scores obtained during the two study visits' baseline assessment from the Bond-Lader, the state STAI, the Stress VAS were computed. Each was subjected to a two-way [Intensity (low, medium) x Assessment (pre, post)] general linear model analysis of variance with both variables as repeated measures factors. Because of the known diurnal rhythm in circulating cortisol [27], and since exactly half of the sample started the sessions at 1130 and 1430 respectively, for this measure only analyses were performed including time of day (morning, afternoon) as a between-subjects factor.

2.7.2 Effects of chewing gum on anxiety, mood and performance during multitasking framework

The primary analysis was as follows. For the mood and cortisol data, each day's change-during-baseline scores were subtracted from change-during stressor ($\Box\Box$ scores obtained during later sessions on that day to obtain change-from-baseline $\Box\Box$ scores. For the performance scores change from baseline scores were computed for each postbaseline assessment. The resulting measures were subjected to a two-way [Chewing (gum, no gum) x Intensity (low, medium)] general linear model analysis of variance with both variables as repeated measures factors.

The above data treatment followed similar processes to our previous studies examining the effects of natural products on stress and adjusts for any day-to-day variations in stress responses [18-20]. In order to confirm that the results in the present study were not influenced by taking into account each day's baseline assessment, further analyses were conducted on the scores from the post-baseline assessment only. Here change scores at both intensity levels and under both chewing conditions were subjected to a two-way repeated measures analysis of variance.

All the above analyses were performed using SPSS v15 for Windows. Additionally effect sizes were computed for relevant change-from-baseline scores using the Cohen's d statistic (difference between means divided by pooled standard deviation).

3. Results

3.1 Gum chewing data

Regarding categorisation of chewers into 'low', 'moderate' and 'heavy' gum users (as defined by the manufacturers); 25% (10) of the sample were low users (1-3 pieces of gum in the previous week), 57.5% (23) were moderate users (4-9 pieces) and 17.5% (7) were heavy users (more than 10 pieces per week). Each type of gum offered was chosen by at least one participant. The specific gums offered were as follows (with number of participants choosing each brand in parentheses): Extra Cool Breeze (7), Extra Spearmint (7), Orbit Professional (6), Extra Ice (5), Cherry Menthol Airwaves (4), Orbit Complete (4), Spearmint (3), Extra Peppermint (2), Black Mint Airwaves (1), Menthol and Eucalyptus Airwaves (1).

3.2 Effects of multi-tasking framework at baseline

Analyses of baseline scores indicated that the stressor was generally effective (see Figure 2 where relevant effect sizes for change from baseline scores are also presented). There were significant main effects of Assessment on all mood items indicating that post-framework scores differed significantly from pre-framework scores. On self-rated mood scores derived from the Bond-Lader VAMS, participants rated themselves as significantly less alert [F(1,39) = 10.714, p = 0.002], calm [F(1,39) = 9.028, p = 0.005], and content [F(1,39) = 19.489, p = 0.001] following the stressor. They also rated themselves as significantly more stressed [F(1,39) = 10.714, p < 0.001] as indicated by responses to the single stress VAS. Participants exhibited significantly more state anxiety as assessed by the state portion of the STAI [F(1,39) = 22.755, p < 0.001]. There was no significant effect of intensity level (low or medium) on the mood measures,

suggesting that both intensities of the stressor were equally effective in modulating mood states.

[Figure 2 around here]

Cortisol is known to follow a marked diurnal pattern, characterised by high levels immediately upon waking, and a decline throughout the day towards nadir in the evening [27]. Since half the sample were assessed in the morning and the other half in the afternoon, the data were analysed taking into account time of day (morning, afternoon) using a three-way mixed model ANOVA (stress x pre-post x time-of-day) with the last as a between-subjects factor. This revealed a significant main effects of intensity [F(1,37) = 6.858, p = 0.013], time (pre-post) [F(1,37) = 4.545, p = 0.040] and time-of-day [F(1,37) = 5.079, p = 0.030], with lower levels in the afternoon than morning. There was also a time-of-day x pre-post interaction [F(1,37) = 10.995, p = 0.002]. To explore these effects further, within-subject ANOVAs (stress x pre-post) were conducted for the a.m. and p.m. sessions separately. These confirmed that cortisol levels were significantly reduced during the morning session [pre-post F(1, 19) = 10.476, p = .004]. During the afternoon sessions there was a significant effect of intensity only F(1, 19) = 9.055, p = .008]. No other main effects or interactions were significant.

3.3 Effects of chewing gum on anxiety, mood and performance during multitasking framework

The mean self-reported mood scores and salivary cortisol levels for each post-baseline experimental condition are presented in Figure 3 (showing change from baseline scores) and Table 1 (showing mean values without adjusting for baseline and effect sizes for change from baseline scores).

The primary analysis involved examining the effects of chewing vs. not chewing on change-from-baseline \square scores in each of these measures at both intensities. There were no main effects of intensity nor any chewing x intensity interactions.

[Figure 3 around here]

There were several main effects of chewing gum (Figure 3). There was a significant main effect of chewing gum on self-rated alertness derived from the Bond-Lader VAMS [F(1,39) = 12.03, p < 0.001]. Ratings of alertness were significantly higher in the chewing condition than the no-chewing condition irrespective of the intensity of the

stressor. Similarly there was a significant main effect of chewing gum on state anxiety. While chewing, anxiety was significantly lower during the framework at both intensities of the stressor [F(1,39) = 5.242, p = 0.028]. Self-rated stress was also significantly affected by chewing with reduced ratings of stress in the chewing compared with the no chewing condition independent of intensity level of the battery [F(1,39) = 4.222, p = 0.047].

There was a significant main effect of chewing on salivary cortisol [F(1,39) = 13.589, p < 0.001], cortisol levels were significantly lower at both levels of the stressor in the chewing condition compared with the no chewing condition (note that these data were not analysed with respect to time-of-day as they are adjusted for baseline changes at the same session).

The above analyses are in keeping with those used in similar studies [18, 19]. A secondary analyses of the same data showed that all significant effects of chewing gum were maintained when the pre-post platform \Box data were analysed without subtracting baseline scores. Chewing was associated with significantly higher selfrated alertness [F(1,39) = 12.903, p < 0.001], lower state anxiety [F(1,39) = 5.324, p = 0.026], selfrated stress [F(1,39) = 4.222, p = 0.047] and salivary cortisol [F(1,39) = 4.222, p = 0.002].

[Table 1 around here]

The framework scores at baseline and in the post-baseline experimental sessions, along with effect sizes for change from baseline scores, at both levels of stressor with the effects of chewing during the post-baseline sessions are presented in Table 2.

[Table 2 around here]

There were no significant main effects of chewing, nor stressor intensity, nor chewing x intensity interactions on any of the scores derived from the individual tasks. (Table 2). However there was a significant main effect of chewing on the total performance score [F(1,39) = 5.242, p < 0.028]. While in the chewing condition, individuals performed significantly better irrespective of the intensity of the battery.

4. Discussion

The data here suggest that chewing gum has beneficial effects on a number of measures during two intensities of laboratory stress. Compared with the no chewing condition, gum chewing was associated with significantly higher alertness coupled with reduced subjective stress and state anxiety. These findings were supported to some degree by physiological responses although caution should be exercised in interpreting the salivary cortisol results. Although no individual task benefited significantly from chewing, total framework performance was higher while chewing.

Before addressing the effects of chewing, it is important to first consider the effectiveness of the multi-tasking procedure in eliciting stress. Examination of the baseline data reveals that twenty minutes of performing the platform resulted in significantly reduced self-reported alertness, calmness and contentment with significantly increased stress and state anxiety. These mood effects are consistent with previous applications of the multi-tasking framework [18]. Previous studies of the physiological responses to the platform have revealed increased secretory immunoglobulin A (S-IgA), a measure of mucosal immunity that responds to acutely stressful stimuli [28], following 5 minute multi-tasking sessions [29]. In the current study, cortisol levels actually decreased during the stressor, although this effect was only observed when it was during morning administrations. This effect was not seen during the afternoon sessions where there was a main effect of stress intensity only implying that the measure was sensitive to the well-documented diurnal cortisol rhythm [27].

The effect of gum chewing on the subjective measures was generally positive, both when change in change from baseline scores were analysed and when post-baseline scores were not adjusted (confirming that the data were not skewed by anomalous baseline scores). Compared with the no chewing condition, chewing was associated with significantly increased alertness, coupled with decreased self-rated stress and anxiety. These effects are subtle and we cannot rule out the possibility that some are Type I artefacts. However any adjustment for Type I error was deemed inappropriate given the exploratory nature of the study [30]. The significant findings here are comparable with those from similar studies into the effects of herbal extracts with stress-relieving properties [18, 19] - where slightly different versions of the platform were used. Unlike here, lemon balm increased calmness ratings (effect size, Cohen's D = .86), and the overall (non-significant) effect on battery performance was not

significant (D = .284 for the most effective dose). A more direct comparison can be made with a valerian-lemon balm combination [19], this produced a significant reduction in state anxiety for one dose (with Ds of .417 and .423 at different time points), compared with a D of .28 in the present study. The same product significantly impaired overall platform performance (D = .338 - .450). This contrasts with the significantly improved performance observed here (D = .27 and .28).

It seems unlikely that the effects seen here result from a direct pharmacological action. Chewing gum can contain glucose which is known to enhance aspects of cognitive function [31-35]. However only one of the gums used in the current study (Spearmint) contained glucose. This was chosen by three participants only and contains approximately 1.9 gm per piece which is around one tenth of the level used to produce psychoactive effects. The other gums are sugar-free and use polyols as bulk sweetener and other high intensity sweeteners (like aspartame). Although we cannot rule out the possibility that these or other components of gum affected mood, or interfered with cortisol levels, any potentially active ingredients were ingested at low levels, and ten different gums were used across the cohort of participants. It appears more likely that the effects are related to either to some aspect of the act of chewing or to the effects of flavour. Regarding mastication alone, it has been reported that chewing paraffin wax (an odourless, tasteless flexible medium which can be used to promote salivary flow) can reduce cortisol levels in a similar fashion to the gum effects observed here. Tahara and colleagues [17] examined the effects of chewing paraffin wax (compared with not chewing) for 20 minutes on salivary cortisol immediately following 20 mins of mental arithmetic. The stress loading intervention increased cortisol in 12 of 17 participants (the other 5 'non-responders' were not included in any analysis) and chewing significantly reduced cortisol levels. The magnitude of the reduction in salivary cortisol in the Tahara study is reported as 15.4% and 24.6% following 10 and 20 mins of chewing respectively (similar figures are reported for cyclic clenching rather than chewing). These figures are comparable with the reductions of 18-19% in the chewing conditions (at both intensity levels) found in the current study, suggesting the cortisollowering effects of chewing are robust and can occur independently of flavour.

While flavour may not be necessary for reductions in cortisol, it may contribute to relevant mood, performance and/or physiological changes associated with gum chewing. Chewing unflavoured gum base with and without flavour and with or without odour all produced positive EEG profiles [16]. However the EEG produced while

chewing flavoured gum was most consistent with a state of 'relaxed alertness' which may contribute to the mood and performance effects in the present study. Thus the different dimensions of chewing a flavoured gum may have additive effects in terms of positive mood and anti-stress properties. These relative contributions of each component of mastication, flavour and odour could be disentangled empirically by a partial replication of the current study but comparing the effects of unflavoured and flavoured gums with the addition of odour inhalation. For example it is known that menthol odour may have differential effects on mood and performance [36, 37].

The results of this study do raise the question as to whether specific properties of gum contribute to the phenomenon described here or whether chewing anything might be an effective anti-stressor. Hollingworth's original paper suggests that chewing may act as a conditioned stimulus which re-evokes the quiet relaxation of meal times, when "...we eat we sit; random restlessness is at a low point; we rest; we relax; and the general feeling tone is likely to be one of agreeableness and satisfaction" [1]. Adapting paradigms from flavour reinforcement studies or by manipulating the consequences of gum chewing may offer some insight into this possibility. It is also very possible that other processes might also be involved, for example chewing may act as some sort of distractor away from ongoing stress. On the other hand it is known that diversion of attentional resources leads to performance impairments as fewer attentional resources are allocated to each task, (e.g.[38], whereas here we found improved performance during chewing.

Turning now to the potential physiological mechanisms which might be affected by gum chewing and lead to both anti-stress and performance enhancement. It has recently been shown that even a brief (5 min) period of mental stress can cause transient impairments to flow-mediated vasodilation lasting around 30 min [39]. It is also known that chewing can lead to an increase in heart rate [4] and blood flow. The functional significance of such acute changes in vaso-activity are not known with regard to stress and anti-stress. However it is possible that reversing the process may help to alleviate subjective stress. The platform used in the current study raises heart rate (Wetherell, unpublished results) and it may be that regular chewers attribute increased heart rate to chewing rather than stress. Additionally we have previously argued that increased delivery and availability of metabolic substrates may result in an increase in cognitive performance which is domain-independent and which can be manifest in a number of ways depending on the task demands [34, 40, 41]. In this case such processes may

contribute to the improved performance seen when taking average performance across all the tasks from the multi-tasking platform.

Chewing did not affect any mood or performance measure differently for the two intensities of the multi-tasking platform. There are several possible reasons for this. One is that the effects of chewing on mood and physiological measures occur independently of stress and may even have been observed in the absence of the framework. This possibility is supported by the recent finding that gum chewing improves mood in the absence of any overt stressor [42]. On the other hand, given the effectiveness of the framework in eliciting negative mood changes it seems more likely that chewing serves to ameliorate the stress response to some degree. The second possibility is that the two intensities of the platform used here were too similar and that chewing is effective in relieving stress only within this range. This notion is supported by the lack of any significant effects of intensity when baseline effects of the framework were analysed. Both these possibilities could be tested by repeating the experiment but with the inclusion of a more demanding version of the multi-tasking paradigm. For example, using the same framework, Wetherell et al., [29] observed increased perceptions of workload, including perceived mental demand, effort and frustration in accordance with increases in workload intensity. Furthermore, whilst only a small increase in perceived stress was reported between low and medium intensities (concomitant with the intensity levels used in the current study), a highly significant increase, double that of the medium intensity response, was reported following the high stress intensity, demonstrating a non-linear relationship between intensity levels, subjective stress and perceived demand.

Differences in baseline levels of cortisol between morning and afternoon sessions with reflected the well-documented diurnal profile of salivary cortisol [43]. Similar effects have been reported by Kudielka and colleagues [44], who compared cortisol responses in different groups administered the Trier Social Stress Test (TSST) in the morning and in the afternoon. They observed no differences in net cortisol reactivity to the stressor, but significant associations between higher basal cortisol levels and lower Hypothalamic-Pituitary-Adrenocortical (HPA) axis stress reactivity. The authors suggest that it may be advantageous to conduct such studies in the afternoon as here, to minimise the effects of baseline values on subsequent stress responses. Furthermore, it should be acknowledged that tasks incorporating social evaluation, such as the TSST, elicit the greatest cortisol responses [45]. The presence of the experimenter in the

current study would have elicited a degree of stress linked to social evaluation. However the primary stress dimension was related to a high mental workload. It could be argued that in the case of stressors that do not incorporate high levels of social evaluation testing in the afternoon, when levels of cortisol are lower and more stable, allows for a more sensitive assessment of HPA reactivity to stress. However given that baseline effects on cortisol here were largely due to the diurnal a.m. fall in cortisol we cannot draw firm conclusions about the effectiveness of the stressor on this measure. We can rule out the possibility that the subjects in the current study did not find the framework stressful since the baseline assessment on each study day was effective in negatively modulating mood states. However it is possible that there were few or no effects of multi-tasking upon cortisol and that this particular physiological response may be dissociated from the subjective stress response to multi-tasking. Alternatively the very act of entering into the psychological experiment and providing saliva samples may have acted as a social stressor and masked any effects of multi-tasking on cortisol. In this context it would be of interest to examine the extent to which these results generalise to other stressors including the TSST and the Cognitive Demand Battery [32, 46, 47].

With regards to the effects of chewing gum, significant reductions in salivary cortisol were observed at both levels of stress whilst controlling for baseline (and therefore time of day) changes. This is consistent with other reports [17]. One concern might be whether decreased salivary cortisol concentrations might reflect increased saliva production or salivary flow related to the act of chewing. This is unlikely for two reasons. Firstly there was a fixed five minute period before the end of chewing and the provision of a saliva sample for determination of saliva samples, an adequate time for serum cortisol to be transferred to saliva [Vinning, 1983 #40;Umeda, 1981

#39]. It is unlikely that any increases in salivation endure to this point. In any case it has been shown that cortisol levels are unrelated to salivary flow. Cortisol, like other steroid hormones, is lipophilic and also relatively small (MW 362) allowing it to diffuse rapidly through cellular membranes including those of salivary gland acinar cells into saliva. Thus as Kirschbaum and Hellhammer [25] state in their review "it is not surprising that saliva flow rate has no impact on salivary cortisol levels". Another question relates to the timing of such effects. Here subjects chewed gum for twenty minutes during the framework. It is possible that the effects of chewing are immediate

and are able to relieve stress over a much shorter period. Equally it is possible that the effects are enduring and may last for longer periods.

Turning to the performance data, chewing gum did not significantly affect any individual task Previous reports have indicated that chewing can improve performance on working memory tasks similar to the one seen here [4]. However in that study the task was presented individually and for a shorter duration. Additionally other reports have indicated that chewing may improve aspects of attentional processing [8]. The one attentional task in the current battery, the Stroop, involved response inhibition and was not similar to any used by Tucha and colleagues (as far as we can tell from the level of detail in that paper). Despite not affecting any single performance measure chewing gum did result in a significant improvement in overall (averaged) performance. This may reflect improvements in performance which do not reach the threshold of significance when individual tasks are scrutinised. Indeed examination of individual task performance reveals that performance on three out of the four modules (with the exception of mathematical processing) were numerically improved by gum chewing. We have previously found that aggregate scores are particularly sensitive to certain manipulations and particularly those which appear to target multiple biological processes, and have argued that these may reflect cumulative or synergistic effects of multiple mechanisms [41]. For example the fact that several varieties of gum were used suggests that the effects seen here cannot be attributed to the effects of flavour alone. It seems feasible that in the absence of clear evidence of a single mechanism underlying the behavioural benefits of chewing gum, multiple processes may be affected.

All of the participants in the current study were regular gum users and a number of individuals indicated that they use gum to relieve stress. For these individuals reductions in perceived stress may, therefore, reflect their expected stress-relieving effects of gum chewing. In one sense they might even be considered as in a state of chewing abstinence. Further work, using individuals who do not regularly chew gum would, therefore, indicate whether chewing gum has generic stress-alleviating effects in individuals who do not chew gum as a stress-coping strategy. However, it is acknowledged that a sample of naïve gum chewers may be hard to come by. At the end of participants' final visit they were asked a number of questions regarding gum chewing. To the specific question "do you chew chewing gum to relieve stress?" 15 people (37.5% of the sample) answered yes and the others answered no. The extent to which gum chewing for stress relief has ecological validity remains to be seen. However

there is some support for its voracity from the recent finding that gum chewing may relieve workplace stress [42].

In conclusion, gum chewing had consistently positive effects on mood during an acute laboratory stressor. These were mirrored by changes in salivary cortisol. There was some weak evidence of benefits to performance when scores from four multitasking modules were averaged. More work is necessary to further delineate these phenomena.

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Figure legends

<u>Figure 1</u> The multi-tasking module (previously called Defined Intensity Stress Simulator) running (clockwise from top left); mental arithmetic, the Stroop task, memory search and visual tracking. The running total is indicated in the centre of the screen.

<u>Figure 2</u> Effects of multi-tasking on self-rated mood and salivary cortisol levels before and after performing the baseline multi-tasking stressor at both low and medium intensity respectively. Bars indicate mean scores (±SEM) for Bond-Lader VAMS mood items (**a.** alert, **b.** calm, **c.** contentment), **d.** a single VAS stress scale and, **e.** state anxiety scores. Probabilities associated with significant changes during the stressor ('PRE-POST') are indicated. Cortisol levels (**f.**) are presented separately for morning and afternoon measures to illustrate main effects of stress level ('INTENSITY') and the three-way (Pre-post x Stress level x Time-of-day) interaction.

<u>Figure 3</u> Significant effects of gum chewing on **a.** alert ratings from the Bond-Lader VAMS, **b.** state anxiety scores from the STAI, **c.** a single VAS stress scale and **d.** salivary cortisol during stress. Bars represent mean change (±SEM) while not chewing or while chewing gum during low and medium intensities of the multi-tasking platform. Values are relative to changes during a baseline assessment at the same level of stress. Probabilities associated with significant effects of chewing ('GUM') are indicated.

<u>Table 1</u> Effect of chewing gum on mood and cortisol during low and medium intensity settings of the multi-tasking platform. Mean values (±SEM) before ('pre-') and after ('post') the platform are presented for each outcome under conditions of chewing and not chewing gum. ^C = significant main effect of chewing. *Effect sizes (Cohen's d) are shown for change from baseline □□scores (as plotted in Figure 3).

<u>Table 2</u> Effect of chewing gum on performance of the multi-tasking platform at low and medium intensity respectively. Mean scores (\pm SEM) while chewing and not chewing gum are presented for each module as is the mean score. Units are points accrued (see text). C indicates a significant main effect of chewing when change-from-baseline performance is analysed (see text). *Effect sizes (Cohen's d) are shown for change from baseline scores.

Figure 1 Figure(s)

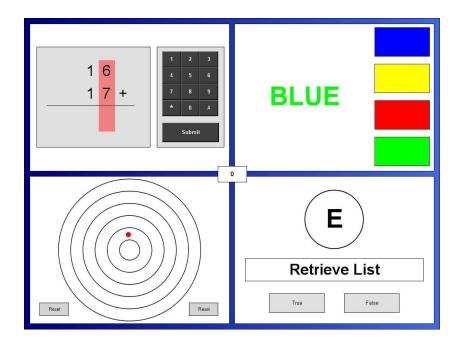
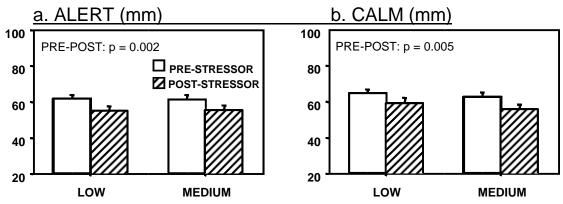
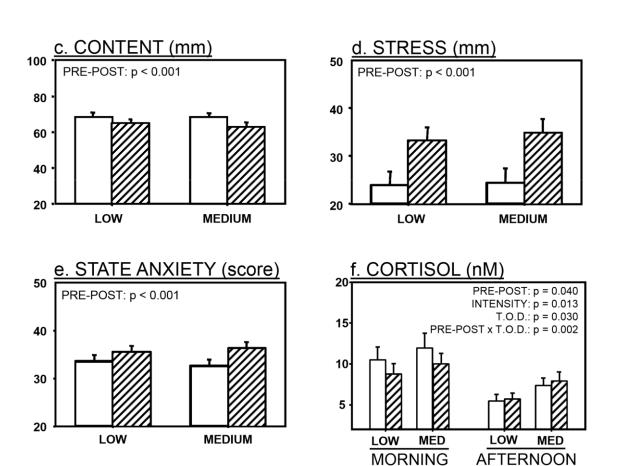
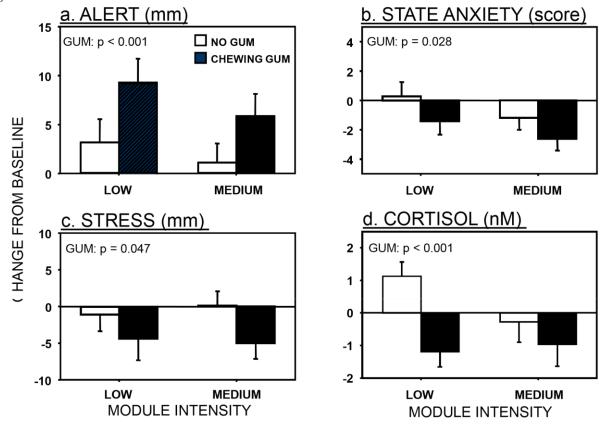


Figure 2







Table(s) Table 1

		Low intensity			Medium intensity			
		pre-platform	post-platform	Cohen's d*	pre-platform	post-platform	Cohen's d*	
ALERT ^C	Not chewing	54.07 ±2.28	50.33 ±2.74		58.01 ±2.032	53.13 ±2.29		
(mm)	Chewing	57.367 ± 2.21	59.73 ± 2.44	0.40	57.49 ±2.293	57.39 ± 2.09	0.36	
CONTENT (mm)	Not chewing	62.23 ±1.95	61.55 ±2.20	0.10	65.60 ±2.190	63.36 ± 2.14		
	Chewing	66.33 ± 2.05	64.48 ± 1.91		64.89 ± 2.038	66.00 ± 2.08	0.28	
CALM	Not chewing	58.61 ±2.33	57.43 ±2.29		59.11 ±2.380	55.25 ±2.45		
(mm)	Chewing	Chewing 63.41 ± 2.30 58.90 ± 2.57	0.16	60.33 ± 2.043	54.99 ± 2.38	0.09		
STRESS ^C	Not chewing	26.24 ±2.60	34.51 ±3.37		28.04 ±2.813	38.65 ±3.38		
(mm)	Chewing	25.03 ± 2.53	30.03 ± 3.38	0.20	27.99 ± 2.970	33.50 ± 3.43	0.39	
STAI-S ^C	Not chewing	34.58 ±1.25	36.65 ±1.23		34.80 ±1.330	37.28 ±1.25	0.28	
(score)	Chewing	33.48 ± 1.19	33.90 ± 1.23	0.28	34.58 ± 1.313	35.63 ± 1.36		
CORTISOLC	Not chewing	5.44 ±0.64	5.52 ±0.61		6.10 ±0.635	6.13 ±0.61		
(nM)	Chewing	5.71 ±0.61	4.64 ± 0.61	0.81	6.60 ± 0.800	5.41 ± 0.61	0.17	
Table 2								

		Low inter	Low intensity Medium intensity					
	Baseline	Not chewing	Chewing	Cohen's d*	Baseline	Not chewing	Chewing	Cohen's d*
MATHS	473.75 ±48.34	501.75 ±56.3	474.00 ±53.76	0.22	295.75 ±38.40	280.000 ±37.55	262.750 ±36.48	0.18

STROOP	3776.25 ±206.03	3959.25 ±223.22	4032.75 ±235.51	0.10	3751.75 ±250.30	3809.25 ±180.43	3894.75 ±237.12	0.06
TRACKING	206.25 ±11.83	203.20 ±10.33	200.10 ±14.04	0.05	356.15 ±36.17	329.300 ±44.27	346.80 ±28.81	0.17
LETTER SEARCH	7744.38 ±640.48	7869.63 ±593.21	8190.75 ±623.81	0.20	5704.25 ±434.35	6264.50 ±434.65	6566.63 ±445.51	0.17
MEAN ^C	3050.16 ±142.11	3133.46 ±38.62	3224.40 ±139.86	0.27	2526.98 ±104.97	2670.76 ±102.38	2767.73 ±102.55	0.29