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Jackson – The Cortisol Awakening Response (CAR) – A feasibility study

Article Title

The Cortisol Awakening Response (CAR) – A feasibility study investigating the use of the Area Under the Curve with Respect to Increase (AUC_i) as an effective objective measure of tinnitus distress.

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Conflicts of Interest

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Purpose: Tinnitus is a chronic medical condition which can result in distress, concentration difficulties, and clinical depression. An effective, objective measure of tinnitus distress does not currently exist. Endocrinal studies into the condition have been few, with those investigating the Cortisol Awakening Response (CAR) limited in scope. It was hypothesised that distressed individuals with tinnitus would awaken, and be unable to effectively prepare for the day ahead due to a blunted cortisol response.

Method: Twenty individuals with varying tinnitus distress were compared with a control group (n=10) in a pilot study which measured salivary cortisol concentrations on awakening. Multiple exclusion variables were applied.

Results: In line with previous studies, total cortisol volume (as measured by Area Under the Curve) was not found to be significantly different in the most distressed individuals with tinnitus [$F(2, 26) = 0.254$; $p = 0.777$ ns.]. However, a separate measure of changing cortisol levels – the Area Under the Curve with Respect to Increase (or AUC_i) – was found to be significantly less robust in those individuals reporting the most severe tinnitus distress [$F(2, 26) = 7.671$; $p = 0.002$]. This indicates that fewer resources would be available to cope with the demands of the day ahead. Additionally, the AUC_i correlated negatively with tinnitus distress later the same day.

Conclusions: Relationships between proposed objective and self-reported components of self-reported tinnitus distress are considered, with some aspects of tinnitus distress more closely related to physiological mechanisms than others. It is suggested that with further research, the CAR (AUC_i) may be put forward as a credible objective biomarker of tinnitus distress.

Introduction

Tinnitus is a common medical symptom best described as the perception of sound in the absence of corresponding external stimulus (Baguley, McFerran & Hall, 2013). It is usually the result of inner ear damage resulting in random and spontaneous signals being perceived as external sound (Saunders, 2007). It is often described as a ringing, buzzing or whistling noise, with roughly 20% of patients unable to describe what their tinnitus sounds like (Aazh, Moore & Glasberg, 2008).

Most individuals with tinnitus either cope well or habituate successfully, but a minority report significant reduction in life quality. Multiple studies suggest that worldwide, 1-2% of the population fall into this latter category (e.g. Khedr et al., 2010), and that tinnitus is known to have a strong comorbidity with clinical depression (e.g. Temugan et al., 2016). Loprinzi et al. (2013) suggest that those bothered by tinnitus are 3.06 times more likely to be clinically depressed, with Khedr et al. (2010) reporting that 65.4% of their Egyptian sample had some degree of clinical depression alongside their tinnitus. However, it is unclear whether tinnitus causes depression or if depression leaves individuals more vulnerable to tinnitus and tinnitus distress.

The methodological issues in ascertaining tinnitus distress are significant. Readily available objective measures – e.g. tinnitus loudness – have no statistical relationship with tinnitus distress (Jastreboff, Grey & Gold, 1996), though Azah and Moore (2017) report a model whereby tinnitus loudness explains 12% of the variance in depression. Instead, researchers and healthcare providers use self-report measures to assess severity. These questionnaires are subjective by nature and vulnerable to individual differences and biases. For example, some individuals prefer scores from the extremes of a given scale whereas others actively avoid these (Austin et al., 1998). Furthermore, the strong co-morbidity between tinnitus and depression (e.g. Zöger et al., 2006) results in significant negative attentional bias (Baert et al., 2010). This is supported by Osihi et al. (2011) who evidenced that the Japanese language version of the Tinnitus Handicap Inventory (Newman, Jacobson & Spitzer, 1996) was vulnerable to influence from depressive symptoms and state anxiety. Furthermore, Azah

and Moore (2017) report a 'high prevalence of depression' (p.566) among patients seeking treatment for tinnitus and hyperacusis, suggested that tinnitus distress accounted for 43% of the variance in depression scores (n=493), and concluded that effective tinnitus management programmes should consider screening for depression. Thus, an objective measure of tinnitus distress would assist diagnosis, aid assessment of treatment effectiveness, and increase our understanding of physiological mechanisms involved.

Cortisol is a stress hormone that brings glucose into the bloodstream to support the 'fight or flight' response (Sapolsky, 1999). Chrousos (2009) discusses how abnormal cortisol levels can provide objective measurement of physiological stress. Here, *Hypercortisolism* indicates anxiety-based disorders (e.g. anorexia nervosa, obsessive-compulsive disorder) while *Hypocortisolism* is linked with depressive disorders such as chronic fatigue syndrome, fibromyalgia, and so on (see Chrousos, 2009; p378). The Cortisol Awakening Response (CAR) is a well-documented phenomenon in which cortisol levels rise in the first 30-45mins after awakening (e.g. Clow et al., 2010). The CAR – see Figure 1 – is a useful measure of the body's response to awakening and individual anticipation of the day ahead. Indeed, Fries, Dettenborn, & Kirchbaum (2009) considered more than 100 published studies relating to the CAR and concluded that the magnitude of the CAR is determined by sudden increases in situation awareness after a long sleep, and on the future demands anticipated. Therefore, the CAR should not be viewed as a simple measure of cortisol concentration, but is instead a dynamic physiological mechanism which evidences how individuals are coping with chronic stress (e.g. Schmidt-Reinwald et al., 1999). Powell and Schlotz (2012) called this the 'Anticipation Hypothesis', stating that the CAR is "linked to reactivation of information from memory [...] throughout the awakening period, and serves the function of preparing the organism to deal with demands of the upcoming day" ('Summary of the CAR Anticipation Hypothesis', para. 2). The role of the hippocampus is well-documented (e.g. Buchanan et al., 2004) with a prime example being that of Rohleder et al. (2007) who evidenced that 44 competitive ballroom dancers had heightened CARs on competition days when compared with non-competition days. Conversely, Nater et al. (2008) evidenced a flattened

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CAR in 75 individuals with chronic fatigue syndrome when compared with 110 controls. In situations where the individual has a chronic condition for an extended period of time, they are no longer able to rise to the challenges of daily demand and the system subsides into hypocortisolism (i.e. a flatter CAR). This has been documented in burnout patients (Kudielka, Bellingrath, & Helhammer, 2006) and the clinically depressed (Dedovic & Ngiam, 2015). Therefore, an abnormal CAR profile indicates that on awakening, either: (a) extra resources are mobilised in anticipation of demanding events; or (b) the existence of a chronic condition results in the mobilisation of fewer resources and a flatter, more lethargic response to the day ahead.

It is noteworthy that cortisol/tinnitus studies are few in number. Of those making use of cortisol measurement, most measured cortisol throughout the day or in responses to acute stressors. Contemporary expert consensus on how to measure the CAR is recent (Stalder et al., 2016) and the author is unaware of any tinnitus studies undertaken since publication of these guidelines. The most relevant contemporary study considering stress and hearing loss (Canlon, Theorell & Hansson, 2013) reports that individuals with tinnitus “display signs of an impaired HPA-axis” (p.11), and that tinnitus distress is enhanced by increasing challenge and emotional exhaustion. The authors suggested a battery of endocrinal measures, but made no mention of CAR methodology nor the value thereof. Previously, Hébert, Paiement and Lupien (2004) measured salivary cortisol at five timepoints throughout the day, including two within thirty minutes of awakening but their analysis incorporated the full day of measurement and did not consider morning measurements separately. In turn, neither Savastano, Aita and Barlani (2007) nor Kim et al. (2014) found evidence of unusual cortisol levels in tinnitus patients during the day. When considering responses to acute daytime stressors, individuals with tinnitus display blunted cortisol reactivity in response to social stress (Hébert & Lupien, 2007), noise exposure (Hébert & Lupien, 2009), and the mental arithmetic component of the Trier Social Stress test (Alsaman, Tucker & Vanneste, 2016). The only tinnitus study to make use of Area Under the Curve (AUC) calculations (see figure 2) has been Simoens and Hébert (2012). Here, it was noted

that AUC values were lower in participants with tinnitus, and that these differences were not related to hearing loss.

When measuring cortisol concentrations, choice of measurement is important. Khoury et al. (2015) noted fifteen different methods and utilised a two factor model to separate them into measures of ‘total cortisol production’ and ‘change in cortisol levels’. The former is best measured by Area Under the Curve (AUC – see figure 2) and the latter by way of Area Under the Curve with Respect to Increase (AUC_i – see figure 3). It is the latter that is of most interest, and it has not been previously considered in tinnitus research. Since the CAR is indicative of the daily demand expected by the awakening individual, and since tinnitus is a chronic condition with strong comorbidity to depression, it is hypothesised that on awakening, individuals with significant tinnitus distress will display hypocortisolism – i.e. a blunted or non-existent increase in cortisol levels – as evidenced by lower AUC_i values. If the hypothesis is accepted, this feasibility study will inform power calculations for future investigation at larger scales. Based on the other reported studies, it is also hypothesised that total cortisol production levels, by way of AUC, will not be related to tinnitus distress. Since AUC_i is a measure an anticipatory demand, it is also hypothesised that AUC_i will also be an effective predictor of tinnitus distress later that same day.

****Figures 1, 2 and 3 near here****

Method

Participants

Thirty eligible participants took part – ten participants without tinnitus and twenty with tinnitus. Those with tinnitus were recruited from local support networks while control participants were recruited from the university at a later stage. There were eighteen males and twelve females, and mean age was 52.20yrs, **with a standard deviation (s.d.) of 12.030**. After participation, tinnitus participants were allocated to one of two experimental groups based on Tinnitus Functional Index

(TFI) scores: one 'habituated' tinnitus group and one 'distressed' tinnitus group. Together, Adam and Kumari (2009) and Stalder et al. (2016) provide interesting reviews of saliva cortisol analysis, and between them, list a significant number of exclusion variables that were replicated here (see Table 1).

Materials

Perceived Stress Scale (PSS-10). The PSS-10 (Cohen et al., 1983), is a popular ten-item scale translated into over twenty languages (Dao-Tran et al., 2017). Each statement is scored from 0 (never) to 4 (always), providing a global score indicating stress as perceived by participants during the last month. Using a sample of 1,236 adults with similar characteristics, Taylor (2015) reports the PSS-10 to be reliable, valid, resistant to gender bias, **and of good internal consistency ($\alpha = 0.90$)**.

Survey of Recent Life Experiences (SRLE). The SRLE (Kohn & MacDonald, 1992) is a measure of stress designed to record 'daily hassles', with 41 items relating to daily experiences that include such statements as "Being let down or disappointed by friends", "Being taken for granted", "Finding your work too demanding", and "Financial conflicts with family members" – and is rated on a four point scale: '0 = not at all part of my life' and '3 = very much part of my life'. High scores indicate more stresses, with Chellew et al. (2015) reporting internal consistency of 0.93. The items cover significant ground and in a confirmation of validity, McIntosh et al. (2010) previously made use of the SRLE to differentiate between number of daily hassles experienced by a 'depression group' and a 'never depressed group' (p.38).

Tinnitus Functional Index (TFI). The TFI (Meikle et al., 2012) is a 25-item questionnaire which has quickly become a popular choice for researchers and clinicians, and has been translated into at least fourteen different languages (Henry et al., 2016). Items include statements such as: "What percentage of your time awake were you ANNOYED by your tinnitus?" and "Over the past week, how ANXIOUS or WORRIED has your tinnitus made you feel?" Each item is rated between 0-10, providing a global score of 250 which is then transformed into a score out of 100 before breaking down into

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eight subscales: Intrusiveness, Sense of Control, Cognitive Interference, Sleep Disturbance, Auditory Difficulties, Relaxation, Quality of Life, and Emotional Distress. Henry et al. suggests scores of 24 or less equate to mild tinnitus (no need for intervention), scores 25-50 indicate some difficulties and scores above 50 indicate severe tinnitus distress. Fackrell et al. (2016) report high internal consistency ($\alpha=0.80$) and very high reliability (0.90).

Audiograms. To control for hearing loss, audiometric data was obtained within six months of study participation. Pure tone audiometry (PTA, 250-8000 Hz) were undertaken, measuring hearing thresholds (dBHL) at 1000Hz, 2000Hz, 4000Hz, 8000Hz, then 500Hz and 250Hz, with a mean value (dB loss) calculated for the better-hearing ear. Standard Hughson-Westlake procedures were followed, making use of a calibrated audiometer and TDH39 supra-aural headphones.

Self-Reported Tinnitus Distress. Participants with tinnitus also completed visual analogue scales (VAS) to indicate how aware they were of their tinnitus (Adamchic et al., 2012), with scores ranging from '0' – 'Not at all noticeable' to '100' – 'As bad as could ever be'. Seeing that tinnitus is known to vary across the day (Henry et al., 2012), this was undertaken at three time points on both testing days: on awakening (+0hrs); six hours later (+6hrs); and twelve hours later (+12hrs).

Cortisol Measurement. The preferred way of measuring cortisol concentration is by passive saliva collection (Dantzer & Kalin, 2016). Since the CAR peaks approximately 30-45mins post awakening (Smyth et al., 2015), expert recommendation is that a four/five sample protocol is used. Stalder et al. (2016) suggest 0mins, +15mins, +30mins, and +45mins, and this four sample protocol was used. As with Oskis et al. (2012), further samples were taken at +6hrs and +12hrs. Participants woke normally – whether spontaneously or by way of alarm clock – between 6am-9am (Federenko et al., 2004) and provided six samples (four morning samples and two afternoon samples) on two typical weekdays within a single week. On testing days (and the evening before), participants were told to refrain from certain behaviours (See Table 1). In every measure, participants placed synthetic swabs under their tongue for 2mins before placing the swab inside a salivette for storage. Saliva samples

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were collected using the SalivaBio Oral Swab (exclusively from Salimetrics, State College, PA), a synthetic swab specifically designed to improve volume collection and increase participant compliance – Swab Device (5001.02) and Swab Storage Tube (5001.05).

Cortisol Storage and Analysis. This was an ambulatory study outside the laboratory setting. Participants were told that on collecting samples, these were to be stored in a freezer at home (-20° C) until collection by the principal investigator. One participant did not follow instruction and had to repeat the process correctly. Samples were transported under cooled conditions to a licensed laboratory (Biomarker Analysis Laboratory, Anglia Ruskin University, UK) then stored at -20° C until assay using commercially available salivary cortisol ELISA kits under standard laboratory protocols.

Participant Adherence. Participant adherence is critical to CAR methodology and any delay to first sample can be disastrous (Stalder et al., 2016). All participants were briefed in person by the author as recommended by Adam & Kumari (2009, p. 1430), emphasising the importance of providing the first sample within two minutes of awakening. If the first sample was not under way by this time, participants were instructed to abandon data collection and try again on a different weekday. To confirm all participants understood what was meant by ‘awakening’, standardised instructions referred to the point at which consciousness was gained. Further, sampling was not to be initiated during nightly awakenings, and that participants had to ‘get up’ once sampling started.

In order to maximise adherence, other strategies were utilised. As suggested by Adam and Kumari (2009) and by Stalder et al., (2016; p.422, Table 2), participants used a sampling diary to note down time of awakening and sampling times. Though truthfulness was not guaranteed, participants were aware these times were to be checked. Participants were briefed as to the importance of study adherence and reminded by email and text messages prior to planned collection days, and participants were encouraged to have everything they needed by the bedside night before collection and to have read all instructions thoroughly in advance. When samples were retrieved from participants, they were encouraged to verbally confirm adherence to procedure, with the option to try again without

penalty. Finally, and partly as recompense for the behavioural changes required, participants were paid £10 once data had been collected and samples analysed. As such, participants were aware unusual results would be identified prior to payment.

Procedure

All participants were briefed by the principal investigator before being given fifteen synthetic swabs (5001.02) and fifteen salivettes (5001.05). Participants provided saliva samples during typical weekdays as requested, setting out the sample kit, instructions and pen(s) beside their bed in the evening prior to collection. The PSS-10, SRLE and TFI (tinnitus participants only) were completed (once only) on non-testing weekdays within the same week so as not to unduly raise tinnitus intrusiveness on testing days. There were no other time constraints. Saliva samples were obtained passively on awakening (+0mins), then at +15mins, +30mins, +45mins, +6hrs and +12hrs, with samples placed in freezing conditions (-20° C) as soon as practically possible. Once all samples were ready (both testing days), the investigator was notified and samples/questionnaires collected.

Statistical Analysis

Based on research by Henry et al. (2016), participants with tinnitus were placed into a 'Habituated to Tinnitus' or 'Distressed by their Tinnitus' group, as determined by their Tinnitus Functional Index score. This median split process, as suggested by DeCoster, Gallucci and Iselin (2011), saw nine individuals with tinnitus being allocated to the habituated group (TFI24-) and eleven individuals with tinnitus being allocated to the distressed group (TFI25+).

After data screening and conformation that confounding variables were controlled for, it was possible to analyse cortisol concentrations. In order to calculate Area Under the Curve (AUC – see figure 2), Fekedulegn et al. (2007) provides a useful discussion of the equations needed (p.654). In this study, reflecting the four time points used, the formula used was:

$$AUC = \left[\left(\frac{0mins + 15mins}{2} \right) \times 15 \right] + \left[\left(\frac{15mins + 30mins}{2} \right) \times 15 \right] + \left[\left(\frac{30mins + 45mins}{2} \right) \times 15 \right]$$

Pruessner et al. (2003) advise that such formula are used that when associations are sought between repeated measures and other variables. Using AUC simplifies the analysis, condensing multiple cortisol measurements into a single value. Additionally, AUC represents the total volume of cortisol produced by each participant. It is hypothesised that there will be no difference in AUC between groups. Calculating Area Under the Curve with Respect to Increase (AUC_i – see figure 3) is slightly more involved and requires an additional formula:

$$AUC_i = AUC - [0mins \times (15 + 15 + 15)]$$

In this formula, the '15' refers to the time (minutes) between each of the four sample points. As stated previously, AUC_i is a measure of cortisol output after awakening, and it is hypothesised that individuals distressed by their tinnitus will produce significantly less cortisol after awakening, and with fewer resources available, will be less able to cope with the demands of their day.

Ethical Permissions

Approval was granted by the ethics panel of the School of Social and Health Sciences, Leeds

Trinity University, UK.

Results

Data Screening (CAR)

CAR data has to be screened for outliers and other unusual results which could affect accuracy of the analysis (Stalder et al., 2016), particularly in a small-scale feasibility study such as this one. Three participants struggled to provide sufficient saliva for analysis, voiding at least one sample each. In each case, inclusion was still possible using results from a single complete day of measurement. Data was then tested for normality and homogeneity of variance and as expected, all cortisol awakening

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response data (i.e. +0mins, +15mins, +30mins and +45mins) was positively skewed (see Stalder et al., 2016). Furthermore, Shapiro-Wilks tests confirmed that none of the four data sets were normally distributed. Visual inspection of histograms, normal Q-Q plots and boxplots suggested that a single participant was a significant outlier at all four sampling points. This participant was trimmed from the data set and normality tests repeated (n=29). The data was much improved with only the samples at +30mins returning a significant Shapiro-Wilk result ($p = 0.016$), including skewness of 1.346 (SE = 0.434) and kurtosis of 2.852 (SE = 0.845). Visual inspection suggested a single outlying data point as being a good candidate for being purposefully winsorized (Schlotz, 2011; cited in Stalder et al., 2016) and reduced to the 95th percentile. Alongside a final visual inspection, new Shapiro-Wilk tests confirmed normality and homogeneity of variance throughout.

Confounding Variables

Multiple variables can confound cortisol levels and tinnitus perception. Those considered here are: age (yrs), hearing loss (dB), 'daily hassles' (as measured by the SRLE), and individual perception of stress (as measured by the PSS-10). Multiple one-way ANOVAs were run utilising Group as an independent variable with three levels (Controls/Habituated (TFI24-)/Distressed (TFI25+)). Descriptives are reported in Table 2. There were no significant main effects of group for age, daily hassles or perceived stress. Hearing loss (dB) tended towards significance [$F(2, 26) = 2.683$; $p = 0.087$ ns.], but confirmatory Tukey's HSD tests did **not** identify any significant differences.

Table 2 near here

Cortisol Awakening Response (CAR): Total Cortisol Production

Confirming the existence of the CAR required use of a 3x4 mixed ANOVA. The independent variable was Group (controls, TFI24- and TFI25+). The repeated measure was Time of Sampling and had four levels (+0mins, +15mins, +30mins and +45mins). The dependent variable was salivary cortisol concentration ($\mu\text{g}/\text{dL}$). Descriptives are reported in Table 3.

Mauchly's Test of Sphericity was violated so the Greenhouse-Geisser correction was utilised. There was a significant main effect of Time of Sampling on cortisol concentration [F (2.206, 57.353) = 41.029; $p = 0.000$; $\eta^2 = .612$). This was a large effect size and evidences that the CAR was present, with Bonferroni post-hocs showing significant differences at all time points except between '+30mins' and '+45mins' ($p=1.000ns.$). This result is to be expected and helps to validate the methodology. There was no significant main effect of Group on salivary cortisol concentrations [F (2, 26) = 0.222; $p = 0.803ns.$; $\eta^2 = 0.017$], but there was a significant interaction between Group and Time of Sampling [F (4.412, 57.353) = 4.836; $p = 0.000$; $\eta^2 = .271$] and this interaction is shown in Figure 4. However, investigation of second-order simple effects found no differences between groups at any time point.

Table 3 near here

Figure 4 near here

As stated previously, it is also possible to make use of Area Under the Curve (AUC) to indicate total cortisol production. Descriptives for Area Under the Curve calculations can be found in Table 4. With no suitable covariates, a one-way ANOVA was undertaken. The Independent Variable was Group with three levels (Control, TFI24- & TFI25+). The dependent variable was Area Under the Curve (AUC). There was no significant effect of Group on Area Under the Curve [F (2, 26) = 0.254; $p = 0.777ns.$; $\eta^2 = .019$]. As such, the hypothesis that tinnitus has no relationship to total cortisol output, as previously supported by other studies, is accepted.

Cortisol Awakening Response (CAR): Changes in Cortisol Levels or AUC;

In the same way, a further one-way ANOVA was used to investigate any significant effect of Group on cortisol production after awakening, as measured by the Area Under the Curve with Respect to Increase (see Table 4 for descriptives). The Independent Variable was Group with three levels (Control, TFI24- & TFI25+). The dependent variable was Area Under the Curve with Respect to Increase (AUC_i). A significant main effect of group was found [F (2, 26) = 7.671; $p = 0.002$; $\eta_p^2 = .371$]. Least

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Significant Differences (LSD) post-hocs reported significant differences between TFI25+ and TFI24- ($p = 0.001$) and between TFI25+ and controls ($p = 0.024$). There was no significant difference between controls and the TFI24- (Habituated) group ($p=0.180ns.$). See Figure 5. As such, the first main hypothesis is accepted. If we consider cortisol output post-awakening then these results suggest that on awakening, those individuals most distressed by their tinnitus anticipate a challenging day ahead and this results in hypocortisolism.

****Figure 5 near here****

Correlations

As Area Under the Curve with Respect to Increase (AUC_i) seems to be an appropriate objective measure of tinnitus distress, it was of interest to investigate the relationship between AUC_i and self-reported tinnitus distress on collection days (i.e. VAS scores at time of awakening, at 6hrs after awakening, and at +12hrs after awakening.) As sample size is too small to consider regression techniques, correlations with respect to AUC_i can be found in Table 5. Most noteworthy are the significant negative correlations between AUC_i and self-reported measures of tinnitus distress. AUC_i correlates significantly with tinnitus distress on awakening ($r = -.507$, $n = 20$, $p = 0.023$), six hours after awakening ($r = -.599$, $n = 20$, $p = 0.005$) and twelve hours after awakening ($r = -.533$, $n = 20$, $p = 0.016$). Specifically, AUC_i has a stronger statistical relationship with later day self-report measures (+6hrs and +12 hrs) than later day cortisol concentrations (+6hrs and +12 hrs). Self-report scales correlated strongly, suggesting good internal validity for visual analogue scale measures.

****Table 5 near here****

Table 6 shows how these measures correlate with hearing loss (dB), daily hassles, perceived stress, and the eight subscales of the Tinnitus Functional Index (TFI). There are significant correlations between self-report measures (i.e. the TFI and tinnitus distress by way of the VAS (mm) on testing days, probably to do with the self-reporting nature of both sets of measures. Of greater interest is that

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AUC_i correlates negatively and significantly with the Tinnitus Function Index (overall) and with four specific subscales, specifically: namely Intrusiveness, Sense of Control, Cognitive, and Auditory. There is also a near-significant correlation between AUC_i and the Quality of Life subscale ($r = -.429$, $n = 20$, $p = 0.053ns$). This suggests that in individuals with tinnitus, reduced production of cortisol post-awakening is related to the belief that tinnitus is intrusive, is not under the control of the individual, significantly interferes with concentration, and increases the challenges of listening to others. This is of real interest and suggests that interventions targeting and acting upon these specific aspects of tinnitus distress may result in significant improvement to physiological wellbeing.

Discussion

Bearing in mind that this is a feasibility study lacking statistical power, several interesting findings can be reported. Firstly, the CAR was in evidence, suggesting some confidence in the collection process and in participant compliance. Secondly, as predicted, measures of cortisol production are not related to tinnitus distress. This supports the findings of Savastano et al. (2007) Kim et al. (2014), and goes some way towards explaining why cortisol has seemingly been discounted as a contending objective biomarker of tinnitus distress. Finally, as the first study which changes in cortisol levels post-awakening, AUC_i levels were significantly lower in participants scoring 25+ or more on the Tinnitus Functional Index (see figure 5). In addition, AUC_i has moderate correlations with self-reported tinnitus distress not just in the morning, but at lunchtime (+6hrs) and in the evening (+12hrs). This was also hypothesised, and illustrates that AUC_i should be seen as a measure of anticipated challenges in the day ahead. Powell and Schlotz (2012) hypothesised that AUC_i is related to stress anticipation and concluded that “...stronger CAR increases are associated with attenuated distress responses.” (p. 9), and are a sign of more effective coping. In turn, Gartland et al. (2014) report that stress negatively affects the cortisol awakening response and that “a lower CAR was associated with more physical symptoms” (p.130). As such, the evidence is mounting that that the Cortisol Awakening Response is more than just a marker of ill-health – it directly moderates coping ability – though great

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care must be taken in choice of how the CAR is measured. In this study, Area Under the Curve with Respect to Increase (AUC_i) was considered to be of paramount importance, based as it is upon the Anticipation Hypothesis. This suggests that more distressed tinnitus participants (TFI25+) are waking, then orientating themselves as they draw upon memories and their perception of their tinnitus to predict the challenges of the day ahead – a day they may feel unable to cope with. This results in the flatter AUC_i illustrated in figure 5, indicating greater lethargy, fatigue, and associated depressive mood. Conversely, more habituated participants (TFI24-) are responding robustly – though not consciously – with a more reactive CAR that ensures provision of the resources necessary to meet the demands of their day. Studies supporting this conclusion are numerous. For example, Kunz-Ebrecht et al. (2004) reported an enhanced CAR on working days rather than weekends, and Leggett et al. (2015) suggest that when caring for others with dementia, caregivers have a more robust CAR when making use of respite care – representing the value of a predictable amount of time away from their charge(s) and the burden of their responsibilities.

Furthermore, AUC_i correlates negatively with specific subscales of the Tinnitus Functional Index – suggesting that particular aspects of tinnitus are more likely to be relevant on awakening. *Intrusiveness* items ask participants to assess how aware they are of their tinnitus, and how annoying they find it to be. *Sense of Control* has items asking how people cope and whether or not they feel able to ignore the sensation. *Cognitive* items ask whether tinnitus interferes with the ability to think clearly and to concentrate on daily tasks. *Auditory* items refer to whether auditory difficulties can be attributable to the presence of tinnitus. The correlation between AUC_i and *Quality of Life* (QoL) – which refers to the effects of tinnitus on social activities and relationships with others – only tends towards significance ($r = -.438$, $n = 20$, $p = 0.053$ ns.) but the scope of this subscale is broad (i.e. work and recreation) so it is probable that the small sample size simply did not generate enough statistical power. Aside from the usefulness of AUC_i as a future objective measure of tinnitus distress, these findings suggest that these aspects of tinnitus distress most effectively determine activation of the HPA Axis and individual ability to cope. As such, this would indicate that these are the best subscales

to be targeted by treatment interventions. For example, the single most significant correlation with AUC_i comes from the *sense of control* subscale. For the audiologist and healthcare practitioner, this suggests that interventions which focus on boosting sense of control over tinnitus may have the greatest effect on AUC_i which in turn, predicts tinnitus distress throughout the day. It is now well understood that feeling in control can help people adapt to significant life events, better process threats to their health, and be more likely to adopt and maintain health promoting behaviours (Robinson & Lachman, 2017). Typical control interventions include cognitive restructuring (e.g. Li et al., 2018), and are most effective when removing barriers and enhancing self-efficacy – belief in one's ability to undertake a given behaviour (e.g. Damush et al., 2016). Conversely, Fackrell et al. (2016) recently considered the psychometric properties of the TFI and suggested that not all TFI subscales contribute to the functional impact of tinnitus. Specifically, they discounted the *Auditory* subscale, concluding that it is measuring something quite different to the rest.

Study limitations

Firstly, this study is limited in scope. Sample size is small but cortisol research is expensive and small scale feasibility studies such as this one are critical in enabling more ambitious studies. The large effect size for AUC_i ($\eta_p^2 = .371$) suggests that sample size may be satisfactory, but intra-personal variability within the Cortisol Awakening Response is well known (e.g. Elder et al., 2016) and making use of structural equation modelling, Hellhammer et al. (2007) illustrated the CAR can be influenced by situational modifiers. Still, drawing on 104,623 salivary cortisol samples obtained from 18,698 unselected individuals, Miller et al. (2016) have produced normative data for salivary cortisol and this data is within acceptable norms. However, Stadler et al. (2016) recommend that six days of sampling are required to have the greatest confidence in findings, so these results must be treated with caution. While the main hypothesis – that AUC_i is significantly lower in participants with greatest tinnitus distress – is accepted, replication is vital and while sample size should be increased where possible,

the number of test days per participant needs to be substantially increased if we are to be more confident that the AUC_c is an objective biomarker of tinnitus distress.

Secondly, it is accepted that the decision to split tinnitus participants into two groups based on TFI scores may be a controversial one. However, this is based on the suggestion by Henry et al. (2016) that individuals will benefit from intervention if they score 25+ on the TFI. The Tinnitus Functional Index is a ratio scale (i.e. zero is possible) but as DeCoster, Gallucci, & Iselin (2011) indicate, there are occasions where this method is justified. Since individuals with tinnitus are either 'coping' or 'not coping', categorisation is possible. However, it is not clear where this line is and practically, can an individual with a TFI score of 24 differ from an individual with a TFI score of 25? This study suggests that some sort of boundary exists, but more work needs to be done to identify the point at which an individual is habituated to the tinnitus sensation and has AUC_c values which are indistinguishable from controls. There is further controversy as to what a clinically meaningful improvement in tinnitus distress looks like. Meikle et al. (2012) proposed that a 13-point reduction in TFI global scores would be clinically meaningful, but Fackrell et al. (2016) suggest a reduction of 23 points or more is required. Whether a reduction in tinnitus distress is clinically meaningful or not, the restoration of a normal Cortisol Awakening Response (AUC_c) in tinnitus patients is a measurable and desirable goal which reduces the effect of tinnitus distress on individual physiology. It remains to be seen where the physiological tipping point lies. As an aside, Henry et al. (2016) also suggested that a TFI rating of 50+ indicated severe tinnitus requiring 'aggressive intervention' (p.64). In this study, four participants provided TFI scores in this higher band – not enough to represent as a distinct experimental group.

Third, even though tinnitus has strong comorbidity with depression, this study did not make use of a depression questionnaire so as to control for this possible confound. Instead, this study made use of the SRLE in the knowledge that previous research has used it to distinguish between depressed and non-depressed experimental groups (McIntosh et al., 2010). That SRLE scores did not differ between groups suggests that depression was not a contributing factor. Furthermore, while Dodovic

& Ngiam (2015) noted flat diurnal rhythms in individuals with clinical depression, they also confirm that the link between depression and the CAR is complicated and not fully understood. They suggest that a flatter CAR is indicative of an increased vulnerability to depression (p.1186) and that the CAR is more attuned 'to experiences of daily hassles' (p.1187). This is of interest, and suggests the possibility that low values for AUC_i in tinnitus patients may result in a vulnerability to depression, rather than tinnitus being the cause of depression. More research is needed. **Furthermore, the literature does not readily agree on how many individuals with tinnitus also have hyperacusis. In their consideration of Danish children, Rosing et al. (2016) report that 15.7% of their sample had tinnitus and hyperacusis, but effective hyperacusis diagnosis is challenging (Aazh & Moore, 2017). Wallén et al. (2012) have concluded that effective hyperacusis diagnosis must also consider emotional exhaustion – indicating that hyperacusis may be a possible experimental confound – and that this is a particularly important consideration in women (Hasson et al., 2013). As such, it is recommended that hyperacusis is controlled for in future studies.**

Finally, this study relied upon a number of measures to ensure participant compliance, as suggested by Adam & Kumari (2009, p. 1430). This including extensive briefing of participants, diaries, a clear experimental protocol, ambulatory methodology (i.e. face-to-face collection of samples from homes) and payment. However, no objective compliance measures were used: for example, use of monitored salivette caps or time-stamped photographs of participants collecting their saliva. There was a significant main effect of time on cortisol secretion, so it is assumed that participant compliance was strong, but this assumption cannot be evidenced.

Conclusions

Previous studies measuring total cortisol volumes (e.g. Kim et al., 2014) were not able to find consistent and meaningful links between tinnitus distress and cortisol secretion. However, by making use of the Area Under The Curve with Respect to Increase (AUC_i), it is suggested that change in cortisol levels post-awakening may be a candidate objective biomarker of tinnitus distress numerous

extraneous variables are controlled for and that participants are tested on multiple days. It is proposed that the more distressing tinnitus is perceived to be, the flatter the curve of the cortisol awakening response once the patient is awake. The relationship between subjective and objective measures of distress is of moderate strength and it is arguable that stronger correlations are undesirable, particularly when we consider the many biases that occur with self-report (e.g. Brenner & DeLamater, 2016).

These results also suggest that several aspects of tinnitus distress are intertwined with participant physiology and indicates that reducing tinnitus distress could have physiological benefits. Furthermore, the ability to measure the CAR (and AUC_i) across time will allow tinnitus interventions to be assessed for efficacy. An example of this is the work of Oskis et al. (2012) who identified Hypercortisolism in a teenage girl with anorexia. When recovered one year after discharge, her CAR profile no longer differed significantly from controls. However, from a practical point of view, salivary cortisol analysis still requires specialist laboratory access and is not likely to be a methodology that can easily enter widespread use. However, further research into this area may confirm certain targeted tinnitus interventions to be better positioned than others. Goldstein et al. (2015) reports that chronic tinnitus costs \$2,110 in healthcare per patient per year (US), and that intervention costs do not correlate with tinnitus satisfaction. Furthermore, Engineer, Rosellini and Tyler (2011) surveyed 439 Americans with tinnitus and found that nearly 40% of respondents had spent between \$500 and \$1,000 on tinnitus therapies. Given the opportunity to purchase a hypothetical tinnitus therapy for \$15,000 (with \$10,000 of the cost met by health insurance and the other \$5,000 met by the individual), 63.2% were either 'willing' or 'absolutely willing' to do so if told that it could eliminate or significantly reduce their tinnitus. Thus it is critically important tinnitus interventions, from the low cost, low-level interventions through to the most expensive – are all tested longitudinally for effectiveness. The author suggests that this could be managed by way of subjective self-report and use of AUC_i methodologies.

Post-awakening perception of tinnitus may drive real physiological change and link to clinical depression. This post-awakening appraisal may also predict tinnitus distress throughout the same day. Most certainly, further research into use of AUC_i should be undertaken. Firm conclusions cannot be made on the basis of a small-scale study such as this, and findings need to be relocated under strict eligibility criteria. But for now, salivary cortisol output (post-awakening) remains an intriguing candidate for objective measurement of tinnitus distress.

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Table 1: Participant Exclusion and Postponement Criteria

Exclusion Criteria	Strategy	Selected citations
<i>On testing day</i>		
Alcohol (within 24hrs)	Postponement	Badrick et al. (2007)
Current illness - <i>This includes Influenza, common cold, etc.</i>	Postponement	Stalder et al. (2016)
Eating and Drinking (within 1hr)	Postponement	Gibson et al. (1999)
Heavy Exercise (within 24hrs)	Postponement	Hill et al. (2008)
Jet lag (last seven days)	Postponement	Doane et al. (2010)
Shift work (last seven days)	Postponement	Harris et al. (2014)
<i>Longer-term influences</i>		
Chronic Pain - <i>Includes chronic back pain and neck pain</i>	Exclusion	Sveinsdottir et al. (2016)
Cushing's Disease	Exclusion	Roa et al. (2013)
Diabetes	Exclusion	Kudielka et al., 2012
Hippocampul Damage	Exclusion	Buchanan et al. (2004)
Known Sleep Disorder - <i>Includes Hypersomnias (e.g. Narcolepsy) and Insomnias (e.g. Restless Leg syndrome)</i>	Exclusion	Elder et al. (2014)
Oral Contraception	Exclusion	Pruessner et al. (1997)
Pregnancy	Exclusion	Buss et al. (2009)
PTSD	Exclusion	Van Liempt et al. (2013)
Smoker - <i>This also includes individuals who have stopped smoking within the last six months</i>	Exclusion	Badrick et al. (2007)
Use of Steroid medication(s)	Exclusion	Granger et al. (2009)
Other conditions/medications - <i>e.g. Steroidogenesis inhibitors</i>	Case-by-case evaluation	

Table 2: Mean values (SD) and [ranges] for confounding variables across all groups (n=3)

	Age (years)	Hearing Loss (dB)	PSS-10	SRLE
Control Group	51.00 (6.964) [41-63]	15.53 (12.886) [3.57-40.71]	11.11 (5.442) [4-22]	57.33 (12.510) [42-84]
Tinnitus (TFI 24-)	50.11 (13.532) [35-70]	42.46 (35.061) [2.14-98.57]	13.67 (6.305) [4-21]	64.33 (11.314) [50-81]
Tinnitus (TFI 25+)	53.82 (14.573) [29-73]	25.96 (22.269) [0.00-77.14]	17.18 (6.570) [7-27]	64.18 (16.400) [44-89]
Overall	51.79 (12.031) [29-73]	27.84 (26.355) [0.00-98.57]	14.21 (6.472) [4-27]	62.10 (13.710) [42-89]
Female	51.18 (12.441) [29-73]	-	-	-
Male	52.67 (11.911) [35-73]	-	-	-

PSS-10 = Perceived Stress Scale; SRLE = Survey of Recent Life Events; TFI = Tinnitus Functional Index

Table 3: Mean salivary cortisol concentrations (SD) across groups (n=3) at all four time-points

	Salivary cortisol concentration ($\mu\text{g/dL}$) at time of sampling			
	On Awakening (+0mins)	+15mins	+30mins	+45mins
Control Group	.2473 (.0624)	.3528 (.1093)	.5172 (.1776)	.4461 (.1592)
Tinnitus (TFI 24-)	.2262 (.0865)	.3794 (.1413)	.5511 (.1975)	.5594 (.1897)
Tinnitus (TFI 25+)	.3296 (.1881)	.3919 (.1797)	.4289 (.1138)	.4027 (.1808)
Overall	.2719 (.1344)	.3759 (.1445)	.4942 (.1664)	.4635 (.1814)

Table 4: Mean values for Area Under the Curve Calculations (SD) across all groups (n=3).

	Area Under the Curve (AUC)	AUC With Respect to Baseline (AUC _b)	AUC With Respect to Increase (AUC _i)
Control Group	18.25 (5.459)	11.13 (2.809)	7.12 (4.186)
Tinnitus (TFI 24-)	19.77 (6.421)	10.18 (3.892)	9.59 (3.900)
Tinnitus (TFI 25+)	17.84 (6.699)	14.82 (8.466)	3.00 (3.408)
Overall	18.57 (6.086)	12.24 (6.048)	6.33 (4.633)

Note: $AUC - AUC_b = AUC_i$

Table 5: Bivariate Pearson’s correlations between self-reported and proposed objective measures of tinnitus distress.

Tinnitus Distress	AUC _i	Cortisol +6hrs	Cortisol +12hrs	VAS +0hrs	VAS +6hrs
Objective Tinnitus Distress					
<i>- Salivary Cortisol Concentration (µg/dL)</i>					
1. AUC _i	-				
2. Cortisol +6hrs	.306	-			
3. Cortisol +12hrs	.138	.351	-		
Subjective Tinnitus Distress					
<i>- Visual Analogue Scale (mm)</i>					
4. Tinnitus Distress +0hrs	-.507*	-.349	-.058	-	
5. Tinnitus Distress +6hrs	-.599**	-.479*	-.090	.871**	-
6. Tinnitus Distress +12hrs	-.533*	-.383	-.073	.827**	.961**

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

Table 6: Correlations between proposed objective/self-reported measures of tinnitus distress, hearing loss, and all three questionnaires. Due to significance, Tinnitus Functional Index subscales (n=8) are also considered.

	Objective Tinnitus Distress			Subjective Tinnitus Distress		
	Salivary Cortisol Concentrations (µg/dL)			Visual Analogue Scale (mm)		
	AUC _I	+6hrs	+12hrs	+0hrs	+6hrs	+12hrs
1. Hearing Loss (dB)	.219	.247	.192	.014	-.062	-.016
2. SRLE (total)	-.184	-.116	-.212	-.207	-.185	-.235
3. PSS-10 (total)	-.328	-.139	-.002	.119	.073	-.078
4. TFI (total)	-.410*	-.082	.060	.703**	.745**	.659**
4a. TFI (Intrusiveness)	-.490*	-.296	-.031	.769**	.770**	.700**
4b. TFI (Sense of Control)	-.580**	-.350	-.115	.749**	.747**	.637**
4c. TFI (Cognitive)	-.452*	-.313	.056	.600**	.677**	.626**
4d. TFI (Sleep)	-.347	-.017	.322	.524**	.531*	.460*
4e. TFI (Auditory)	-.453*	-.130	-.020	.509*	.598**	.546*
4f. TFI (Relaxation)	-.372	-.233	.099	.630**	.643**	.547*
4g. TFI (Quality of Life)	-.439	-.220	.055	.639**	.692**	.604**
4h. TFI (Emotional)	-.372	-.357	.020	.674**	.692*	.605**

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

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Figure 1: Depiction of a typical Cortisol Awakening Response (CAR).

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Figure 2: Indicating the Area Under the Curve (AUC) beneath a typical Cortisol Awakening Response.

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Figure 3: Indicating the Area Under the Curve with Respect to Increase (AUC_i) beneath a typical Cortisol Awakening Response.

Figure 4: Illustrating the Cortisol Awakening Response across experimental groups (n=3)

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Figure 5: Illustrating the Area Under the Curve with Respect to Increase (AUC_i) for TFI25+ only.

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