Maxi Research Proposal Example.

***Salivary alpha-amylase as a biomarker for sleepiness and depression: Proposal***

**Background Information**

The Problem of Poor Sleep

The hectic work environments and busy lifestyles of today’s society make it easy to see why so many individuals suffer from sleep disturbances. Insomnia is a common and yet serious problem, which refers to an individual’s inability to fall or maintain asleep, or nonrestorative sleep for over a month (Neubauer, 2003; DSM-IV). It can present itself as a symptom, or a disorder which leads to significant distress or functional impairments for an individual (Espie, 2002). Not only is it an inconvenience for the sufferer, and can lead to a range of other problems, but it also has consequences on society. These include increased healthcare costs, lower productivity rates, and higher likelihood of traffic accidents (Vigil et al, 2010).

Correlates with Depression

It is common for insomnia to present itself alongside other conditions. Comorbidity rates with psychiatric conditions are as high as 40-50% (Roth & Roehrs, 2003), and depression is especially common (Espie, 2002; Ancoli-Israel, 2006). Insomnia may present itself at the same time, or after the development of a depressive episode (Roth & Roehrs, 2003; Jansson-Fröjmark & Lindblom, 2008), though much research has looked at its role as a risk factor for subsequent depression (Baglioni et al, 2009; Riemann & Voderholzen, 2003; Roth & Roehrs, 2003; Breslau et al, 1996; Ford & Kamerow, 1989; Buysse et al, 2008; etc.). It is also used as diagnostic criteria for depressive and anxiety disorders (DSM-IV; Roth, 2007; Ancoli-Israel, 2006), and it has been suggested that the two have a common pathology which thus causes an individual to be at a high risk for developing both conditions (Roth & Roehrs, 2003).

Having major depression can affect an individual in similar ways as insomnia. Aside from difficulties sleeping, personal and professional relationships suffer, as do eating habits and general health. An individual’s functioning declines, and can lead to problems with concentration and memory.

The Utility of Biomarkers

Because the two are potentially so debilitating, it is of great interest to study whether there may be a biological marker which links the two, and thus can be used as an indicator for possible development. Biomarkers often used in sleep research are salivary, due to their ease of collection, non-invasiveness, and reflection of current levels of biomarkers (Soo-Quee Koh & Choon-Huat Koh, 2007). Often used is cortisol, which is related to stress, with lower levels correlating with greater amounts of stress (Hellhammer et al, 2009). Saliva chromogranin A, secretory IgA, lysozyme, and α-amylase have also been shown as biomarkers for acute stress (Soo-Quee Koh & Choon-Huat Koh, 2007; Rohleder et al, 2004; Rohleder et al, 2004), and other studies have linked α-amylase to physiological and psychological stress (Noto et al, 2005; Nater & Rohleder, 2009; Van Stegeren et al, 2006; Chatterton et al, 1996; Rohleder et al, 2004; Bosch et al, 2002; Bosch et al, 1996). Higher levels of this enzyme have been found in patients with generalized social anxiety disorder (gSAD) (Van Veen et al, 2008). Vigil and colleagues (2010) report higher levels of α-amylase in females after Hurricane Katrina, which may reflect gender differences in depression. They also suggest that α-amylase may be used as a marker for depressive symptoms among adolescents; however their research was not sufficient to claim such a link. As α-amylase exhibits a circadian patter similar to salivary cortisol, it has been recommended for use in sleep research (Rohleder, 2008; Rohleder et al, 2004). However, not much investigation has yet gone into the link between α-amylase and insomnia, and none has looked at the interaction together with depression. For this reason, it would be of interest whether there is a relationship between two very overlapping disorders and α-amylase.

A 2006 study (Seugnet et al, 2006) discovered salivary amylase as a possible biomarker to indicate sleep drive in humans. Drosophila melanogaster (fruit flies) were examined, and it was found that levels of amylase mRNA extracted from flies sensitive to sleep-loss increased progressively with the duration of waking (3, 6, 9, and 12 hours of sleep deprivation [SD]), thus it is responsive to increasing levels of sleep debt. Furthermore, this effect was not present in flies resistant to SD, or induced waking not followed by periods of sleep homeostasis. Lastly, amylase was not found to be involved in sleep regulation, rather sleep debt. In a small sample of human participants (*n = 9*), these researchers also found that amylase activity increased (around 2-fold) after 28 hours of sustained waking, compared to controls. Total salivary protein and volume were not significantly altered, and salivary cortisol was also not altered by SD, suggesting that changes in amylase are not due to stress. However this study only utilized a sample of 9 human participants, and thus did not allow an investigation into the impact of individual differences in amylase. Lastly, subjective measures of sleepiness were lacking from this study.

**The Current Study**

There seems to be a gap in research investigating the link between depression, insomnia, and amylase. Any research conducted so far has failed to establish a clear link, often as the result of too small sample sizes and insufficient measures or focus. The study at hand aims to address these issues, by looking at the possible link between amylase together with depression and insomnia, in a larger-scale study.

This will provide some insight into whether amylase could potentially be used ad a biomarker to link depression and sleepiness, which results from insomnia. The current study will test individuals on their sleeping habits, as well as depressive symptoms, while gathering salivary samples for the analysis of amylase.

*Hypothesis:*

It is hypothesized that individuals with high levels of depression (measured with the HADS) and insomnia (measured with the ISI/PSQI) will have higher levels of amylase, compared to individuals who do not score highly on insomnia measures.

*Design:*

A cross-sectional design will be used to investigate this question, in an attempt to find a correlation between variables.

*Subjects:*

Data will be gathered from 300 individuals, both male and female, who are over 18 years of age. They will not be excluded on the basis of nationality or ethnicity, providing they have sufficient English skills to fulfill the questionnaires. Subjects will be recruited from the Glasgow Science Centre and Glasgow University, as part of a public engagement study.

*Stimulus/Apparatus:*

All participants will be given sleep questionnaires to fill in, including the SSS, MEQ, ISI, PSQI, and a Visual Analogue Scale (VAS) for Sleepiness. This VAS allows for the use of another subjective measure of sleepiness to examine besides the SSS, which current amylase levels can be compared against in analysis. The HADS will allow for assessing levels of depressive symptoms, and obtaining oral saliva swabs allows for determination of current amylase levels.

*Procedure:*

Participants will be recruited as volunteers from the Glasgow Science Centre and Glasgow University, during June-August 2010. Subjects fitting inclusion criteria will be asked to take part, and will first be given an explanation of the study, and any questions that arise will be answered. Once providing written consent, they will take 10-20 minutes to fill in an online or paper version of the above measures, as well as providing demographic information about themselves. During the testing period, they will be provided with a drink of water to rinse their mouths, and shortly afterwards, an oral saliva swab to test for amylase levels (Salimetrics Ltd). Participants completing the online version will also have access to their results of the MEQ and ISI, which will be explained to them upon completion.

*Statistical Analysis:*

One group for analysis will consist of subjects scoring highly on the HADS (scores ≥8, a recommended cut-off used by many studies in the area; Jansson-Fröjmark & Lindblom, 2008; Neckelmann et al, 2007; Zigmond & Snaith, 1983), as well as insomnia from the ISI or PSQI (scores 15-28, or a global score of ≥5, respectively). These individuals will be contrasted with those who score highly on the HADS, but low on the ISI/PSQI (scores 0-14, and <5, respectively). A comparison of amylase levels will be conducted using an independent-samples t-test.

Furthermore, the link between depression and amylase can be investigated using a Pearson Correlation, to explore a possible relationship between the two.

Amylase levels will be looked at alongside scores on the SSS and VAS, expecting that lower enzyme levels will be present among scores from 0-3. The SSS and VAS scores can be analyzed with a Pearson Correlation as well.