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Stress, Appraised Control, and Salivary Immunoglobulin A

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ABSTRACT

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Salivary IgA is the primary antibody of mucosal immunity. It has been suggested that chronic stress may lower levels of IgA and lead to an increased susceptibility to respiratory illness. It is also suggested that acute stress increases IgA during active coping (that involves mental effort or controllability, such as time-based mathematics or memory tests) and decreases it during passive coping tasks (with no mental effort required or uncontrollable, such as the passive viewing of disgust images). However, tasks often classed as stressors have produced inconsistent IgA effects in areas of passive coping and chronic stress. These inconsistencies might be a consequence of methodological issues, such as sampling procedures, or may reflect individual differences, for example how a task is appraised. This thesis examined appraisal effects with a focus on control over a stressful event and a potential relationship with salivary IgA.

Three different study designs were used to examine stress, appraised control and salivary IgA. To alter appraisal during passive coping, disgust images were presented as either real pictures or as fake effects from fictional films to change the participant's perceptions of control during the image presentations. The role of appraised control during a chronic stress situation was explored in caregivers, and finally, appraised control and subjective stress were investigated in relation to IgA daily for a week in undergraduates, alongside perceived stress and hassles from the prior month.

Viewing disgusting images increased perceived stress, irrespective of whether the images were presented as real or fake. Crucially, control was lower and salivary IgA increased only in the group that were told the images were real. Appraised control over a chronic stressor of caregiving did not affect IgA, but neither did perceived stress. Finally, in undergraduates, stress measured at the same time as sampling showed a lower level of IgA on days rated the highest compared to lowest on stress, and appraised control had a negative correlation with IgA when averages were used over the week, but only in a sub-group of participants. Perceived stress or hassles from the prior month did not relate to IgA.

The main conclusions are that a participant's appraisal of passive coping tasks can be altered and that this may lead to a change in their IgA response. The overall results challenge the view that IgA is a stress marker, as the only consistent effect of stress on IgA was its inconsistency. Yet inconsistent IgA responses are likely to be a recurring issue in research due to the sensitivity of IgA to a number of different methodological practises that may cause a direct effect, or may alter appraisals.

KEY WORDS: Salivary Immunoglobulin A (IgA), Stress, Appraisal, Control, Methodology

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Introduction

1 – Brief Overview

Psychoimmunology research has shown that psychological states can affect many health-related conditions and measures of the human immune system. For example, chronic stress has been significantly associated with lower levels of various immune markers (Segerstrom & Miller, 2003), including immunoglobulin A (Ng, Koh, Chan, Ong, Chia & Ong, 1999; Phillips, Carroll, Evans, Bosch, Clow, Hucklebridge & Der, 2006). However, the impact of stress on this specific measure is not always consistent across research paradigms, with reports of decreases, increases, and no changes in its response to certain stress events. For example, in the acute passive coping literature the emotion of disgust has resulted in all three outcomes with the measure of salivary immunoglobulin A (IgA) and within the chronic stress research, salivary IgA has been suggested to be a weak marker of stress (Mouton, Fillion, Tawadros & Tessier, 1989). Such inconsistency and poor associations with stress might be a consequence of methodological issues, such as direct confounds through saliva sampling numbers or time of day, or even through individual differences in the appraisal of an event. The concept of control has been examined within much prior stress research and has been shown to have an impact on physiological responses, such as heart rate and blood pressure (e.g. Lovallo, Wilson, Pincomb, Edwards, Tompkins & Brackett, 1985; Weinstein, Quigley & Mordkoff, 2002). Given that control has been seen to affect other systems, it was one aim of this thesis to examine appraised control in relation to stress and a potential relationship with salivary IgA. However, before this is described it is important to introduce the systems the body uses in its fight against infection.

2 - The Immune System

The human immune system consists of a number of integrated structures to maintain our biological health. These structures have the principal function to defend the "cellular integrity of the body" whereby they ward off external attack from pathogens and destroy those internal cells that are damaging to our body (Hucklebridge & Clow, 2002). However, it is necessary to focus on one aspect of our immunity, the mucosal immune system.

a) The Mucosal Immune System

The body's first protection against virus invasion is known as the mucosal immune system (Brandtzaeg, Baekkevold, Farstad, Jahnsen, Johansen, Nilsen & Yamanaka, 1999), which tries to destroy an infectious agent before it even enters the body's tissues. This 'firewall' is dedicated to protecting the largest portal of entry into the human body. The respiratory, digestive and uro-genital tracts are prime candidates for contamination due to the fact that their barriers are in contact with the outside world. Consequently they have a huge defensive range, including anatomical barriers such as the cilium of the epithelial layers (Rastogi, Ratner & Prince, 2001), though the predominant constituent of mucosal immunity is called secretory immunoglobulin A (s-IgA) (Lamm, 1997; Russell, Kilian & Lamm, 1999; Woof & Mestecky, 2005).

b) Secretory Immunoglobulin A

Approximately 80% of the B cells located beneath the mucosal layers produce s-IgA (Brandtzaeg, 1992). It has been reported that the non-inflammatory nature of s-IgA makes it most suitable to protect delicate mucosal surfaces (Brandtzaeg et al, 1999). Secretory IgA can be located within secretions such as tears and sweat (Bowry, 1985; Goldblum, 1990) with the major glandular site being that of the salivary glands (Marcotte & Lavoie, 1998). Secretory IgA differs from its serum counterpart in its larger size (Tomasi, 1976) and in its probable ability to bind to antigens more effectively (Valdimarsdottir & Stone, 1997).

The main function of s-IgA has been termed 'immune exclusion' (Brandtzaeg & Johansen, 2005; Lamm, 1997) which refers to noninflammatory surface protection, and is accomplished via the s-IgA capacity to bind to pathogens, preventing them from penetrating the mucosal epithelium. The function of s-IgA within the mucosal regions comes mainly from this ability to bind to invading pathogens. Woof and Mestecky (2005) report that on mucosal surfaces, s-IgA uses four 'hands' to bind to pathogens and inhibit penetration of the epithelial layers. These can be clumped together and carried out of the body with the mucus. Secretory IgA can also neutralise antigens by binding to specific epitopes while their Fc 'tail' region attaches to the mucus covering the epithelial lining (Lamm, 1997). A further role may involve intracellular defence during its transportation to the surface. In these intracellular areas s-IgA may be capable of neutralising viruses that have already managed to penetrate the epithelial layers. Here the s-IgA molecule can bind to the virus and carry it back to the lumen (Lamm, 1997) or block its replication (Mazanec, Kaetzel, Lamm, Fletcher & Nedrud, 1992).

While s-IgA is an important part of our mucosal defence it does not act alone and there exists a range of other mechanisms that help prevent pathogen infection. Some IgA-deficient individuals remain healthy and this has been attributed to a greater level of s-IgM (Natvig, Johansen, Nordeng, Haraldsen & Brandtzaeg, 1997), which may be able to functionally replace s-IgA (Bowry, 1985; Brandtzaeg et al, 1999). Other mucosal defence extends beyond the immunoglobulin group and includes α-amylase (Scannapieco, Torres & Levine, 1993), and the mucins (Nieuw Amerongen, Bolscher & Veerman, 1995). Given the complexity of the interconnecting systems that defend our cellular integrity, it is unlikely that a single measure of one immune function could truly represent the body's immune competence, and many of the studies investigating s-IgA do not claim that it is more important than other mucosal mechanisms. However, an immune parameter like salivary IgA has practical and ethical reasons for use in human research and it is an easy and unobtrusive measurement to collect. Secretory IgA is produced daily in gram amounts across the human mucosal surfaces (Holmgren, Czerkinsky, Lycke & Svennerholm, 1992) and salivary IgA may represent the responsiveness of the entire mucosal immune system (Bosch, Ring, de Geus, Veerman, & Amerongen, 2002). Externest, Meckelein, Schmidt and Frey (2000) state that specific s-IgA reactions within one area may spread (via IgA plasma cells) to other more remote mucosal sites and that strong correlations should allow sampling from one area to mimic the status of another.

There are a number of studies that advocate the importance of salivary IgA for health. For example, the IgA deficient population display a higher occurrence of respiratory infections, asthma, allergic rhinitis, and allergic disorders (Edwards, Razvi & Cunningham-Rundles, 2004; Tomasi & Grey, 1972). It has also been suggested that a deficiency may cause a diminished priming of the macrophages found within the lungs (Lawn, Butera & Shinnick, 2002), and research on IgA deficient mice has provided evidence for its protective factor against tuberculosis (Rodriguez, Tjarnlund, Ivanji, Singh, Garcia, Williams, Marsh, Troye-Blomberg & Fernandez, 2005). A meta-analytic study conducted by Jemmott and McClelland (1989) indicated a negative correlation between salivary IgA and the occurrence of upper respiratory infections (URI). This association has been further supported as low salivary IgA has been linked with a concurrent increase in acute otitis media in children (Hess, Kugler, Haake & Lamprecht, 1991) and the incidence of recurrent URIs in Down's syndrome children (Chaushu, Nof, Becker, Shapira & Chaushu, 2003). Drummond and Hewson-Bower (1997) investigated salivary IgA levels of children with recurrent URIs and found a significantly lower IgA:albumin ratio than in healthy children (albumin is a plasma protein that passively leaks into saliva). These authors recorded this plasma protein as a measurement of mucosal membrane permeability and claim that the ratio "provides an indication of the local secretory immune response, controlling for any serum leakage of IgA" (p.273). Students on an RAAF survival training course had a salivary IgA:albumin ratio that was significantly negatively correlated with health symptom reporting and URIs (Carins & Booth, 2001). Fahlman and Engels (2005) reported that football players displayed a significant decrease in salivary IgA concentration and secretion rate in connection with a higher incidence of URIs compared to controls. Though both concentration and secretion rate were associated with URIs, regressional modelling showed secretion rate to be the best predictor. At the end of an ultramarathon, nearly a quarter of the runners reported a URI during two weeks after the race, and a decrease in salivary IgA secretion rate was significantly greater in those reporting an illness compared to those not (Nieman, Henson, Dumke, Lind, Shooter & Gross, 2006).

However, there are some reports that do not find the IgA and health link. For example, not all IgA deficient individuals display an increased risk of illness (Edwards et al, 2004). No association has been reported between salivary IgA and URI over a 12-week training period in elite swimmers (Gleeson, McDonald, Pyne, Clancy, Cripps, Francis & Ricker, 2000) or training duration for the commonwealth games (Pyne, McDonald, Gleeson, Flanagan, Clancy & Ricker, 2001). There are similar reports of no relationship between salivary IgA and the occurrence of URIs in the average population over a three-month period (Evans, Hucklebridge, Clow & Doyle, 1995). However, given the relatively low number of acute URI episodes that most adults suffer in a year, three months may not yield a reliable measure of vulnerability in these studies, and later data has found different results. In the general population nine individuals were examined during a three-month period of moderate exercise training (Klentrou, Cieslak, MacNeil, Vintinner & Plyley, 2002). When compared to ten sedentary controls, daily records of symptoms for total light URIs was significantly reduced in the exercise group with a significant increase in salivary IgA secretion rate. This was also significantly related to the number of days showing symptoms of influenza and the total number of sickness days.

Despite some inconsistencies the general evidence suggests "low levels of salivary IgM and IgA, particularly the IgA1 subclass, are associated with an increased risk of respiratory illness" (Gleeson, 2000, p.5). Overall the findings suggest an important role for IgA in keeping us healthy, though the exact mechanisms outside a test-tube may not be as direct as the laboratory results have implied.

However, recent evidence raises doubts about the validity of some salivary IgA data as a consequence of a diminished concentration from the use of the Salivette technique, which is repeatedly used within psychoimmunology (e.g. Cieslak, Frost & Klentrou, 2003; Phillips et al, 2006; Zeier, Brauchli & Joller-Jemelka, 1996), where saliva is preferable to other potential measures. Saliva collection is an easy and noninvasive technique that "lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears" (pg. 125, Mandel, 1990), whereas the process of blood collection can be perceived as a stressful experience by many potential volunteers (Bauer, Vedhara, Perks, Wilcock, Lightman, & Shanks, 2000), which could bias opportunity samples. Yet, for over a decade there has been some doubt as to the validity of the secretory parameters from samples acquired through the Salivette. Firstly, salivary IgA concentration can be significantly diminished in Salivette samples compared to the 'spitting-method' (Aufricht, Tenner, Salzer, Khoss, Wurst & Herkner, 1992). While this study involved only six children, later evidence has repeated the finding that Salivettes are associated with significantly lower levels of IgA (Shirtcliff, Granger, Schwartz & Curran, 2001) when compared to other techniques (Strazdins, Meyerkort, Brent, D'Souza, Broom & Kyd, 2005). Salimetrics$_{LLC}$ have recognised "tests have indicated that the use of cotton-based absorbent materials to collect saliva samples interferes with the assay of several other markers including sIgA…" in their 2005 revised advice sheet. Though in defense, it has been suggested that while salivary IgA may be diminished by Salivettes it is "reasonable to consider that any such reduction would be a constant error" (Phillips et al, 2006 p.193).

However, the cotton wadding of the Salivette may cause a systematic bias in the volume of saliva that can be extracted dependent upon how much is added. Li and Gleeson (2003) examined the effects of cotton wadding on varying amounts of saliva and found the volume extracted by centrifugation was significantly affected by the volume originally put in. The cotton wadding seemed to retain a larger percentage of saliva with the smaller the initial amount. While IgA concentration represents the amount of total IgA protein that is measured from a limited amount of saliva, the secretion rate is the concentration level recorded per unit of time ($\mu g.min^{-1}$) (Valdimarsdottir & Stone, 1997). According to Valdimarsdottir and Stone (1997), there exists an issue of flow rate for both measures of salivary IgA. Particularly when saliva is stimulated they report that decreased levels of concentration are found concurrently to an increase in flow rate, but also an increase in secretion rate (Brandtzaeg, 1971) and such findings have been extended to unstimulated collections (Kugler, Hess & Haake, 1992). Valdimarsdottir and Stone state that "relations between saliva flow rate and s-IgA concentration and secretion rate are particularly important, since autonomic arousal associated with stress or negative affect may influence saliva flow rate" (1997, p.462). For the experiments of this thesis the measure of secretion rate was chosen over concentration to compensate for an impact of salivary flow rate and to follow from previous secretion rate findings regarding URI vulnerability. This index is often used and may be more responsive during acute psychological states where concentration may not change (e.g.

Burns, Ring, Harrison, Carroll & Drayson, 2004; Willemsen, Ring, Carroll, Evans, Clow & Hucklebridge, 1998). The issues associated with Salivettes will be addressed later in the thesis.

Another potential confound is one that applies to any data regarding IgA and comes from the influence of specific IgA increases in response to infection. Gleeson, Cripps and Clancy (1995) have stated that the polyclonal activation of B cells is produced in response to many viral invasions. When infected by a pathogen that causes a respiratory infection there is a significant increase of secretory IgA, peaking by the fourth day of symptoms. It has been suggested that the specific IgA to a pathogen may go up while total IgA levels remains the same, but analyses cannot differentiate these and confound measures of total IgA. Stone, Cox and Valdimarsdottir (1987) examined salivary IgA antibody response to rabbit albumin, stating it should parallel that observed for a virus. While the specific salivary IgA antibody response increased, the level of total IgA protein actually decreased over an eight-week period. Stone, Neale, Cox, Napoli, Valdimarsdottir & Kennedy-Moore (1994) gave participants rabbit albumin over a 12-week period and report a significant effect in increased IgA over this daily sampling. Valdimarsdottir and Stone (1997) have criticised IgA and health research for having a limited understanding of the conceptual implications of total IgA as an index of immune protection and question whether it would be more appropriate to employ a measurement of specific antibody over total IgA. Furthermore, there may be a temporal issue where an infection could alter normal IgA levels for a greater length of time than is considered in many experiments. When infants were infected with respiratory syncytial virus, one month following the illness the levels of IgA from nasopharyngeal secretions were significantly raised up to 42 days after onset (Welliver, Sun, Hildreth, Arumugham & Ogra, 1989). However, it has been seen that over a five week baseline period there were no differences in once-weekly saliva samples in the s-IgA secretion rate between healthy subjects and those with respiratory infection symptoms (Reid, MacKinnon & Drummond, 2001). Yet additionally, for the rhinovirus (which accounts for 40-60% of the adult population cold infections) an entire third of cases are asymptomatic (Gwaltney, 1983). Research regarding psychological variables and the impact on IgA must consider that one-off samples may be potentially confounded by respiratory illnesses that are either asymptomatic, or occurred weeks prior to the sampling, and can mask findings by artificially elevated specific IgA levels. One such psychological variable is that of stress.

3 – Stress: Acute and Chronic

While the term stress has been defined in a variety of ways, for the purposes of introduction and in broad terms, psychological stress will refer to events/situations that are seen to be threatening and where demands exceed the perceived coping response of the individual (Lazarus & Folkman, 1984). Stress requires a stimulus (referred to as a stressor, which is environmental or internal) and is linked to a subjective negative state (e.g. Agarwal & Marshall, 2001; Herbert & Cohen, 1993) activating physiological systems within the organism (e.g. Carrasco & Van de Kar, 2003). When examining the impact of stress on immunity, many psychoimmunology researchers split the study of stress into brief time-restricted (acute) and more long-standing events (chronic) (Anisman, 2002). Acute stressors (the event/situation that evokes the negative affect and physiologic reactions) last only minutes or hours at

the most while chronic stressors can extend from months into years (Agarwal & Marshall, 2001). Research in acute stress generally uses laboratory tasks with a definite time limit. Chronic stress is less controlled and studies have encompassed situations such as stressful job experiences and the burden of caregiving (Dimsdale, 2008). For chronic stress, the stressor may be a more internal state rather than an objective and persistent environmental event, such as apprehension and anxiety over weeks of exam periods where the tests actually last only a few hours. The acute-chronic subdivision is essential within psychoimmunology as reviewers often state that chronic stressors have a detrimental impact on illness susceptibility (e.g. Cohen, Miller & Rabin, 2001). This does not deny the importance of acute stressors within the experimental domain. Brief laboratory-based experiments allow investigation of the psychological and physiological moderating factors of the immune system in a more controlled environment, reducing confounding variables and enabling greater precision on the direct cause of immunological changes during an event, such as the fight-or-flight response.

It is generally argued that acute stressors activate the fight-or-flight response (Dhabhar & McEwen, 2001) where it may be adaptive that threatening events activate the defence systems that enhance an organism's survival. They may cause redistribution of immune cells into the specific areas in which they can act the most quickly and efficiently against pathogens that enter through increased respiration, wounding or infection. In contrast, it is generally regarded that chronic stress has a negative influence on the immune system with effects that can translate to an increased risk of many disorders including the incidence of infectious diseases such as colds and flu (Plotnitkoff, 2007).

Segerstrom & Miller (2003) have meta-analysed immune regulation following acute stressors and conclude that in general, laboratory stress tasks increase numbers of innate immune measures, such as NK cells, T cells, peripheral blood neutrophils, as well as affecting the functioning of such cells, for example in increased cytotoxicity of NK cells. They argue that acute stress "upregulates" innate immunity, but can also dysregulate adaptive immune measures (reporting decreased mitogen-stimulated proliferative responses after acute stress). Another meta-analysis regarding chronic stress has found generally opposing effects, with overall decreases in counts of circulating B cells, T cells, and NK cells and the functional measure of lymphocyte proliferation to mitogens (Herbert & Cohen, 1993). It is generally regarded that chronic stress suppresses while acute stress potentiates immunity (e.g. Dhabhar, 2009; McEwen, 2004).

However, when it comes to the mucosal immune system, and specifically that of salivary IgA, the assumptions about stress and immunity do not always hold true. Briefly, all three outcomes of increases, decreases and no change in salivary IgA have been reported following similar stressors from both acute and chronic stress research. While this inconsistency will be explored further, it is first necessary to separate discussion of the two major categories of acute and chronic stress as they are seen to affect immune measures via different pathways.

a) Physiological Pathways between Stress and Immunity

Researchers have examined pathways to delineate *how* stress affects immunity via the homeostatic balance of the central nervous, autonomic nervous, endocrine, and immune systems. When a threatening event occurs the body responds instantaneously with an adaptive reaction to perpetuate survival, and researchers argue the existence of a prototypical stress response (Sapolsky, Romero & Munck, 2000). Psychoimmune routes have been divided into two major pathways from the brain via the sympatho-adrenomedullary (SAM) axis and hypothalamic-pituitary-adrenal (HPA) axis (Cacioppo, 2000; Elenkov, 2002).

b) The Sympatho-adrenomedullary (SAM) axis

The SAM axis involves the autonomic nervous system and a release of the catecholamines (adrenaline and noradrenaline) and acetylcholine (Berntson, Sarter & Cacioppo, 2003). The autonomic nervous system consists of the sympathetic and parasympathetic branches that work to maintain the homeostasis of the body, each with links to immune organs (Felten & Olschowka, 1987; Friedman & Irwin, 1997; Lawrence & Kim, 2000).

Regarding salivary IgA there is research that shows influence of both sympathetic and parasympathetic nerve mediation. While both "increase the movement of IgA into saliva" in rats there was a significantly greater (2-fold) secretion rate following sympathetic activation, though saliva flow rate was four times larger with a parasympathetic drive (Carpenter, Garrett, Hartley, & Proctor, 1998). A differential effect of the two branches of the autonomic nervous system is important regarding acute stress, as laboratory stressors have been divided into two sub-divisions, known as active and passive coping tasks, by means of their physiological reactivity (e.g. Beh & Harrod, 1998; Bosch, de Geus, Kelder, Veerman, Hoogstraten, & Amerongen, 2001; Bosch, de Gues, Veerman, Hoogstraten & Nieuw Amerongen, 2003; Dishman, Jackson & Nakamura, 2002; Obrist, 1982; Willemsen, Carroll, Ring, & Drayson, 2002). For example, active tasks have been defined by an increase in sympathetic excitation (e.g. Willemsen et al, 2002) while passive coping stressors have been accepted (e.g. Ring et al, 2000) as dominated by a parasympathetic drive (Bosch et al, 2001).

The SAM axis potentially plays a role during acute stress when saliva may be the main source of immune secretion, as in salivary IgA (Garrett & Proctor, 1998), though it may be a limitation of some previous research to have classified tasks based on their physiological templates. As discussed by Dickerson and Kemeny (2004) not all tasks reliably elicit such responses and a simplistic focus on the physiological response takes no consideration of individual coping resources, bypassing exploration as to why the same events can cause a specific response on one occasion but not another. This is also true of salivary IgA reactivity that shows increases, decreases, and no change to specific acute stress tasks, which are covered in more depth later in the chapter. There are also some inconsistencies evident in the chronic stress and salivary IgA literature, where other causal pathways play a dominant role, such as the hypothalamic-pituitary-adrenal (HPA) axis.

c) The Hypothalamic-pituitary-adrenal (HPA) axis

When a potentially stressful event continues the body must adapt to the elevated activation and is required to cope with the prolonged demands made upon it. Within a matter of seconds of the increased secretion of the catecholamines, a concurrent secretion of corticotrophin-releasing hormone from the hypothalamic paraventricular nucleus (PVN) creates a cascade of hormonal effects. The PVN of the hypothalamus is the central regulatory device of the hypothalamic-pituitary-adrenal axis (Herman & Cullinan, 1997), which has been classed as one of the most important stress neuroendocrine pathways (Hucklebridge, Clow & Evans, 1998; Carrasco & Van de Kar, 2003). When the PVN is activated a chain of events occur that trigger the expression and release of glucocorticoid hormones from the adrenal cortex (Habib, Gold & Chrousos, 2001). Of these, cortisol is the most important in the human body (Carrasco & Van de Kar, 2003). Secreted cortisol is employed by the body to break down muscle protein which results in the release of amino acids into blood circulation. These amino acids are then employed by the liver to synthesise glucose for energy, enabling a sustained level of activity. At heightened levels they also serve an anti-inflammatory purpose around infection or injury to enable free movement of the body (Wilson, 2001) - an essential aspect of escape.

Psychological stress (where no life-threatening event is actually present) has been seen to induce increases in cortisol (Biondi & Picardi, 1999). Such increases are reported following both acute stress tasks (e.g. Kern, Oakes, Stone, McAuliff, Kirschbaum & Davidson, 2008; van Stegeren, Wolf & Kindt, 2008) and during chronic stress (e.g. Bauer et al, 2000; Steptoe, Cropley, Griffith & Kirschbaum, 2000). Increases to acute stress generally display a peak at about 20 minutes after the stressor (e.g. Gaab, Blattler, Menzi, Pabst, Stiyer & Ehlert, 2003; Hammerfald, Eberle, Grau, Kinsperger, Zimmermann, Ehlert & Gaab, 2006), so it is probably unlikely that cortisol has a major role to play in the salivary IgA reactivity to acute stress, where changes can be measured within minutes. However, it is argued that cortisol responses to acute stress may be potentially mediated by psychological aspects of the stress event, which have been suggested to include predictability and controllability (de Kloet, Joels & Holsboer, 2005; Dickerson & Kemeny, 2004). This suggests that stress response markers (which may or may not include salivary IgA) could be moderated by psychological factors beyond the physical demands of the stress task. In response to the marked variability in individual differences in the stress response, Biondi & Picardi (1999) have stated that "the subjective perception of the situation is probably a main determinant of the psychoendocrine response pattern ... the 'objective' characteristics of a given event are not the only determinants of reaction to the event itself" (pg. 114). Consequently, cortisol was measured alongside IgA secretion rate in this thesis to determine when a stressor activates the core stress response via the HPA axis.

HPA axis stimulation results in a significant increase in the amount of cortisol that circulates the human body, and it has been suggested that elevated levels of cortisol have a dysregulatory influence on immunity (Flinn and England, 2003). All immunological cell types display receptors for stress hormones (Schedlowski & Benschop, 1999) including glucocorticoids (Blalock, Bost & Smith, 1985). In rats a synthetic glucocorticoid (dexamethasone) has been linked to a significant reduction in salivary IgA secretion after 24 hours (Wira, Sandoe & Steele, 1990) though also associated with an

increase in the transcription mechanism of secretory component (Wira & Rossoll, 1991). While Bosch et al (2002) caution the extension of such findings to human effects, there is evidence of a significant negative relationship between salivary cortisol and IgA levels during 30 minutes post-awakening (Hucklebridge et al, 1998). There is little human research in this area, especially regarding salivary IgA during a chronic situation when cortisol levels would be expected to be high (e.g. de Vugt, Nicolson, Aalten, Lousberg, Jolle & Verhey, 2005). It is inconclusive what role cortisol may play regarding the pattern of salivary IgA reactivity to stress events, which is not always identical to the same stressor.

4- Stress and Mucosal Immunity

The concept of stress has been regarded as a key element in the psychological impact of events on illness susceptibility (Segerstrom & Miller, 2004) and has been linked to changes in the body's defense mechanisms, being cited as a leading influence in numerous psychological ill-health variables (e.g. Agarwal & Marshall, 2001). Much research has focused on salivary IgA as the way in which stress can lead to vulnerability to infectious respiratory illnesses. Deinzer, Kleineidam, Stiller-Winkler, Idel and Bachg (2000) have said "salivary IgA became a focus of interest in psychoimmunological research since it has been shown to be sensitive to variations in subjective and objective stress levels" (pg. 220). However, this claim is not entirely accurate and an inconsistency in the salivary IgA response to stress is evident when the literature is reviewed.

A contrasting salivary IgA effect of both increases and decreases was initially thought to be consolidated by a temporal division of stressors into short-term and longer-term events. Indeed it is broadly accepted that salivary IgA goes up transiently to short-term (acute) stressors and decreases in response to longer-term (chronic) stress (Bosch et al, 2002). However, the effect of acute stress has had further distinctions made due to the more recent findings that an increase in IgA to acute stress does not always occur, with some events producing decreases. Acute stress has been divided into active and passive coping tasks and as an event usually starts short-term, we will begin the review of stress and salivary IgA by focusing on the acute stress literature.

a – Acute Stress and IgA

Within the mucosal immunity literature, acute laboratory stress tasks are frequently divided into those requiring active and passive coping (Bosch et al, 2003; Dishman et al, 2002; Keay & Bandler, 2001). This is an important distinction as some researchers (e.g. Bosch et al, 2002) state that active coping tasks cause an increase in salivary IgA while passive coping tasks induce decreases, albeit both transient changes.

Physiologically, active coping tasks have been suggested to activate a sympathetic dominance (Bosch et al, 2001) and psychologically, either require either mental effort for their completion (Bosch et al, 2001; 2003) or engage a perception of control (Lovallo et al, 1985; Bongard, 1995; Weinstein et al, 2002). Within mucosal immunity research, such stressors have included timed mathematics tasks

(Winzer et al, 1999), time-restricted memory tests (Bosch et al, 2001), and multi-tasking tests (Wetherell, Hyland & Harris, 2004). Acute laboratory stressors have been found on average to increase subjective levels of rated stress (Segerstrom & Miller, 2003), and cortisol levels (Dickerson & Kemeny, 2004), and it is generally accepted that these types of stressors tend to elicit fairly reliable increases in salivary IgA (Segerstrom & Miller, 2003).

However, there are also some questions as to the psychological nature of such tasks and potentially to whether such increases are solely attributable to the induction of stress. For example, in relation to cortisol, a meta-analysis by Dickerson & Kemeny (2004) determined that the variance of cortisol increases to acute laboratory stressors was such that it "suggests that there is variance in cortisol changes that could be predicted by other factors" (pg. 363) than those attributed to the tasks themselves or the rating of stress. Also, a number of psychological states other than stress seem to produce increases in salivary IgA. For example, salivary IgA reacts with increases to humour and excitement (Harrison, Carroll, Burns, Corkill, Harrison, Ring & Drayson, 2000), relaxation (Reid et al, 2001), exercise (Ring et al, 2000), and novelty (Willemsen, Ring, McKeever & Carroll, 2000). Although the psychological cause for increased reactivity to active stress tasks is not completely known, they do result in fairly consistent increases in salivary IgA (whether this is stress-induced or not), yet there are a separate group of acute stressors that do not produce reliable reactivity from salivary IgA, known as passive coping stressors.

In the human mucosal immune literature, passive coping stressors are associated with parasympathetic activity (Bosch et al, 2001) and psychologically require no mental effort (Bosch et al, 2001; 2003), or have little controllability over their course (Lovallo et al, 1985; Bongard, 1995; Weinstein et al, 2002). Passive coping tasks have included the cold pressor task, which typically involves the immersion of a hand into ice cold water (Willemsen et al, 1998; Willemsen et al, 2002; Winzer et al, 1999; Ring et al, 2000; Isowa et al, 2004), and the elicitation of disgust (Bosch et al, 2002). Some researchers have stated that passive coping stressors cause decreases in salivary IgA (e.g. Bosch et al, 2002), yet the most striking feature is that the influence of the cold pressor is highly erratic with increases (Willemsen et al, 1998), decreases (Burns et al, 2004; Ring et al, 2000; Willemsen et al, 2002), and no change (Isowa et al, 2004; Winzer et al, 1999) in salivary IgA.

However, while many cold pressor investigations do not measure subjective stress they still discuss the results in terms of the salivary IgA response to acute laboratory stressors (e.g. Willemsen et al, 1998; Willemsen et al, 2002; Winzer et al, 1999). One study investigated the issue of inconsistent salivary IgA responses to cold pressor in the context of sample timings and concluded this does not account for the mixed findings (Burns et al, 2004). Yet they ignored the fact that most previous studies make assumptions that the cold pressor task can increase stress ratings, and that a variance in the psychological consequence of the cold pressor may be a potential factor that explains such salivary IgA diversity. The question arises as to whether the cold pressor does induce the psychological experience of stress? Including research from other fields, the cold pressor task has significantly increased cortisol (Gregg, James, Matyas & Thorsteinsson, 1999; Isowa et al, 2004), and in one of the salivary IgA studies, did increase a scale of stress intensity and state anxiety (Isowa et al, 2004). This

would suggest that the cold pressor task can induce increases in both physiological and psychological stress. So then, the literature contains some stressors that consistently produce an increase in salivary IgA (active coping tasks) and others a far less consistent response (passive coping tasks). The question is why? To address this issue, we can compare two common psychological components that have been associated with the active-passive stressor definition – mental effort and control.

i - Mental Effort

The use of mental effort is a difficult concept to define between two tasks, where it is hard to say whether a task requires or does not require mental effort. For example, Bosch et al (2001) employed a timed memory test as an active coping stressor, which clearly contains mental effort, while their passive viewing of a surgical video may not obviously include an effort element (in the sense of having to work things out), it could initiate coping strategies which may still constitute mental effort. Furthermore, the concept of mental effort is quite difficult to describe and many investigations make use of the term without providing an acceptable definition (e.g. Peters, Godaert, Ballieux, Brosschot, Sweep, Swinkels, van Vliet & Heijnen, 1999). However, it has been suggested that where a task engages mental effort it initiates active coping (Bosch et al, 2001), which has a consequence of an increased salivary IgA response. Yet, in relation to salivary IgA, Willemsen et al (2000) have suggested that novelty in fact may play a stronger role in regulating increases in salivary IgA than the level of mental effort involved. During a mathematics stressor, task difficulty was manipulated through easy, hard and impossible maths tests in a counterbalanced order. These authors state that the novelty of the first presentation to the task elicited the greatest elevation in salivary IgA, irrespective of the mental effort required through task difficulty. Altogether, such findings make mental effort quite a hard concept to successfully distinguish between active and passive coping stressors. Although it may be possible to measure an element of mental effort during such tasks as mathematics and memory tests, it is harder to assess a level of mental effort in the cognitive coping strategies that may be based on individual differences during passive tasks, such as the cold pressor and the induction of disgust. In these tasks, mental effort would be difficult to resolve the inconsistent responses of salivary IgA and a better approach could be to focus on control.

ii - Control

A second approach to the psychological categorisation of a stressor as active or passive involves the notion of control. Control has been advanced as a determinant of negative biological and affective consequences to a threatening event (e.g. Levine & Ursin, 1991). It can be defined as the ability (actual or perceived) to guide the physical or emotional course of an event so that its outcome is a consequence that helps achieve the end goal, whether this is physical (such as successful completion of a mathematics task) or emotional (such as the avoidance of unpleasant negative states). For Levine & Ursin (1991) control is essential to enable active responses to combat negative stimuli and reduce a detrimental reaction. Control separates active and passive coping tasks (Lovallo et al, 1985; Bongard, 1995; Weinstein et al, 2002) with an active coping stressor being a controllable event that includes a

behavioural demand (a physical response required) while a passive coping stressor has little or no controllability, with or without a behavioural demand to the task. There is some good and long-standing evidence in the literature that control is related to stress and the stress response (e.g. Dickerson & Kemeny, 2004; Glass & Singer, 1972; Manuck, Harvey, Lechleitter & Neal, 1978; Peters, Guido, Godaert, Ballieux, Brosschot, Sweep, Swinkels, van Vliet & Heijnen, 1999; Sieber, Rodin, Larson, Ortega & Cummings, 1992; Weinstein et al, 2002). Previous stress research has used a control aspect to examine some immunological consequences. For example, Brosschot, Benschop, Godaert, Olff, de Smet, Heijnen & Ballieux (1994) used a "potentially uncontrollable" puzzle-explanation task and reported significant differences compared to a neutral condition in a measure of blood-lymphocytes. However, there are some limitations in the way the control concept has been adopted within psychoimmunology as investigations can regard everyone identically in their appraisal of the controllability of an event, without clarifying whether the manipulation was subjectively successful in the appraisal of control. An appraisal approach to control may provide a better framework for the study of acute stress.

Appraised control over physically aversive stimuli has been studied in relation to physiological arousal. In an early study, Lovallo et al (1985) implemented control over noise or shock via a button-press and compared reactions to a 'passive' experience condition. The procedure was subjectively successful as participants rated their perceived/appraised control higher in the 'active' avoidance condition, which coincided with significantly elevated muscle tension, heart rate and blood pressure measures compared to the 'passive' condition. Weinstein et al (2002) used a video game task to manipulate control over an aversive noise. They standardised the performance for everyone and reported a change in perceived control over the noise. This control manipulation was associated with significantly greater blood pressure and higher total peripheral resistance changes during the low control level. Given evidence that appraised control may affect physiological responding to stress, it could be explored in the context of the discrepancy in the acute stress and salivary IgA literature.

While prior active and passive coping research has some interesting avenues for investigation, a limitation should be noted from the physical aspect (e.g. shock, physical cold) that are part of the tasks involved. Not only are the control components artificially manipulated but they involve systemic events (which involve immediate physiological threat) in shock or loud noise. Systemic stressors have been separated from processive stressors (which require cognitive processes to become meaningful) in the neurocircuitry of the stress response (Herman & Cullinan, 1997). Immediate systemic stressors activate the PVN directly while the interpretation of the processive threat involves limbic forebrain channels prior to PVN stimulation. In the salivary IgA literature, tasks usually involve a systemic cold pressor task for 'passive' coping and a somewhat processive mathematics test for the 'active' coping stressors. Bosch et al (2002) have proposed an emotional state of disgust as a "passive coping challenge" and which has the propensity to be more processive in nature. This could make it a better vehicle to examine the effect of appraisal on stress, control and salivary IgA reactivity.

Acute Stress and IgA Summary

Broadly speaking, it has been suggested that acute active stress leads to an increased salivary IgA, though there may be some debate as to the psychological cause of such increases, be it stress or novelty, for example. There are also types of stress tasks that have been argued to cause a decreased IgA response, known as passive coping tasks. While researchers are aware of potential appraisal impacts, they use these paradigms to elicit differentiated coping responses on average to explore the pathways to immune regulation. However, there is inconsistency in the salivary IgA response to these tasks with all three outcomes of increases, decreases and no change being reported following similar events. Such inconsistency has not been investigated in relation to individual stress perceptions over the passive tasks, which may be a further consequence of specific appraisal processes in relation to mental effort and control.

b – Chronic Stress, Health and Salivary IgA

A chronic stressor refers to a stable and prolonged stimulus (environmental or internal) that can last from weeks, months to years (Agarwal & Marshall, 2001) and pervades a person's life (Segerstrom & Miller, 2004), though it may also reflect a cumulative load of day-to-day negative events (McEwen, 1998). Again it should be noted that for chronic stress, the stressor may be a more internal state rather than an objective and persistent environmental event, such as the stress felt over weeks of exam periods where the tests are only a few hours. Chronic stress has been linked to changes in the body's defense systems and is often cited as a leading influence in numerous psychological ill-health variables (e.g. Agarwal & Marshall, 2001).

Within stress and immunity research, there are a variety of situations that have been investigated as chronic stressors, including academic exam periods (Bosch, de Geus, Ring and Amerongen, 2004; Kim & Maes, 2003), negative life-events (Cohen, Kearney, Zegans, Kemeny, Neahaus & Stites, 1999), the burden of caregiving (Bauer et al, 2000), and the cumulative effect of negative daily events (Kubitz, Peavey & Moore, 1986). Overall, chronic stress has been attributed to immunosuppressive effects. Specific to mucosal immunity, negative life-stressors have been associated with increases in oral diseases (da Sliva, Newman & Oakley, 1995), and upper respiratory and rhinovirus symptoms in participants experimentally exposed to the influenza A and common cold viruses, respectively (Cohen, Doyle & Skoner, 1999; Cohen, Frank, Doyle, Skoner, Rabin & Gwaltney, 1998). There seems to be a broad agreement that chronic stress either leads directly to a susceptibility of infectious disease (Cohen et al, 1999; Cohen et al, 1998) or inhibits mechanisms that could protect against such illness, like the influenza vaccine (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey & Sheridan, 1996; Vedhara, Cox, Wilcock, Perks, Hunt, Anderson, Lightman & Shanks, 1999). While this suggests chronic stress can lead to a greater risk of respiratory infection, there is research that has focused on salivary IgA as the way in which chronic stress can lead to vulnerability to infectious respiratory illnesses.

However, when it comes to salivary IgA the explicit assumptions about chronic stress and its immunosuppressive effects do not always hold true, with outcomes of both decreases and no change. For example, it has been reviewed and argued that academic stress generally causes a decrease in IgA secretion rates (Bosch et al, 2004). However, these authors have stated that academic stress should be studied in relation to the timing of measurements, where samples collected close to the actual exam test could initiate an acute stress response with increases in salivary IgA. In contrast, those taken around the extended study period may be associated with chronic stress decreases. There is evidence from many experiments with increases in IgA secretion rate immediately after or close to the exam (Bosch, Brand, Ligtenberg, Bermond, Hoogstraten & Nieuw Amerongen, 1996; 1998; Bristow, Hucklebridge, Clow & Evans, 1997; Evans, Bristow, Hucklebridge, Clow & Pang, 1994; Huwe, Hennig & Netter, 1998; Takatsuji, Sugimoto, Ishizaki, Ozaki, Matsuyama & Yamaguchi, 2008) and decreases at sampling times that are further around the exam period (Deinzer & Schuller, 1998; Jemmott, Borysenko, Borysenko, McClelland, Chapman & Meyer, 1983; Jemmott & Magloire, 1988; Mouton et al, 1989).

However, both distinctions of academic stress have shown some inconsistent effects with the variable of salivary IgA. For example, there are studies that report higher state stress in students from baseline to after exams, without a concurrent effect on salivary IgA (e.g. Ng, Koh, Mok, Chia & Lim, 2003; Murphy, Denis, Ward & Tartar, 2010), and when samples are collected in the more chronic stress phase, there are also studies that show no change in salivary IgA secretion rate, despite higher stress ratings (Deinzer et al, 2000; Mouton et al, 1989). Yet while these studies are the minority, there are other factors to consider. Potentially not all exam periods initiate a chronic psychological stress response for all students, and Stowell (2003) has stated that examining the academic data "in terms of individual differences may reveal what makes some individuals more vulnerable to the effects of examination stress than others" (pg. 1056). This issue may be present in other chronic stress areas, such as life-event research.

Academic exam periods have been classed as objective and discrete negative life-events (Herbert & Cohen, 1993) where the stressor may be similar for those under investigation. The category of life-events can encompass a more subjective aspect to stress, which may be reflected through self-reports with checklists or cumulative stressful events, such as daily hassles (Herbert & Cohen, 1993). These subjective events may be quite different from person to person, and the emphasis is on the appraisal of an event as instigating a stress response. Life-event research can be divided by time-frame, such as major life-events that can extend their influence to years after the event itself (e.g. Phillips et al, 2006), minor monthly life events such as those measured by checklists or questionnaires that extend back to the prior weeks or months (e.g. Bosch et al, 1998; Murphy et al, 2010) or those of daily occurring events (e.g. Stone et al, 1994; Stone et al, 1996; Evans, Bristow, Hucklebridge, Clow and Walters, 1993).

Subjective appraisals of major negative life-events were investigated with a large sample of 1222 participants (Phillips et al, 2006). Here it was reported that a stress load (from single item rating scales) was negatively correlated with salivary IgA secretion rates. However, this type of design limits

the number of people that can be examined - out of eight potential life-event areas the median number reported was only one. Furthermore, they do not examine the stress effect in relation to a control group. To incorporate a way of assessing chronic stress in everyday life, daily hassles have been used as a way to assess a chronic stress effect of minor monthly events. Bosch et al (1998) looked at a checklist of daily hassles in the prior two months of an exam and found that increases in IgA response to the academic stressor were positively correlated with both the number and intensity of daily hassles. While this did not show immunosuppressive effects of daily hassles, it suggested a potential impact of the stress of everyday life on the way the immune system reacts. A more general stress approach was used by Ng et al (1999) who looked at a Self-Assessment Score from the past month that assessed stress in terms of the physiological and emotive consequences, without a reference to the event that caused them (job-related events were not the only source of stress). They found a weak negative correlation between salivary IgA and self-perceived stress (Spearman's r = -0.18). This type of design does not assess people that are actually undergoing a chronic stressor, only subjective stress levels from a retrospective measure. Herbert and Cohen (1993) find some limitation with the use of life-event research as each participant may undergo a variable mix of experiences over the retrospective time-frame of reflection.

One way to counter this is to take repeated measurements from a similar population regarding the current state of daily events, and there are numerous studies that have adopted such a design. Stone et al (1994) recorded daily saliva samples in response to ingestion of a novel protein capsule over 12 weeks. In addition they measured the frequency of both desirable (e.g. accomplishing a goal) and undesirable (e.g. an argument) daily events. Here desirable events correlated with higher IgA antibody production, while undesirable events were associated with lower levels. In more detail, Stone et al (1996) further investigated undesirable events to find that a lower IgA level was enhanced by an accompanying negative mood. However, these studies have used a specific antibody response, rather than a total level of salivary IgA secretion rate. A total index of salivary IgA was examined by Evans et al (1993) who used desirable to undesirable events that may have occurred every day over two weeks. Here a between-subjects comparison of aggregated data showed a negative correlation between undesirable events and IgA secretion rate. In a within-subjects analysis with day-to-day samples the reverse trend was observed with those days containing more than average undesirable events having higher salivary IgA levels. Other research incorporating a measure of accumulating hassles has found no effects (Kubitz, Peavey & Moore, 1986; Graham, Bartholomeusz, Taboonpong & Brooy, 1988) or a lagged effect where hassles reported at one time are seen to negatively relate to the level of IgA at a later time (Farne, Boni, Gnugnoli & Corallo, 1994; Martin & Dobbin, 1988). In relation to specific stress ratings, daily stress has been related with a lower IgA (Miletic, Schiffman, Miletic & Sattely-Miller, 1996), though these authors were very unclear about how the data was handled (i.e. whether it was aggregated). Some of these designs are limited by use of single sampling and the different methodologies used to investigate stress and salivary IgA make it quite difficult to fully conclude its effects.

However, collectively the chronic stress literature tends to show a decreased response of IgA to stress, but there are inconsistencies present, which suggest that salivary IgA may be sensitive to factors

beyond the simple concept of stress. Before we conclude that salivary IgA is an inconsistent stress marker, it must be recognised that such discrepancies may be attributable to other factors, and there are methodological issues that should be brought to attention. Some of the research may be confounded by the use of single-sample collections (e.g. Kubitz et al, 1986; Phillips et al, 2006; Ng et al, 1999), which may leave the level of IgA under the influence of many variables other than the stress measured in the study. Even in the daily event data, there is a potential for other events not covered by specific exemplars (e.g. undesirable events) to mute the chronic stress effects on immunological parameters. This is especially true of salivary IgA, which is responsive to many things, such as relaxation (Reid et al, 2001), exercise (Ring et al, 2000), and novelty (Willemsen et al, 2000), among others. This could be further complicated by fairly small participant sizes (e.g. Evans et al, 1993; Farne et al, 1994; Kubitz et al, 1986), and even when multiple sampling is undertaken, a small number of participants could negate a potential increase in power. There is also an issue regarding the operationalisation of the stress under investigation, which can be quite different and ranges from single items (e.g. Ng et al, 2003) to scales in which the psychometric properties are unknown (e.g. Miletic et al, 1996; Ng et al, 1999). Furthermore, the perceived resources available to cope with an event differ and "across individuals, appraisals of a particular stressor…vary widely" (Kemeny & Laudenslager, 1999, p.73). Yet this seems to have been limited in the way it is examined in relation to salivary IgA.

Chronic Stress and IgA Summary

In concurrence with the majority of the chronic stress literature, there is a fairly good argument that chronic stress could induce decreases in salivary IgA (Bosch et al, 2002). However, there are inconsistent results. But before salivary IgA is discarded as a reliable stress marker, this literature is limited by methodological differences where sample timings (morning versus afternoon) and sample numbers (single versus aggregated data) may not show a complete picture of the standing of IgA as a chronic stress index. Furthermore, many of these studies examine chronic stress through subjective questionnaires without including a control group, or using a population that is actually experiencing a chronic stress event. A better way to assess the direct impact of a stress situation would be to compare groups that are thought to be under chronic stress to those that are not. To attempt to understand the relationship between salivary IgA and chronic stress, it would seem practical to start an exploration with an objective event. Caregiving has been classed as such an event (Herbert & Cohen, 1993), and this could be contrasted to a control group of non-caregivers to ensure that potential stress induced changes are above and beyond increases caused by non-stressful confounds. A further dimension could be added to such a design that may tap into the resources available to cope with such a specific chronic event. In many studies the perceived stress rating is based on scales (e.g. Ng et al, 1999; Phillips et al, 2006; Deinzer et al, 2000) that assess the psychological state response to some event or environment. While other methodological differences may account for the inconsistent findings it should be noted that even when differences in IgA secretion are significant, the relationships between self-perceived stress and salivary IgA are described as weak (Mouton et al, 1989; Ng et al, 1999) or that the variance explained is small (Phillips et al, 2006). It may be beneficial to the research field to attempt to find a specific appraisal process that best relates to immunological consequences in salivary

IgA. It is possible that an underlying appraisal process could partially account for some of the inconsistent effects and determine why some individuals do not show identical response patterns.

6 – Appraised Control as a Potential Mediator of IgA Effects

Torres and Pritchard (2006) have stated the appraisal of a stressful situation can influence the perception of control, which makes it an important part of efficient stress management. Indeed, Vitaliano et al (2003) have stated that appraised control may be an important cognitive mediator in the stress and caregiver relationship, and "stressors that are resistant to behavioural coping, particularly stressors perceived as unpredictable and uncontrollable, may continue to be associated with elevated stress hormones" (Kiecolt-Glaser, McGuire, Robles & Glaser, 2002, pg.538). Objective control refers to the relationship between an individual's action and its contingent outcome, while appraised control describes a person's belief in this contingency and its positive outcomes (Peterson, Maier & Seligman, 1993). Wallston, Wallston, Smith and Dobbins (1987) suggest that it is "the belief that one can determine one's own internal states and behaviour, influence one's environment and/or bring about desired outcomes" (p.5). If an individual believes they can end a negative event or state by their own response it will no longer cause a negative response.

In an appraisal approach stress is conceptualised as the product of an interaction between the environment and the psychological factors of the individual. The focus is on the individual's ability to judge their coping responses to be adequate based on the situational characteristics and personal skills (Baum, 1990). In this thesis the focus is more specific incorporating the psychological concept of appraised control as a potential mediator of salivary IgA responses to a stressful situation. Appraised control is defined as a person's belief in the contingency between an action and its outcome (Peterson, Maier & Seligman, 1993). This is the belief that it is one's own attributes and actions that can influence the environment and outcomes (Wallston et al, 1987). This appraisal of a stressful event could determine its psychological and physiological impact. In the Murphy et al (2010) exam period study, although state stress was increased, the perceived stress (as measured by the Perceived Stress Scale-10) over the prior month was not and there was not an impact on salivary IgA levels. This could suggest a potential influence for controllability as the Perceived Stress Scale-10 (PSS-10) contains items that explicitly address this component and leads to investigation of appraised control effects on salivary IgA.

However, the issue of circularity between stress and control should be addressed. It is not disputed that many stress definitions use some controllability as part of the definition itself, and by the thesis' focus on appraised control, it may be suggested that this is investigating a response (rated/perceived control) that is already part of the overall stress concept. Yet what this thesis is attempting to do is specifically focus on appraised control, which may best distil part of the stress appraisal that potentially better relates with IgA.

a) Appraised Control and Caregiving

There are some chronic stress events that have been investigated in relation to appraised control over the situation, and caregiving is one. Caregiving involves a stressor that is an ongoing act that may require certain coping mechanisms to diminish the negative psychological and health effects of the ongoing situation. In fact, an objective event like caregiving provides a good avenue to explore the potential relationship between stress, appraised control and salivary IgA. However, this immune mechanism has not been studied often in the area of caregiving. Where it has, again inconsistent results are reported with a significantly lower rate only in older caregivers (Gallagher et al, 2008) and no difference in caregivers compared to matched controls (Bristow, Cook, Erinzlioglu & Hodges, 2008). Appraised control could be investigated as a possible reason for these different results.

Wallhagen (1992) has shown that elderly caregivers report better life satisfaction and lower depression when perceived control is high. Furthermore, caregiver burden is associated with the experience of a loss of control over the situation (Dupuis, Epp & Smale, 2004), and a lack of control over the instigation of the caregiving experience has been connected with negative health outcomes (Robinson-Whelen & Kiecolt-Glaser, 1997). Interestingly, the expected distress associated with a family member having a degenerative disease is actually worse in those not involved in the caregiving experience. Here Amirkhanyan and Wolf (2003) reported significantly greater rates of depression in siblings not directly involved in caring for disabled parents compared to the child caregivers. Schulz and Beach (1999) examined caregiving in three subgroups of caregivers with (1) a disabled spouse but not providing care, (2) a disabled spouse for whom they were primary caregiver and reported strain, and (3) a disabled spouse with care but not reporting strain. An interesting result was seen in those not responsible for care (group 1) who had significantly higher rates of prevalent cardiovascular disease than all other groups studied at intake, though the authors do not clarify whether the heightened ill-health was a cause of non-caregiving. Mortality risks over four years were significantly greater in primary caregivers reporting strain compared to non-caregivers (63% greater risk) who did not differ from primary caregivers not reporting strain. The authors point to a lack of time in group 2 for general rest or recovery when sick and no time for leisure or exercise, potentially mediating the caregiver-strain and mortality link, and implicating an element of 'control' regarding time.

b) Appraised Control and Acute Stress

Lazarus and colleagues (e.g. Lazarus & Folkman 1984) have suggested that an event requires cognitive interpretation to become a stressor, which is formed and mediated in two stages. Primary appraisal reflects the acknowledgement of a threat from the environment and secondary appraisal refers to the individual's availability of coping mechanisms for the situation. Early studies (e.g. Lazarus & Alfert, 1964) emphasised the appraisal of an event as threatening, and consequently whether stress would manifest both psychologically and physically. They stated "we define coping as constantly changing cognitive and behavioural efforts to manage specific external and/or internal demands that are appraised as taxing or exceeding the resources of the person" (1984, pg 141). The

coping process is the attempt made to manage psychological stress and may be associated with the appraisal of their control over an event (Lazarus, 2006).

Lazarus & Alfert (1964) investigated coping mechanisms made available by differing cognitive appraisals of an operation film through an altered soundtrack. This was done to promote either a 'denial and reaction formation' condition (where the pain and harm were denied, and the positive aspects accentuated), or a threat condition (where no such denial was offered). They reported lower physiological arousal in heart rate and skin conductance in 'reappraisal' participants compared to those offered none. While these results have shown that appraisal can affect the way an individual responds to a stimulus, the experiments did not go further to explore the potential cognitive mechanisms (e.g. appraised control) that altered such responses.

In a linked area regarding emotion, Gross (2002) has discussed the process of emotion regulation to an event. Gross (1998) also accepts the need to recognise an event as an emotional cue by stating that "how we think about an event has a profound effect on the emotions we have" (Gross, 1999, p.531) and describing two ways for emotion regulation in antecedent- and response-focused strategies. Antecedent approaches are utilised before the event has taken place and depend on appraisal processes (i.e. seeing a vive voce as an exciting chance to discuss the thesis topic or as a terrifying ordeal) while response-focussed strategies are used when an emotion has been triggered and aim to influence the reactions that occur. These "cognitive control of emotion" (Gross, 1998) strategies were investigated in relation to an emotional film used to elicit a disgust response. In particular reappraisal (antecedent) and suppression (response) were compared to a watch condition where significant differences were observed between the watch and suppression (but not reappraisal) groups in many sympathetic nervous system parameters – finger pulse amplitude, finger temperature and skin conductance. While these results further support the importance of appraisal mechanisms, they also suffer similar limitations as they do not specifically assess whether the appraisal process involved control.

7 - Thesis Aims, Objectives and General Hypotheses

The concept of stress has been regarded as a key element in the psychological impact of events on illness susceptibility (Segerstrom & Miller, 2004) and is cited as a leading influence in numerous psychological ill-health variables (e.g. Agarwal & Marshall, 2001). One way in which stress could lead to an increased risk of infectious respiratory illnesses is through suppression of the mucosal defence mechanisms. Salivary IgA is the primary constituent of mucosal immunity and, due to its ease of collection through non-invasive techniques, has been widely investigated in relation to health and stress. While there is a general consensus that salivary IgA levels are associated with respiratory health, its relationship with stress is less defined. Tasks often classed as stressors have produced inconsistent effects on salivary IgA in both the passive coping and chronic stress domains.

The sub-division of acute stress into active and passive coping tasks has not completely eradicated contradictory findings. Even when the increase in salivary IgA seems a fairly consistent response to active stressors, there is some question as to the psychological variable behind such increases, be it

stress or even novelty. Furthermore, there are greater problems in the passive coping literature where there are reports of all three potential responses from salivary IgA in increases, decreases, and no change to a number of similar tasks (e.g. Willemsen et al, 1998; Burns et al, 2004; Winzer et al, 1999). Stress ratings are rarely measured and as such these studies can not determine the relationship between acute stress and mucosal immunity. However, where identical tasks can cause such different effects, it is hypothesised that the inconsistency is linked to differences in the individual appraisal of the stressor. Given some evidence that appraised control may affect physiological responding to acute stress (in heart rate, for example), appraised control has the potential to explain the apparent discrepancy in the acute stress and salivary IgA literature. It was one aim to address an appraisal process in particular reference to a proposed "passive coping challenge" in an emotional state of disgust (Bosch et al, 2002). Here it was hypothesised that a different appraisal of the same disgust stressor would produce different salivary IgA responses.

This thesis also explores the extent to which a person appraises a chronic situation to examine an impact on salivary IgA. The overall literature has produced some inconsistent effects with decreases and no change, and there are a number of methodological issues that should be resolved before any conclusions can be drawn regarding chronic stress effects on salivary IgA. This thesis aimed to investigate an objective negative event in a caregiving situation and compare this to a control group not undergoing the stress event. It further aimed to assess appraisal as a possible variable in the response of salivary IgA. The impact of caregiving on health has the potential to be influenced by appraised control. Therefore, it is one aim to examine appraised control effects on caregiver health status, with specific reference to salivary IgA. Here it was hypothesised that while caregivers would report greater stress, this rating and its relationship with salivary IgA will be mediated by appraised control over the situation. Finally, the more subjective approach to chronic stress was investigated with daily ratings being collected at the same time as saliva samples over a seven day period. Such ratings included stress and appraised control over the day's events. Here it was hypothesised that a relationship between daily stress and salivary IgA could be influenced by the control each participant appraised themselves as having over the day's events.

2

Disgust: Stress, Control and Salivary IgA in Response to a Passive Coping Task

To investigate the role of appraised control in relation to stress and salivary IgA it was necessary to choose a paradigm in which to run the first experiments. Acute stress research not only has a benefit of being conducted in a controlled laboratory environment (where you can keep confounding factors to a minimum) but it is within this area that most inconsistent salivary IgA responses arise, particularly within passive coping stress. Two common laboratory tasks (the cold pressor and a state of disgust) have resulted in increases, decreases, and no change in salivary IgA reactivity, despite authors suggesting that passive tasks generally cause decreases. An appraisal process involving control has the potential to explain the inconsistent salivary IgA reactivity, where differences in the way a task is perceived may alter the physiological response to it.

As discussed in the introduction, there is a division of acute stressors into active and passive coping tasks. Within the stress-immune literature many tasks have been classified as active stressors including memory tests (Bosch et al, 2001; Bosch et al, 2003), public speaking (Bristow et al, 1997), and mental arithmetic tests (Isowa, Ohira & Murashima, 2004). For mucosal immunity research most active tasks have been reported to produce transient increases in IgA secretion rate (e.g. Ring et al, 2000; Ring, Drayson, Walkey, Dale & Carroll, 2002), in combination with a sympathetic excitation of the body resulting in increases in cardiac contractibility (Willemsen et al, 2002), shorter pre-ejection periods (Dishman et al, 2002), greater total peripheral resistance (Winzer, Ring, Carroll, Willemsen, Drayson & Kendall, 1999), and increases in blood pressure (Lovallo, Wilson, Pincomb, Edwards, Tompkins & Brackett, 1985; Dishman et al, 2002; Ring et al, 2000; Ring et al, 2002). These reactions reflect a fight-or-flight response as the sympathetic drive mobilises energy via supply of glucose and oxygen to the heart and muscles, accompanied by a strengthening in resistance to infection that may occur as a result of injury (Segerstrom & Miller, 2004).

However, there are acute laboratory tasks that do not produce increases in IgA and these have been termed passive coping stressors. These tasks can also rely on the autonomic nervous system response for classification, which has been accepted (e.g. Ring et al, 2000) as being dominated by a parasympathetic drive (Bosch et al, 2001). While researchers are aware of appraisal influences, they have used these paradigms to elicit active and passive coping on average. However, not all results in this field show the same reactions to identical tasks. For example, the cold pressor task (the immersion of a hand into ice water) has been widely accepted as a passive coping stressor (Willemsen et al, 1998; Willemsen et al, 2002; Winzer et al, 1999; Ring et al, 2000; Isowa et al, 2004). Yet results vary on indices such as heart rate, even when the task (water at $10^{o}C$) procedures are identical at 8 minutes, with both decreases (Ring et al, 2000) and no change (Winzer et al, 1999), or at 4 minutes with again reports of both decreases (Willemsen et al, 1998) and no change (Willemsen et al, 2002). Investigating this very issue, Mourot, Bouhaddi, Boussuges and Regnard (2008) have stated that heart rate responses to the cold pressor are generally associated with high inter-individual variability.

Potentially, the discrepancies in the physiological response to passive tasks could be explained if the current division was merged to form a continuum where a task may evoke a more sympathetic or parasympathetic response depending on the appraisal of the task at hand. For example, Lovallo et al (1985) used control over an aversive stimulus via a button-press and compared reactions to a passive

experience condition. A higher level of appraised control from the active avoidance condition was associated with significantly elevated muscle tension, heart rate and blood pressure measures compared to the passive group. Weinstein et al (2002) found that differences in appraised control during a video game task produced significantly greater blood pressure and higher total peripheral resistance changes during a low control (over aversive noise) group. However, these experiments have a focus on artificially manipulated control over a physiological consequence (such as a loud noise), which could reflect a systemic stressor. In the salivary IgA literature, tasks can involve a systemic cold pressor task for a passive coping and a somewhat processive mathematics test for an active coping stressor, where there is no obvious threat from the task and requires some interpretation to evoke a stress response. Research could involve a more processive stressor as a passive task, to examine individual differences in appraised control during a stimulus that requires cognitive processing to become meaningful. The second passive stressor widely used is that of a state of disgust, where there is a visual stimulus without the presence of a physical threat. It may be possible to manipulate the processing of such a stimulus to examine appraisal effects on stress, control and physiological responding.

With regards to salivary IgA there are varied results from passive coping research, which could also suggest that some tasks may be causing an active mucosal response. Some researchers state that passive coping stress causes decreases in salivary IgA (e.g. Bosch et al, 2002), yet the influence of the cold pressor is highly erratic, with reports of increases (Willemsen et al, 1998), decreases (Ring et al, 2000; Willemsen et al, 2002), and no change (Isowa et al, 2004; Winzer et al, 1999) in this measurement. While it could be argued that such differences are attributable to a few odd results, this type of passive stressor is not alone in its varying effect on salivary IgA as disgust research also contains inconsistencies. Given that the same passive task can produce such variable results it was one aim of this thesis to test an idea that individual differences in the reactivity of salivary IgA to tasks may be attributable to the appraisal of a task.

Suggestion that appraisal may impact on the physiological response has been supported by research regarding a second passive coping stressor, that of disgust. Bosch et al (2002) have proposed an emotional state of disgust as a "passive coping challenge" following the report of significant decreases in salivary IgA after participants viewed a film of oral surgery (Bosch et al, 2001). Although it was reported that a significant increase in anxiety occurred after viewing the gruesome film, they did not assess whether the target emotion of disgust had been induced. While the induction of disgust has lead to increases in negative affect (e.g. Schafer, Schienle & Vaitl, 2005) the specific rating of stress has not been addressed within the research, particularly that investigating disgust and salivary IgA (e.g. Farley & Bristow, 2003; Hennig & Netter, 1997). When cortisol levels are measured following what is termed 'gruesome video clips' significant decreases have been reported (Nyklíček, Bosch & Nieuw Amerongen, 2005). While this is an unusual finding, methodological problems exist as out of two film clips shown in succession only one is described as fitting a 'gruesome' classification.

The mucosal immune response to disgust has also shown contradiction. Some investigations report significant decreases in salivary IgA following 'gruesome' film clips of surgery (Bosch et al, 2001)

and an accident involving a hand and mixer (Nykliček et al, 2005). Others have reported significant increases in salivary IgA after the successful subjective induction of disgust through video clips of fictional content (Farley & Bristow, 2003; Hennig, Possel & Netter, 1996). These results warranted further examination of the stimulus qualities. Those stimuli used in the investigations reporting increases (Hennig et al, 1996; Farley & Bristow, 2003) were fictional film-clips and the participants were aware of the nature of the images. In contrast, the Bosch et al (2001) stimulus was a film of a real-life surgical procedure. It is not clear whether participants knew the nature of the clip used by Nykliček et al (2005). It is hypothesised that when participants were told what they viewed was fake, this appraisal allowed them to control their negative emotional response. A real-appraisal of an event refers to the presentation of a stimulus as containing images that are from the real-world, whereas during a fake-appraisal stimulus the content would be perceived as being created by special effects and unreal. One approach is that greater control leads to active coping and consequently induces increases in salivary IgA while low control is passive and creates an opposite IgA reaction. From the previous disgust and salivary IgA results it is suggested that a fake-appraisal enabled participants to control their emotional response and made the event an active, controllable task. However, when the stimulus depicted real events a different appraisal prevented such control making it a passive task.

This thesis examined whether a manipulated appraisal regarding the real and fake content of a visual disgust stimulus could alter the emotional control and salivary IgA response to it. The following studies aimed to explore a manipulation over the appraisal of emotional control to the same disgust stimulus to assess if its influence could lead to different responses in IgA. Control has been advanced as a defining mechanism of active versus passive coping (e.g. Lovallo et al, 1985; Bongard, 1995) and this was expanded through a focus on appraisal processes on salivary IgA reactivity.

Aims and Objectives

The varied results regarding salivary IgA reactivity to passive tasks have raised one question of whether such tasks have been correctly classified, but also necessitated research being conducted to try and understand why the same task can have a different response. More specifically, the findings from the passive coping stress literature led this research to address whether a passive task could in fact become more active in salivary IgA response based on the appraisal of the same event. To test this, the appraisal of an identical disgust stimulus was altered to assess the effects of emotional control on salivary IgA responses.

Hypotheses

Passive coping stressors may produce differentiated responses from the way a task is appraised. In this experiment it is hypothesised that the immunological and endocrine reaction to disgusting images would be altered by a manipulation of the appraisal of the images. This appraisal manipulation should affect the perception of emotional control over the images, where the perception of stress and disgust will not vary. A differential physiological response will not be caused by a different perception of stress or disgust but by their differing perception of control.

1) The participant's rating of emotional control over disgust images can be altered by the images being described as either real (real events, wounds and diseases) or fake (special effects and make-up).
2) The negative psychological reactions (from SACL stress, arousal, and disgust) will not differ from this appraisal manipulation of the images as real or fake.
3) There will be a different physiological reaction to the disgust images from the real and fake appraisals of the images.

Method

Participants: Sampling and Demographics

A total of 120 participants volunteered through one of the following recruitment methods: Signs were placed around the campus and an e-mail was sent to the faculty mailing list. Participants were drawn from the University student and staff population, and those studying a degree in psychology were given $1^1/_2$ hours of participation credit. The inclusion criteria asked that all volunteers were healthy and not currently suffering a respiratory infection, or had done within the past two weeks. It was also stated that those who were blood or needle phobic should not take part. All participants gave written consent, and the study received prior University ethical approval (UREC0417). See **Table 1** for participant demographic information. Of the 120 participants, 76 provided saliva samples by Salivettes and 44 through the spitting method of collection.

Table 1: Participant demographic information.

	NEUTRAL	REAL APPRAISAL	FAKE APPRAISAL
N	25	48	47
Male:Female	9:16	18:30	18:29
Mean Age in Years (SD)	24.5 (7.7)	24.8 (6.2)	25.3 (6.6)
Age Range	19 – 56	18 – 42	18 – 43

Materials

i – Questionnaires and Forms

Detailed information sheets made it clear that the images were of a potentially disgusting nature but made no reference to whether the pictures were real or fake. Participants were required to sign consent forms and complete a baseline questionnaire pack. This pack contained questionnaires that were chosen to record demographic information and assess the trait and state variables described below.

Demographic information was recorded, such as date of birth, sex, smoker status and a question regarding the number of colds over the past year. The 29-item Rotter (1966) Locus of Control Scale was used to assess an effect of the stable trait belief in the control over general life-events. This trait is either a more internal control orientation where the person feels in control of the consequences of their

behaviour, or a more external perception where things are seen to be beyond personal control and power is seen in others, fate and chance. It has been suggested that people with a strong internal locus of control could find themselves vulnerable to the effects of uncontrollable situations as their coping strategies are rendered useless (Burger, 1989; Phares, 1976). The scale contains 29 forced-choice items, 23 to measure locus of control expectancies and 6 as filler items. Each item consists of a pair of statements and the respondents choose between an internal and an external alternative. Scores range from 0 to 23 and a high score represents a strong external trait where the person perceives other people, fate and chance to be in charge of the reinforcements they acquire through life. An internal scoring individual sees themselves in control over events. This test has shown reliability in corrected split-half values of .65 (males) and .79 (females), test-retest results ranging from .49 to .83, and Kuder-Richardson coefficients between .69 to .76 (Rotter, 1966). This construct has shown external validity from its predictability in goal-directed behaviour over the physical environment (Joe, 1971), greater negative reactions both psychologically and physiologically to an uncontrollable life event (Baum, Cohen & Hall, 1993), and to work stressors (Krause and Stryker, 1984). The final questionnaire measured the subjective stress and arousal response to the immediate situation via the 30-item Stress Arousal Checklist (Mackay, Cox, Burrows & Lazzerini, 1978; Cox & Mackay, 1985). Factor analysis has identified two bipolar factors, stress and arousal (Fischer & Donatelli, 1987; Mackay et al, 1978). The stress dimension consists of the subjective response to the immediate situation and includes terms from pleasant to tense. The arousal dimension represents a sense of alertness and consists of descriptors ranging from lively to drowsy. The higher the individual's score the greater the stress and arousal (from 0 to 12). While no data exist on its reliability (Fischer & Corcoran, 1994), there is evidence of internal validity with alpha values of .89 and it shows external validity as the stress component has been significantly correlated with stress-induced increases in urinary cortisol (Doyle, Pang, Bristow, Hucklebridge, Evans & Clow, 1995).

A second questionnaire pack assessed the more situational/state variables evoked by the stimulus and contained a modified version of the Differential Emotions Scale (Izard, 1972) to examine the emotions of interest, joy, surprise, sadness, anger, disgust, fear, shame and guilt. Each emotion was assessed by three items, averaged to provide a score from '1 = Not at all' to '5 = very strongly felt'. This scale is reported to have good discriminate ability between the emotion states (Philippot, 1993). It was modified so the time-frame reflected the emotions felt while viewing the image presentations, which is in line with previous research indicating that psychometric instruments are robust to modifications such as measurement scales or wording (Villani & Wind, 1975). This is also in line with previous disgust research that has modified the scale to fit the time frame with images just viewed (e.g. Sawchuk, Lohr, Westendorf, Meunier & Tolin, 2002). A simpler emotion scale containing 24 emotional adjectives was created to further assess the emotions evoked by the image presentations. This was to assess a neutral and bored rating not present in existing emotion scales. Participants were asked to rate each adjective as they had felt during the presentations from 0 being "Did not feel the slightest bit of the emotion" to 5 being "The most I have ever felt in my life". It aimed to cover the emotion categories of happiness, anger, disgust, fear, boredom, sadness, surprise, and neutral with three adjectives per category (*Appendix III*). This second questionnaire pack also

included a second copy of the Stress Arousal Checklist to compare the ratings to baseline levels, and an emotional control scale which was constructed by the researcher and supervisor.

The Emotional Control scale consisted of 10 questions designed to assess the level of emotional control felt during the image presentation (See *Appendix IV* for the final layout and instructions). The measurement scale used a seven-point scale from 1 = not at all to 7 = very much so, with three of the items in a reverse scoring format. A high score reflected greater control. According to Folkman and Lazarus (1980) any stressful event will be evaluated in relation to both the physical actions required and the emotional reactions. There seem to be two main divisions regarding stress and control in 'Primary' and 'Secondary Control' (Rothbaum, Weisz and Snyder, 1982). However, during the presentation of disgust images, there seem to be no behaviours that are under the control of the participant to determine the outcome of the stressor (Wallston et al, 1987) and so this aspect was unrequired to be covered by questions in the Emotional Control Scale. The concept of 'Secondary Control' guided the overall construction of the items for this Emotional Control scale. This is a widely adopted feature of appraised control and is more cognitive in nature, representing control over thoughts, appraisals (Kobasa, 1985), and emotions (Wallston et al, 1987). This aspect of control concerns the emotional and cognitive reactions to a stressful event.

During the presentation to what extent did you feel:

1. you were in control of your emotions?
2. able to govern the way you felt during the presentation?
3. you could influence how you felt during the presentation?
4. there was no way you could make the presentation any less disgusting
5. that by changing the way you thought about the pictures you made them less disgusting
6. felt 'out of control' in the presentation
7. you were able to think of things that made the presentation less disgusting
8. you tried to think about other things during the presentation
9. that changing the way you thought about the pictures made them less unpleasant
10. the pictures were disgusting and nothing you could do or think could make them less disgusting

Scale Analysis: It was investigated whether the items on the scale were measuring one or more underlying constructs (i.e. emotional control). The Emotional Control scale provided good reliability with Cronbach's alpha being 0.81 (n=95). It was also possible to assess some construct validity by correlating the Emotional Control scale with other instruments accepted as standardised measures of similar concepts. Rotter's (1966) Locus of Control scale has been recognised as measuring a general tendency to perceive rewards in life as being controllable or not. Consequently it was predicted that the Emotional Control scale would correlate with this questionnaire to show some construct validity. Furthermore, as this questionnaire was designed to assess the ability to control the emotional response to the disgust stimulus it was predicted that this scale would correlate with specific disgust ratings from the Differential Emotions Scale and simpler Emotions Scale, and potentially with the Stress Arousal Checklist. Such correlations were run and these data were displayed in **Table 2**.

Table 2: Pearson correlation values for the Emotional Control scale (n=95)

Locus of Control (r value)	DES Disgust (r value)	ES Disgust (r value)	SACL Stress (r value)	SACL Arousal (r value)
-0.36, $p<0.001$	-0.46, $p<0.001$	-0.38, $p<0.001$	-0.47, $p<0.001$	ns

The personality dimension of Locus of Control showed a negative correlation, where a person who rated themselves more internal had a higher score on the Emotional Control scale. This meant that people who view themselves as being more in control over life and events also score higher on being able to control their thoughts and emotions to negative stimuli. The state-dependent results also suggested that the scale showed validity in the direction of the correlations observed. A higher rating of emotional control was associated with lower levels of disgust and stress following the image presentations. The people that rated themselves as more in control over the experience did in fact rate the individual negative responses at a lesser level than people who reported lower emotional control during the stimulus presentation.

ii – Stimuli

A stimulus was needed that would induce a state of disgust and be capable of being appraised as real and fake. A search of the literature suggested that such appraisal manipulations for disgust had not been conducted before and so a brief pilot study was run to ensure that this could be successfully achieved. Bosch et al (2001) used film-footage of a surgical operation, while Hennig et al (1996) employed disgust clips taken from a commercially available film. However, neither type was able to pass as both real and fake. Memory recollection was unsuitable as the same stimulus could not be employed and so the method of static images seemed the most appropriate. For example, the image shown in **Figure 1** could be described as being a real medical event, or a special effect.

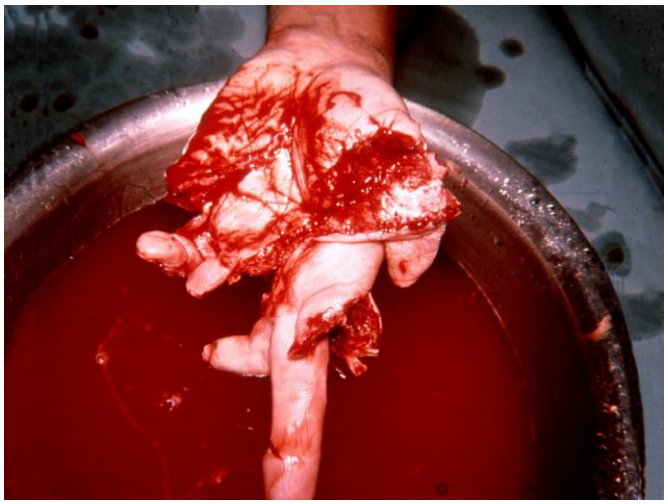

Figure 1: One of the International Affective Picture System (IAPS) images used in the disgust stimulus

A number of disgust-inducing images were obtained from one of the most frequently used collection of pictures within emotion literature. The International Affective Pictures System (IAPS) (Lang, Bradley & Cuthbert, 1999) is a large collection of images produced as a set of standardised, normative pictures specifically for use in emotion experiments. A search of the literature focused on the IAPS database and revealed a number of studies that incorporated particular images to invoke a specific state of disgust (e.g. Schafer et al, 2005; Yartz and Hawk, 2002). Added to these were a smaller number of other images chosen due to their negative content. A total of 39 images were collated into a PowerPoint presentation with each image as one slide automatically displayed for seven seconds. This made a total display time of 4-minutes and 33-seconds.

Eighteen participants rated their emotional reaction via the modified version of the Differential Emotions Scale (Izard, 1972) to examine the emotions of interest, joy, surprise, sadness, anger, disgust, fear, shame and guilt. To ensure the images could be appraised as either real or fake, a five-point scale was used as a single statement with the question "Did you believe the images in the presentation just shown to be: 1 as fake to 5 as real".

One group of nine participants were told that the images were special-effects and the other group (of nine) were informed that they contained real events. The belief rating of the images as real and fake ranged from 1 (fake) to 5 (real) and average values were computed between the appraisal groups. Mann-Whitney U test was used to determine that the real-appraisal group rated the stimulus with a significantly higher belief score (mean = 4.33, SD = 0.7) than the fake-appraisal (mean = 2.78, SD = 0.9) group ($Z=-2.81$, $p<0.01$). This meant that the appraisal manipulation may have been successful with the different descriptions of the images leading to a significant difference between belief ratings. From the Differential Emotions Scale the category scores for each emotion were averaged between the two appraisal conditions and analysed by ANOVA, followed by simple contrasts. Multivariate statistics displayed only a significant main effect of emotion category ($F_{(8,9)}=6.26$, $p<0.01$,$eta^2=0.85$) and meant that the emotion of disgust was induced to a similar degree by images appraised as fake or real. As disgust was expected to be the highest rated emotion, the simple contrast analysis compared this level to all others for both groups and found it to be significantly greater than six of the emotion ratings (all $p<0.017$), except interest ($F_{(1,17)}=0.21$,$p=0.75$) or surprise ($F_{(1,17)}=0.53p=0.42$). Yet the most important finding was that the presentation of the stimulus as real and fake images produced significantly different belief scores. This was essential as the appraisal manipulation to perceive the images as real and fake was the crux of the main research design.

In the main study, it was important to add a neutral image condition to compare the effects of the image appraisals to a stimulus that should not evoke a disgust response, allowing the results from the saliva data to be compared to a neutral stimulus. The disgust image presentations for the main study were identical to the 39 images employed in the pilot. Each appraisal manipulation was first mentioned in verbal instructions and then emphasised in an introductory written slide that preceded the presentations (see *Appendix V*). For the control group the neutral image presentation was constructed from a different set of 39 IAPS images all of which had been employed by previous research as neutral stimuli. All images were displayed automatically by the PowerPoint programme

for seven seconds via a projector onto a screen. In line with the disgust stimuli the total running time was 4-minutes and 33-seconds.

iii – Saliva collection

Saliva samples were collected by two different techniques across the study duration, firstly via the Salivette method (Sarstedt, UK) (see *Appendix II* for protocol), and later samples were collected by a modified Navazesh (1993) spitting-method. This was due to possible limitations involving the impact of the Salivette collection technique (see pages 106-107). For the spitting-method the participant is seated upright with their head tilted slightly forward towards the chest and eyes open. Participants were asked to allow saliva to collect in the bottom of the mouth for one minute intervals the end of which they were told to expel the saliva into 30ml tubes. This occurred for three minutes, after which the tubes were sealed. Following saliva collection, all samples from both techniques were put into a ziplock bag and stored in a –80^{o}C freezer until analysis.

Design

A mixed factorial design was employed. The consistent between-subjects factor were the appraisal conditions of real versus fake images, with a control condition of neutral images. The within-subjects factor were the timings for the collection of the salivary indices of flow rate, IgA secretion rate, and cortisol which were taken at three occasions – baseline, immediately after, and recovery at 20 minutes after the stimulus presentation. Though samples taken at the beginning of each study may have been in storage between three to six months, Gleeson (2000) has determined that such long-term storage is not associated with IgA degradation. The effects of the diurnal rhythms of IgA and cortisol (Miletic et al, 1996; Hucklebridge et al, 1998) were taken into consideration by ensuring that all session times fell between 12 noon and 4pm. Participants were allocated to each condition in a pseudo random manner by blindly picking a condition token out of a box (where appropriate numbers of tokens were present for each condition).

Procedure

The experimental sessions followed almost identical procedures except that on occasion one participant was run alone and at other times groups (average of three) were used. The participants were taken to the testing laboratory where they were seated opposite the projection screen and given consent forms to sign and date. They were then given the first questionnaire pack to fill in, the three Salivettes, and a ziplock freezer bag. After approximately five minutes they were then asked to provide the baseline sample. Once this had been collected the lights were turned off to ensure the images could be seen clearly. Participants were asked to remain still and silent during the presentation time and participants were read one of three descriptive passages regarding the images they were to view, depending on the condition allocation (see *Appendix V* for descriptions).

There was a written slide that briefly re-iterated the spoken instructions, and then the 39 images were automatically displayed for seven seconds each by the PowerPoint programme. Immediately

following the end of the presentation the lights were turned on. The participant was instructed to provide a second saliva sample and then complete a second questionnaire pack. At exactly 20 minutes after the stimulus had ceased, and usually at a point when the participant was just completing the second questionnaire pack, the third saliva sample was provided. The experiment ended and the participant was fully debriefed.

Salivary Analysis

Salivary Flow Rate: All samples were removed from the –80°C freezer and thawed for $1^1/_2$ hours before being centrifuged at 1500RCF (3000rpm) for 10 minutes (ALC55 Centrifuge, CWSystems). For the Salivettes the insert tubes and cotton wadding were removed. Salivary flow rate was determined gravimetrically, using analytical scales accurate to four decimal places.

Salivary cortisol: Dickerson & Kemeny (2004) found that cortisol measurements taken 21-40 minutes after a stress event began were significantly greater than any other time of assessment and refer to this as the 'peak' period (p. 368). Consequently salivary cortisol levels were only assessed at baseline and the recovery time of 20 minutes after the presentations. During initial laboratory analysis of salivary cortisol the first four plates failed due to very high variability in the standards and unknowns. This resulted in the loss of a substantial amount of saliva samples. A brief investigation revealed that the automatic plate washer (at the recommended settings) was introducing the error. It was identified that the wells were not treated identically with wash solution (in amount and pressure) and so a hand-washing system was introduced. This involved shaking the contents into a container and pipetting 360µl of wash buffer, Phosphate buffered saline (Disodium hydrogen phosphate (3.34g), Monosodium Phosphate (1.14g), Sodium Chloride (17g) with 2 litres ultrapure water and 1 millilitre of tween), into each well and then shaking empty again for a total of three cycles.

Salivary cortisol was analysed following IgA as cortisol has been reported to be stable for two freeze-thaw cycles (Gröschl, Wagner, Rauh & Dörr, 2001). This was analysed in duplicate via high sensitivity cortisol Enzyme-linked immunosorbent assay (ELISA - Salimetrics, USA). This assay follows the competition principle - the cortisol levels from 25µL of known standards (1.8, 0.6, 0.2, 0.067, 0.022, 0.007 ng/ml) and the unknown test samples compete with cortisol (conjugated to horseradish peroxidase) for the antibody binding sites of the microtitre plate wells, which are coated with anti-cortisol rabbit antibodies. After a 55-minute incubation period at 23°C, the competition was halted and the plate was washed by hand. The substrate tetramethylbenzidine solution was added to react to the bound cortisol peroxidase and incubated at room temperature for further 25-minutes. Following this period the stop solution was added (2M sulphuric acid) and the optical density was read by a MXR_{II} plate reader (Dynex Technologies, Chantilly) at 450nm with a reference at 490nm. The concentration of cortisol (µg/dl) was determined by first subtracting the average optical density of the non-specific binding wells from those of the standards and unknown test samples (the non-specific binding wells contained no antibodies), then the percent bound was assessed by dividing these figures by the average of the zero wells. The 4 parameter sigmoid minus curve fit, utilising a series of six standards (in a synthetic saliva matrix), was calculated by the Revelation software (Dynex

Technologies, Chantilly) to determine the cortisol concentration of the unknown test samples. Two internal controls (high at 1.052 and low 0.103 µg/dl) were run on each plate, and all were within the manufacturer's recommended pass range. For any unknown sample with a CV above 10% both samples were re-run (n=5). The sigmoid curve was applied to the standards (0.012 to 3.0 ng/ml) and each r^2 value was >0.99.

Salivary IgA: An in-house Enzyme Linked Immunosorbent assay (ELISA) was used to measure salivary IgA. The ELISA method is used to detect the presence of antibody in a test sample using two antibodies, of which one is specific to capture the antibody and the other linked to an enzyme that reacts to the antigen-antibody complexes (Engvall & Perlman, 1971). The 96 wells of medium bind plates were coated with 100µl of the polyclonal anti-human IgA antibody (STAR92) diluted at 1:1000 with a carbonate coating buffer of pH 9.6 (Sigma C-3041) and left overnight to incubate at 4^{o}C. This allowed the capture antibody to coat the wells and this dilution factor was pre-determined to provide the most accurate concentration to prevent stacking or limited receptor site availability. There were some minor changes to the protocol during the analysis phase where the plates were initially covered by firm lids and later covered by disposable ones after each phase to minimise carry-over from previous stages. The following day the plate was washed to separate bound and unbound IgA antibodies. To begin the salivary analysis, plates were washed with a plate-washer on fully automatic mode (4 times) with no stop between cycles. However, a number of these (five) failed both internal and external quality controls through the high variability caused by the plate-washer. Consequently, the hand-washing technique was again employed. Following the wash procedure plates were then blotted and dried. A total of nine IgA standards (using Sigma l1010 human secretory IgA) were prepared ranging from 10ng/ml to 350ng/ml, and the sigmoid curve was applied to the data. All unknown saliva samples were initially diluted to 1:1000 with assay diluent (3% BSA in wash solution). If this dilution factor did not bring the unknown sample values within the range of the standards, then different factors were applied and the final multiplication level adjusted. Two external control samples were also created and stored in the –80^{o}C to be run on each plate to test interplate reliability. Each well received 100µl of the appropriate standards, controls or unknown samples in triplicate. The plates were covered, incubated and mixed with a desk-top shaker at 37^{o}C for one hour. They were then washed. The conjugate again changed slightly over the course of the saliva analysis with a Sigma conjugate diluted with AD to start, but then due to depleted stock this changed to STAR92P. The conjugate was added at 100µl to each well, following which the plates were again incubated and shaken for a further hour. After a third wash procedure 100µl of TMB (Pierce) was added to each well and shaken at room temperature for 3-minutes. The plate was finally incubated at 22^{o}C (LKM incubator) for 15-minutes, after which 100µl of stop solution (either 2M sulphuric acid or a combined 1M sulphuric acid with 8M acetic acid) was pipetted into each well. After 3-minutes of shaking, each plate was read at 450nm with a 490nm reference. With implementation of the hand-wash procedure all plates conformed to the acceptance criteria of a CV less than 10% between triplicate repeats, and the standard correlation coefficient was greater than 0.98. External inter-plate controls (two control saliva samples) were examined and did not differ more than 10% between plates.

Results

Statistical Analysis: Data Assumptions

Unless specifically stated, the data sets were reasonably normally distributed, and met the homogeneity of variance assumptions in both the EXPLORE function and as Levene's test for equality of error variances in ANOVA. Where the sphericity assumption was breached, the multivariate table was consulted following the recommendations of both Howell (2007) and Pallant (2005). If normality tests showed significantly skewed data, when the direction was the same for all groups and the sample size ratio (largest/smallest) was less than 1.5, an ANOVA was also considered acceptable (Stevens, 1996). For two sample comparisons, the data (or difference scores for paired samples) were normal and did not breach the homogeneity of variance.

Personality, Demographics and Baseline States

There were no differences between the real, fake and neutral groups in locus of control and baseline stress states. Scores were averaged between the appraisal groups (real, fake and neutral) and analysed by oneway ANOVAs, which revealed no significant differences in any of the trait variables or stress states between the conditions. There were also no differences in average age and Chi-square analysis found no significant gender differences. These results enabled more confidence in the appraisal manipulations producing any later results rather than individual differences prior to testing.

Hypothesis One: The participant's rating of emotional control over disgust images can be altered by the images being described as either real (real events, wounds and diseases) or fake (special effects and make-up).

Belief Ratings

To ensure that the images were perceived to be real or fake in nature, each participant was asked to mark a percentage of the images (0…25…50…75…100%) they believed to be real and then fake in two scales. These ratings were compared via paired Wilcoxon tests within each appraisal (real and fake) group. In the real appraisal condition, the images were perceived to be significantly more real than fake ($Z=-4.78, p<0.001$). The images were not significantly believed to be more fake than real ($p>0.3$) from the fake-appraisal group. However, when each scale was compared by Mann-Whitney U tests between the real and fake groups, there was a greater score of the perception that the images were real from the real appraisal group ($Z=-4.83, p<0.001$) compared to a higher rating of fake from the fake appraisal group ($Z=-4.62, p<0.001$). What these results mean is that although the images were perceived to be real in the real appraisal group and believed less as fake in the fake appraisal conditions, they were rated with significantly different belief ratings where it mattered between the groups – those told the images were real, perceived them to be more real than the group told they were fake. In the fake appraisal condition participants rated their belief of the images to be fake as significantly higher than participants in the real appraisal condition. This meant a successful manipulation of the image appraisals, where more images described as fake were believed to be

unreal. This meant that emotional control could be assessed in terms of the affects of the appraisal manipulations, to test the first hypothesis.

Emotional Control

Psychometric data were collected regarding the level of emotional control during the disgust presentations. An independent t-test between real and fake images was significant ($t_{(93)}$=-2.74,p=0.007). This indicated that emotional control was higher in the fake-appraisal (mean = 4.76, SD = 1.01) compared to real-appraisal condition (mean = 4.14, SD = 1.16) and supported the first hypothesis.

Hypothesis Two: The negative psychological reactions (from SACL stress, arousal, and disgust) will not differ from this appraisal manipulation of the images as real and fake.

Emotions

To check the experimental stimuli had induced a state of disgust, and a high neutral rating from the neutral-image stimulus, the emotions experienced were assessed via the Differential Emotions Scale (modified) and Emotions Scale. Both disgust groups were amalgamated and compared to the neutral-image group. These emotion category ratings were analysed via ANOVA, followed by simple contrasts comparing the specific rating of disgust/neutral to the other categories. ANOVA showed significant main effects for condition (disgust versus neutral) which meant that an overall higher average rating of emotion was found in the amalgamated disgust group compared to the neutral-image group ($F_{(1,118)}$=42.5,p<0.001,eta^2=.27). Additionally, the emotion category main effect was also significant ($F_{(8,109)}$=23.17,p<0.001,eta^2=0.63), indicating higher emotion ratings for one or more categories. A significant interaction ($F_{(8,109)}$=19.97,p<0.001,eta^2=0.59) was due to a higher disgust rating for the disgust groups. Removing the neutral-image condition from the simple contrasts found that the rating of disgust was significantly higher than most other categories (all p≤0.001), except interest (p=0.63) or surprise (p=.18).

The emotion scale results showed a similar pattern with significant main effects for condition ($F_{(1,118)}$=16.69,p<0.001,eta^2=.13), and for the main effects of emotion category ($F_{(7,109)}$=14.88,p<0.001,eta^2=0.49). Again a significant interaction ($F_{(7,109)}$=17.50,p<0.001,eta^2=0.53) was observed. For the disgust rating, all simple contrast comparisons were significant (all p≤0.001). For the neutral-image condition only, where the neutral rating of the Emotion Scale could be assessed all simple contrasts were significant (all p<0.03), suggesting the neutral images elicited a higher rating of neutral than any other emotion category.

It was necessary to assess whether the real versus fake appraisal altered the emotional experience of the presentations. These analyses were limited to the disgust groups and found that both the Differential Emotions Scale and emotion scale ANOVAs showed only one significant result in the main effects for emotion category (all p<0.01) which can be explained by the results above (i.e. disgust rated higher). These results indicated that the appraisal manipulation of believing the images to be

either real or fake did not affect the emotional response of the presentation experience – both groups were equally disgusted, fearful, surprised, etc.

Stress and Arousal

It was also necessary to examine the subjective states of stress and arousal. The neutral group were excluded from the ANOVA which was run with the factors of appraisal (real versus fake) x stress assessment time (from baseline to after the presentations), see **Figure 2**. Only one significant main effect for time was discovered ($F_{(1,93)}=50.26, p<0.001, eta^2=0.35$). Subjective stress was significantly increased from baseline in both disgust groups ($p>0.2$) and was unaffected by the image nature ($p=0.10$). The arousal ratings did not change from baseline for either of the disgust groups (all $p\geq0.09$). These results suggested that the disgusting images induced a significant rise in subjective stress. This meant that the emotional experience induced could be described as being subjectively stressful. Furthermore, the disgusting images viewed as fake caused the same psychological response as those seen to be real.

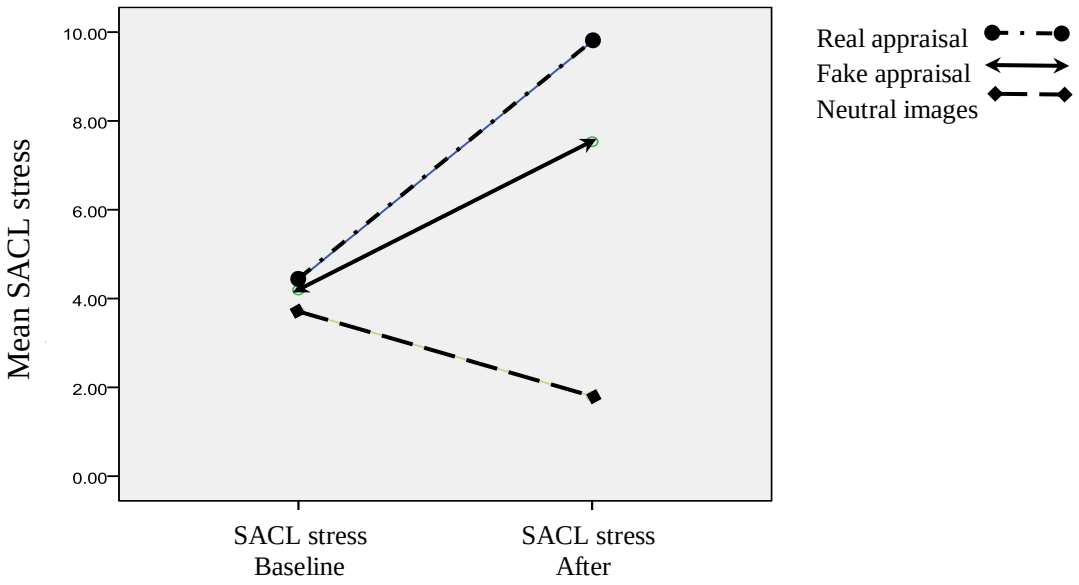

Figure 2: Mean SACL stress ratings from baseline to after the stimulus separated by appraisal group and neutral image condition.

Hypothesis 3: There will be a different physiological reaction to the disgust images from the real and fake appraisals of the images.

Flow Rate

Fifteen participants did not produce enough saliva to be weighed at one of the three collection times. The frequency of these missing samples was analysed via Chi-square and was not significantly different between the three experimental groups (all $p \geq 0.68$). Salivary flow rate across the three collection times was not affected by appraisal condition ($F_{(2,102)}=0.80, p=0.45$) nor changed over time ($F_{(2,204)}=0.23, p=0.79$). These results indicated that salivary flow rate was not affected by disgusting images appraised as either real or fake, or neutral images. It is important to note that when the collection device was added into ANOVA as an extra between-subjects factor these results did not change and it did not interact at any level.

Salivary Cortisol

The salivary cortisol data suffered a number of laboratory problems which left 74 samples with both baseline and recovery (20 minutes after) results. Repeated ANOVA was run with the two collection times (baseline and 20-minutes after) and appraisal groups (real versus fake, neutral) as the factors. This test showed only one significant main effect result with a decrease in cortisol between the two collection times ($F_{(1,71)}=6.32, p=0.014$, $eta^2=0.08$). There was no effect from appraisal condition ($p=0.35$) and no interaction ($p=0.24$). Again the addition of saliva collection technique as a between factor did not interact with these findings. A Spearman's rho analysis determined no relationship between cortisol change (baseline minus the later sample) and the rated emotional control score ($r_{(74)}=-0.05, p=0.7$).

Salivary IgA Secretion Rate

There were numerous problems with the IgA data from the Salivette samples of this study. Insufficient saliva volume was extracted from 13 participants and the initial plate washer procedure caused disastrous effects on the ELISA and too much of the IgA data was lost. Consequently, the IgA results are limited to the data available from the spitting method of saliva collection.

Five participants did not have data available at all three collection times and the remaining data for the salivary IgA secretion rate was examined by an appraisal x collection time ANOVA. This analysis showed one significant result in an interaction between appraisal and time across the three collection points ($F_{(4,72)}=2.79, p=0.033, eta^2=0.13$), see **Figure 3**. Separate repeated ANOVA analysis for each condition revealed a significant change in secretion rate for the real-appraisal group only ($F_{(2,15)}=7.19, p=0.003, eta^2=0.32$), and post-hoc analysis determined a significant increase from baseline to immediately after ($t_{(15)}=-3.32, p=0.005$) the presentation followed by a significant decrease 20 minutes later ($t_{(15)}=2.76, p=0.014$) back to baseline levels ($t_{(15)}=-0.94, p=0.36$). Salivary IgA secretion rate was not affected by neutral images ($F_{(2,14)}=1.05, p=0.38$) or disgust images appraised to be more fake in nature

($F_{(2,28)}$=1.56,p=0.23). Spearman's correlation between the Emotional Control scale score and secretion rate change from baseline revealed no significant relationship within an amalgamated disgust group of both real and fake appraisal groups ($r_{(30)}$=-0.22,p=0.23). Though in the opposite predicted direction, the graphical depiction and statistical results determined a differential effect of appraisal on salivary IgA secretion rate.

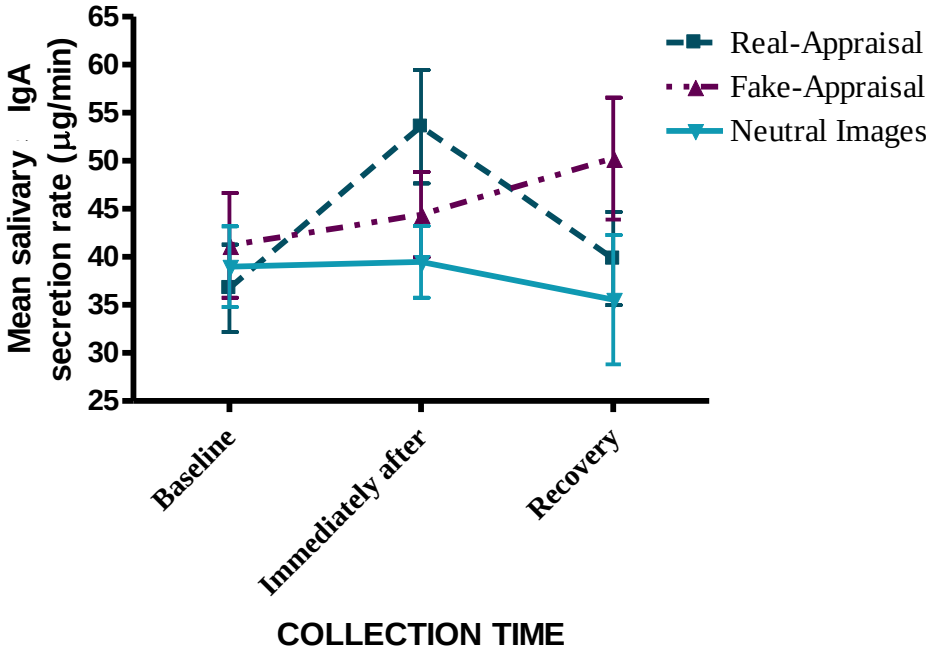

Figure 3: Mean (+/-SE) salivary IgA secretion rate from the *Disgust Spitting* study across the three collection times and separated between the appraisal (real, fake and neutral) conditions.

Discussion

A real appraisal (participants told pictures were of real surgery, disease and wounds) of disgust inducing images created an increased salivary IgA level that was absent following the same images appraised as fake (participants told the pictures were special effects and unreal), and a set of neutral images. The real-appraisal of these images also produced a significantly lower rating of emotional control when compared to the fake appraised images. While this latter finding supported the first hypothesis, the increased salivary IgA result after the real-appraisal of disgust images, and no change from the fake-appraisal of the images, did not support the second hypothesis. Furthermore, there was no statistical association between the emotional control rating and salivary IgA reactivity.

These results suggest that an appraisal of disgust images can alter a state of emotional control felt during an image presentation, and can also cause a different IgA response to the same set of images. This is despite the fact that a state-stress rating (from the SACL) increased to a similar level following the two appraisals (real versus fake). These results suggest: 1) an emotional state of disgust may not

be the "passive coping challenge" it has previously been classed (Bosch et al, 2001), and 2) emotional control over disgust may not be the mediator of a salivary IgA response to this emotional state.

Disgust as a 'Passive' Stressor

In relation to the previous literature regarding disgust and salivary IgA, this research has at least established that a presentation of 'gruesome' images can significantly induce psychological states of stress, disgust, interest, and surprise. Furthermore, these emotion ratings were unaffected by the appraisal of the images as being either real or fake. While the induction of the three emotional states (disgust, interest and surprise) means that the results can be less clearly defined in terms of disgust alone, the results are important in terms of the standing of a disgust stimulus as a stressor. The images significantly induced a rise in the subjective state of stress (not arousal) that was similar for both real and fake appraisal groups, and so disgust images can at least be classified as a psychological stressor. However, the significant increase in salivary IgA following a real appraisal, that was absent in those who viewed identical images as more fake or those who saw neutral pictures, discourages a categorisation of disgust as a passive coping stressor. Indeed, the salivary IgA increase was in the opposite direction from those predicted, and it adds to the slowly accumulating evidence that puts the Bosch et al (2001; 2002) literature in the minority with their reported salivary IgA decreases.

Disgust and Emotional control

Gross (1998; 2002) suggests that the process of emotion regulation to an event requires the event to be recognised as an emotional cue, making specific reference to "cognitive control of emotion" (Gross, 1998) strategies, that he investigated in relation to a disgust film. However, Gross did not specifically assess whether the appraisal process explicitly involved control. The Emotional Control scale used in this thesis was developed to assess the potential cognitive strategies that may be adopted to control the reactions to an emotional stimulus. In support of the validity of this scale, it was seen that a higher rating of emotional control was significantly associated with lower levels of disgust and stress following the image presentations. Participants reporting themselves as more in control over the experience did in fact rate the individual negative responses at a lesser level.

The average rating from the Emotional Control scale was significantly higher from those who were told the disgust images were fake and unreal, suggesting that a more fake appraisal of the picture content enabled participants to gain a sense of control over the emotional reaction to the images. However, this was not seen in the subjective ratings of stress or disgust as these were increased to a similar level following both the real and fake appraisal of the images. Appraisal processes over a disgust stressor do not affect the feelings of disgust and stress felt towards the images, but do increase the perceived control the viewer feels they have over their emotional reaction.

Stress, Emotional control and Salivary IgA

The manipulated appraisal of disgust did not change the rating of the images as a stressor, with similar significant increases in stress (SACL) from both groups. However, the appraisal of the images altered the emotional control rating and salivary IgA was significantly increased following the lower emotional control rated stimulus of real appraised images only. The SACL stress rating was not associated with the IgA increase from the real appraisal group as this stress rating was similarly increased after the fake appraised images, where salivary IgA did not change. While the significant difference in emotional control suggested this psychological construct had the potential to impact on the salivary IgA response, there were no significant correlational findings. This suggests that appraisal over a disgust stressor may influence its effect on salivary IgA, but is more complicated than merely a change in emotional control felt during the state of disgust and may extend further than the cognitive control strategies that were assessed by the scale constructed for this research.

The biological stress response of cortisol was not affected by either stress or emotional control during the state of disgust. In fact, the cortisol results showed a decrease following the induction of disgust (with interest and surprise) from both appraisal groups, which partially parallels findings from Hennig et al (1996). To be more specific, there was an overall significant decrease in salivary cortisol following all image presentations (disgust and neutral) which replicated those of Nykliček et al (2005), who reported a significant decrease in cortisol following a disgusting video clip (in addition to one showing a stressed mechanic). Yet these researchers did not compare this to a control condition and provided no psychological data. These cortisol results could be showing the natural diurnal pattern of cortisol secretion, as it declines over the course of the day.

While the subjective ratings of stress increased, the classic 'stress response' of HPA activation was absent, which indicates that a state of disgust is not a HPA stressor. Following from prior research, it is suggested that an increase in salivary IgA could be a result from differentiated SAM axis activation from the real appraisal group. Where salivary flow rate was not different between the three groups (real, fake and neutral images), but salivary IgA increased in response to the real-appraisal of disgust, it could suggest that real appraisal induced a greater sympathetic activation than a fake appraisal or viewing of neutral images. This follows from the Carpenter et al (1998) study where both sympathetic and parasympathetic nerve mediation "increased the movement of IgA into saliva" (pg. 572) yet there was a significantly greater secretion rate following sympathetic activation. The absence of an effect on saliva flow rate would suggest a limited parasympathetic response as this branch of the nervous system has been related to a much greater increase in flow (Carpenter et al, 1998). Again this would indicate that disgust is not the "passive coping challenge" it has been classed under as the required parasympathetic activation could seem to be absent. It would be interesting in the future to incorporate the measurement of alpha-amylase from saliva as an index of changes in plasma noradrenaline (Chatterton, Vogelsong, Lu, Ellman & Hudgens, 1996) to assess the potential influences of SAM axis activation during the appraisal of real and fake disgust stimuli. Though whether such potential differences are driven by emotional control remains an open question.

However, there are other factors to discuss before emotional control is disregarded as a potential mediator of salivary IgA effects, as this rating was the only one to be different between the real and fake appraisal groups. Therefore, the following discussion will provide some potential explanations for the lack of significant association between emotional control and salivary IgA.

It is possible that the basic emotion of disgust may have survival properties (i.e. the rejection or avoidance of harmful substances) strong enough to negate an impact of higher-order cognitive effects (potentially such as emotional control) on the mucosal defence for oral and digestive protection. This may explain why the biologically protective mechanism of salivary IgA increased in response to the disgust events appraised as real only. Disgust has a gustatory emphasis and its adaptive evolutionary past means that it protects the individual from ingesting any pathogen and protects against disease contamination (Haidt, McCauley & Rozin, 1994; Rozin & Fallon, 1987). It has been suggested that disgust's primary function may be to act as a guardian of the mouth (Haidt, Rozin, McCauley & Imada, 1997) and is linked to the rejection of "bad taste" with a consequence of nausea (Davey, 1994). This is one possible explanation why the mucosal defence of salivary IgA may increase in response to a potential infection from rotting food through to the touch of a contaminated agent and its pathway to the mouth. Images perceived as real may have a greater threat relevance that initiate mucosal defence properties, while fake images are not real events that do not have the potential of contagion. However, given that flow rate was not affected by the images of any type (disgust or neutral) it could diminish this possibility. Salivary flow rate is increased with nausea (Olatunji & Sawchuk, 2005) and is the vehicle for many immunological indices in addition to IgA, including lysozyme and lactoferrin. It seems unlikely that real appraisal differentially activated a mucosal defence over a fake appraisal as salivary flow rate was not changed in any of the three groups, yet it may be interesting to examine other mucosal mechanisms to examine this prospect in more detail.

Another potential explanation could involve novelty. A definition of novelty states that it is unfamiliar, represents new information, and depends entirely on the past experience of the individual (Webb, 1999). Through our experience with media gore we may have learnt to become desensitised to fake portrayals of blood and injury, which could mean that less experience with real images may initialise a greater novelty induced increase in IgA from the real appraisal group. It has been reported that novelty can cause increases in salivary IgA (Willemsen et al, 2000; Burns et al, 2004). Novelty is when a discrepancy occurs from what we expect of our environment and what actually happens, and when this is great enough then we become surprised (Webb, 1999). Another aspect to novelty has also included interest (Cassidy, 1997). Thus novelty could be partly assessed via surprise and interest. The Differential Emotions Scale used in this thesis had both a 'surprise' and 'interest' sub-scale. These were analysed post-hoc with independent t-tests to examine a potential difference in novelty between real and fake appraisals of disgust images. Neither of these were significantly different for this comparison ($p=0.42$; $p=0.76$ respectively). However, both of these states were repeatedly significantly increased together with disgust (for both real and fake appraisals), which could suggest that the negative emotion of disgust may not be elicited alone and may be a novel event irrespective of whether the images are perceived as being real or fake in content. When other disgust-inducing stimuli have been rated both interest and surprise are also reported to be high (Valentiner, Hood & Hawkins,

2005). In the future one possible way to minimise a surprise reaction may be to use a repeat technique. For example, participants could be shown the images described as real or fake at a time prior to testing and shown them again later when physiological responses are measured. This would enable a more definitive testing of whether the results were due to emotional control, or novelty, as currently it is somewhat difficult to do.

In summary, the differential effect of real versus fake appraisal of disgust images on salivary IgA reactivity is not clearly attributable to the emotional control felt during the stimulus presentation. Where other possible causes have been discussed, such as the rejection of a harmful pathogen or the potential that real images are more novel, there is still no clear conclusion that can be drawn as to why the real appraisal of disgust images induced a significant increase in salivary IgA. Yet it is interesting that emotional control was significantly lower from the real appraisal group and so it should be addressed that some methodological factors may have overshadowed a relationship between control and salivary IgA reactivity to disgust.

Study Limitations

A potential confound may arise from the procedure where participants were run alone for the first half, but due to time restrictions during the second half (when the spitting-method replaced Salivette collection) they were run in groups of three. An issue of audience effects should be mentioned, where the presence of other participants may alter the control experience of viewing the images. Without assessing such factors directly it's impossible to determine but it seems plausible that other people may have provided support that could affect biological reactions to stressful events (Christenfeld, Gerin, Linden, Sanders, Mathur, Deich & Pickering, 1997). The fact that this corresponded to a change in saliva collection technique should also be mentioned. Here the significant effect of appraisal on salivary IgA was discovered from the samples collected via the spitting-method. Bosch et al (2001) employed the spitting-method of saliva collection and reported significant decreases in salivary IgA secretion rate, while Hennig et al (1997) and Farley and Bristow (2003) used Salivettes and discuss significant increases. Though Bosch et al (2003) did not record IgA they do report significant increases in the salivary proteins of MUC7, lactoferrin, and total protein output in response to both an active timed memory task and the passive surgical video when compared to a neutral video condition. The spitting-method used during this chapter also displayed an increase (restricted to the real-appraisal condition) in IgA secretion rate with no sign of a decrease at all. This suggested that the method of collection was not a factor in the salivary IgA response to disgust.

The results are also not attributable to the stimulus characteristics as the two prior studies reporting increases used film-clips. This thesis contained the only studies to utilise static images and was the only design to show that disgust had actually been induced with a concurrent increase in subjective stress. Yet there are some issues with the stimulus. Firstly, with hindsight it may have been better to use the pilot study to determine which images gave the best range and spread of real and fake ratings. Where the images were not significantly believed to be more fake than real from the fake-appraisal group, this different approach could have allowed the images to have been more easily perceived as

being either real or fake in the main study. However, the crucial difference did exist in belief ratings between the appraisal groups. Secondly, there could be differences due to the stimulus length. The Bosch et al study used an 11-minute film, while the image presentations of this chapter were four and a half minutes long. However, the footage used by Farley and Bristow (2003) involved a sequence of fictional clips that lasted 12-minutes and they also reported significant increases. The impact of stimulus length does not seem to be a factor. Yet a further design-related explanation comes from gender differences. While it was important to ensure a similar gender ratio between the groups of each study, what had gone unnoticed was a larger number of males participating in the first half of the study compared to the overall female dominance of the second, where the spitting-method saliva samples were used for analysis. As Drushel and Sherman (1999) have reported females are significantly more "disgust-sensitive" than males. However, this influence was addressed by a dissertation project (Farley & Bristow, 2003), which found no gender differences within an increase of salivary IgA to fictional film-clips used to induce disgust.

Summary and Conclusions

In summary, the increased salivary IgA result from the real appraisal of disgust images has questioned whether the emotional state of disgust has been classified correctly under the passive sub-division of acute stress. The results have shown the potential that appraisal processes can significantly impact the physiological reactivity to the same passive stimulus. Specifically to disgust, an appraisal effect was seen on both emotional control and salivary IgA reactions to an identical stress stimulus. However, these effects may have occurred independently and may have some underlying cause that is yet to be identified, such as novelty. Consequently, the conclusion regarding the relationship between control and salivary IgA is left an open but still interesting question, and one that was to be addressed during a different type of stressor, a chronic stress situation, where control may act in a different manner over salivary IgA during a longer time-frame.

3

Caregiving: Perceived Stress, Appraised Control and Salivary IgA

> "Clinically, caregivers are considered to be subjected to chronic stress and serve as good models to study chronic stress in humans" (pg. 159, Plotnikoff, 2007).

The experience of caregiving was used to examine whether appraised control may interact with stress to mediate salivary IgA secretion rate during a chronic stress event. A common way to explore stress and salivary IgA has been to use between-subjects correlational designs, yet a better approach could be to compare a high stress group with a low stress group. This chapter examines the relationship between appraised control, perceived stress, health, and salivary IgA in individuals who care for a partner with a debilitating illness, such as dementia and multiple sclerosis.

There is an increasing trend for people with deteriorating health to be cared for at home by relatives (Kinsella, Cooper & Picton, 1998). The term informal caregiving refers to the task undertaken by unpaid relatives or friends who provide direct care for people with a chronic illness. Such informal caregivers are usually spouses or other relatives, such as siblings or children, and home-based care may involve a number of demands made upon the caregiver, such as assistance in daily living tasks. The caregiving situation has often been cited as an exemplar of a chronic stressor (e.g. Bauer et al, 2000; Vedhara, McDermott, Evans, Treanor, Plummer, Tallon, Cruttenden & Schifitto, 2002; Vitaliano, Zhang, & Scanlan, 2003), and is associated with increased ratings of stress in caregivers of parents with dementia (Dura, Stukenberg, & Kiecolt-Glaser, 1991), spousal caregivers of partners with multiple sclerosis (Vedhara et al, 2002), and the more elderly spousal caregivers for Alzheimer disease sufferers (Glaser, MacCallum, Laskowski, Malarkey, Sheridan & Kiecolt-Glaser, 2001). These psychological aspects could show as negative health effects (e.g. Kiecolt-Glaser et al, 1996).

Yet caregiving has been mostly examined with regard to systemic immunity, with indices of lower T lymphocytes (Kiecolt-Glaser, Glaser, Shuttleworth, Dyer, Ogrocki & Speicher, 1987) and NK cells (Esterling, Kiecolt-Glaser & Glaser, 1996; Kennedy, Kiecolt-Glaser & Glaser, 1988), and a dampened response to influenza vaccines (Kiecolt-Glaser et al, 1996). As a collective body, research in this area has led many to conclude that caregiving tends to suppress the immune system (e.g. Kiecolt-Glaser, McGuire, Robles & Glaser, 2002; Segerstrom & Miller, 2004). In other research that investigates stress and mucosal immunity, there is a body of evidence to suggest that chronic stress may diminish levels of salivary IgA (e.g. Deinzer & Schuller, 1998; Jemmott et al, 1983; Jemmott & Magloire, 1988; Mouton et al, 1989). However, the predominance within this literature seems to be a between-subjects correlational design, which does not "properly conceptualise chronic stress" (Bristow et al, 2008, pg. 601) and there seems to be no reason why such groups would experience an ongoing stressful situation, where exam periods last for weeks, for example. It would seem useful to the area to conduct a study that compares a high stress group with a low stress group, and comparing caregivers to non-caregivers certainly seems to fit this category.

Furthermore, despite a general association between caregiving and poor health, research seems to have ignored individual variation, where not all caregivers display a suppression of immunity. A number of studies report no differences between caregivers and non-caregivers in many indices, such as T-Cell counts (Glaser et al, 2001), NK cell percentages (Kiecolt-Glaser et al, 1987) and T

lymphocytes and monocytes (Kiecolt-Glaser et al, 1996). In fact, Vitaliano et al (2003) suggest a need to improve health responses to the caregiving experience by taking individual differences into consideration, where neither stress nor depression has been consistently correlated with immunological measures (e.g. Bauer et al, 2000; Kiecolt-Glaser et al, 1991; 2003). It is also noted that the caregiving research examining stress and mucosal immunity has focussed on dementia caregivers who are an older cohort (Ory, Hoffman, Yee, Tennstedt & Schulz, 1999). These may be a more immune-vulnerable population with regards to salivary IgA, where older individuals may have lower levels than a younger cohort (Miletic et al, 1996), even when they report fewer stressful life events (Phillips et al, 2006). While there is obvious cause to conduct research with the older populations of caregivers, the younger cohorts should not be ignored as their immunity may also be affected by the caregiving experience.

Research has focused on caregivers of those with Alzheimer's disease, with relatively few studies regarding other types of dementia, such as frontotemporal dementia (Bozeat, Gregory, Ralph & Hodges, 2000). Frontotemporal dementia is caused by focal atrophy within the frontal and/or temporal lobes, and mainly affects the middle aged (Snowden, Neary & Mann, 2002). This earlier age of onset may make caregivers more susceptible to the negative effects of the caregiving experience as they are unprepared for such rapid ageing changes from their partner (Hatada, Okazaki, Yoshitake, Takada & Nakane, 1999). To fill a gap in the caregiver and immunity literature, it was the aim of the project undertaken by researchers at the University to examine the impact of frontotemporal dementia caregiving on salivary IgA levels (Bristow et al, 2008). This larger study is from which this research was developed, to examine the potential influence of appraised control in relation to stress and salivary IgA in frontotemporal dementia caregivers.

Furthermore, caregivers of relatives with multiple sclerosis could be examined as they are a younger age group and also have significantly greater levels of stress (Vedhara et al, 2002) and distress (Figved, Myhr, Larsen & Aarsland, 2007). Yet the two groups caring for people with frontotemporal dementia or multiple sclerosis have received very little attention in the psychoimmunology research field, and very few studies have examined the impact of caregiving on mucosal immunity. There was only one within the Vitaliano et al (2003) meta-analysis that assessed plasma IgA levels, which was an unpublished dissertation abstract (i.e. Giefer, 1994). Recently one study has been published that looked at the effects of caregiving on salivary IgA, though the nature of the caregiving situation (e.g. dementia sufferers) was unspecified. Here a significantly lower salivary IgA level was seen in caregivers compared to non-caregivers only in an older cohort (Gallagher, Phillips, Evans, Der, Hunt & Carroll, 2008). Only one study to date has been published regarding the specific effects of frontotemporal dementia caregiving on salivary IgA, which is the larger project from which this research was undertaken (Bristow et al, 2008). This part of the larger project focused specifically on appraised control over the situation of caregiving with respect to stress, health and mucosal immunity, where previous argument has advocated appraised control as a potential cognitive mediator in the stress and caregiver relationship (Vitaliano et al, 2003).

In this thesis, appraised control is the focus of attention with regard to salivary IgA, to examine the potential to reduce the negative psychological, physiological and health related effects of chronic stress. It was expected to show a good relationship to psychometric scales that assess negative states, and may potentially show a relationship to health variables associated with a chronic stressor of caregiving. Furthermore, it was examined whether appraised control could be an aspect of stressor appraisal that could better relate to salivary IgA. Individual differences in appraised control were to be assessed in relation to psychological morbidity and salivary IgA levels during the situation of caring for a relative with either frontotemporal dementia or multiple sclerosis. Based on the prior research and theory, three hypotheses are formed and tested:

1. Caregivers will have lower appraised control than a group of non-caregivers.
2. Caregivers will have lower salivary IgA secretion rates than non-caregivers, which will be mediated by appraised control.
3. When compared to perceived stress (PSS-10), appraised control will show a stronger relationship with negative states (Stress Arousal Checklist), distress (General Health Questionnaire), and general health ratings (RAND Short-Form-36 Health Survey).

Methods and Materials

Participants

Participants were twenty-five caregivers of people with frontotemporal dementia, with a mean age of 63 (SD = 6), belonged to a caregiver support group at the MRC Cognition and Brain Sciences Unit. Each participant was an informal caregiver for a spouse/partner with frontotemporal dementia at home. A control group of 36 non-caregivers also with a mean age of 63 (SD = 5) was recruited from the Cognition and Brain Sciences Unit research participant panel. All participants were paid £15 to compensate for their time. A year later a group of 60 multiple sclerosis caregivers with a mean age of 53.2 (SD = 12) were recruited via adverts printed in a multiple sclerosis support magazine. Most were spousal/partner caregivers though some were other relatives (e.g. daughter). Three individuals with Frontotemporal dementia had gone into nursing homes at the time of the study. The frontotemporal dementia investigation was approved by the National Research Ethics Service (NRES) while the multiple sclerosis project was approved by Anglia Ruskin University Research Ethics Sub Committee (RESC).

Materials

To investigate appraised control during the chronic situation of caregiving a literature search was performed to examine existing questionnaires. While there are different scales to measure consistent and trait-like concepts of control (such as Locus of Control Scale, Rotter, 1966; Generalised Self-Efficacy Scale, Tipton & Worthington, 1984) there are fewer that assess more state-dependent aspects of control. This state aspect was the focus of the investigations here as the aim was to examine the impact of appraised control on salivary IgA, during a chronic stress situation. It was necessary to apply the concept of appraised control to the specific situation of caregiving and the questions of such

a scale needed to reflect this. Therefore trait aspects of control (e.g. "I am a very determined person" from the Generalised Self-Efficacy Scale, Tipton & Worthington, 1984) were not situation-specific to the chronic stress of caregiving.

The existing state-control scales were again unsuitable for use here as none assessed the specific events that may occur during everyday caregiving duties. Rosenbaum's (1980) Self-Control Schedule addresses an individual's trait towards emotional and psychological control, the way in which they use self-control behaviours and thoughts to regulate internal reactions to everyday life and events (e.g. "When I am feeling depressed, I try to think about pleasant events"). It also measures problem-solving strategy use, gratification delay, and perceived self-efficacy. None of these were relevant as they were too general to be of use for a caregiver situational measure of appraised control. Pallant (2000) has also criticised this scale for being biased in the focus of the concept of control to the person's behaviour as a solution to a problem and ignoring emotional reactions. The Negative Mood Regulation Scale (Catanzaro & Mearns, 1990) was limited by a focus on the behavioural reaction to negative states without consideration to control over the environment. In fact, the scale does not assess direct perceptions of control at all, rather behaviours seen to be effective at regulating negative mood. The Perceived Control Scale (Nayyar, 1993) addresses control over environmental factors and again refers to a general disposition of control. The Perceived Control of Internal States Scale (Pallant, 2000) was created to record the perceived level of control over an individual's internal state, which would moderate the effects of aversive situations on mood, cognition and physical well-being. While this test initially seemed of interest, it was noted that the scale was designed with a clinical setting in mind - the author describes its intentions as a "way of monitoring the effectiveness of therapeutic interventions" (p.213). It also reflected a generalised belief in the control the participant perceived themselves to have over their internal states. There is no appropriate measure that addresses the concept of appraised control, one that assesses situational factors and more specifically the appraisal of events in everyday life as being controllable or not. As a consequence, a scale was constructed to encompass aspects of control dependent on daily and situational events.

Control Questionnaire Construction

According to Folkman and Lazarus (1980) any stressful event will be evaluated in relation to both the physical actions required and the emotional reactions. In research regarding "hardiness" (the trait of individuals who experience high stress but do not become ill), three aspects of control have been hypothesised to diminish the detrimental effects of stress - decisional control, cognitive control, and coping skills (Kobasa, 1985). Overall, there seem to be two main divisions regarding stress and control of Primary and Secondary control (Rothbaum et al, 1982) and these guided the overall construction of the items for this Appraised Control scale.

Primary control: This refers to an individual's environment and physical requirements of the situation, and is linked to the behavioural component of Wallston et al's (1987) definition "the belief that one can determine one's own … behaviour (and) influence one's environment " (p.5). This construct deals with the perceived ability of the individual to choose a course of action or behaviour to

deal with the situation. Questions relating to Primary control were created to assess the appraisal of control over the environment, events, time, and own behaviour. They were created by expanding aspects of items from other control questionnaires. For example, the Perceived Stress Scale (PSS-10) item "How often have you felt that you were unable to control the important things in your life?" was given more detail to be specific about things that may be affected by the caregiving task, such as changes in planning own time, predictability of daily events, and general routine. These questions are shown below:

1. Sometimes I feel that there are too many events in my life in which I have no control over.
2. I have command over most aspects of my life.
3. Fate usually conspires against me.
4. My time is my own to organise as I please.
5. My days are filled by unpredictable situations.
6. I have the ability to plan and organise my day so that I have time for myself
7. I feel that I have enough time for my friends and family.
8. I feel a great loss of control in my daily life.
9. I feel that I can control any unpredictable situation that occurs in my life.
10. I have the time for the things that I enjoy doing.
11. My life is controlled by other people.
12. Uncertainty dominates my day.
13. I have a successful routine to my day.
14. When I wake in the morning I know how my day will go.

Secondary Control: This second widely adopted feature of appraised control is more cognitive in nature and represents control over thoughts, appraisals (Kobasa, 1985), and emotions (Wallston et al, 1987). Also associated with the response-directed control of Neufield and Paterson (1989), this aspect of control concerns the emotional and cognitive reactions to a stressful event. Kobasa (1985) has suggested that cognitive control represents the capability of an individual to interpret stress as part of a greater life-plan, which thereby diminishes its negative impact. Questions were created to measure the 'Secondary control' associated with the emotional and cognitive reactions to stressful events.

15. When upsetting events occur I am capable of staying calm.
16. I let my emotions get the better of me.
17. I cry myself to sleep
18. I am happy
19. I find myself repressing my emotions for the good of other people.
20. It is hard to make new friends.
21. I have ways to relieve the stress and strains of my day.
22. I have trouble sleeping.
23. I find it difficult to concentrate on tasks that need my attention.
24. I feel helpless.

Six additional questions were created to directly tap into the appraised control of the caregiving experience. Within this field, review studies have suggested that the demands associated with the caregiving experience can also be separated into two categories (e.g. Kinsella, Cooper, Picton & Murtagh, 1998; Lim & Zebrack, 2004). The first are the physical demands of the care experience or primary stressors (Lim & Zebrack, 2004). These include help with daily living tasks (like meal preparation or transportation), the duration and intensity of care, and problem behaviours from the care recipient. Secondary care stressors involve the emotional demands placed on the caregiver when

dealing with the concerns of the recipient as well as their own distress (Kinsella et al, 1998). The following six items were created to directly address these types of specific caregiver demands.

a. When something unexpected happens I find I have the ability to change the outcome.
b. Outbursts from others around me cannot be influenced and I leave them to run their course.
c. Due to my responsibilities I am rarely able to do what I want.
d. If erratic behaviour occurs from people around me I manage to calm the situation immediately.
e. When my environment becomes turbulent and uncertain I take a step back and assess the best way to handle the situation.
f. I feel that I'm blamed too often for other people's behaviour/actions.

This rating scale (see *Appendix I* for the final layout and instructions) was created with 30-items to measure appraised situational control from the two caregiver groups. The measurement scale used a five-point scale from 1 = never to 5 = always for each item, with half the items in a reverse scoring format.

Reliability Analysis: The caregiver scale was completed by a total of 120 participants (25 frontotemporal dementia caregivers, 60 multiple sclerosis caregivers, and 35 non-caregivers). This sample statistics provided excellent reliability with Cronbach's alpha being 0.93. Internal consistency was also very good using the split-half method (odd versus even items) with Spearman-Brown coefficient at 0.92. Statistical tests of normality were run on the scale, and Shapiro-Wilk's value was not significant ($p=0.071$) which meant a normal distribution of the appraised control scale score. However, the near to significant value warranted a further assessment, where the scale was examined by a caregiver (n=85) to non-caregiver (n=35) group split. Here, the scale response remained as a normal distribution for caregivers ($p=0.23$) but became significantly and negatively skewed in response by the non-caregiver group ($p=0.05$). This may be related to the fact that six of the scale's questions are directed to actions and behaviours specific to a caregiving role, where the non-caregivers would all respond to such questions in the same way to squash the spread of ratings into one end of a normal distribution.

Construct Validity: It was possible to assess construct validity by correlating the Appraised Control scale with other instruments accepted as standardised measures of similar concepts. Rotter's (1966) Locus of Control scale has been recognised as measuring a general tendency to perceive rewards in life as being controllable or not. The Perceived Stress Scale-10 (Cohen, Kamarack & Mermelstein, 1983; Cohen & Williamson, 1988) (PSS-10) assesses elements of a general stress state with one aspect being controllability (e.g. "How often have you felt that you were unable to control the important things in your life?"). Consequently it was predicted that the caregiver appraised control scale would correlate with these two questionnaires, yet would display discriminant validity with the separate constructs not being so correlated to suggest that they measure exactly the same underlying concept. The data for all 120 participants were calculated by Pearson's correlational assessment and displayed in **Table 3**.

Table 3: Pearson's correlations to examine the Caregiver Appraised Control scale construct validity in concurrent and discriminant validity.

	Perceived Stress Scale-10 (PSS-10)	Rotter's Locus of Control Scale
Appraised Control Scale	-0.76, p<0.001	-0.27, p=0.003
Perceived Stress Scale-10 (PSS-10)	- -	0.22, p=0.02

The results in **Table 3** showed that the Appraised Control scale was significantly negatively correlated with Rotter's Locus of Control scale which suggested concurrent validity. The negative direction was confirmatory in that the more external an individual's trait (control in life from other people, fate and chance) the less appraised control they rated over their situation. Given that stress controllability is one part of the PSS-10 scale it was to be expected that there were significant negative correlations with the PSS-10, meaning that people with a low appraisal of control also scored higher on this stress scale. The PSS-10 was correlated to the Locus of Control scale at a similar level to the Appraised Control scale. The more external an individual scored on the Locus of Control the higher they rated their perceived stress over the past month. Where an individual believed life is generally more controlled by other people and fate they had a higher stress score, so here low personal control was associated to high perceived stress. Both scales were related with the trait Locus of Control of the individual and both correlate well to each other. While this classes the three scales into a similar category, the PSS-10 contains only three explicit references to control in its items, and is said to assess how "unpredictable, uncontrollable, and overloaded respondents find their lives" (pg. 33-34, Cohen & Willliamsen, 1988). However, six of the Appraised Control scale items were designed to cover details specific to a caregiving situation, therefore this specific control component may best distil the part of the caregiver stress appraisal that relates to its negative consequences.

Other Questionnaires

Each frontotemporal dementia caregiver was sent two questionnaire packs, one for the assessment of trait and more general variables and the other to record psychometric data at the time of saliva sample collection. Rotter's (1966) Locus of Control Scale was used to measure a trait of control in the belief of causes of events throughout life, and details of this can be found in the preceding chapter. To assess the impact of appraised control over the caregiving situation on psychological health, the General Health Questionnaire was used to identify distress and mental health (Goldberg & Williams, 1988). For the frontotemporal dementia caregivers the General Health Questionnaire-30 (Goldberg, 1972) version was employed. It was designed to detect current diagnosable psychiatric disorders and has also been stated to assess mental well-being and health (Jackson, 2007; Whittington & Huppert, 1998). It has very good internal consistency at .82 to .92, reliability at .73 (Goldberg & Williams, 1988) and has displayed external validity in its predictive value in the number and severity of psychiatric episodes in newly-registered general practice patients (Corser & Philip, 1978). To measure stress the Perceived Stress Scale (Cohen et al, 1983; Cohen & Williamson, 1988) was used. The 10-item version (PSS-10) measured the level of perceived stress over the past month. It was designed by

Cohen and Williamson (1988) to measure their three central components of a stress experience - predictability, controllability and overload. The 10-item version is recommended over others as it has produced the highest reliability (Psychosocial Working Group, The MacArthur Network, 2000). This scale has very good internal reliability with Cronbach's alpha ranging from .84 to .86 and test-retest correlations of .85. Its validity has been shown through strong correlations with measures of depressive symptoms, social-anxiety and life-event scores (Cohen & Williamson, 1988). A second booklet sent only to the Frontotemporal dementia caregivers contained questionnaires to assess saliva collection information and ratings at the time of collection. Stress and arousal levels were measured through the 30-item Stress Arousal Checklist (Mackay, Cox, Burrows & Lazzerini, 1978; Cox & Mackay, 1985), detailed in the previous chapter where the range of scores is 0 to 14 for stress and 0 to 11 for arousal.

A year later the multiple sclerosis caregivers were also sent in the post one questionnaire pack. These included Rotter's (1966) Locus of Control Scale, the Appraised Control scale, and the PSS-10. The 28-item version of the General Health Questionnaire (Goldberg & Hillier, 1979) was used to detect the existence of psychiatric symptoms that relate to distress. This 'scaled' version was developed on the basis of principal components analysis, from which four sub-scales were derived, each of seven items: somatic symptoms, anxiety and insomnia, social dysfunction and severe depression. Validity for this version has been suggested through a relationship with the categorisation of a depressive episode (Thomas & Lincoln, 2006), and in physiological measures significant correlations with 3-Methoxy-4-hydroxyphenylglycol (a metabolite of noradrenalin that is seen to be higher in people with anxiety disorders) in women (Li, Ueki, Yamamoto & Yamada, 2006). The multiple sclerosis caregiver's pack also contained questions that asked about the number of daily tasks that were involved in the caregiving situation where they were asked to indicate from a list of forty items that covered the areas of hygiene, dressing, continence, eating, meal preparation, telephoning, going on an outing, finance & correspondence, medications, leisure & housework. Additionally they answered questions from the four 'Physical Health' sub-scales of the RAND Short Form-36 (SF-36) Health Survey (Ware & Sherbourne, 1992). These sub-scales are said to measure the concepts that are most frequently assessed in widely-used health surveys, which are also those commonly affected by disease and treatment (Ware, Kosinski, Bayliss, McHorney, Rogers & Raczek, 1995). The overall 'Physical Health Summary' scale (a combination of the four sub-scales) has shown strong reliability at 0.92. Validity of this scale as a measure of physical health has been shown with its ability to predict disease burden from over 200 diseases and conditions, including arthritis, cancer, cardiovascular disease, among many others (e.g. Turner-Bowker, Bartley & Ware, 2002). It has also predicted the future use of health care services (Ware, Kosinski & Keller, 1994), 180-day survival after coronary surgery (Rumsfeld, MaWhinney, McCarthy, Shroyer, Villa Nueva, O'Brien, Moritz, Henderson, Grover, Sethi, & Hammerstein, 1999) and five-year survival rates from all-cause mortality (Ware et al, 1994).

Saliva Sampling and Handling

For saliva collection a number of established guidelines were implemented: all participants were asked not to brush their teeth for at least an hour prior to providing a saliva sample as oral bleeding

can lead to inaccurately high levels of salivary IgA (Shirtcliff et al, 2001). Unstimulated flow rate can be affected by a number of factors, including the position of the body during saliva collection. In fact higher flow has been found when standing and lower rates when lying down, when compared to sitting (Shannon, Suddick & Dowd, 1974). Navazesh (1993) suggests that volunteers do not eat, drink (bar water), or smoke for at least one hour before saliva collection. Every participant was asked to follow these guidelines and asked to provide details.

Saliva samples were collected from the frontotemporal dementia caregivers at home using the Salivette technique. Each volunteer was given three Salivettes (and two practice ones) with detailed instructions for their use and asked to store them immediately in the freezer until sent back to the University. The Salivette consists of four parts - a stopper, a cotton dental swab, a suspended insert, and a centrifuge vessel. The procedure is easy and hygienic and fully detailed in *Appendix II*. Once the three collections had been made, the Salivettes were posted to the University laboratory using a Royal Mail Safebox. These Safeboxes were designed for the safe transport of biological samples and comply with United Nations directive UN3373, which governs the transport of diagnostic specimens including saliva. When received by the University the samples were immediately frozen at –80^{o}C until assayed.

A freezer failure occurred with a small number of participant's samples in storage (n = 6). The power was lost to the –80^{o}C freezer for a total of 48-hours though the internal temperature did not rise above 0^{o}C. Comparisons between the caregiver samples contained in the freezer during the disruption and those returned after the failure found no difference in salivary IgA. Furthermore, external quality controls were no different in IgA levels from samples analysed before the power failure to those afterwards, suggesting that this storage interim did not have a detrimental effect.

Design

The study used an opportunity sample of frontotemporal dementia caregivers, multiple sclerosis caregivers, and non-caregivers, who volunteered to take part. These groups were tested in a between-subjects design, where the frontotemporal dementia caregivers and non-caregivers had been matched on demographic variables, such as age, gender and employment status. This independent variable of caregiver versus non-caregiver was tested on the dependent variables of perceived stress (PSS-10), appraised control, and psychiatric disorder symptoms related to distress (GHQ). For the matched groups of frontotemporal dementia caregivers and non-caregivers, they were also tested on the with-subjects variables of three daily state stress ratings (SACL), salivary flow rate, and salivary IgA secretion rate. Additionally, the effects of both perceived stress (PSS-10) and appraised control were to be tested against each other to determine independent effects on perceived health, salivary flow rate, and IgA secretion rate. To account for the effects of the diurnal rhythms (Miletic et al, 1996; Hucklebridge et al, 1998) all participants provided samples at the same time during the evening between 5 and 8pm for both studies.

Procedure

Each frontotemporal dementia caregiver was sent a letter from the Cognition and Brain Sciences Unit support group manager asking if they would take part in the research project. Individuals from the Cognition and Brain Sciences Unit volunteer panel were matched to the caregivers who agreed to participate on age and sex to form a control group of non-caregivers. To ensure anonymity all participants received the research packs from the support group manager and only the fully completed and anonymous questionnaires were returned to the University. Each frontotemporal dementia caregiver and non-caregiver was sent two questionnaire packs (one 'Trait' and a 'Daily Saliva Collection' booklet) with a saliva collection kit. They completed the 'Trait' pack over a few days prior to the sample collection time. They were asked to provide three evening saliva samples between 6pm and 9pm on consecutive weekdays, and to complete the appropriate daily questionnaires at the same time. Each Salivette was labelled for the day and each participant was allocated a number to ensure anonymity. Participants were asked to store the samples immediately in heavy-duty zip-lock bags in their freezer. Once all three samples had been given they were asked to be removed from the domestic freezer on the morning of posting and immediately returned in the Royal Mail Safebox. Once received, the date and time was noted and the saliva samples were stored at -80ºC until assayed. Questionnaire packs arrived separately via a pre-paid envelope to the University laboratory. Once all documentation had been returned to the University each participant was sent a £15 postal order.

During the follow-up multiple sclerosis caregiver study one year later, an advert was placed in a support magazine asking for volunteers to take part in this research study. Any multiple sclerosis caregiver that responded to the advert was sent more detailed information. Once they agreed to participate they received a 'Trait Questionnaire Pack' through the post. To ensure anonymity all participants received the research packs with an ID number and no reference to names. In the instructions that accompanied these packs, all participants were asked to complete the 'Trait' packs in their own time and advised that they could complete them over a few days. The multiple sclerosis caregivers then returned the finished and anonymous questionnaires to the University laboratory with a pre-paid, self-addressed envelope. No saliva data was collected for these caregivers.

Saliva Analysis

Salivettes were thawed for an hour and a half before being centrifuged at 1500 RCF for 15 minutes to extract saliva from the cotton insert, which was then removed. All samples were vortexed to homogenise the saliva and returned to the centrifuge for a further 10 minutes to break down any solid particles.

Flow Rate: Salivary flow rate was determined gravimetrically. The Salivettes were weighed using analytical scales accurate to four decimal places (ALC Series, Acculab) and saliva density was assumed to be 1 g/ml^{-1} (Li & Gleeson, 2003).

Salivary IgA: Salivary IgA concentration was measured via the commercial IgA Single Radial Immuno Diffusion kits (SRID – Dade Behring, Germany), which is based on the complexing process

between an antigen and antibody (Mancini, Carbonara & Heremans, 1965). A RID plate uses agarose gel containing an even distribution of appropriate antigen. The LC Partigen immunodiffusion plate used here utilised monospecific antiserum to IgA (from rabbit, sheep, or goat) in the agar gel, and contained twelve wells. The antibody from the test samples diffuses into the gel from its well where the complexing process produces a visible precipitin ring around the well. The diameter of the ring is proportional to the concentration level of the antibody and when this is compared to the precipitin rings of known standards it provides the unknown sample's concentration. The IgA standard was a protein standard LC-V (human, Dade Behring) that required reconstitution with 0.5ml ultrapure distilled water for 30-minutes prior to use. Three standards were prepared a) undiluted for a final concentration of 104µg/ml^{-1}, b) 50:50 standard to isotonic saline (0.9% NaCL/150mM) to give 52µg/ml^{-1}, and c) 25:75 standard to saline for 26µg/ml^{-1}. These standards filled the first three wells of every plate. All standards and neat unknown saliva samples were pipetted at 20µl into the wells and left until the fluid had diffused into the agar (20-minutes). Plate lids were then replaced and the plates were incubated at 22^{o}C (LKM incubator) for 72 hours. Following incubation the precipitin rings were measured. The RID was originally developed to assess serum IgA and employs a correction factor. The data were analysed with the Brandtzaeg, Fjellanger & Gjeruldsen (1970) correction factor of 3.25 because salivary IgA differs in its physicochemical properties from IgA in serum due to a sedimentation effect of salivary IgA compared to its monomeric counterpart. Internal plate quality control was via inspection of the ordinate intersection of the reference line created by the three standards and for IgA this is 21 ± 4.5mm, all plates passed this internal quality control. An external control sample was placed in well 4 in all plates to ascertain plate reliability, this was required to be within 0.1mm across all plates and ensured consistency in plate analysis. The concentrations of the IgA standards were regressed against the squared diameter of the resulting precipitate rings to calibrate each plate. The correlation between the standard IgA and squared precipitate rings was >0.99 for all plates. Any plate that failed to meet these criteria had its samples re-analysed. Each resulting ring diameter was read separately by two researchers by a 10x magnified eye-piece to the nearest .1mm and a value agreed. Salivary IgA secretion rate was determined by multiplying the final IgA concentration by salivary flow rate per minute (µg.min^{-1}).

Results

Participant Demographics

Analyses were run to assess for differences between the overall caregiver group and the non-caregiver controls (see **Table 4**). An Independent t-test was run for age, while Pearson's Chi-square test assessed differences for gender distribution, percentage of smokers, reported frequency of current illness, and frequency of participants taking medication.

Table 4: Differences in age, gender, percentage of smokers, illness, and medication between caregiver and non-caregiver groups.

	CAREGIVER	NON-CAREGIVER	TEST	RESULT
N=	85	35		
Mean age (SD)	56.08 (12.02)	63.25 (5.49)	t-test	$t_{(116.67)}$=-4.5,p<0.001
Gender ratio (female:male)	49:36	25:10	Chi square	p=0.16
Percentage of smokers	23%	15%	Chi square	p=0.38
Frequency of current illness (ill)	48%	46%	Chi square	p=0.80
Frequency of medication taken (on med)	58%	68%	Chi square	p=0.27

While gender distribution and the percentage of smokers were equal between the two groups, the non-caregivers were a significantly older population than the caregiver group. This was due to the multiple sclerosis caregivers being a younger sample (as the frontotemporal dementia caregivers were matched to the non-caregivers). Age has been seen to be related to salivary IgA (Miletic et al, 1996; Evans, Der, Ford, Hucklebridge, Hunt & Lambert, 2000) and so this variable was included in the following analyses. The two groups did not differ on the numbers of people reporting a current illness, or the number of people currently taking medication.

Appraised Control and Perceived Stress

Hypothesis One: Caregivers will have lower appraised control than a group of non-caregivers

Assessing the possible impact of the caregiving situation on appraised control required comparisons between the caregivers (n=85) versus the non-caregiver control group (n=35). Ratings showed that the non-caregiver group had a higher appraised control mean of 3.59 (SD = 0.52) than the caregivers mean of 3.14 (SD = 0.53) and a t-test confirmed this to be significant ($t_{(118)}$=-4.29,p<0.001), supporting the first hypothesis (see **Table 5**).

The appraised control variable was also examined in relation to potential differences between the frontotemporal dementia caregivers, multiple sclerosis caregivers and the non-caregiver group. A oneway ANOVA ($F_{(2,117)}$=9.14,p<0.001) confirmed significantly different appraised control ratings, and post-hoc analysis determined a significantly larger rating of control from the non-caregivers to both frontotemporal dementia caregivers (p=0.002) and multiple sclerosis caregivers (p<0.001), who did not differ from each other (p=0.96). This again supported the first hypothesis. However, a oneway ANOVA also found that perceived stress (PSS-10) was different ($F_{(2,116)}$=13.98,p<0.001) where the score was significantly lower from the non-caregivers compared to both the frontotemporal dementia caregivers (p=0.011) and multiple sclerosis caregivers (p<0.001), who did not differ (p=0.31). Finally, an independent t-test was used to compare average state-stress (from the SACL) over the three days of collection, between the frontotemporal dementia caregivers and non-caregivers (this scale was not used in the multiple sclerosis research). This test determined a significantly higher average state-stress (SACL) from the frontotemporal dementia caregivers (Mean = 8.0, Std. Dev = 6.2) than non-caregivers (Mean = 4.4, Std. Dev = 4.4) ($t_{(40.38)}$=2.48,p=0.018). The following analyses needed to examine whether there was an independent effect of appraised control compared to stress on the number of psychological variables recorded.

Table 5: Means (SD) and t-test statistics for locus of control, appraised control, and perceived stress (PSS-10) between caregivers and non-caregivers.

	CAREGIVER	NON-CAREGIVER	TEST	RESULT
Mean Locus of Control (SD)	10.86 (2.76)	10.74 (3.86)	t-test	p=0.87
Mean Appraised Control (SD)	3.14 (0.53)	3.59 (0.52)	t-test	$t_{(118)}$=-4.29,p<0.001
Mean Perceived Stress Scale-10 (SD)	20.6 (5.58)	14.4 (7.12)	t-test	$t_{(115)}$=5.06,p<0.001
General Health Questionnaire-30 (SD)	8.44 (8.0)	2.91 (5.35)	t-test	$t_{(38.9)}$=2.99,p=0.005
General Health Questionnaire-28 (SD)	6.13 (6.2)	-	n/a	n/a

Hypothesis Two: Caregivers will have lower salivary IgA secretion rates than non-caregivers, which will be mediated by appraised control.

Salivary Analysis Data

Only the frontotemporal dementia caregivers and non-caregivers gave saliva for IgA secretion rate assessment. For this data a number of participants missed a sample, or provided insufficient saliva for immunological analysis. Four caregivers and four non-caregivers had all three samples missing. Four participants (all non-caregivers) provided only enough saliva on one day, and eleven on only two days (seven caregivers and four non-caregivers). To minimise the impact of removing these participants from the data, participants with at least two days of measurements were included in the following analyses (caregivers n=21; non-caregivers n=27). For each salivary index the average was calculated over the three days, where a minimum of at least two samples were available.

Salivary Flow Rate: The first analysis required a direct comparison between the two groups of caregivers versus non-caregivers. For salivary flow rate this comparison showed no significant differences via an independent t-test ($t_{(47)}$ = 0.06, p= 0.99). Next Spearman's correlational assessment examined a relationship between salivary flow rate and the variables of appraised control ($r_{(48)}$ = -0.31, p= 0.03), perceived stress from the prior month ($r_{(48)}$ = 0.22, p= 0.13), and average state-stress over the three days of sampling ($r_{(48)}$ = 0.15, p= 0.32). Flow rate showed only one significant negative correlation with appraised control.

Salivary IgA Secretion Rate: One of the non-caregivers had a mean IgA secretion rate almost four SDs above the group mean and the whole data set displayed significant heterogeneity of variance between the groups. A Log10 transform was applied to these data to bring the distributions to normality and create a homogeneous variance. The first analysis required a direct comparison between the two groups of caregivers versus non-caregiver controls. For Mean (Log10) salivary IgA secretion rate this comparison showed no significant difference with an independent t-test ($t_{(47)}$ = -0.21, p= 0.95). Next Spearman's correlational assessment examined a relationship between salivary IgA secretion rate and the variables of appraised control ($r_{(48)}$ = -0.21, p= 0.15), perceived stress from the prior month ($r_{(48)}$ = 0.17, p= 0.26), and average state-stress over the three days of sampling ($r_{(48)}$ = 0.12, p= 0.43). There were no significant correlations for the independent variables and salivary IgA secretion rate.

Overall, belonging to the caregiver group did not have an effect on the level of salivary IgA compared to a matched non-caregiver control group. There were no relationships between salivary IgA and the variables of perceived stress, appraised control, or state levels of stress rated at the time of sampling. These results did not support the second hypothesis.

Hypothesis Three: When compared to perceived stress (PSS-10), appraised control will show a stronger relationship with negative states (Stress Arousal Checklist), distress (General Health Questionnaire), and general health ratings (RAND Short-Form-36 Health Survey).

Negative States and Distress

To initially test the third hypothesis, analyses were focused on the psychometric variables, though this meant that the caregiver groups (frontotemporal dementia caregivers and multiple sclerosis caregivers) were assessed independently as the questionnaires employed were not the same across the two studies. The frontotemporal dementia caregivers (n=25) were compared to their matched non-caregivers (n=35) on the SACL scales, which were completed on three consecutive weekdays to assess the state levels of stress and arousal at the time of saliva collection. Initially a mixed factor ANOVA was run with the three day ratings as the within factor and the frontotemporal dementia caregiver versus non-caregiver as the between factor. For state-stress, this showed only one significant result in the between groups main effect ($F_{(1,58)}=6.88, p=0.011, eta^2=0.11$), with caregivers reporting higher state-stress over all three days (mean=8.0, sd=6.56) than non-caregivers (mean=4.43, sd=5.40). None of the ANOVA results were significant for the arousal ratings (all $p \geq 0.3$). The three daily state-stress scores were averaged for the next assessment to determine if there was a greater effect of appraised control or perceived stress (PSS-10) on state-stress (SACL) scores.

To account for a potential age affect, a hierarchical multiple regression technique was used. Data assumptions were tested, where each independent variable held some relationship with the dependent variable under investigation of 0.3 or above (Pallant, 2005) and the collinearity diagnostics did not show multicollinearity evident between the independent variables (tolerance value above 0.1 and the VIF value less than 10). For the independent variables, age was always entered at the first step and then the Appraised Control Scale and the PSS-10 at the second step.

The initial dependent variable assessed was the average state-stress (SACL) score from the three consecutive days for the 25 frontotemporal caregivers and the 35 non-caregivers. The ANOVA table showed the first model with age was not significant ($p=0.79$), but the whole second model was significant ($F_{(3,58)}=15.09, p<0.001$) with the R^2 value at 0.45. The R^2 change value for the effects of the second step independent variables remained at 0.45, meaning that appraised control and perceived stress (PSS-10) explained 45% of the variance in state-stress (SACL) ratings. However, specific variable analysis determined that appraised control was not a statistically significant contributing factor and only perceived stress (PSS-10) produced a significant and unique contribution to the average state-stress (SACL) score, see **Table 6** for the statistical values.

The next dependent variable examined was distress as measured by the General Health Questionnaire-30 (GHQ-30). The ANOVA table showed the first (age) model was significant ($F_{(1,58)}$=7.53,p=0.02) and the whole second model was also significant ($F_{(3,58)}$=100.75,p<0.001) with the R^2 value at 0.85. The R^2 change value for the effects of the second step independent variables was 0.81, meaning that appraised control and perceived stress (PSS-10) explained a massive 81% of the variance in the ratings from this scale. More specifically, all three independent variables were statistically significant contributing factors in this second model, and in order of importance they were the perceived stress (Beta = 0.55), appraised control (Beta = -0.44), followed by age (Beta = 0.38), see **Table 6** for the statistical values.

Table 6: Hierarchical linear regression statistics for age (1st step), perceived stress (PSS-10) and appraised control (2nd step) in the frontotemporal dementia caregiver and non-caregiver group (n=60) on the dependent variables of average state stress (SACL) and general health (GHQ-30). Significance values relate to the unique significance of the predictor variable within the second model.

	Age	PSS-10	Appraised Control
Average state-stress (SACL)			
B	0.09	0.36	-2.81
Std. Error	0.05	0.13	1.48
Beta	0.18	0.44	-0.29
Sig.	p=0.09	p=0.006	p=0.06
Distress (GHQ-30)			
B	0.50	1.18	-11.14
Std. Error	0.07	0.17	2.07
Beta	0.38	0.55	-0.44
Sig.	p<0.001	p<0.001	p<0.001

In summary, the combined group of 60 frontotemporal caregivers and non-caregivers results did not support the second hypothesis in terms of negative states, where perceived stress (PSS-10) was the only predictor variable over state-stress (SACL) ratings across three days, though a clear trend was evident for appraised control. While perceived stress (PSS-10) was a stronger predictor of the level of psychiatric symptoms that reflect distress (GHQ-30), appraised control was a significant independent predictor variable in these GHQ-30 scores. This means that appraised control can predict distress levels independently of perceived stress. However, the analyses were run on a combined group of both frontotemporal caregivers and non-caregivers so secondary analyses were run where the caregiver / non-caregiver status (1/0) was added at step 1 (with age) of the model.

For the analysis of distress scores from the GHQ-30, the ANOVA table was significant for the first ($F_{(2,58)}$=5.9,p=0.005) and second models ($F_{(4,58)}$=30.71,p<0.001). The first model summary found that both age and caregiver status significantly explained 17% of the variance in distress scores (GHQ-30) with an R^2 of 0.17, p=0.005. With the addition of the independent variables of perceived stress (PSS-10) and appraised control the whole model explained nearly 70% of distress scores (R^2 = 0.695, p<0.001). The R^2 change of the second model was 0.52 after the effects of age and group were removed, meaning that perceived stress and appraised control predicted an additional 52% of the ratings on distress (GHQ-30). The independent effects of these variables in the second model are summarised in

Table 7. For the second model, only perceived stress and appraised control were significant contributing factors to the GHQ-30 scores.

Table 7: Hierarchical linear regression statistics for caregiver versus non-caregiver grouping and age (1st step), perceived stress (PSS-10) and appraised control (2nd step) on the dependent variables of average state stress (SACL) and general health (GHQ-30). Significance values relate to the unique significance of the predictor variable within the second model.

	Group	Age	PSS-10	Appraised Control
Distress (GHQ-30)				
B	-2.99	0.26	0.97	-7.17
Std. Error	2.39	0.19	0.25	3.72
Beta	-0.10	0.10	0.53	-0.28
Sig.	p=0.21	p=0.19	P<0.001	p=0.05

	Group	Age	PSS-10	Appraised Control
SACL average stress score				
B	-1.4	-0.03	0.32	-1.45
Std. Error	1.25	0.10	0.13	1.95
Beta	-0.13	-0.03	0.47	-0.15
Sig.	p=0.27	p=0.77	p=0.018	p=0.46

For the analysis of average state-stress (SACL), the ANOVA table was significant for the first ($F_{(2,58)}$=3.38,p=0.041) and second models ($F_{(4,58)}$=9.74,p<0.001). The first model summary found that both age and caregiver status explained only 11 % of the variance in state-stress (SACL) with an R^2 of 0.11, p=0.041. With the addition of the independent variables of perceived stress and appraised control the whole model explained 42% of average state-stress (R^2 = 0.419, p<0.001). The R^2 change of model two was 0.31 after the effects of age and grouping were removed, meaning that perceived stress and appraised control predict an additional 31% of average state-stress (SACL). The independent effects of these variables in the second model are summarised in **Table 7**. As can be seen, for the second model perceived stress was the only significant contributing factor to the variance in state-stress (SACL) ratings and the previous trend for appraised control was not replicated when the caregiver grouping was added at step 1. Overall, while perceived stress (PSS-10) was again a stronger predictor of distress, the appraised control scale remained a significant contributing factor to these scores when the division of caregiver versus non-caregiver was added.

Age was a significant predictor for distress in the whole group of caregivers and non-caregivers, but this lost significance when the division was added. The average age for the frontotemporal dementia caregivers matched non-caregivers was 63.14 (5.65), and given that age was a potential predictor variable for distress levels it was necessary to examine the influence of perceived stress and appraised control in the significantly younger ($t_{(81.30)}$=5.51,p<0.001) group of 60 multiple sclerosis caregivers. These caregivers completed the General Health Questionnaire-28 (GHQ-28) version to assess for distress. Regressional analysis was again run with age in the first step and the PSS-10 and Appraised Control scale scores at the second. The GHQ-28 ANOVA table showed the first model (age) was not a significant finding (p=0.07) which suggested that age did not explain the variance in these distress

scores. The whole second model was significant ($F_{(3,57)}$=15.63,p<0.001) with the R^2 value at 0.47. The R^2 change value for the effects of the independent variables at the second step was 0.40, meaning that appraised control and perceived stress explained 40% of the variance in the scores. However, in contrast to the previous analysis, only the Appraised Control scale gave a statistically significant contribution to this variance in the second model with a Beta of 0.56, see **Table 8** for the statistical values. It should be mentioned that state-stress (SACL) ratings were not available for this group.

Table 8: Hierarchical linear regression statistics for age (1st step), perceived stress (PSS-10) and appraised control (2nd step) in the multiple sclerosis caregiver group (n=60) on the dependent variable of distress (GHQ-28). Significance values relate to the unique significance of the predictor variable within the second model.

	Age	PSS-10	Appraised Control
Distress (GHQ-28)			
B	-0.07	0.10	-6.20
Std. Error	0.06	0.14	1.68
Beta	-0.12	0.11	-0.56
Sig.	p=0.24	p=0.49	p=0.001

In summary, within this group of multiple sclerosis caregivers, appraised control gave the only unique and significant contribution to predicting the variance in distress scores from the GHQ-28. These results supported the second hypothesis, and warranted a little further exploration. Correlations were run to assess associations between appraised control and certain objective aspects to the caregiving task, such as the number of daily tasks that required help and the number of years the caregiver had been caring for. These are displayed in **Table 9**.

Table 9: Correlation coefficients for multiple sclerosis caregivers

	Appraised Control r (n)	Perceived Stress Scale-10 r (n)	Distress (GHQ-28) r (n)	Number of daily tasks r (n)
Appraised Control				
Perceived Stress Scale-10	-0.59** (58)			
Distress (GHQ-28)	-0.68** (59)	0.57** (58)		
Number of daily tasks	-0.23* (57)	-0.18 (58)	-0.17 (59)	
Care duration in years	0.22* (59)	-0.17 (58)	-0.33** (59)	0.07 (56)

*p<0.05
**p<0.01
NOTE: Pearson's values are for most analyses but Spearman's were adopted for GHQ-28 and Duration data due to significant positive skews in the distributions.

Table 9 shows that appraised control was the only construct to be associated with both objective aspects to the caregiving situation in number of daily tasks, and care duration in years. Perceived stress (PSS-10) was not related to these objective measures at all, and distress was negatively related with the duration of caregiving, where fewer years of caregiving were linked with higher distress.

In summary, the results suggest that distress levels assessed via both versions of the General Health Questionnaire were significantly and independently predicted by the level of appraised control when compared to perceived stress (PSS-10). This means that appraised control is measuring a component of a stress experience above and beyond that of perceived stress (PSS-10) alone. Though distress was better predicted by perceived stress than appraised control in the frontotemporal caregivers and non-

caregivers (GHQ-30), in the younger group of multiple sclerosis caregivers this result was not replicated and appraised control was the only significant factor to contribute to distress (GHQ-28) scores. Due to this finding, and in addition to the previous literature that has found 'stress' does not always correlate well with immunological measures, including salivary IgA, the two independent variables of appraised control and perceived stress were next examined in relation to physical health ratings from the multiple sclerosis caregivers (as this group was the only one with such items recorded).

General Health Ratings

For the multiple sclerosis caregivers, general health ratings were measured from the 'Physical Health Summary' of the RAND Short-Form-36 Health Survey (RAND SF-36). For this overall general health score hierarchical linear regression was run with age at the first step, followed by perceived stress (PSS-10) and appraised control at the second step. The first model (age) ANOVA did not show a significant finding for age (p=0.75) which suggested that age did not explain the variance in these health related scores. The second model with age at the first step, and then perceived stress (PSS-10) and appraised control at the second step, was significant ($F_{(1,57)}$=5.28,p=0.003) with an R^2 value of 0.23, which remained at this level in the R^2 change value 0.23. This meant that perceived stress and appraised control explained a total of 23% in the variance of the general health ratings. To examine the individual contribution of these variables, the second model results for the independent variables of perceived stress and appraised control are presented in **Table 10**.

Table 10: Second step linear regression statistics for perceived stress (PSS-10) and appraised control in the multiple sclerosis caregiver group (n=58) on the dependent variable of the 'Physical Health Summary' (RAND SF-36).

	PSS-10	Appraised Control
'Physical Health Summary'		
ANOVA	$F_{(2,57)}$=7.43,p=0.001	
B	0.89	23.85
Std. Error	0.56	6.61
Beta	0.29	0.66
Sig.	p=0.12	p=0.001

These results show that appraised control was the only variable to provide a unique significant contribution to predicting the level of general health rated by multiple sclerosis caregivers. The part correlation value of 0.43 meant that appraised control uniquely contributed 18% of the variance explained by the model for 'Physical Health Summary' scores. Perceived stress did not show any effect here and these results support the hypothesis.

Overall analysis of the third hypothesis generally supported its prediction. In support the frontotemporal dementia caregivers and non-caregivers who had a higher rating of appraised control also had lower average SACL stress score ($r_{(48)}$= -0.59, p<0.001), though it was not a significant predictor variable, and a lower rating on the GHQ-30 ($r_{(48)}$= -0.77, p<0.001) where it was. For the

multiple sclerosis caregivers, appraised control was significantly associated with the GHQ-28, with those higher in appraised control having lower scores of distress ($r_{(59)}$=-0.68,p<0.001). These results supported the hypothesis with a relationship between higher appraised control and a lower level of state-stress (SACL), and a predictive relationship with a scale of distress (GHQ-28 and GHQ-30). Furthermore, the multiple sclerosis caregivers with higher appraised control scored better on the scale ($r_{(60)}$ = 0.41, p=0.001) that assessed general health, i.e. were healthier caregivers than those reporting lower appraised control over the caregiving situation.

Discussion

Caregivers of individuals with frontotemporal dementia and multiple sclerosis rated their appraised control over the situation significantly lower than a group of non-caregivers, which supported the first hypothesis. Caregivers were also significantly higher in perceived stress (PSS-10) rated over the past month. In the group of frontotemporal dementia caregivers and non-caregivers, appraised control was a significant and unique variable that contributed to scores for distress (GHQ-30), though this was not a stronger relationship than that found with perceived stress. While appraised control showed a trend to predict daily state-stress levels (SACL) over three consecutive days, perceived stress over the prior month was the only significant predictor of these state-stress scores. While this did not fully support the third hypothesis, appraised control did stand as a significant independent predictor of distress. Furthermore, in a group of multiple sclerosis caregivers the rating of appraised control was the only unique and significant contributing factor to the level of distress as rated by the General Health Questionnaire-28 (GHQ-28). It was also the only significant predictor variable for the RAND Short-Form 36 Health Survey (RAND SF-36) scale of the overall 'Physical Health Summary', where a higher level of appraised control predicted a participant to have a better physical health score. In fact, perceived stress did not provide any contribution to the variance in these health scores. These results supported the hypothesis, that when compared to perceived stress, appraised control did show a stronger relationship with distress and physical health. Psychologically, it can be summarised that appraised control may assess an aspect of the caregiving situation beyond that of perceived stress (discussed later). However, with regards to the immunological results, there were no significant findings. Not even age or caregiver status affected salivary IgA secretion rates, and there was no influence from either perceived stress, or appraised control, and such results are discussed.

Caregiving: Perceived Stress and Appraised Control

The experience of caregiving for an individual with frontotemporal dementia and multiple sclerosis was perceived as a stressful situation in both cases. A rating of perceived stress from the past month and three daily state-stress scores (from the frontotemporal dementia caregivers) were significantly higher in the group of caregivers compared to a control group of non-caregivers. This replicates previous research where dementia and multiple sclerosis caregivers have reported significantly greater levels of stress (recorded using the PSS-14) compared to non-caregivers (Bauer et al, 2000; Esterling, Kiecolt-Glaser, Bodnar & Glaser, 1994; Vedhara et al, 2002). It further supports the categorisation of caregiving as a chronic stressor.

This research also examined a caregiving situation with a focus on appraised control. There was a significantly lower rating of appraised control from both caregiver groups compared to the non-caregiver group. Being a caregiver meant having a lower appraisal of control over their situation, and there were no differences between the two caregiver types of individuals with frontotemporal dementia or multiple sclerosis. With regard to daily state-stress levels, only perceived stress from the past month was a significant predictor. Where the Appraised Control scale was focussed on the individual appraisal of control over the situation, it is suggested that this specific controllability aspect is not linked to a negative state-stress response. This could mean that appraised control assesses something different to perceived stress, and given the difference in appraised control between caregivers and non-caregivers it may be asked what feature of the caregiving situation the scale measures. One aspect may relate to the behavioural and physical demands of the actual caregiving experience, which could vary between the different types of caregiving situation.

The appraised control rating was compared between those caring for an individual with frontotemporal dementia and those with multiple sclerosis. It has been suggested that there is a great burden in dementia caregivers (Messinger-Rappoport et al, 2006), especially when a care-recipient with frontotemporal dementia can show alterations in character and social conduct (Snowden et al, 2002). Perceived stress and appraised control over the situation were not different between the frontotemporal dementia caregivers and multiple sclerosis caregivers where the care-recipient can show more physically disabling symptoms (Rodgers & Bland, 1996). This may suggest that appraised control does not assess the effect of potentially different behaviours from the care-recipient, though neither does perceived stress. Yet, data was not collected on the severity or presence of symptoms from the people being cared for, where the dementia illness may be quite mild without obvious changes in character, for example. However, the similarly low levels of appraised control from both caregiver groups could suggest that the one-on-one experience of caregiving is not the cause of changes in appraised control over the situation, even when the symptoms are mild.

A different potential explanation may be that the caregiving situation could use up the caregiver's time and resources from other activities. Indeed, more hours of daily care have been related to a higher score from single item measure of caregiver stress (Tsai & Jirovec, 2005), and here the appraised control variable was significantly correlated with the situation-specific variables in the number of daily tasks requiring help from the caregiver over the past two weeks and the number of years spent being a caregiver, associations not found with perceived stress. Some support that appraised control may relate to something other than physical caregiving aspects can be found in a study by Dennis, O'Rourke, Lewis, Sharpe and Warlow (1998). Regarding caregivers (Mean age = 60) of stroke survivors, it was reported that while distress levels (GHQ-30) from caregivers were predicted by the level of disability, there was not one single disability that could be picked out to contribute the greatest to the variance in scores. Rather, it was the number of daily activities that required help that had a strong relationship with distress, which suggests that a greater demand for help (that may use up more of the caregivers time) is associated with worse mental health. Caregiver burden has been associated with the experience of a loss of control over the situation (Dupuis et al, 2004) and in this Chapter appraised control significantly predicted distress levels.

Caregiving: Perceived Stress, Appraised Control and Distress

Frontotemporal dementia caregivers scored with significantly higher levels of distress (GHQ-30) than non-caregivers. This was significantly predicted by both perceived stress and appraised control. Where perceived stress was higher the level of distress increased, and where appraised control over the situation was lower then distress also increased. While the latter effect of appraised control on distress maintained significance in the multiple sclerosis caregivers ratings (from the GHQ-28), perceived stress was not a predictor variable. Overall the results follow from Wallhagen (1992) where it was shown that elderly caregivers reported better life satisfaction and lower depression when perceived control was high.

Other research has shown the greater the number of tasks that require help, the higher the distress (Dennis et al, 1998). However, given that appraised control was a predictor of distress (GHQ-28) in this chapter, the relationship between care duration and distress could potentially be a by-product of the correlation with appraised control. Although cause and effect are not possible in such a design, it was seen that a longer duration of caregiving in years was significantly correlated with a higher rating in appraised control. Other findings have shown a shorter duration of caregiving being associated with significantly higher caregiver burden (Zainuddin, Arokiasamy & Poi, 2003), a greater likelihood of the carer institutionalising the care-recipient (Gaugler, Kane, Kane, Clay & Newcomer, 2003), and lower scores in the World Health Organisation quality of life (Fatoye, Komolafe, Adewuya & Fatoye, 2006). It may be possible that more years of caregiving experience can lead to higher appraised control over the caregiving situation. Yet another interpretation views appraised control as a measure for which a chronic stress situation exacerbates risky health behaviours, or limits the utilisation of good health practises. For example, "in addition to depression, other reactions may increase the risks of caregiver illnesses . . . including sleep problems, poor diets, and sedentary behaviours" (p. 947, Vitaliano et al, 2003). This is a limitation of the Appraised Control scale, as it may be that some of the questions merely reflected these behaviours and not actual appraised control. Certainly further research is necessary to determine if different factors exist within the scale, whereby it may be made redundant if such behaviours can be recorded by simple questions. However, this does not detract from the finding that the Appraised Control scale did predict health.

Caregiving: Perceived Stress, Appraised Control and Physical Health

The number of participants reporting a current illness and the frequency of participants taking medication was equal between frontotemporal caregivers and non-caregivers. This would suggest that the act of frontotemporal dementia caregiving does not lead to a greater likelihood of being ill or of requiring medication, despite the fact that here perceived stress and distress measured by the General Health Questionnaire-30 were both significantly higher from the caregiver group. This contrasted a meta-analysis review where it was found that caregivers report significantly more health problems than non-caregivers, and take a greater number of medications (Vitaliano et al, 2003). However, there are other studies that also show similar health status between caregivers and non-caregivers (e.g

George & Gwyther, 1986; Haley, West, Wadley, Ford, White, Barrett, Harrell & Roth, 1995; Kiecolt-Glaser et al, 1987). Interestingly, in the multiple sclerosis caregivers the health scale of the RAND-SF-36 was significantly predicted by appraised control.

Appraised control was the significant predictor of the general physical health (RAND SF-36) of the caregiver in comparison to perceived stress, which showed no impact. A lack of control over the instigation of the caregiving experience has previously been connected with negative health outcomes (Robinson-Whelen & Kiecolt-Glaser, 1997). While one argument could be that a poorer state of health has the consequence of less appraised control, a counter argument comes from post-hoc assessment that found the 'physical functioning' sub-scale of the RAND SF-36 was not related to appraised control. The everyday activities that could be hindered by ill-health were not associated with appraised control, thus suggesting that the physical ability of the caregiver is not a factor in appraised control of the situation. Yet while appraised control did predict health ratings, this did not translate into an effect on salivary IgA.

Caregiving: Perceived Stress, Appraised Control and Salivary IgA

Neither perceived stress nor appraised control related to salivary IgA in frontotemporal dementia caregivers or non-caregivers. Being as there are only two other caregiver studies that have investigated salivary IgA, this result is difficult to assess within this specific prior literature. Yet in other areas, research has tended to focus on dementia caregivers who are an older (Ory, Hoffman, Yee, Tennstedt & Schulz, 1999) population where salivary IgA may be lower (Evans et al, 2000). In the prior Gallagher et al (2008) study a significantly lower level of salivary IgA was seen in caregivers compared to non-caregivers only in an older cohort. However this older group had a mean age of 63 years, similar to the age of the frontotemporal dementia caregivers studied here, which would prevent an argument that perceived stress may only affect salivary IgA in much older people.

Where stress was high there was no effect on the level of salivary IgA, from either a state-stress (SACL) rating taken at the time of saliva collection, or a more general perceived stress level (PSS-10) from the past month. In light of previous literature that can show a decreased mucosal immune response to other negative life-events, such as academic stress (Bosch et al, 2004), this finding may support the assertion that salivary IgA is a weak and inconsistent index of stress (Mouton et al, 1989), and adds to the minority of studies that have found no differences in salivary IgA during life-event stress periods (e.g. Deinzer et al, 2000). However, these are the minority and some discussion should turn to methodological differences in the literature.

A potential argument was that single sample collections in the null finding studies (e.g. Kubitz et al, 1986; Graham et al, 1988) were confounded by the numerous variables that can impact on salivary IgA, such as real-disgust images, exercise (Ring et al, 2000), and novelty (Willemsen et al, 2000). In the Evans et al (1993) study, day-to-day analysis revealed a reverse effect with days containing more than average undesirable events having higher salivary IgA levels, which suggests a number of factors could hide a general chronic stress effect when relying on single sample measurements. However,

other single sample designs have also reported significant effects (Phillips et al, 2006; Ng et al, 1999), though it should be noted that the sample sizes were large at 1222 and 124, respectively. This caregiver data used a minimum of two samples and found no effects, though potentially two days may not be enough to determine the full effect of state-stress on salivary IgA: two significant studies used an aggregation of a longer time-frame of 14 (Evans et al, 1993) and 7 days (Miletic et al, 1996).

Overall, it seems necessary to collect more than two samples to ensure a confounding effect of state variables is averaged out over a longer period. State-stress was recorded over the three days, which remained at a similar level for each participant. Does this mean that state-stress was not sensitive enough to daily variations possibly associated with salivary IgA? Also, with a large enough sample it would be interesting to compare higher and lower state-stress in an interaction with chronic perceived stress. When a large sample size may not be possible then a greater number of multiple samples could provide enough of a range in state scores to allow a within-subjects comparison of high to low state-stress on salivary IgA.

Study Limitations

The relatively small number of participants who provided immunological measures in this study does necessitate some caution in the reliability of the findings. The Phillips et al (2006) results are described as showing a small effect of life-event stress on IgA and they state that large samples may be needed to achieve statistical significance in measures of salivary IgA. The potential impact of stress or appraised control on salivary IgA may have been further confounded by a lack in data on prior URI occurrences during the five weeks prior to study participation. A larger sample size and more extensive questioning would have minimised this potential impact, yet it can be stated for this research that no participant had reported a cold within two weeks prior to saliva collection.

The advantage of comparing the caregiver chronic stress to a control condition not experiencing the same stress also created a limitation. Studies that use such naturally occurring stress situations mean that participants are not randomly assigned to the high and low stressful conditions (Cohen, Evans, Stokols & Krantz, 1986). Certainly, Cohen et al (2001) have argued that a consequence of this design may be that other factors (e.g. environmental conditions or personal dispositions) exist that may predispose selection of people to high stressed conditions, confounding immune effects. Where some of the more obvious factors can (and were) controlled for via matching with the control group on age, gender, and race (for example), even in the most controlled studies there is potential for less obvious and unmeasured confounds to lead to confusion in the conclusions regarding stress and immune responses. Yet it would be impossible to record every single potential influence on salivary IgA as these have included personality characteristics in neuroticism (Hennig, Possel & Netter, 1996), locus of control (Kubitz et al, 1986), Type's A and B personalities (Ohira, Watanabe, Kobayashi & Kawai, 1999), among others, and there are potential state effects that have been examined, such as choir music (Kreutz, Bongard, Rohrmann, Hodapp & Grebe, 2004), mental imagery (Rider, Achterberg, Lawlis, Goven, Toledo & Butler, 1990), humour (Lefcourt, Davidson-Katz & Kueneman, 1990), to name but a few. Therefore, such a criticism seems redundant as it is necessary to determine the

psychological factors that generally cause a diminished immune system, despite the transient increases that may occur in response to the number of potential states that can induce this. Yet here, the chronic stress of caregiving and appraised control over this situation were not such factors to affect salivary IgA.

However, other interpretations can also be explored with regard to the specific measure of salivary IgA. Certainly within the context of chronic stress there have been variations in procedure and measurement of IgA. For example, in the previous caregiver research the Bristow et al (2008) study used SRID whilst the Gallagher et al (2008) procedure involved the ELISA technique. One potential consequence may be seen in the limited sensitivity of SRID compared to the ELISA, which could preclude the accurate quantification of IgA (e.g. Harley, Gruffydd-Jones & Day, 1998). This becomes a particularly relevant problem for secretions in which the IgA concentration may be relatively low, such as saliva collected via the Salivette (Aufricht et al, 1992; Shirtcliff et al, 2001). However, both procedures are well recognised and it is thought unlikely that these standard assays would be a potential source of different results in studies regarding salivary IgA (Burns et al, 2004). Yet the use of the different saliva collection methods does warrant some caution, and the effects of the two different methods in Salivettes and the Spitting-method needs further investigation.

Summary and Conclusions

Appraised control added a dimension to the assessment of the chronic stress of caregiving that extended beyond that of perceived stress, where appraised control was the only significant contributing variable to distress as measured by both versions of the General Health Questionnaire (GHQ28 & GHQ-30), and it was the only significant predictive variable of subjective health status. Yet there was a lack of effect of either appraised control or perceived stress on salivary IgA. However, there are a number of methodological issues that may have muted an effect on salivary IgA, including a small sample size that was confounded by only two successful days of collections. Furthermore, the appraised control variable measured an aspect of the stress experience in a very general manner over the caregiving situation. Where salivary IgA can be responsive to more state-dependent effects, it may be appropriate to examine appraised control over a shorter reflection time, for example over daily events. Though state-stress levels were not different across the three days of collection in caregivers, it is possible that appraised control needs to be more time-specific to show an independent effect on salivary IgA. Overall, it is suggested that appraised control remains a variable to investigate and the following research chapter covers the effects of daily appraised control over seven days of saliva collections.

4

Perceived Stress, Hassles, State-Stress, Appraised Control and Salivary IgA

The immunological effect of chronic stress may be confounded by the more transient reactivity of salivary IgA secretion rate to a number of acute states. This study examined the impact of perceived chronic stress, whilst taking into consideration the more acute events that may occur on an everyday basis. To do this, perceived stress over the past month was analysed with state-stress measured of an evening, and compared to appraised control over a day's events in a design where samples were collected every evening for seven consecutive days.

A chronic stressor usually refers to a stable and prolonged stimulus that can last from weeks, months to years (Agarwal & Marshall, 2001). Chronic stress effects on salivary IgA have been examined in the context of academic stress through exam periods, or workloads (Murphy et al, 2010; Ng, Koh, Mok, Lim, Yang & Chia, 2004), caregiving (Bristow et al, 2008; Gallagher et al, 2008), and occupational stress (Ng et al, 1999) to name a few. Such events would fit under the category of objective and discrete negative life-events and are contrasted to subjective life-events that may be reflected through self-reports with checklists or cumulative stressful events, such as daily hassles (Herbert & Cohen, 1993). The chronic stressor may be a more internal state rather than an objective and persistent environmental events and appraisal processes are important. The situations may be quite different from person to person, and the emphasis is on the appraisal of the event as instigating a stress response. In relation to salivary IgA the area of subjective life-event stress research has shown contradictory findings in salivary IgA with both lower secretion rates (Ng et al, 1999; Phillips et al, 2006) and no change (Kiecolt-Glaser et al, 1984). It should also be noted that even when differences in IgA secretion are significant, the relationships between self-perceived stress and salivary IgA are described as weak (Ng et al, 1999) or that the variance explained is small (Phillips et al, 2006). In fact some authors have argued that salivary IgA is not a good marker for stress (Mouton et al, 1989). Yet this may be confined to the chronic stress research where a dysregulatory effect may have been disguised by an increase associated with an acute event immediately prior or close to the time of sample collection (Tsujita & Morimoto, 1999). Such effects would be more detrimental to those studies that only collected one saliva sample. Daily sampling may prevent confounds on chronic stress effects, where states can also be recorded at the time of saliva collection.

Yet, multiple consecutive days sampling has also produced different effects, with decreases in IgA to daily stress (Miletic et al, 1996) and a proxy for perceived stress through aggregation of undesirable daily events (Evans et al, 1993), and no association with an aggregation of data from seven days over weeks of academic stress (Deinzer et al, 2000). Where one-off sampling has been used, there are also different results with accumulating hassles (Kubitz et al, 1986), or a lagged effect where hassles reported at one time are seen to negatively relate to the level of IgA at a later time (Farne et al, 1994; Martin & Dobbin, 1988). Certainly these results do not allow a definite conclusion about subjective stress and salivary IgA at a daily level. However, while the perception of stress has been considered by this research, it lacks a more specific direction to account for an appraisal process, and there is a tendency to ignore coping mechanisms that may reduce the negative impact of events on immunological responses. While research has investigated some moderators of the stress effect on salivary IgA, such as a locus of control (Kubitz et al, 1986), a repressive-style personality (Farne et al, 1994), or negative mood (Stone, Marco, Cruise, Cox & Neale, 1996), this does not look at a specific

appraisal approach where control may play a role. There is evidence that control is related to stress and the stress response (e.g. Dickerson & Kemeny, 2004; Glass & Singer, 1972; Manuck, Harvey, Lechleitter & Neal, 1978; Peters, Guido, Godaert, Ballieux, Brosschot, Sweep, Swinkels, van Vliet & Heijnen, 1999; Sieber, Rodin, Larson, Ortega & Cummings, 1992; Weinstein et al, 2002), and in acute stress research control has even been proposed to distinguish active and passive coping, with possible differential effects on salivary IgA. It was a potential avenue to investigate appraised control in a more time-definitive manner over a day.

In the preceding chapter appraised control over the general caregiving situation was not associated with salivary IgA, though nor was perceived stress over the prior month. However, it has been suggested that a possible effect from either stress (or appraised control) should "be superimposed with the immediate stress effect, which increases slgA secretion in saliva immediately" (Tsujita and Morimoto, 1999, pg. 6). This was partially incorporated in the caregiver design with state-stress measures taken at the time of saliva sampling. However, this was only over a three day period where most participants were missing at least one day of data. Where daily samples are taken over a limited time-frame, it could be difficult to compare high and low effects of state-stress where ranges (lowest to highest) may be small. For example, in the caregiver research, the three days of measurements were equal in the level of state-stress so it was impossible to use the more powerful statistical technique of a within-subjects comparison between high to low state-stress days. Furthermore, the appraised control variable was assessed in a way that reflected its impact over a general caregiving situation and was not specific to events that may have occurred each day over the three collection days. Where control has been suggested to play a role in acute stress (e.g. Lovallo et al, 1985; Bongard, 1995; Weinstein et al, 2002) it would seem possible to examine appraised control in a shorter time-frame, such as that over a day's events. As an additional point, some authors (e.g. Kanner, Coyne, Schaefer & Doyle, 1981) have argued against the use of life-events to examine stress, and many of the previous subjective stress studies utilise a measure of hassles to examine minor events that may accumulate to become stressful. Yet it is within this type of design that the most inconsistent salivary IgA results prevail. Therefore, it would be of interest to accommodate a measure of hassles within research that investigates daily stress and daily appraised control.

A design that measures perceived stress over a month, hassles over a month, daily state-stress, and daily appraised control with salivary IgA and cortisol over a week of collections could allow a comparison of all these potential effects. Furthermore, day-to-day comparisons as a within-subjects factor would be preferable for such a measure as salivary IgA that displays such high inter-individual variability (Kugler et al, 1992). Appraised control could be applied within a daily sampling design, and compared to both chronic stress, and daily state-stress, to examine an effect on salivary IgA. Here appraised control over a day could be assessed alongside state-stress to see if an interaction occurred with a measure of more chronic perceived stress. Analysis could include a subjective chronic stress effect, an aggregated daily effect, an interactional effect, or a specific one-off daily effect of states at the time of sample collections.

Aims and Objectives

It was necessary to take into account the quick responsive nature of salivary IgA by using multiple sampling over consecutive days to ensure any reactivity to confounding states could be covered by a an aggregated value. This encompassed the idea that non-specific acute event reactivity of IgA could hide either a general stress or an appraised control effect. It was also necessary to examine and compare the effects of stress and appraised control within a more time-definitive format, such as daily recording and sampling, over a longer period than three days. This would enable assessment of general perceived stress (from the Perceived Stress Scale-10) and hassles of the previous month, state stress (from the Stress Arousal Checklist) of each day, and appraised control over the day's events, to compare potential effects on salivary IgA and cortisol levels. Not only can this design allow examination of perceived stress with an aggregated data set, but it also enables a comparison of single days which can be used in interactional analyses. The different ways of examining the effects of stress and appraised control would allow a more comprehensive analysis on the impact on salivary IgA, and would allow better comparison between daily stress and daily appraised control.

Hypotheses

The experiments of this chapter used multiple consecutive days sampling to examine the impact of subjective chronic stress, state-stress and daily appraised control over a single day to assess the relationship of these with salivary IgA. Three hypotheses were formed and tested.

1. A comparison between groups of high and low chronic stress over the past month (PSS-10 and hassles) will produce an overall reduction of salivary IgA in individuals with high levels of stress compared to those with low levels of stress
2. An average daily appraised control over the week will show a stronger effect on salivary IgA than the average of state-stress ratings. This will be more pronounced when comparing individual days of low to high daily appraised control in comparison to days of low to high daily state-stress ratings (from the SACL).
3. An interaction will occur between perceived stress over the prior month (PSS-10) and the daily effect of appraised control on salivary IgA secretion rate.

Methods

Participants: Sampling and demographics

An opportunity sample of fifty-five undergraduate students participated in the first study that involved the Salivette collection technique (*Salivette-method study*), though nine withdrew over the study duration (n=46). Approximately one-year later a total of thirty students took part in a second study that used the Spitting-method (*Spitting-method study*) (n=30). The different collection methods were due to later findings that Salivettes had the potential to impact on salivary IgA levels, see pages 106-107. The first study was independently run by this researcher. The participants volunteered their time following a recruitment phase of signs and posters placed around the Psychology Department at Anglia Ruskin University, and were awarded two and a half hours of participation credit. There were

thirty-nine female to eight male participants with an age range of 18 to 45 years (mean = 21.7, SD = 8.8). The *Spitting-method study* was part of a larger investigation run by the Stress and Health Research Group. First year psychology undergraduates were approached by a member of the research team during the first few weeks of seminars and were asked to take part as a control group, to be paid a sum of £50 on return of the saliva samples and forms. Twenty-six were female and five were male with an age range of 18 to 42 years (mean = 23.6, SD = 6.9). The inclusion criteria for both studies involved all volunteers being healthy, not currently suffering a respiratory infection, and having access to a home freezer. All participants gave written consent and the study had received approval from the University Research Ethics Committee (UREC).

Materials

i – Questionnaires and Forms

Information sheets and consent forms were signed prior to completion of a 'Trait Questionnaire' pack. For the *Salivette-method study* this asked for general demographic and health status information (sex, age, whether they were ill, etc.) and also contained questionnaires to measure personality traits, and emotional states. Rotter's (1966) Locus of Control Scale determined the level of this internal-external control trait. The General Health Questionnaire-30 (Goldberg, 1972) was used to assess the level of psychiatric disorder symptoms that reflect a state of distress. The Perceived Stress Scale (PSS-10) of Cohen and Williamson (1988) was utilised to quantify a global level of stress at study onset. All questionnaires have been discussed in preceding chapters with reference to reliability and validity. In addition, the 117-item Hassles Scale of Kanner, Coyne, Schaefer and Lazarus (1981) was completed as a measure of the way in which people may feel hassled through annoying, irritating or frustrating common events, and provides not only the number of such events, but also the degree of severity (scale 1, moderately to 3, extremely) for each experienced hassle. This scale has been found to predict psychological health better than life event measures (Lazarus, 1984) and has been used in stress and salivary IgA research (e.g. Kubitz et al, 1986; Martin & Dobbin, 1988). While a variety of questionnaires were handed out by the University Research group to *Spitting-method study* participants, the relevant questionnaires analysed in this thesis were Rotter's (1966) Locus of Control scale, the General Health Questionnaire-28 (Goldberg & Hillier, 1979), and the Perceived Stress Scale (PSS-10).

For both studies, during a seven-day saliva collection period a number of daily questionnaires and scales were completed at the same time as the sample was provided. This 'Baseline Questionnaire' pack contained written instructions to follow for both questionnaire completion and sample collection at home, following the sample collection guidelines as set by Navazesh (1993) with specifications for the times to provide the saliva samples. The daily forms completed were the Stress Arousal Checklist (Mackay et al, 1978), a symptom health assessment checklist to ensure they were not experiencing a current URI, and a Daily Appraised Control scale (see below).

Daily Appraised Control Scale

Due to the finding that the caregiver appraised control scale showed discriminant predictive value for physical health status, and distress measures from the General Health Questionnaires, this scale was used in this study. The 30-item caregiver scale gave excellent reliability statistics for the non-caregiver group (n=35) and showed a strong alpha of 0.92, and Spearman-Brown at 0.93. This suggested that the scale could be employed in a more generalised setting to explore appraised control within a population of individuals that were not experiencing an objective negative event, such as caregiving. Five of the specific caregiver questions were removed and one re-worded to read "I feel that I have been blamed for things that were not my fault". These items were excluded as the questions were directed at the behaviour of the care recipient (e.g. outbursts from others). It was also necessary to change the tense of the questions to reflect the time-frame from the general situation to the more specific control over daily events. This is in line with previous research indicating that psychometric instruments are robust to modifications such as the number of items or wording (Villani & Wind, 1975). The final Daily Appraised Control scale consisted of 25 items chosen to assess perceived control over a day's events (see *Appendix VI*).

Reliability Analysis: Repeated analysis was conducted across the seven baseline collection days from the *Salivette-method study* and Cronbach's alpha ranged from 0.88 to 0.92. These values ranged from 0.83 to 0.95 over the fourteen days of the *Spitting-method study*. It was also possible to run test-retest analysis and comparisons were made between the first and final collection days with a week for the *Salivette-method study* ($r_{(46)}$=0.46, p=0.004) and a fortnight during the *Spitting-method study* ($r_{(29)}$=0.44,p=0.008). To counter accumulating practise effects the two groups were amalgamated and the minimum and maximum daily control values were correlated (these would be randomly distributed for each participant). The two values were significantly correlated ($r_{(76)}$=0.41,p<0.001) and suggested that while the scale displayed some test-retest reliability, the scores were not so strongly associated for it to be measuring a completely stable and trait-like concept. This larger combined group was used to test the scales validity measures.

Construct Validity: To assess the construct validity of this scale it was correlated with the Rotter (1966) Locus of Control scale and Perceived Stress Scale-10 (PSS-10) as both are established questionnaires that address some form of controllability. It was predicted that the Daily Appraised Control scale would correlate with these two questionnaires to show construct validity but also display discriminant validity. Both groups were amalgamated to form one large sample from which to run analysis, and the values are shown in **Table 11**.

Table 11: Appraised Control scale construct validity: Pearson's one-tailed correlations between the first valid appraised control rating, perceived stress (PSS-10) and Rotter's locus of control for the amalgamated group (n = 76)

	Appraised Control (alpha)	Locus of Control	PSS-10
Appraised Control	(0.825 - 0.946)	-0.07, p>0.2	-0.39, p=0.001
Locus of Control	-	-	0.27, p=0.018
PSS-10	-	-	-

The results in **Table 11** showed that the Appraised Control scale was not significantly correlated to Rotter's Locus of Control scale in this group of undergraduates. The change in time-specificity of the appraised control scale to reflect daily events could explain the lack of association with this trait measure. In the more global measure of stress, where controllability is an aspect of stress appraisal measured in the PSS-10, it was to be expected that there were high negative correlations, meaning that people with a high levels of perceived stress scored lower on appraised control. Yet the fairly small correlation value may suggest that these scales are able to measure different things, which would be expected where the appraised control scale was designed to focus specifically on control. The shared variance could be a product of the few items in the PSS-10 that explicitly refer to control. In light of the previous chapter's findings that a version of this appraised control scale was a unique predictor of health status outcomes, this scale was used again within the more time-definitive format of daily collections.

ii – Saliva Collection and Storage

The saliva samples from the *Salivette-method study* were provided by the participant at home using the Salivette (Sarstedt, UK) (see *Appendix II* for protocol), technique for two minutes. Once the cotton roll had been returned and the tube sealed, the Salivette was placed within a heavy-duty ziplock bag and stored in the participant's home freezer until all samples had been provided and could be returned to the University laboratory. The samples from the *Spitting-method study* were collected used polypropelene plastic test tubes. They were given instructions to allow saliva to accumulate for exactly two minutes, following which they were told to dribble into the 30mL tube, replace the cap and store the tube in their home freezer until all samples had been collected. Though domestic freezer temperatures range from -18 to -22^{o}C, the majority of prior psychoimmunology studies have used storage temperatures of -20^{o}C (e.g. Evans et al, 2000; Willemsen et al, 2002).

Design

Two studies shared the same design, but one used Salivettes and the other the spitting-method for the collection of saliva samples. Both studies used opportunity samples of undergraduate students. There were four measures related to stress: Cohen and Williamson's (1988) perceived stress scale (PSS-10), Kanner et al's (1981) hassles from the past month, Mackay et al's (1978) Stress Arousal Checklist (SACL) and daily appraised control. These were assessed in terms of their effects on the dependent variables of salivary flow rate, IgA secretion rate, and cortisol. The independent variables were examined in three ways. The first involved a between-subjects contrast in perceived stress and hassles effects, the second used a within-subjects contrast between single days rated highest and lowest on state-stress and appraised control. The third was looking for an interaction between perceived stress and the daily state variables of stress and appraised control. To account for the effects of the diurnal rhythms (Miletic et al, 1996; Hucklebridge et al, 1998) all participants provided samples at the same time during the evening between 6 and 8pm for both studies. While the *Spitting-method study*

participants provided 14 days of samples, only the first seven were used in this thesis to make the data comparable between the studies, where a week was collected in the *Salivette-method study*.

Procedure

Initial contact with the *Salivette-method study* participants was made by an e-mail, which had attached an electronic version of the information sheet. They were asked meet at the research office to collect the 'Trait Questionnaire' pack (containing demographic and health related questions, Rotter's Locus of Control scale, the Perceived Stress Scale-10, and the General Health Questionnaire). On arrival they were handed this pack and given instruction to complete the forms by the following Monday. When this was returned, the participants collected the 'Baseline Questionnaire' pack, containing daily questionnaires (items relating to sampling date, time of collection and when frozen, symptom checklist, the SACL, and Appraised Control scale), seven labelled Salivettes in a ziplock bag, and detailed instructions (see *Appendix* II for the Salivette instructions). They were asked to fill out the forms at the same time they provided the seven evening saliva samples, and this time was set between 6 and 8pm. At home that evening the participant provided the first sample (labelled Day One), placed it in the ziplock bag and stored it in their domestic freezer. They completed the questionnaires at the same time. This procedure continued each of the following evenings until all seven samples had been collected. On the day of the samples to be returned, the participant was asked to be as quick as possible in getting the samples from their freezer into the University, where they handed them over to the researcher at a previously determined time. The samples were then stored in University laboratory at –80^{o}C until assayed.

For the *Spitting-method study* first year psychology students were asked during their first week of seminars to participate in a research project as a control group. They were asked to take the 'Trait Questionnaire' pack (containing demographic and health related questions, Rotter's Locus of Control scale, the Perceived Stress Scale-10, and the General Health Questionnaire) if they were willing to volunteer. Inside this pack were the information sheet, two consent forms, instructions, and the trait questionnaires in booklet form. They were asked to return the pack by the following Monday when the 'Baseline Questionnaire' pack was waiting for them to collect. This pack contained daily questionnaires, fourteen labelled saliva tubes (e.g. Day one), and a couple of ziplock freezer bags. The participants were required to complete forms at the same time as the sample was given which recorded the time of sampling (set between 6 and 9pm), freezing, symptom checklist, the SACL, and the Appraised Control scale. This time the samples were collected via the *Spitting-method* for two minutes and for a total of fourteen consecutive days. Each sample was frozen immediately after being provided and stored in the domestic freezer until all fourteen had been given. On return to the University the fourteen samples were immediately stored at –80^{o}C until assayed.

Salivary Analyses

Salivary Flow Rate: All samples were removed from the –80°C freezer and thawed for $1^1/_2$ hours before being centrifuged at 1500RCF (3000rpm) for 10 minutes (ALC55 Centrifuge, CWSystems). For the Salivettes, the insert tubes and cotton buds were then removed and the remaining outer tube with sample was re-capped. Salivary flow rate was determined gravimetrically.

Salivary IgA: The in-house ELISA was used to detect the level of salivary IgA in the samples from the *Salivette-method study*. This protocol is detailed in the previous chapter. All plates were covered by disposable lids after each phase to minimise carry-over and wash procedures were by hand. The nine IgA standards used (using Sigma l1010 human IgA) and were diluted to provide a range from 10ng/ml to 350ng/ml. The conjugate was STAR92p diluted to 1:30k. All plates conformed to the acceptance criteria of a CV less than 10% between the standard and control triplicate repeats, and the standard correlation coefficient was greater than 0.98. External inter-plate controls (two control saliva samples) were examined and did not differ more than 10% between plates.

For the salivary IgA levels in the *Spitting-method study* a Tecan Genesis Freedom (150/8) automatic ELISA was used. While the principle remains the same for analysis, the main differences must be noted. All samples, including standards and controls were run in duplicate and each plate had a 37 unknown sample capacity (as opposed to 36 by hand). The standard range had a lower cut-off point of 200ng/ml and any unknown sample (diluted at 1/2000) above this was re-assayed with a greater dilution factor (1/10k). The wash procedure was conducted by a Tecan Columbus Washer (16 channel head) for four cycles. The Tecan Genesis Freedom does not use lids at any stage as the incubation/shaking phases occur within sealed compartments (37°C and 22°C). The conjugate was STAR92p diluted to 1:1000 and the stop solution used was 8M acetic/1M sulphuric acid. The curve-fitting was the 4-parameter sigmoid option and a Tecan Sunrise reader used a 450nm filter with a reference at 620nm. All plates had an average sample duplicate CV below 8% and the standard correlation coefficient was greater than 0.98.

Salivary Cortisol: Cortisol was analysed following IgA as cortisol has been reported to be stable for two freeze-thaw cycles (Gröschl et al, 2001). This was analysed in duplicate via high sensitivity cortisol Enzyme-linked immunosorbent assay (ELISA - Salimetrics, USA). This assay follows the competition principle - the cortisol levels from 25µL of known standards (1.8, 0.6, 0.2, 0.067, 0.022, 0.007 ng/ml) and the unknown test samples compete with cortisol (conjugated to horseradish peroxidase) for the antibody binding sites of the microtitre plate wells, which are coated with anti-cortisol rabbit antibodies. After a 55-minute incubation period at 23°C, the competition was halted and the plate was washed by hand. The substrate tetramethylbenzidine solution was added to react to the bound cortisol peroxidase and incubated at room temperature for further 25-minutes. Following this period the stop solution was added (2M sulphuric acid) and the optical density was read by a MXR_{II} plate reader (Dynex Technologies, Chantilly) at 450nm with a reference at 490nm. The concentration of cortisol (µg/dl) was determined by first subtracting the average optical density of the non-specific binding wells from those of the standards and unknown test samples (the non-specific binding wells

contained no antibodies), then the percent bound was assessed by dividing these figures by the average of the zero wells. The 4 parameter sigmoid minus curve fit, utilising a series of six standards (in a synthetic saliva matrix), was calculated by the Revelation software (Dynex Technologies, Chantilly) to determine the cortisol concentration of the unknown test samples. The standards were run in triplicate and unknown samples were entered in duplicate. The two standard controls were within the manufacturer's acceptance range, CV% for duplicate samples across the plate were all < 7% for standards, controls, and unknowns, and the sigmoid r^2 fit value was >.99.

Results

Statistical Analysis: Data Assumptions

Unless specifically stated, the data sets were reasonably normally distributed, and met the homogeneity of variance assumptions. If normality tests showed significantly skewed data, when the direction was the same for all groups and the sample size ratio (largest/smallest) was less than 1.5, an ANOVA was considered acceptable (Stevens, 1996). Where the sphericity assumption was breached, the multivariate table was consulted and noted. For two sample comparisons, the data (or difference scores for paired samples) were normal and did not breach the homogeneity of variance.

During the first study nine individuals withdrew. Demographic information, Rotter's Locus of Control, perceived stress (PSS-10), and the General Health Questionnaire (GHQ-28 and GHQ-30) were assessed between the two saliva collection method conditions. Data were tested for normality and the means (standard deviations) presented in **Table 12** for both studies.

Table 12: Demographic information, Locus of Control, perceived stress (PSS-10), General Health Questionnaires for the *Salivette-method study* and *Spitting-method study* groups: Means (Standard Deviations)

	Salivette-method study	*Spitting-method study*	Test	Sig.
n=	46	30		
Gender ratio (female:male)	38:9	26:5	Chi-square	p=0.92
Age	26.7 (8.8)	24.4 (6.9)	t-test	p=0.22
Rotter's Locus of control	11.1 (3.7)	13.1 (3.3)	t-test	p=0.02
PSS-10	16.1 (7.2)	25.29 (6.6)	t-test	p<0.001
General Health Questionnaire-28	-	4.61 (4.6)		
General Health Questionnaire-30	0.85 (0.35)	-		

Note: Gender distribution run by Chi-square, all other distributions were normal and comparisons were run by independent t-tests.

Though the groups were equal in gender distribution and age, participants in the *Spitting-method study* scored significantly higher on Rotter's Locus of Control scale ($t_{(75)}$=-2.4,p=0.02), and in ratings of perceived stress (PSS-10) ($t_{(75)}$=-5.64,p<0.001) than those in the *Salivette-method study* group. In addition to the fact that the two saliva collection methods can cause a different level of salivary IgA to be measured from the samples (see below), and to encompass any potential problems that psychological differences in locus of control and perceived stress could cause between these groups, the two studies were combined. However, before this analysis followed it was of interest to explore the saliva collection technique, incorporating certain dependent variables of interest.

Salivette versus Spitting Method

There were seven days of repeated measures of flow rate, and salivary IgA that could be entered as a within-subjects factor into ANOVA with the study collection method (*Salivette* versus *Spitting-method*) as a between-subjects factor. However, only 31 *Salivette-method* and 20 *Spitting-method* participants actually gave all seven days of flow rate data. To maximise the numbers, an average weekly value was calculated for each participant (minimum of four days). The Mann-Whitney U test showed no difference in average flow rate between the two saliva collection methods (p=0.93). For salivary IgA, only samples from 25 *Salivette-method* and only 7 *Spitting-method* participants were able to be measured from every day's sample, and so an average value was also calculated from a minimum of four days. As expected there was a lower level of salivary IgA from the *Salivette-method* (Mean = 19.81 μg/min, Std. Dev = 19.4) than the *Spitting-method* (Mean = 78.49 μg/min, Std. Dev = 64.53) and the Mann-Whitney test showed this to be significant (Z=-5.5,p<0.001).

To examine the potential that the effect of collection method on IgA secretion rates could interact with a psychological variable of interest (e.g. where different methods have been used in stress research) an assessment was run with ANOVA, where a within-factor of single days of highest and lowest rated state-stress (SACL) were compared with the between-factor of saliva collection method. For the variable of salivary flow rate (n=76), this analysis did not find any significant results for the main effect of high versus low stress day (p=0.22), main effect of collection method (p=0.73), or an interaction (p=0.80). The same ANOVA analysis was run for salivary IgA secretion rate (n=58) on the day rated the highest versus the day rated lowest on SACL stress. As expected there was a significant main effect of collection device, where levels of IgA were lower from the *Salivette-method* ($F_{(1,56)}$=18.52,p<0.001,eta^2=0.25). There was also a significant main effect of stress rated day ($F_{(1,56)}$=4.40,p=0.04,eta^2=0.07) with higher levels of salivary IgA secretion rate on days rated the lowest on SACL stress (Mean IgA = 45.26 μg/min, Std. Dev = 62.59) compared to the highest on SACL stress (Mean IgA = 32.53 μg/min, Std. Dev = 34.68). The ANOVA did not find that the collection method interacted with the state stress (SACL) factor under investigation (p=0.22). In summary, whilst the method of saliva collection affected the IgA secretion rate, it did not appear to interact with the factor of stress under investigation and this stress effect was examined further within the larger group amalgamation in the following analyses.

Group Amalgamation

The difficulty of huge absolute differences in IgA according to collection method was countered by first standardising the scores around the particular mean and standard deviation of the method used for saliva collection in each case. These standardised Z scores were then used to examine the potential effects of perceived stress (PSS-10) and hassles over the past month, daily state-stress (SACL), and daily appraised control.

Hypothesis One: A comparison between groups of high and low chronic stress over the past month (PSS-10 and hassles) will produce an overall reduction of salivary IgA in individuals with high levels of stress compared to those with low levels of stress

The amalgamated group was divided by the mean of perceived stress (PSS-10) over the previous month (mean = 19.87, Std. Dev = 8.27), which split the group into 37 high PSS-10 and 38 low PSS-10, with one missing classification due to the score being too close to the mean at 20. To examine an impact on salivary flow rate and ZIgA secretion rate, two independent t-tests were performed using the week mean for flow rate (mean = 0.44, St. Dev = 0.25) and standardised salivary IgA (Zmean = 0.018 µg/min, Std. Dev = 0.83), where a minimum of four days were available. There were no differences between high and low perceived stress groups found in the week means for flow rate (p=0.45) or salivary ZIgA (p=0.40). There was also no correlation between the PSS-10 score and mean ZIgA ($r_{(76)} = 0.10$, $p = 0.37$).

The number and intensity of hassles experienced from over the previous month were only recorded in the first (*Salivette-method, n=42*) study, and consequently these were assessed through correlational analysis with mean flow rate and raw salivary IgA. Spearmans's Rho analysis revealed no relationship between the number of hassles recorded from the prior month and mean flow rate (p=0.25) or salivary IgA secretion rate (p=0.14), and no correlation for hassles intensity with flow rate (p=0.46) or raw IgA (p=0.30). Neither of these findings supported the first hypothesis. A level of chronic stress measured as either perceived stress or monthly hassles was not associated with the expected decrease in salivary IgA. Cortisol results could not be examined in these analyses due to the lack of data in the low PSS-10 (n = 7) group as cortisol was only assessed in the second study, which precluded examination with the hassles scale that was only completed in the first study.

Hypothesis Two: An average daily appraised control over the week will show a stronger effect on salivary IgA than the average of state-stress ratings. This will be more pronounced when comparing individual days of low to high daily appraised control in comparison to days of low to high daily state-stress ratings (from the SACL).

The week average was computed for both appraised control (mean = 3.66, Std. Dev = 0.42) and state-stress (SACL) ratings (mean = 5.34, Std. Dev = 3.63). Spearman's Rho correlational analysis revealed no associations between the week's mean salivary flow rate and either mean appraised control ($r_{(76)} = 0.14$, p=0.21) or state-stress ($r_{(76)} = 0.08$, p=0.49). The same analysis revealed no associations between the week's mean salivary ZIgA and either mean appraised control ($r_{(76)} = -0.14$, p=0.23) or state-stress ($r_{(76)} = 0.15$, p=0.20). In contrast to the hypothesis, there was no effect of appraised control on mean ZIgA secretion rate.

The week's worth of data were inspected to find the two days rated with the highest and lowest levels of state stress (SACL) and appraised control. Where two days were rated the same, the first of these days was used. For appraised control, there was a significantly greater level of rated appraised control on the high-control days compared to that for the low-control days ($t_{(75)}=17.16, p<0.001$). A significant

correlation was also found between these days ($r_{(73)}$ = 0.43, p<0.001). Other variables of week day, state stress (SACL), salivary flow rate, ZIgA secretion rate, and cortisol were compared between these days. The means, Standard Deviations, and test results are displayed in **Table 13**.

Table 13: Single days of high-control compared to low-control on the dependent variables of week day, state stress (SACL), salivary flow rate, IgA secretion rate, and cortisol.

	High Control Mean (Std. Dev)	Low Control Mean (Std. Dev)	Test	Sig
Appraised control rating (n=76)	4.15 (0.37)	3.08 (0.59)	t-test	p<0.001
Week days	n=51	n=61		
Weekend days	n= 25	n=15	Chi-square	p=0.24
SACL stress rating (n=76)	3.28 (3.57)	8.39 (5.76)	t-test	p<0.001
Saliva flow rate (ml/min) (n=76)	0.4406 (0.394)	0.4255(0.268)	t-test	p=0.73
ZIgA secretion rate (µg/min) (n=73)	0.018 (1.00)	-0.03 (0.95)	t-test	p=0.64
Salivary cortisol (n=26)	0.0945 (0.07)	0.0867 (0.07)	t-test	p=0.58

In comparing high-control and low-control days, the crosstab statistic of Chi-square found that these days were not differently distributed between week and weekend days. State stress (SACL) ratings were significantly higher on low-control days, yet there were no differences found in salivary cortisol. For the variables of salivary flow rate and salivary ZIgA secretion rate, there were no appraised control effects when comparing the single highest and single lowest days of rated appraised control.

The next assessment required identical analysis of the state stress (SACL) scores. A paired t-test showed significantly higher state stress (SACL) on high-stress days compared to low-stress days ($t_{(75)}$=17.83,p<0.001). Again there was a significant correlation between these days ($r_{(58)}$= 0.42, p<0.001). The high-stress day and low-stress day were then compared on week day, appraised control, salivary flow rate, IgA secretion rate, and cortisol, the Means, Standard Deviations, test and results of which can be seen in **Table 14**.

Table 14: Single days of high-stress compared to low-stress on the dependent variables of week day, appraised control rating, salivary flow rate, IgA secretion rate, and cortisol.

	High Stress Mean (Std. Dev)	Low Stress Mean (Std. Dev)	Test	Sig
SACL stress rating (n=76)	10.93 (5.0)	1.62 (2.5)	t-test	p<0.001
Week days	66	48		
Weekend days	10	28	Chi-square	p=0.24
Appraised control rating (n=76)	3.24 (0.61)	3.79 (0.56)	t-test	p<0.001
Saliva flow rate (ml/min) (n= 76)	0.4212 (0.285)	0.461 (0.299)	t-test	p=0.19
ZIgA secretion rate (µg/min) (n=58)	-0.06 (0.82)	0.16 (1.12)	t-test	p=0.14
Salivary cortisol (n=27)	0.0789 (0.035)	0.0761 (0.062)	t-test	p=0.82

In comparing high-stress and low-stress days, the crosstab statistic of Chi-square found that these days were not differently distributed between week and weekend days. Appraised control ratings were significantly higher on low-stress days ($t_{(75)}$=-7.79,p<0.001). There were no differences found in salivary cortisol or salivary flow rate. Though differences were previously found in the analysis comparing saliva collection methods, the standardised salivary IgA secretion rates were not significantly different between days of low-stress and high-stress (p=0.14). Neither of these results supported the second hypothesis, where it was expected that appraised control would show some

effect on the biological dependent variables. In fact neither state-stress (SACL) nor appraised control impacted on evening salivary IgA levels.

Hypothesis Three: An interaction will occur between perceived stress over the prior month (PSS-10) and the daily effect of appraised control on salivary IgA secretion rate.

The high and low perceived stress (PSS-10) grouping was entered into ANOVA as the between-subjects factor with the high-control and low-control days as the within-subjects factor for the dependent variables of salivary flow rate, ZIgA, and cortisol. These psychological factors did not affect salivary flow rate at all (all $p>0.20$) and the effects on salivary ZIgA secretion rate were also not significant (all $p>0.15$). There were not enough cortisol samples on both days in the low perceived stress group ($n=4$) to allow the ANOVA to be run. The next statistics needed to look at a similar analysis with a PSS-10 grouping, but with the high-stress day as a within-subjects comparison to the low-stress day. The ANOVA for salivary flow rate did not show any effects (all $p>0.13$), and neither did the ANOVA for ZIgA secretion rate (all $p>0.20$).

In summary, perceived stress and hassles from the previous month, and daily appraised control did not show any effect on salivary flow rate or IgA secretion rate. These results did not support the hypotheses, yet there was one significant result where a comparison between the high state-stress and low state-stress day revealed a significantly lower IgA secretion rate on the high-stress day, only when the untransformed data was assessed in relation to the saliva collection technique. This result was not repeated with the standardised Z scores. However, given the results from the previous Caregiver chapter where appraised control was a significantly unique predictor of general health outcomes, some additional analyses were run.

Additional Analyses

Participants completed a URI symptom checklist every day of saliva collections, which meant that appraised control could be examined in relation to the objective and current rating of physical symptoms, which were averaged over the week. They also answered a retrospective question regarding the number of colds they had suffered in the previous year. Spearman's Rho correlational analysis (the symptom checklist was significantly positively skewed) examined these in relation to perceived stress (PSS-10), the number and intensity of hassles, average daily state-stress, and average daily appraised control. These results are presented in **Table 15**.

Table 15: Spearman's Rho correlations for the week mean of URI symptoms, perceived stress (PSS-10), number and intensity of hassles form previous month (only available in the Salivette study), week mean of state-stress, and week mean of appraised control.

	Mean symptoms r (n)	PSS-10 r (n)	Number of hassles r (n)	Hassles intensity r (n)	Mean state-stress r (n)	Mean appraised control
Number of URIs in past year	0.30** (74)	0.02 (73)	-0.03 (41)	-0.02 (41)	-0.08 (74)	-0.2 (74)
Mean Symptoms	-	0.08 (76)	0.07 (42)	0.09 (42)	0.1 (77)	-0.26* (76)
Perceived stress (PSS-10)	-	-	0.61** (41)	0.38* (41)	0.30** (76)	-0.35** (76)
Number of hassles	-	-	-	0.36* (42)	0.51** (42)	-0.61** (42)
Hassles intensity	-	-	-	-	0.30 (42)	-0.27 (42)
Mean state-stress (SACL)	-	-	-	-	-	-0.59** (76)
Mean appraised control	-	-	-	-	-	-

All two-tailed *p<0.05 **p<0.01

The weekly mean appraised control value was the only measure to be correlated with the mean number of symptoms over the week, and it should be noted that the result for the number of URIs in the past year only just missed significance (p=0.09). Appraised control also showed a stronger correlation with perceived stress (PSS-10), number of hassles, and intensity of the hassles when compared to the state-stress (SACL) measure. The average flow rate and ZIgA secretion rate were also added to these assessments but only correlated with each other ($r_{(76)} = 0.72$, $p<0.001$).

It was thought that distress measures from both the GHQ-28 and GHQ-30 would correlate well with the week average for appraised control. Pearson's statistics for the GHQ-30 showed this to be the case ($r_{(46)} = -0.55$, $p<0.001$) and so did Spearman's Rho for the GHQ-28 ($r_{(30)} = -0.32$,$p=0.04$). For both measures, a higher average of appraised control over a week was associated with a lower level of distress. Again neither average flow rate nor ZIgA secretion rate correlated with the level of distress.

Where cortisol was only collected in the second study (n = 30), this was examined with further Spearman's Rho (average cortisol values were significantly positively skewed) correlational analyses between the average over the week and perceived stress (PSS-10), average daily state-stress (SACL), average daily appraised control, average flow rate and average raw salivary IgA secretion rate (standardised scores were unnecessary here as only *Spitting-method* samples were involved). These results are presented in **Table 16**.

Table 16: Spearman's Rho correlations for the week average salivary cortisol, perceived stress (PSS-10), average week of state-stress, average week of appraised control, average week of salivary flow rate, and average week of salivary IgA secretion rate (n = 30)

	PSS-10	Average state-stress	Average appraised control	Average flow rate	Average IgA
Average salivary cortisol	-0.29	-0.38*	0.36*	-0.23	-0.35*
Perceived stress (PSS-10)	-	0.40*	-0.63**	-0.21	0.17
Average state-stress (SACL)	-	-	-0.48**	0.18	0.22
Average appraised control	-	-	-	-0.03	-0.44*
Average salivary flow rate (ml/min)	-	-	-	-	0.75**
Average IgA secretion rate (µg/min)	-	-	-	-	-

All two-tailed *p<0.05 **p<0.01

These results presented some interesting and contradictory findings to those reported above. In this sub-group (n = 30), where saliva was collected via the *Spitting-method*, there were significant results regarding the average week daily appraised control and the average salivary IgA secretion rate. This was above that seen for the average state-stress (SACL) values, which did not show any significant association. This supported the second hypothesis: where appraised control was higher then IgA was lower, and showed a stronger effect than state-stress. Furthermore, the average salivary cortisol was significantly correlated with both the state-stress and appraised control averages, and salivary IgA. Where appraised control was high, cortisol was also high but salivary IgA was low. Overall, where cortisol levels were increased these were associated with lower levels of salivary IgA.

Discussion

The average standardised salivary IgA secretion rate from a week of sampling (minimum of four days) was not affected by either of two measures of subjective chronic stress: through a general level of perceived stress (PSS-10) from the past month, or the number and intensity of hassles reported from the prior month. This did not support the initial hypothesis where it was expected that a measure of chronic stress would be inversely related to average salivary IgA.

For the entire sample (n = 76), salivary IgA was standardised to account for a reducing IgA effect from Salivettes. The state-stress (SACL) that was rated concurrently to saliva sample collection was examined through an average over the week, and compared to the salivary IgA effects of an average appraised control rating provided at the same time. Here, neither of the assessments showed significant findings. Neither did comparison of the more state-dependent examination of single high to low days for both state-stress and appraised control. These results did not support the second hypothesis where it was predicted that the state-dependent variables would show some effect on salivary IgA. However, what must be noted was the significant result when comparing the raw salivary IgA values between high to low days of state-stress, within the ANOVA that focused on examining a potential affect of two saliva collection methods. This found that the collection method (Salivette versus Spitting) did not differentially affect salivary IgA on days of high compared to low state-stress, where salivary IgA secretion rates were significantly increased on days of low stress than on days of high stress. Yet this result was not seen again when the IgA values were standardised to allow the two studies to be amalgamated, and this will be addressed in the main discussion.

There were no interactions present between the global measure of perceived stress and the more state-dependent variables, which did not support the final hypothesis. However, when cortisol analyses involved the raw IgA data from the Spitting-method samples, there was a significant negative correlation between appraised control (averaged over the week) and salivary IgA which was not seen in the analysis regarding either perceived stress or daily state-stress ratings. This did support the second hypothesis. Furthermore, a negative relationship was observed between the week average cortisol and salivary IgA secretion rates.

Perceived Stress, Hassles and Salivary IgA

The design of this study included both a global measure of stress and hassles, and used an average salivary IgA secretion rate measure over a week of collections to minimise the problems of a single IgA value. These global measures were compared to the state measures completed concurrently at the time saliva samples were collected over seven consecutive days. These different ways to analyse the data have made the results comparable to a number of previous studies. The average standardised IgA secretion rate value from these collections was not seen to be affected by either perceived stress (PSS-10) or hassles from the preceding month, where they were examined between-groups and as a correlational analysis.

Given the results were from undergraduate students who participated at different times during the academic year, they could be compared to a number of studies in which this population is utilised. The lack of perceived stress effects support the findings of Mouton et al (1989) who report no relationship between stress and IgA during exam periods, MacLaughlin, Wang, Noone, Liu, Harazduk, Lumpkin, Haramati, Saunders, Dutton and Amri (2010) who found no difference in IgA at final exams in May compared to baseline in January (though cortisol increased), and Deinzer et al (2000) who used daily sampling throughout an exam week in comparison to a baseline four weeks previously to report no effect on IgA secretion rate despite a higher level of rated stress. However, these results are inconsistent with the decreased levels of salivary IgA secretion rate in response to perceived stress over the academic year (Jemmott et al, 1983), during exam periods (Dienzer & Schuller, 1998; Jemmott & Magloire, 1986), and perceived stress in dental students (Ng et al, 2004), and attention should be focused as to why.

Obviously this study did not specify a high stress event in terms of exams but it did measure the global level of perceived stress (and the levels of hassles experienced) from the student population and split the group into those with high and low perceived stress. Psychology students must face a regularity of academic pressures from essay deadlines to oral presentations across the year. In this population, the overall events (in and outside university life) that were experienced created the high perceived stress (PSS-10) group mean of 26.83 (4.9), which is actually greater than the caregivers mean of 20.6 (5.6). Consequently it can be argued that despite the lack of a specific negative life event, these students were under high perceived stress. Yet this level of stress did not differentially affect salivary ZIgA compared to a group of low perceived stress students, mean 13.08 (4.4) which was lower than the non-caregiver group 14.4 (7.1). Combined with the other inconsistent findings, this could lead to a suggestion that salivary IgA is not a good marker of global perceived stress. However, the stress scales used by former research vary in form and detail, where some merely ask for single-item ratings and others delve into further depth by varying the style of the stress outcome in terms of mood, energy, pressure, for example. Yet it is unlikely to be the case that different scales are the sole cause of inconsistent IgA effects where identical scales have been associated with differing outcomes (e.g. the Recovery-Stress Questionnaire used by Deinzer & Schuller, 1998 and Dienzer et al, 2000; or the Perceived Stress Scale employed by Ng et al, 2004 and this research).

The number and intensity of hassles from the prior month was also examined. The lack of effect on salivary IgA supports some research (e.g. Kubitz et al, 1986; Graham et al, 1988) but not others (e.g. Evans et al, 1993), though it can not be compared to potential delayed effects as reported by others (e.g. Martin et al, 1988). This style of stress examination also needs review in terms of study design, where some looked prospectively at daily hassles (Evans et al, 1993) while others took a more retrospective approach (as with this thesis) of hassles from the prior month (e.g. Farne et al, 1994; Kubitz et al, 1986; Martin & Dobbin, 1988). Evans et al (1993) only found a negative association between an aggregated set of undesirable events, which could mean that this result is not a consequence of the design that measured daily events: salivary IgA appears to respond to a more global average of such daily events. Evans et al (1993) only used 12 participants, and is the only of the above hassles studies to use IgA secretion rate as an outcome variable (the others are based on concentration). Yet, the null finding of this chapter does not suggest that the effects of hassles may only be found in this measure. If methodological differences do not obviously account for such inconsistencies, then we look to other areas, and the main aim of this thesis was to investigate the potential for appraised control to relate with salivary IgA secretion rates.

Daily Stress, Daily Appraised Control and Salivary IgA

Over the seven days of collections, both state-stress and appraised control ratings were recorded at the same time the saliva sample was provided. Initial high versus low day analysis only revealed one effect where a decreased IgA secretion rate was seen on the day rated the highest in state-stress. Yet this result was not repeated when the scores were standardised to account for the impact Salivettes can cause in lowering the level of IgA measured. This could represent a greater likelihood of a Type I error due to the significantly skewed raw data and large variability between the saliva sampling techniques. The transformation was necessary to compare the overall group without the issue of collection method affecting the analyses. If the significant result reflected a genuine effect of state-stress, then it potentially delineates a time-frame of one day for a diminishing effect of stress to occur, and supports the results of Miletic et al (1996) who used a single-item stress scale to show a lower IgA secretion on days of high stress. However, the average state-stress over the week was not associated with salivary IgA in the amalgamated group, and any decision regarding this result needs to be tentatively made. While salivary IgA *may* be responsive to short-term stress over a day it is somewhat clearer that it is unreliable as an index of longer-term stress effects. Crucially, however, there were different results regarding the daily appraised control variable and salivary IgA.

When the untransformed data was used, the results showed that daily appraised control impacted on both cortisol and salivary IgA levels. Here, in the Spitting-method samples, an averaged value of daily appraised control over the week was positively correlated with averaged cortisol, and negatively associated with salivary IgA. To counter the argument from before (that significantly skewed and variable data could lead to a Type I error here) the data was Log10 transformed to bring the distribution to normality, and exactly the same correlations were observed (appraised control and average salivary IgA(Log10) $r_{(30)} = -0.45$, $p=0.013$). There is a predominant use of transformed data in the literature (e.g. Ng et al, 1999; Ng et al, 2004; Phillips et al, 2006), although raw data is also used

(e.g. Miletic et al, 1996; Deinzer et al, 2000), and both still report opposing IgA results in reaction to stress. The significant finding regarding appraised control could mean that appraisal processes may play a role in the response of IgA to daily events, and could imply an underlying variable that may work towards explaining the inconsistent stress and IgA results. Here participants higher in appraised control had higher evening cortisol and lower salivary IgA secretion rates averaged over the week.

This direction was not expected, being that control has been regarded as an essential resource that enables active responses to combat negative stimuli and reduce a detrimental reaction (Levin & Ursin, 1991). In fact, it has even been stated that "stressors … perceived as unpredictable and uncontrollable, may continue to be associated with elevated stress hormones" (Kiecolt-Glaser et al, 2002, pg.538). Low job control has been linked to greater levels of salivary cortisol over the working day in men (Kunz-Ebrecht, Kirschbaum & Steptoe, 2004). Yet the reverse is true of the results here, where appraised control was high so was the core stress hormone of cortisol. Additionally, the global perceived stress score did not correlate with the level of cortisol, though this also follows from other research (van Eck, Berkhof, Nicolson & Sulon, 1996). Interpretation of these results is difficult, but there is a possibility that a high level of perceived stress prevents a habituation effect to recurring events (van Eck et al, 1996) that is affected by the level of appraised control over those events. Certainly, this sub-group of participants had very high PSS-10 scores (mean = 25.3, std. dev = 6.6). All the participants were psychology university students and will have undergone the pressure of essay deadlines, homework and examinations. It may be that the appraised control effects were in response to such student pressures. If a student felt a higher sense of control over the results they could achieve then this may cause a higher negative state at critical times of deadlines and could explain why higher appraised control was associated with higher cortisol. In work-related stress, it has been reported that individuals of an extreme internal control orientation have shown greater negative reactions to job-stress over a three year period (Krause & Stryker, 1984).

While the increased cortisol may allude to the physiological reasoning as to how high appraised control was linked with lower salivary IgA secretion rates, some alternative explanations should be advanced, especially when we are directed to the fact that the students in which these results were found were first years and participated across the beginning semester of University, which potentially minimises some of the academic stress they faced. Although interestingly it may suggest that the first experience of University life is a stressful one.

There are very few field studies that the salivary IgA results can be compared with, but there are some other findings that potentially concur with those reported here. For example, Zeier et al (1996) found salivary IgA to increase following two working sessions of air traffic controllers, who are described as "not able to regulate their workload" (p. 414) which may suggest increases in response to a more uncontrollable situation. In the Kubitz et al (1986) study, they found that individuals with high internality in locus of control showed a significant negative correlation with salivary IgA, indicating high control individuals had lower salivary IgA. It could be suggested that in every day life, events that are generally appraised as being uncontrollable cause an increase in salivary IgA. However, increases in salivary IgA seem ambiguous in cause with such responses to so many different states

and events. These are described, and interpretation of this finding is detailed in the next chapter. Yet as with any research the limitations that are present in this study must be addressed before any final conclusion is drawn.

Study Limitations

The spitting-method results depict that when average appraised control was lower over the week it was associated with a significantly greater secretion rate of salivary IgA. However, the cause and effect can not be identified. The sense of appraised control over daily events could be lowered by a state of unwell and there was certainly a significant correlation between this scale and daily URI symptoms. We can not completely rule out an underlying factor that moderates changes in all variables, such as a physical state of unwell. When the body is beginning to fight some infection it may trigger chemical messengers in the immune system (cytokines) that can affect the brain, resulting in both changes to immune markers and psychological feelings and states (Maier & Watkins, 1998). However, none of the symptom data suggested actual infection and none of the participants recorded having a cold over the study duration. Although it should be mentioned that an entire third of respiratory infections can be asymptomatic (Gwaltney, 1983), it must be assumed that this was not the case for all 76 participants.

A second issue pertains to the possible impact of varying environmental factors at the time of saliva collection, where a decreasing appraisal of control could have meant a poor consistency to compliance with the procedural demands for home collection. Despite explicit instructions given to each participant it is impossible to know that every sample had been provided at least an hour after eating or smoking, within the specific time-frame set, or frozen immediately after collection. While these aspects were asked in questions completed at the same time as saliva collection it is impossible to know the accuracy of the responses. There are other factors, such as a predominance of female participants, the time of evening sample collection, the lack of information regarding potential confounds such as Body Mass Index, exercise, nutritional status, among others. However, these potential limitations are common to the three research chapter and will be addressed in context of the entire thesis in the following main discussion.

Summary and Conclusions

This study examined different measures of subjective stress, including perceived stress from the prior month, an index of hassles over the past month, state levels of stress and appraised control every day over seven days. Here, only one stress and salivary IgA effect emerged where the day rated highest on state-stress (from the SACL) had significantly lower salivary IgA levels than the day lowest in state-stress, and one appraised control effect occurred with an average weekly value being negatively correlated with average salivary IgA. This means there is a possibility for appraised control effects to exist in the current literature regarding stress and salivary IgA. However, neither result was replicated in the standardised data to compensate for a difference caused by Salivettes and Spitting-methods of saliva collection. Although this makes it difficult to interpret, what it may suggest is that salivary IgA

is not a measure of enduring stress but potentially something that marks daily effects of a state. This interpretation is with some caution, and a number of issues arise with it, including questions as to the best way to measure chronic stress, a potential unknown impact of the saliva collection method, and many others that will be addressed in the following main discussion.

5

Discussion

Synopsis of thesis aims

Salivary IgA is the primary antibody of mucosal immunity (Lamm, 1997; Russell et al, 1999; Woof & Mestecky, 2005) and is argued to have health-protective functions that may help the body's resistance to colds and flu (Brandtzaeg & Johansen, 2005; Lamb, 1997; Woof and Mestecky, 2005). Stress has been regarded as a key element in the psychological impact of events on illness susceptibility (Segerstrom & Miller, 2004) and has been cited as a leading influence in numerous psychological ill-health variables (e.g. Agarwal & Marshall, 2001). Much research has focused on salivary IgA as the way in which stress can lead to vulnerability of infectious respiratory illnesses. Yet while there is general consensus that salivary IgA levels are associated with respiratory health (Gleeson, 2000), its relationship with stress is somewhat less conclusive. Tasks classed as stressors have produced some inconsistent effects on salivary IgA in both passive coping acute stress and chronic stress. While active tasks seem to produce a fairly consistent increase in salivary IgA (though whether this is due to stress, novelty, mental effort, or other factors is not known), the sub-division of passive coping tasks contains reports of all three responses from salivary IgA in increases, decreases, and no change to a number of similar tasks (e.g. Willemsen et al, 1998; Burns et al, 2004; Winzer et al, 1999). While the inconsistencies are not obviously caused by direct methodological factors, as identical designs have found different results, there was a potential for the salivary IgA response to be an effect of individual differences. Where control has been advanced as a defining aspect of active and passive coping stressors (Bongard, 1995; Weinstein et al, 2002), control was examined through an appraisal process over a specific "passive coping challenge" in an emotional state of disgust. Appraisal over a longer-term stress event has also been related with control (e.g. Vitaliano et al, 2003; Kiecolt-Glaser et al, 2002), and there is inconsistency in the salivary IgA response to some chronic stress. In contrast to the similar methodologies used in acute stress experiments, review of the chronic stress and salivary IgA literature suggests there are methodological differences (discussed in detail later, but including between-subjects correlational designs, lack of control groups, and differences in the saliva collection approaches) that could potentially cause the different IgA results. Some of these were examined in the thesis, including a potential that the way a person appraises a stress event could impact on salivary IgA. Initially the research investigated a state of disgust as a passive coping stressor with a manipulated appraisal of disgust images to alter perceptions of control. The research then investigated an objective chronic stress event through a caregiving situation and compared this to a control group, with a further aim to assess appraised control as a possible mediator of salivary IgA. Finally, saliva samples were collected every day over a week with concurrent levels of state stress and appraised control in undergraduates. These variables were compared to the effects of the subjective levels of perceived stress and hassles from the prior month to examine the different approaches that are used in the literature. The aims of these studies were to asses stress, appraised control and salivary IgA.

Thesis Results

The first study explored the inconsistent results within the passive coping literature of acute stress by showing that an appraisal manipulation could affect the response of salivary IgA to disgust-inducing images. While state-stress was increased to both disgust appraisals, a rating of emotional control was

significantly lower from the images appraised to be real. Although emotional control was not significantly correlated to a rise in IgA, an increase in salivary IgA was only seen from a group that viewed the images to be real. A group told the same images were fake showed equal non-reactivity of IgA to a control group that were shown neutral images. This showed that a task used to evoke passive coping is open to the effect of individual differences in the way that it is appraised, meaning that not all tasks will induce passive coping. Such results may lead to a number of important revisions that could be made to the current acute stress model. When appraisal effects are not considered in the method of presentation of tasks (such as the description of the stimulus or procedure involved), then a passive task will only be passive to some individuals. Identical tasks may be capable of evoking a different emotional control via an altered appraisal, where it is not stress that appears to be crucial. Consequently, rigid protocols should be standardised for any passive coping task to ensure that even slight differences in the presentation of identical tasks do not affect the response to it. However, the appraisal process may not be solely attributed to control, where altered appraisals could also change the novelty status of the task, and future research should address this. It is clear that appraisal is one methodological aspect that could be considered during stressor events, particularly those assumed to induce passive coping and it is important to set clear and precise methodological procedures for tasks that are classified as passive coping events to attempt to create identical appraisals of the same event.

In the second study, in a chronically stressed group of caregivers both perceived stress from the prior month, and a level of state-stress were higher than a matched control group of non-caregivers, indicating a high stress event. Additionally, appraised control over the situation was significantly lower in the caregiver group. However, there was no significant difference in salivary IgA between these two groups, despite the differences observed in the psychological findings. This potentially meant two things, firstly, that chronic stress in this form of a negative life-event did not affect salivary IgA, and secondly, that appraised control did not mediate the stress and salivary IgA relationship.

The third study examined different measures of subjective stress, including perceived stress from the prior month, an index of hassles over the past month, and state levels of stress every day over a seven day period. Here, only one stress and salivary IgA effect emerged where the day rated highest on state-stress (from the SACL) had significantly lower salivary IgA levels than the day lowest in state-stress. However, this result was not repeated when IgA levels were standardised to compensate for a difference caused by Salivettes and Spitting-methods of saliva collection. There was one appraised control effect, where an average over a week was negatively correlated with an average salivary IgA, yet this too was found in untransformed data of a sub-group of participants. Although this makes the results difficult to interpret, what it may suggest is that salivary IgA is not a measure of enduring stress but potentially something that marks daily effects of a state, and a more enduring appraised control effect.

Stress and Salivary IgA

A large proportion of the prior literature produces results that show a decreased level of salivary IgA when stress is higher (e.g. Deinzer & Schuller, 1998; Jemmott & Magloire, 1988; Mouton et al, 1989;

Ng et al, 1999). However, there is a predominance within this literature to use between-subjects correlational designs, which may not "properly conceptualise chronic stress" (Bristow et al, 2008, pg. 601). The common populations in which this research is conducted are undergraduates, where the reliance is on academic situations to investigate the effects of stress (e.g. Deinzer & Schuller, 1998; Deinzer et al, 2000; Jemmott & Magloire, 1986; Jemmott et al, 1983; Kiecolt-Glaser et al, 1984; Li et al, 1997; MacLaughlin et al, 2010; Mouton et al, 1989). However, there is no reason to suspect that such circumstances induce enduring perceptions of stress where academic events are time-dependent with a definitive ending (Anisman, 2002). Indeed, when the PSS-10 has been used in the context of an academic exam, there were no significant differences in this level of stress between the baseline and exam period, and no effect on salivary IgA (Murphy et al, 2010). What this could mean is that academic stress is not a chronic stress situation, and any effects of perceived stress on salivary IgA may be due to other factors. Certainly there are inconsistent IgA effects from the academic stress research with four reports of decreases (Deinzer & Schuller, 1998; Jemmott et al, 1983; Jemmott & Magloire, 1986; Li et al, 1997) and four studies with no change (Deinzer et al, 2000; Kiecolt-Glaser et al, 1984; MacLaughlin et al, 2010; Mouton et al, 1989).

Where an event may be more long-standing, there is a slightly more consistent effect. Occupational stress research has focused on female nurses and there are at least three studies that report significant negative associations between stress and salivary IgA (Ng et al, 1997; Ng et al, 1999; Yang, Koh, Ng, Lee, Chan, Dong, Goh, Anantharaman & Chia, 2002). However, the stress involved in this research is linked to the nursing occupation, which does not necessarily pervade the individual's life, and this research relied on between-subjects correlational designs that use single sampling. While this is an issue covered in detail later, briefly, where repeated measures have been used with nurses they have shown a contrasting effect with the low objective and subjective stress group to have lowest levels of both total IgA and specific IgA (Lee, Kang, Yoon, Kim, Kim, Yoon, Trout & Hurrell, 2010).

It was useful to this area of research to conduct a study that compared a high stress group with a low stress group. The Caregiver study is one of few that has compared chronic stress effects on salivary IgA to a group not undergoing a chronic stress situation, and actually recorded a measure of chronic stress. The null finding regarding caregiver stress and salivary IgA are those reported by Bristow et al (2008), and replicate those found in the younger cohort of caregivers examined by Gallagher et al (2008). Only the older caregivers in the Gallagher et al (2008) study showed a lower level of salivary IgA to those of a younger age. However, this older group were of a similar age to the caregivers that volunteered here, which means that stress may not limit a dysregulatory affect in an older population. Overall these results suggest that where chronic stress (and state-stress) levels are high in a group of individuals currently undergoing a chronic and pervading event, there is no decreased effect on salivary IgA. This expands the former argument that salivary IgA is a weak marker of stress (Mouton et al, 1989). Here salivary IgA was a weak marker of both a specific chronic stress event in caregiving, and a weak index of subjective stress from undergraduates. There was no effect on salivary IgA from perceived stress and hassles from the prior month in undergraduates, again supporting some of the literature (e.g. Deinzer et al, 2000; MacLaughlin et al, 2010; Mouton et al, 1989) where there is no relationship between stress and IgA in student samples during exam periods.

However, they are inconsistent with the decreased levels of salivary IgA secretion rate in response to perceived stress over the academic year (Jemmott et al, 1983), during exam periods (Dienzer & Schuller, 1998; Jemmott & Magloire, 1986), and perceived stress in dental students (Ng et al, 2004). The null hassles result also supports some research (e.g. Kubitz et al, 1986; Graham et al, 1988) but not others (e.g. Evans et al, 1993) and collectively, what the results suggest is that salivary IgA is an inconsistent responder to stress, whether this is measured subjectively through scales at the time of a specific academic stressor, generally over the past month, or as more objective daily hassles.

However, one significant result that emerged in the expected direction was that on day-to-day analysis of high to low state-stress levels, salivary IgA was lower on the day rated higher in stress. What this could suggest is that salivary IgA may be a stress marker for specific days, not as a more enduring stress index. However, even this is inconsistent with some research, for example Evans et al (1993) found the reverse effect regarding undesirable events, where these were higher than average on a day they were associated with high IgA levels. This could suggest that other factors than stress or the undesirability of events has a role to play in salivary IgA secretion rates. In fact the overall results found in this thesis would suggest that something beyond stress may relate with salivary IgA, specifically in chronic stress, and perceived stress or hassles from the prior month. Where the salivary IgA inconsistencies were examined in relation to appraised control, there was one result.

Appraised Control and Salivary IgA

With regards to appraised control, there was no effect of the single highest to lowest appraised control day in the daily study, and when the standardised scores were used there were no significant findings from an averaged value. Appraised control was also not seen to produce an effect during the chronic stress of caregiving. However, when the untransformed data from the spitting-method was employed in the daily study, this showed one result where average appraised control over the week was significantly negatively correlated with average salivary IgA levels, and perceived stress and hassles from the prior month, and average state stress did not. While its specificity to this one group makes it difficult to explain with any certainty that these are valid results, it should be briefly discussed.

Where high and low appraised control days were not different, it may be suggested that appraised control may work in a cumulative manner where a number of days of high or low control are required for an effect to occur. There could be a prolonged effect where the level of appraised control has an influence three to four days later. For example, other daily research has found hassles to relate with somatic symptoms three to four days later (Sheffield, McVey & Carroll 1996). This four-day lag has also been seen with specific URI symptoms that relate with increased hassles (Stone, Reed and Neale 1987) or decreased uplifts (Evans and Edgerton 1991; Evans, Pitts and Smith 1988; Stone et al. 1987). Interestingly, this four day dip is said to reflect an incubation period of many URI viruses, and in this thesis URI symptoms were significantly correlated only with the level of appraised control, not stress from either the preceding month or daily state levels. In relation to some of the prior hassles and IgA literature, it is possible that a level of appraised control over a week may be a consequence of rising daily hassles (or decreased uplifts), or the perception of events as hassles may be influenced by the

appraisal of control over the day's events. Although neither hassle number nor intensity were related with URI symptoms or appraised control, the hassle measurement of this thesis was from the prior month and may not be sensitive enough where concurrent daily events could be of importance. It may be of interest for future prospective research to use a measure of both daily hassles and appraised control to examine possible effects. Yet what must be pointed out is that the appraised control result with salivary IgA was not found in the larger amalgamated group. This may be due to an undiscovered problem within the Salivette data and this issue is addressed shortly, yet first another explanation is proposed.

In the spitting-method group of first year students, the perceived stress rating was much higher than expected; in fact, it exceeded those from the caregiver groups. What this could suggest is that for appraised control to show some effect on salivary IgA, a high experience of perceived stress is a pre-requisite. Potentially, the caregiver group was not stressed enough to allow appraised control to show an influence. Though for a counter argument it could be seen that the non-caregiver control group were lower in stress than both student groups, and there is a possibility that undergraduates could over-report stress. There is a future direction to specify groups of participants that are extremely high and very low in perceived stress to examine appraised control effects. Another possible reason is proposed by Anisman (2002) who has suggested categorical differences in the characteristics of the stress under investigation, which include the stressor in terms of duration where academic stressors may be scheduled events with a time-limit (such as deadlines), while a situation such as caregiving is ongoing and has no definitive ending. Also, these events differ in the predictability of the tasks involved, academic stressors are set on a specific date and are usually about exact topics, while the act of caregiving may have both predictable and unpredictable components. Appraised control may only play some role when a stressor is at least objectively controllable. Another potential could be from the specificity of the items belonging to the Appraised Control scale. In contrast to the PSS-10 (which is a general measure that requires retrospective ratings of items from the past month), the version in the daily collection data assessed each day individually. This may help minimise memory confounds and may be more useful for a dependent variable such as salivary IgA that has a quick response rate. Salivary IgA may even be responsive to memory recollection (Hucklebridge, Lambert, Clow, Warburton, Evans & Sherwood, 2000), which could have implications for the memory process used when answering a retrospective questionnaire, potentially producing confound between the individual's current state to that of the past month.

Although the above factors do not explain the lack of the relationship in the larger group when standardised scores were used, these results suggest a potential for individual differences in appraised control to play a role in salivary IgA reactivity to some stress. Just as appraisal should be measured during acute passive tasks it may be recommended that appraised control be measured during experiments that aim to examine stress and salivary IgA beyond the laboratory. However, there are some methodological considerations across the literature that must be addressed first.

Methodological Issues

Salivary IgA is a Non-Specific Responder

There is one major aspect to all salivary IgA research that needs discussion, as it may be the sole reason why neither stress nor appraised control will ever be consistently linked to salivary IgA. Salivary IgA is a highly reactive mucosal protein that increases in response to a huge number of states and experiences. For example, it can react within minutes to a number of situations including something watched on television (Harrison et al, 2000; Hennig & Netter, 1997), hypnosis (Benham, Nash & Baldwin, 2007), exercise (e.g. Ring et al, 2000; Winzer et al, 1999), relaxation (Reid et al, 2001), humour (Lefcourt et al, 1990), mathematics tasks (Winzer et al, 1999), memory tests (Bosch et al, 2001), multi-tasking tests (Wetherell et al, 2004), a level of interest during lectures at University (Tsujita & Morimoto, 2002), choir music (Kreutz et al, 2004), mental imagery (Rider et al, 1990), and most importantly novelty (Burns et al, 2004; Willemsen, 2000). It seems quite difficult to require participants to avoid all these states (and those not yet discovered) at least an hour prior to participation, yet unless this is done, research in this area will continue to be compromised. Such confounds may be reduced in the laboratory situation where an hour's wait could be monitored before baseline measures are collected, but they are impossible to control in field designs, which is where stress may show its chronic effects. One state that could be common across all research designs is that of novelty, and while this at least has the potential to be minimised a definition should be provided.

Something novel is unfamiliar, represents new information, and can not be referenced to a previous experience (Webb, 1999). Consequently, novelty may have a base in our survival instinct. During a threatening situation a focused attention towards unexpected events may allow for quick responses towards potential harm (Garcia-Garcia, Yordanova, Kolev, Domínguez-Borràs & Escera, 2010). Novel events, by their nature, are unknown and unpredictable and could potentially cause a heightened state of alert to threats that may occur. Indeed the processing of unexpected novel stimuli is enhanced within a negative emotional context (Garcia-Garcia et al, 2010). Novelty could initiate the sympathetic drive to mobilise energy via supply of glucose and oxygen to the heart and muscles, accompanied by a strengthening in resistance to infection (increased IgA) that may occur as a result of injury during fight or flight action (Segerstrom & Miller, 2004). Certainly, novelty has been shown to play a role in blood pressure (Carroll, Ring, Shrimpton, Evans, Willemsen & Hucklebridge, 1996) and salivary IgA reactivity to laboratory events (Burns et al, 2004; Carroll et al, 1996; Willemsen, 2000) and a further variable to consider is that the collection of saliva itself may induce novelty to a situation. It is not a common occurrence in everyday life to have to stick a cotton roll under the tongue or to spit into a tube in front of other people. This novelty factor may be emphasised in a laboratory environment and creates many possible confounds to the research that investigates acute stress tasks within such surroundings. While this may not impact on active coping where increased IgA is to be expected, it will certainly create problems for passive coping research, and in fact any study that predicts lower IgA. This is also true of the field-based stress research, where there is a predominance of single sampling within the literature.

Single Sampling Designs

Both areas of academic stress and life-event research may be confounded by the predominant use of single sampling. One-off sampling techniques could allow an impact of states or acute events prior to the collection, and could even induce novelty via procedural aspects. Yet many studies regarding stress and salivary IgA have used one-off samples and report a negative relationship between stress levels and IgA (e.g. Ng et al, 1999; 2004; Phillips et al, 2006; Yang et al, 2002), though some do not make the data handling clear (e.g. Miletic et al, 1996), and others have reported no such effects (e.g. Kubitz et al, 1986). Although the majority would suggest that such acute events may not hide a general stress effect, it should be considered that some underlying and unrecorded variable could affect both stress and IgA levels. Certainly the correlations reported are far from perfect. For example, boredom or interest during the study procedure could be higher or lower in individuals that report greater perceived stress, so where boredom is high this could cause a higher level of salivary IgA in those individuals of high stress. This is obviously the case for any number of variables, but what could to be taken from this argument is that single sampling is not a good way to measure an index such as salivary IgA that is responsive to many various states and does not necessarily show a definite stress link. Averaged data with repeated sampling should provide a more sensitive index of the general effects of stress, where the potential confounding states may be subdued by an overall effect and any clear outliers from an individual could be detected. Although averages of data also produce inconsistent results with stress levels (e.g. Deinzer et al, 2000; Henningsen, Hurrell, Baker, Douglas, MacKenzie, Robertson & Phipps, 1992; Lee et al, 2010), this design is preferable to single sampling. Though quite costly to conduct, experiments that collect consecutive daily samples over a longer time-frame could allow a better assessment of the effects of daily and general states on such an index as salivary IgA that can show quite large intra-individual variations. However, even with such a design, another factor that should be addressed is the time of day for sample collection.

Saliva Collection Times

Additional procedural issues arise in the time of day that many saliva collections were taken. Previous research (e.g Hucklebridge et al, 1998; Stiller-Winkler et al, 1998) has shown that salivary IgA peaks upon awakening and then shows a steep decline for the next 4 hours, after which it seems to remain at a constant. It would seem that the spike of salivary IgA in the morning may be dependent on the time of awakening as opposed to the actual time of the day (Hucklebridge et al, 1998). This would certainly instil potential confounds in the prior literature that either makes no mention of the time at which collections were made (e.g. Gallagher et al, 2008; Phillips et al, 2006) or limits the samples to early day without taking note of awakening times (e.g. Ng et al, 1999). For a study that required immediate awakening collections there is no significant stress and salivary IgA results (Deinzer et al, 2000). This could suggest such studies with collection times earlier in the day may have been hampered by differing waking times of participants, especially when students are involved. However, there may also be difficulties associated with evening samples and these are addressed later in reference to the limitations of the thesis. Consequently, any research that investigates salivary IgA should minimise such confounds by explicit instruction to have awoken at least four hours prior to participation and be

clear about what time range samples are collected between. Such instructions should further cover another problem, that of a URI.

Upper Respiratory Tract Infections

In much of the previous literature there is no note of URI tracking in many of the studies (e.g. Phillips et al, 2006; Deinzer et al, 2000; Gallagher et al, 2008). While Miletic et al (1996) asked about health during the study, they make no point to disclose this with regard to URIs. This could be an issue regarding the one-off sampling collections (which tend to dominate), where a respiratory infection can raise levels of IgA that would be measured (Gleeson et al, 1995), and could remain elevated for a month (Welliver et al, 1989). In experiments that show no stress and IgA link, it is possible that feeling or being ill may increase stress ratings and concurrently increases the specific IgA response. However, this may be countered by the results of this thesis where URI occurrence was recorded by questions, and each participant was required not to have experienced a cold or flu at least two weeks prior to their participation. Additionally, some of the other issues may have been at least partially encompassed in the studies of this thesis.

Comparisons to the Results of the Thesis

Although the methodological limitations cited above are not an exhaustive list of possible confounds, many of these potential problems were countered by the experiments of this thesis. A question was asked about respiratory health in the previous two weeks for every study, and inclusion criteria were set to have been free from illness two weeks prior to participation. Even if this may not have been quite long enough it at least minimises such confounds. These are also reduced by the measurement of daily symptoms during the final study where no participant showed enough signs to exhibit a URI. Such use of repeated sampling further diminishes the issues associated with single sampling. Where the single day comparisons were examined in the daily study, they were spread out over a seven day collection period, which at least reduces a potential confound of novelty as these were not automatically from the first day of sampling, and were collected in the participant's familiar surroundings of home.

Where the samples were evening collections, this creates both advantages and disadvantages. It is possible that the events of the day may show more impact on evening collections, and differing awakening times would not be an issue. However, it is at the lowest point of the diurnal cycle that samples are collected and as such may hide a general stress effect. Yet at least this stress effect was unlikely to be obscured by state-dependent factors, where the average over seven days should allow variability caused by transient states to be covered by the longer time-frame, and the overall effect could show through an average score. This was attempted in three days of caregiver collections, though only two samples were finally used. However, the caregiver data was one of the only studies to incorporate a control group to investigate the effects of a chronic stressor and enabled greater reliability that the findings were a cause of the stress situation under investigation.

Another factor to be addressed in the literature is the variety of different stress measurements used, and as Bosch et al, (2002) have stated, some of which are not confirmed as reliable or valid questionnaires. Though this is also true of the Appraised Control scale employed here, the stress scales that were used in this thesis are long-standing and reputable, with years of validity and reliability testing. Overall, the methodological considerations that were implemented in the designs across this thesis allow the final conclusions regarding stress, appraised control and salivary IgA to seem more reliable. Though there are obviously limitations to the thesis research, these will be addressed later, and the methodological considerations here make the following summary and conclusions seem appropriate, though far from definitive.

Stress, Appraised Control and Salivary IgA: Summary and Conclusions

During the chronic stress of caregiving, salivary IgA was not found to be affected by reliable and valid measures of perceived stress from the prior month (PSS-10) or state-stress (SACL) levels at the time of saliva collection. These results were seen in relation to a matched control group not undergoing such a negative life event, and show no relationship between a chronic stress event and salivary IgA. In the daily collection study, stress recorded globally (PSS-10) and as hassles (The Hassles Scale) over the prior month did not affect salivary IgA. Although there was a significant difference in salivary IgA on days rated the highest state-stress compared to lowest over a week, this was not replicated when data were standardised and leaves the result difficult to interpret. However what is clear is that stress did not impact on salivary IgA in any consistent or reliable manner. Examination of the role of appraised control only showed an effect in students that were coincidentally highest in perceived stress. Here, appraised control was negatively correlated with salivary IgA. However, these results are difficult to concretely interpret as a number of remaining issues regarding salivary IgA exist.

It is inherently difficult to test stress or appraised control effects on salivary IgA as each participant may bring into the testing room a number of states or appraisals that will immediately produce a different level of salivary IgA, independent to the concept under investigation. This is even more of an issue when testing is run outside the laboratory, where field research can be compromised further by events that happen even closer to the sampling time. Yet it is these field designs the give the greatest ecological validity and within these types of study no relationship between stress and salivary IgA were found. However, such criticism can be applied to some extent to all psychological research, and studies with a large enough sample size can hope to average out these effects and get some indication of the causal factors. For example, in the disgust study the results have provided some convincing evidence of how a manipulation can alter IgA independently to stress. While the challenge to the common perception that salivary IgA is a marker for stress is not new (e.g. Mouton et al, 1989), the evidence has been expanded upon by considering stress in varying designs, in addition to assessing its effects in terms of a potential appraisal focus with control. While there was a possibility that the inconsistency with stress might be a consequence of methodological issues, this thesis attempted to reduce some of the methodological confounds to suggest a very inconsistent response of salivary IgA to numerous measures of stress, from a global perspective, through daily hassles, to state-dependent

ratings. It is one conclusion that future research should veer away from salivary IgA as a consistent index of stress or appraised control (where cortisol would be a better index of HPA activation), or at the very least future experiments need to minimalise the potential problems.

These could be minimised by producing standardised procedures that are used across the board, to ensure methodological rigidity. In terms of the task descriptions in laboratory stress this could reduce an appraisal effect, and they would also need to use a rest period (though future research could determine how long is required) before a sample is collected either in the laboratory or in the field to reduce the number of confounding states a participant can bring with them. Many acute stress studies describe the experimental procedure as starting upon arrival of the participant, without considering the impact of acute states just prior to their arrival, or the novelty effect that may be present in the immediate baseline collections (e.g. Willemsen et al, 1998; Willemsen et al, 2002). Although many do ask participants to refrain from certain activities prior to testing, such as exercise, alcohol and food consumption, few can give any certainty that these requests were adhered to (e.g. Burns et al, 2003; Isowa et al, 2004). To counter a novelty effect from saliva collection, at least one practise should be done, as was the case in the Bosch et al (2001) study. Encompassing standards for research in the chronic stress area too, the sampling time of day should always be replicated across studies to factor the diurnal rhythm of both cortisol and salivary IgA. A control group should be employed to ensure the results are the consequence of the stress under investigation, and when the research is field-based then at least more than one sample should be collected over consecutive days. Finally, appraised control may show an effect of individual differences on salivary IgA and so this too could be measured. Only after further research that could adopt such guidelines, can any real conclusions regarding stress, appraised control and salivary IgA be made. However, interesting results were found regarding appraised control and health status, where such methodological issues are not quite such a complication. Perceived stress measured in this thesis was not a predictor variable of health status, yet appraised control significantly predicted health status outcomes in caregivers, and was significantly associated with the weekly URI symptoms reported by undergraduates in the daily study.

Appraised Control and Health Outcomes

People with a higher level of appraised control reported greater overall health status using the RAND SF-36, and had lower URI symptoms over a week of collections. Before this is discussed in detail, quick mention should be returned to the IgA results. If the negative relationship between average daily appraised control and lower levels of salivary IgA is valid, then this seems counter to the finding that high appraised control meant lower URI symptoms. However, this could be reconciled following Ursin's (1994) suggestion that **"**low values may not signal low resistance; they may only signal that this particular individual has not been challenged by infectious agents for a while; in fact, it may signal an extraordinary degree of health" (pg. 207). Furthermore, the positive relationship with cortisol could be supported by the findings of Cobb, Rixon and Jessop (2010) where higher evening cortisol from children starting school was associated with fewer episodes of URI over the next 6 months. They suggest that a greater diurnal decrease over the day suggests higher susceptibility to URIs. This could be linked to the negative trend between appraised control and the number of URIs

from the past year. Cohen and Hamrick (2003) have stated that while stress is linked with risk of URI susceptibility "only a fraction of those with high stress develop illness" (pg. 410) and this thesis could suggest a psychological resilience may be through appraised control. However, there are criticisms that need to be addressed regarding both the measure of appraised control and stress that were used and these will be discussed in the next section.

Thesis Limitations and Future Directions

The main criticism regarding the appraised control variable under investigation is the scale that was developed for the purposes of this research. As such it is undocumented and the results are impossible to compare to any previous literature. Without further exploration of this scale it is certainly impossible to state with any true confidence that it measures appraised control alone, although the correlations observed with other scales that contain an aspect of controllability (such as Rotter's Locus of Control, and the PSS-10) do suggest it may be doing something like this. Factor analysis should be run with a large number of respondents to determine if there is a single concept measured, or as mentioned in the previous chapter, whether it merely correlates to health-related behaviours. The scale could be further tested, for example, in occupational research where it could be given to those with high-control over their jobs and compared to those with little control over their day's work (Karasek, 1990), in addition to a list of health-related behaviours that may impact on IgA. It would be interesting to see which of these measures could relate better with a level of salivary IgA (or health status) and could determine whether the Appraised Control scale assesses a different dimension to merely behavioural aspects of a stress response.

Another issue regarding the psychometric instruments employed are for those used to measure chronic stress. Is perceived stress from the past month a good reflection of chronic stress in studies that don't incorporate a group of individuals expected to be stressed over a long duration? Even the caregivers used here may not have been quite as stressed as expected. An alternative explanation to the conclusion that salivary IgA was not a good marker for chronic stress is that the caregivers were not sufficiently stressed for an effect to occur on salivary IgA. One way to examine chronic stress could be to use a prospective design where individuals are assessed for a week at the beginning of a chronic event (such as new caregivers) and then repeated weekly data in the form of saliva samples concurrently with state-stress and appraised control collected every six months for a number of years. Individuals that show extremely high stress to the caregiving situation could be compared to matched controls to determine a clearer finding regarding the effects of a chronic stress situation on mucosal immunity. However, such a study would require a very large starting sample size given the likelihood of high drop-out rates (difficult with a caregiver population). An alternative design could be to use undergraduates and record a level of perceived stress at the end of each month (alongside at least three consecutive daily saliva samples) over a year and run a within-subjects comparison on individuals that have three consecutive months of high stress to three months of low stress. Both possible studies could allow exploration of the influence of chronic stress, and appraised control in those reporting high stress.

A further limitation to this thesis is seen in the subjective measures of health, which as they are called, are subjective and may be open to other confounds. For example, the retrospective accounts of colds over the past year may be open to inaccurate memory. However, research has at least corroborated the accuracy of subjective health ratings via reports from self-reported colds compared to physician ratings (Maclntyre & Pritchard, 1989) and people can accurately recall previous health symptoms over a 3-month period (Cohen & Java, 1994). In following from the health data, another question arises as to whether it would be more appropriate to measure the specific IgA to a particular virus, or antigen challenge (e.g. Stone et al, 1987). Future research could look to follow a similar procedure to Stone et al (1987) where rabbit albumin is used as a novel protein to increase a specific antibody response, then stress and appraised control effects could be compared to see whether the health implications observed for appraised control could relate to more functional measures. However, there are some limitations to this approach. Firstly, there is some question as to whether rabbit albumin can threaten the biological integrity of the body and therefore act as a pathogen to assess viral challenge. Secondly, by dividing specific and non-specific IgA levels it will always be difficult to know which is causing any effect. Following from other research (e.g. Kiecolt-Glaser et al, 1996; Vedhara et al, 1999), measuring the specific antibody response following an influenza vaccine may be a more ecologically valid way to determine functional effects of stress (and appraised control) on mucosal immunity. However, this is a secondary issue when considering that current health and IgA data may already contain confounds through the use of different methods for saliva collection.

In the initial projects of this thesis the Salivette technique was employed to collect saliva. This method was chosen in line with numerous prior psychoimmunology investigations measuring salivary IgA (e.g. Clow, Lambert, Evans, Hucklebridge & Higuchi, 2003; Phillips et al, 2006). It was chosen over other devices, such as 'plastic transfer pipettes', due to its ease and cost-effective benefits which were especially relevant for the studies involving multiple collections within the participant's home. However, as described in the introduction, the popular Salivette method has come under some scrutiny and questions have been raised regarding the accuracy of the IgA results drawn from it. Salivary IgA has been reported to be diminished from samples collected by Salivettes (Aufricht et al, 1992; Shirtcliff et al, 2001; Strazdins et al, 2005) and it was of interest to examine the effects within this thesis as the caregiver study relied solely on the Salivette method for saliva collection.

The daily study used both Salivettes and the spitting-method of collection. This provided an advantage with data to compare the two methods within identical research protocols. This was particularly useful as prior research has used both methods without mention of their possible impact. For example, in the disgust literature Bosch et al (2001) found salivary IgA from the spitting method to significantly decrease, while others have found increases with Salivette samples (e.g. Hennig et al, 1996). This is an issue raised by Strazdins et al (2005) who have commented on the current literature by stating that variations in the saliva collection techniques mean results across studies may not be comparable. However, in the disgust experiments of this thesis it was the spitting-method design that showed a significant increase in salivary IgA following real-appraised disgust images, which contrasts the decreases described by Bosch et al (2001) who also used this technique. While it has been suggested that the cotton roll of the Salivette may cause a bias in the volume of saliva that can be extracted (Li &

Gleeson, 2003), saliva flow rate was not affected by disgust in either saliva collection method of this thesis. Furthermore, a comparison between the highest and lowest stress days from the daily study data sets were compared and did not differ in salivary flow rate values.

One finding that was replicated was the impact of the Salivette on salivary IgA levels. Using the highest and lowest stress days from the Daily study data the range fell between 3 to 76 μg/min from the Salivettes, and 5 to 147 μg/min from the spitting-method, and again the mean differences were significant with the spitting-method providing a greater amount. However, while salivary IgA may be diminished by Salivettes it is "reasonable to consider that any such reduction would be a constant error" (Phillips et al, 2006 p.193). The ANOVA run with the collection types as an independent factor on days of high to low stress showed no interaction. Another relevant observation is that different salivary indices seem to vary in response to active coping tasks despite the consistent use of Salivettes. For example, following a timed maths task (PASAT) increases have been seen without an effect on flow rate (Ring et al, 2000; Willemsen et al, 2000), with two opposing flow rate results with one increase (Willemsen et al, 2002) and one decrease (Willemsen et al, 2002) and finally limited to salivary IgA concentration with no flow rate effects (Ring et al, 2000; Winzer et al, 1999). Overall the prior results, and those presented here, suggest that any differences within similar experimental paradigms may be due to other factors than the saliva collection methods used. This supports the findings from the first research project of this thesis involving caregivers where only the Salivette method was employed. However, the fact that the state-stress result and the appraised control effect from the daily study were limited to the untransformed data necessitates some observation that while there were no direct findings here, there is cause to consider Salivettes may obscure significant trends. As a consequence there is potential for the caregiver data to be confounded and any stress or appraised control impact to have been obscured. There is a need for this to be examined in future research, but also leads to a further stipulation for future stress and IgA research where it is advised to employ the spitting-method for saliva collection.

The average effect of appraised control was investigated in a fairly small sample size (n=30) in comparison to prior life stress and salivary IgA research such as that conducted by Phillips et al (2006) where 1,222 participants were used. When compared to small number of caregivers that provided adequate saliva samples (n = 21), this could suggest that a null stress effect was due to poor power from such a small size. This is slightly less of an issue regarding the larger number of participants in the daily study, where additionally multiple collections were taken. Furthermore, there was a good sample size for the disgust research chapter, with seventy-seven participants in the first study and forty-four in the second. Bosch et al (2001) used thirty-four and Hennig et al (1996) used thirty-two participants with sixteen in each of a disgust and control condition. However, the Bosch et al study used a within-subjects design to compare conditions of neutral stimuli to the disgust film clips, which enhances the statistical power of the research design. Although the appraisal manipulation would have made that impossible in this thesis, it would have been better to show each participant one of the disgust appraisal conditions with the neutral set of images in a counterbalanced order. The reason for not adopting such an approach was time-restriction; it was felt it would add too much time and limit the number of participants that volunteered. However, a within-subjects design

was used in the daily study to investigate the highest and lowest state days, where a stress effect did emerge from the untransformed data. Again, this issue needs to be addressed by future research which could incorporate another methodological point that may be an issue in the current studies, that of gender.

In the design of the Phillips et al (2006) study, near equal numbers of men and women were used. In this thesis the participant groups were mostly females in all three experimental paradigms. This female dominance is not so apparent in other research where studies use balanced ratios (e.g. Nykliček et al, 2005) or limit the sample to one gender (e.g. Brosschot et al, 1994). In fact, both the Bosch et al (2001) and Hennig et al (1996) studies used only male participants. This gender bias may have implications for all three investigations, with a particular problem for the disgust research where females are repeatedly found to be more subjectively disgust sensitive than males (e.g. Druschel & Sherman, 1999; Schienle et al, 2005; Rohrmann, Hopp & Quirin, 2008). However, prior research designed specifically to test such effects found an identical increase in salivary IgA after disgust from both men and women (Farley & Bristow, 2003). A more recent article by Rohrmann, Hopp and Quirin (2008) examined gender influence on a number of biological reactions to disgust IAPS images and found no effect. Gender has not previously been found to affect salivary IgA responses to a passive cold pressor task (Willemsen et al, 2002).

Though gender may not have caused a major problem for the disgust studies, a gender influence could extend to produce more general complications across all three experimental paradigms. At a biological level, the hormonal balance during the menstrual cycle may influence the secretion of salivary IgA from parotid glands, with higher levels during the follicular phase (Gomez, Ortiz, Saint-Martin, Boeck, Diaz-Sanchez & Bourges, 1993) and lower levels at the premenstrual phase (Kubitz, peavey & Moore, 1989). However, this study used small sample sizes and the impact of the menstrual cycle on salivary IgA has been a relatively untouched field of study, leaving any conclusion impossible. The menstrual phase was not recorded here as a female prevalence in the participant samples was not expected during study design; though it could be a useful variable to employ in future research.

Final Conclusions and Summary

In this thesis the only consistent effect of stress on salivary IgA was its inconsistency. The results challenge the view that salivary IgA is a stress marker where there were no consistent effects found regarding chronic stress, with no effects seen in caregivers who reported high levels of stress, nor from high perceived stress and hassles over the prior month in undergraduates. The only result that emerged in the predicted direction occurred on single day comparisons of highest to lowest state-stress levels in undergraduates, but this result is tempered by its specificity to the untransformed data, leaving it difficult to interpret. In the passive coping experiments, the IgA increase was in the opposite direction predicted and only from the group that appraised the images to be real. Furthermore, this increase seemed to be independent of the rating of state-stress, where appraisal seemed to be the important factor. Regarding appraised control, the final conclusions can only be quite modest in suggesting the potential importance of measuring differences in appraisal in IgA research. Passive

coping may not be passive for everyone as there is evidence for tasks to be modified by appraisal. In field based research it is suggested to incorporate appraised control as a variable that may influence salivary IgA, under conditions of high subjective stress. However, inconsistent IgA responses are likely to always remain a problem in anything but clear active coping tasks, due to the likely sensitivity of salivary IgA to methodological differences that may cause a direct effect (e.g. collection time of sampling) or may alter appraisals and potentially novelty. Precise methodological factors need to be considered in any research and include many that currently exist in the literature, such as one-off sampling at different times of the day, without reference to potential URI confounds. What should be taken from this thesis is that future salivary IgA research needs to incorporate rigorous methodological procedures that minimise as many of the potential confounds as possible. These include standardised procedures for saliva collection (in time of day, number of samples, URI identification, among others) but also in the way a task or situation is presented to account for potential effects of appraisal. When a study incorporates such factors then we may stand to have a better understanding of stress, appraised control and salivary IgA.

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APPENDICES

List of Appendices

Appendix I – Caregiver Appraised Control Scale

Instructions: The statements set in this questionnaire are designed to assess your current perception of the control you feel you have over your situation. There are no right or wrong answers. Please rate each sentence from 1 to 5 indicating how often you experience the events covered by the statements. Please respond to all 30 statements.

1 = never, 2 = seldom, 3 = sometimes, 4 = often, 5 = always

1. When something unexpected happens I find I have the ability to change the outcome. _____
2. Sometimes I feel that there are too many events in my life in which I have no control over. _____
3. Outbursts from others around me cannot be influenced and I leave them to run their course. _____
4. I have command over most aspects of my life. _____
5. Fate usually conspires against me. _____
6. My time is my own to organise as I please. _____
7. Due to my responsibilities I am rarely able to do what I want. _____
8. If erratic behaviour occurs from people around me I manage to calm the situation immediately. _____
9. My days are filled by unpredictable situations. _____
10. I have the ability to plan and organise my day so that I have time for myself. _____
11. I feel that I have enough time for my friends and family _____
12. I feel that I'm blamed too often for other people's behaviour/actions. _____
13. It is hard to make new friends. _____
14. When my environment becomes turbulent and uncertain I take a step back and assess the best way to handle the situation. _____
15. I feel a great loss of control in my daily life. _____
16. When upsetting events occur I am capable of staying calm. _____
17. I feel that I can control any unpredictable situation that occurs in my life. _____
18. I let my emotions get the better of me. _____
19. I have the time for the things that I enjoy doing. _____
20. I cry myself to sleep _____
21. I am happy _____
22. My life is controlled by other people. _____
23. Uncertainty dominates my day. _____
24. I find myself repressing my emotions for the good of other people. _____
25. I have ways to relieve the stress and strains of my day. _____

26. I have trouble sleeping. _____

27. I have a successful routine to my day. _____

28. I find it difficult to concentrate on tasks that need my attention. _____

29. I feel helpless. _____

30. When I wake in the morning I know how my day will go. _____

Appendix II – Salivette Procedure

The principle behind the Salivette saliva collection is that the saliva produced is absorbed into the cotton swab. Because chewing alters the type of saliva that is produced it important that the participant did not chew on the cotton swabs and just allowed them to rest under the tongue.

- The Salivette cap was removed and the cotton swab was taken out of the inner tube.
- The participant's mouth was cleared of any saliva by spitting or swallowing.
- The cotton swab was then placed under the tongue for exactly two minutes.
- Following two minutes the cotton swab was either 'spat' or placed back into the Salivette inner tube and the cap was replaced firmly, ensuring the inner tube was firmly in place in the Centrifuge tube.
- The Salivette was then placed into a zip-lock bag provided.

<u>Appendix III</u> – Disgust Study Emotions Scale

Instructions: Below you will find a list of emotion terms which persons might use to describe how they felt during the slide presentation. Read each word and decide whether it represents an emotion that you felt was induced by the pictures just viewed. Please rate each emotion term with 0 being "Did not feel the slightest bit of the emotion" to 5 being "The most I have ever felt in my life".

"The contents of the photo presentation I have just viewed made me feel…."

Please rate **each** of the following words from

0 = "Did not feel the slightest bit of the emotion" to
5 = "The most I have ever felt in my life".

Amused	Repulsed	Downhearted . . . . .
Joyful	Fearful	Blue . . . . .
Merry	Scared	Surprised . . . . .
Angry	Afraid	Amazed . . . . .
Irritated	Bored	Astonished . . . . .
Mad	Apathetic	Neutral . . . . .
Disgusted	Uninterested	Impartial . . . . .
Nauseated	Sad	Disinterested . . . . .

<u>Appendix IV</u> – Disgust Study Control Scale

For each statement below, please indicate your response. Do so by filling in the blank in front of each item with the appropriate number from the following rating scale:

1	2	3	4	5	6	7
Not at all						**Very much so**

During the presentation to what extent did you feel:

_____ 1. you were in control of your emotions?

_____ 2. able to govern the way you felt during the presentation?

_____ 3. you could influence how you felt during the presentation?

_____ 4. there was no way you could make the presentation any less disgusting

_____ 5. that by changing the way you thought about the pictures you made them less disgusting

_____ 6. felt 'out of control' in the presentation

_____ 7. you were able to think of things that made the presentation less disgusting

_____ 8. you tried to think about other things during the presentation

_____ 9. that changing the way you thought about the pictures made them less unpleasant

_____ 10. the pictures were disgusting and nothing you could do or think could make them less disgusting

Appendix V – Disgust Presentation Introductory Slide Descriptions

Real appraisal groups

For the real appraisal groups this slide read:

> "*The images you are about to see are taken from the real-world and depict scenes from accidents, diseases, and wounds. It is therefore important that you remember you may withdraw from this experiment at any time. If you wish to stop the experiment ask the researcher to turn off the images at any time.*"

Fake appraisal groups

For the fake appraisal groups this slide read:

> "*The images you are about to see are fake and depict scenes of accidents, diseases, and wounds, taken from special effects exhibitions, hospital dramas and commercially available films. It is important that you remember you may withdraw from this experiment at any time. If you wish to stop the experiment ask the researcher to turn off the images at any time*."

Neutral image groups

The 39 control (neutral) images were also taken from the IAPS as each had been previously employed as a neutral picture (e.g. Larson, Ruffalo, Nietert & Davidson, 2005; Schupp, Junghofer, Weike & Hamm, 2004). The introductory slide for the neutral images read as follows:

> "*The images you are about to see depict scenes, objects, and events from everyday life. They have been selected because of their neutral content. You may withdraw from this experiment at any time. If you wish to stop the experiment ask the researcher to turn off the images at any time*."

Appendix VI – Daily Appraised Control Scale

The statements set in this questionnaire are designed to assess your current perception of the control you feel you have had **over your day**. There are no right or wrong answers. Please rate each sentence from 1 to 5 indicating how often you experience the events covered by the statements today. Please respond to all 25 statements.

1 = never, 2 = seldom, 3 = sometimes, 4 = often, 5 = always

1. I feel that there have been many events today in which I have had no control over. _____
2. I have had command over most aspects of my day. _____
3. Fate has conspired against me today. _____
4. My time has been my own to organise as I please. _____
5. I feel that my responsibilities have prevented me from doing what I wanted today. _____
6. My day has been filled by unpredictable situations. _____
7. I have been able to plan and organise my day so that I have had time for myself. _____
8. I have had enough time for my friends and family _____
9. I feel that I have been blamed for things that were not my fault . _____
10. I have felt a great loss of control today . _____
11. I feel that I have been capable of remaining calm to upsetting events. _____
12. I feel that I have been able to control any unpredictable situation that occurred today. . _____
13. My emotions have got the better of me today. _____
14. I have had the time for the things that I enjoy doing. _____
15. I will cry myself to sleep later. _____
16. I have been happy today. _____
17. My day has been controlled by other people. _____
18. Uncertainty has dominated my day. _____
19. I have had to repress my emotions throughout the day. _____
20. I have successfully employed ways to relieve the stress and strains of my day. _____
21. I had trouble sleeping last night. _____
22. I have been able to use a successful routine today. _____
23. I have found it difficult to concentrate on tasks that needed my attention today. _____
24. I have felt helpless today. _____
25. When I woke this morning I knew for certain how my day would go. _____