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SECRETORY IMMUNOGLOBULIN A AND HEART RATE REACTIONS TO
MENTAL ARITHMETIC AND HYPNOTIC SUGGESTIONS.

A Dissertation

Presented for the

Doctor of Philosophy Degree

The University of Tennessee, Knoxville

Grant Benham

December 2000

DEDICATION

I dedicate this work to the following people:

My wife, Chelse. Without her continued love and support, none of this would have been possible. She has been my safe harbor when the stresses have seemed overwhelming.

My parents, Sue and Rob. Between them they nourished both my curiosity about the world around me, and the patience needed to deal with upsets along the way.

My mother-in-law, Lauren. For her unquestioning financial and emotional support in times of need, and for treating me like a son, rather than her daughter's husband.

My son, Gage. For making me smile each morning, and providing me with the excuse to behave like a child again.

ACKNOWLEDGEMENTS

First, I would like to acknowledge the continued support of my mentor, Dr. Michael Nash. Even before I began working with him, he went out of his way to welcome me to the University. He has allowed me to be driven by my own scholastic passions, constantly available for support and guidance. He has encouraged curiosity in all areas of psychology (even in history and literature, though in vain). Additionally, for the last 3 years I have had the pleasure of working with him on the International Journal of Clinical and Experimental Hypnosis. Perhaps more importantly, I am grateful for his friendship which has made my time in Tennessee that much more valuable.

I would also like to acknowledge the support of my other committee members. Both Dr. Debora Baldwin and Dr. Kathleen Lawler have been invaluable resources during both the design and interpretation of my dissertation research. Additionally, Dr. Baldwin has been most generous in providing me access to her lab space and equipment. I would like to thank Dr. Schuyler Huck for making sure that I am able to distinguish the forest from the trees. He has been most helpful in making sure that the “big picture” is both understandable and meaningful, and that my interpretations are not absurdly esoteric.

Finally, I would like to acknowledge the financial support of the Psychology department, spearheaded by Dr. James Lawler. Now in my 6th year, the department has worked hard to provide me financial support via assistantships well past the guaranteed 4-years. Funded via alumni donations, the department also provided me with a \$500.00 dissertation support award, without which this research would not have been viable.

ABSTRACT

The current study replicates and extends previous research on the effects of both specific hypnotic suggestions and a mental arithmetic task on secretory immunoglobulin A (sIgA). Participants (14 males, 16 females) were shown a short video on immune functioning and then sat quietly for 8 minutes in order to obtain an initial baseline measure of sIgA. Participants were then administered an 8-min mental arithmetic (stress) task and a 16-min hypnosis task in a counterbalanced order and separated by a second 8-min baseline period. During the hypnosis condition, participants received a taped hypnotic induction followed by specific suggestions for increasing immune components in their saliva. During the mental arithmetic condition, participants received a taped 8-min paced auditory serial arithmetic test (PASAT). During the procedure, heart rate was continuously recorded at 5-sec intervals, and 4-min timed saliva samples were obtained immediately following both resting baselines and both tasks for subsequent sIgA analysis. Results showed a non-significant increase in sIgA levels following the mental arithmetic task, but a significant increase in both sIgA concentration and sIgA secretion rate following the hypnosis task. Results also demonstrated that the hypnosis task and mental stress task could be significantly distinguished by both physiological (heart rate) and experiential (questionnaire) measures. sIgA concentration following the hypnosis task was significantly greater than following the PASAT, suggesting that hypnosis is at least as powerful a technique as a mental stress task for increasing sIgA levels, without the corresponding increases in heart rate and subjective stress. However, within the 8-min

resting condition immediately following the hypnosis task, sIgA levels decreased to below initial baseline levels, indicating that the effects of the hypnosis task are short-lived. Potential mechanisms underlying the observed increases in sIgA are presented and implications about the clinical significance of these findings are discussed.

TABLE OF CONTENTS

CHAPTER

I	REVIEW OF THE LITERATURE.....	1
	Immunosuppressive effects of psychological stress.....	2
	Psychological techniques for immunoenhancement	5
	Modulation of secretory IgA (sIgA) levels.....	5
	Hypnosis and immune functioning.....	8
	Anomalies.....	13
	Conclusion.....	16
	Hypotheses	17
II	METHODS	18
	Participants.....	18
	Mental Arithmetic and Hypnosis Tasks	18
	The Paced Auditory Serial Arithmetic Task (PASAT)	18
	Hypnosis Task	19
	Quantitative Measures.....	21
	Hypnotic Susceptibility Measure	21
	Secretory IgA measures	22
	Post-experiment Questionnaire	23
	Procedure.....	23
	Data Reduction and Analysis	28
III	RESULTS	29
	Salivary Measures	29
	PASAT	29
	Hypnosis.....	31
	PASAT vs. Hypnosis.....	31
	Order Effects	31
	Heart Rate Measures	33
	PASAT	33
	Hypnosis.....	33
	PASAT vs. Hypnosis.....	33
	Order Effects	33
	Supplemental Analyses	35
	The Relationship of Hypnotic Susceptibility to Changes in sIgA.....	35
	Subjective Ratings of the PASAT and Hypnosis Task	35
	Relationship Between Subjective Ratings and Changes in sIgA	36
	Differences Between Sexes	36
	Baseline Differences.....	38
IV	DISCUSSION	41
	Replication and Extension of Previous Hypnosis Research.....	41

Longevity of Increases in sIgA Following Hypnotic Suggestions	42
Failure to Replicate the Effects of the PASAT	43
Methodological Differences	43
Issues of Baseline	45
Mechanisms Underlying the Observed Increases in sIgA	48
Clinical Significance	50
Conclusions	52
REFERENCES.....	54
APPENDICES.....	68
APPENDIX A: Informed Consent.....	69
APPENDIX B: Mental Stressor (PASAT) Stimuli.....	70
APPENDIX C: Hypnosis Script	71
APPENDIX D: Questionnaire	74
VITA	75

LIST OF TABLES

Table 1. Means and Standard Deviations of sIgA and heart rate measures pre- and post-task.....	29
Table 2. Sex differences between measures of heart rate, sIgA, and subjective experiences.	37

LIST OF FIGURES

Figure 1. Mean sIgA concentration ($\mu\text{g/ml}$) and secretion rate ($\mu\text{g/min}$) from samples collected immediately following the PASAT and the pre-PASAT baseline.	30
Figure 2. Mean sIgA concentration ($\mu\text{g/ml}$) and secretion rate ($\mu\text{g/min}$) from samples collected immediately following the hypnosis task and the pre-hypnosis baseline.....	32
Figure 3. Mean heart rate levels (bpm) during the two tasks (PASAT and hypnosis) and their respective pre-task baselines.....	34

CHAPTER I

REVIEW OF THE LITERATURE

Stress has been variously defined in terms of a stimulus (Cannon, 1932), a non-specific physiological response (Selye, 1955) and, more recently, in terms of the reaction to a situation following cognitive appraisal (Lazarus & Folkman, 1984). An established definition still remains elusive, but it is perhaps by reason of stress's intangible nature that such a variety of studies on this topic have been conducted.

Knowledge of the physiological indices of stress, such as the role of the catecholamines and the corticosteroids, has been expanding rapidly since the beginning of the nineteenth century, but it is only in the last few decades that investigation into the effects of both acute stress (e.g., see Glaser, Kiecolt-Glaser, Speicher, & Holliday, 1985) and chronic stress (e.g., see McKinnon, Weisse, Reynolds, Bowles, & Baum, 1989) on the immune system have really gained impetus. Immune responses are generally divided into two types, cell-mediated and humoral. Cell-mediated immune responses such as defense against cancer cells, parasites, or intracellular viral infections, are effected primarily by T-lymphocytes. Humoral immunity is carried out primarily by B-lymphocytes and involves producing and releasing immunoglobulins (antibodies) which combine with and neutralize foreign substances (such as bacteria) or coat them to assist phagocytosis. Although some studies have examined the interaction of both psychological and physical stressors in relation to immune functioning (e.g., Baldwin, Wilcox, & Zheng, G., 1997; Zama, Papa, Marinozzi, & Caroli, 1992), the majority of studies

examining the effects of stress on immune functioning have focused exclusively on either psychological stress such as exam stress (e.g., Halvorsen & Vassend, 1987) and bereavement (e.g., Pettingale, Hussein, & Tee, 1994) or on physiological stress such as exercise (e.g., Fry, Morton, Crawford, & Keast, 1992).

Immunosuppressive effects of psychological stress

Probably the greatest evidence for the effect of naturalistic psychological stressors on cellular immune function comes from a series of studies investigating the impact of medical school examinations on students' cellular immune function. Students typically reported more stress during the examination period with a corresponding decrease in cellular immune response measured on a variety of indices including decreased natural killer (NK) cell activity, (Kiecolt-Glaser, Garner, Speicher, Penn, & Glaser, 1984; Glaser, Rice, Speicher, Stout, & Kiecolt-Glaser, 1986), decreased lymphocyte proliferation (Glaser, Rice, Sheridan, Fertel, & Stout, J.C., 1987), and increased production of antibody to herpesvirus (Glaser, et al., 1985).

The immunosuppressive effect of stress on humoral immunity has also been extensively studied. Several studies have indicated that changes in blood immunoglobulin correlate with chronic stress (e.g., see Ursin et al., 1984). However, due to its relative ease of collection and assessment, secretory IgA (sIgA) is an index of immune functioning often preferred over blood sampling techniques. Secretory IgA is one of five classes of immunoglobulins found in serum and secretory fluids, including saliva, breast milk, and nasal, gastrointestinal, and bronchial secretions. Secretory IgA is the major

class of immunoglobulins in mucosal secretions, and is considered to be a major effector of host defense against microorganisms causing illnesses such as increased upper respiratory tract infections (Tomasi & Plaut, 1985). The presence of sIgA antibodies in the fluids bathing mucosal surfaces provides an important first line of defense against infection (Tomasi & Grey, 1972), and the concentrations of sIgA in mucosal fluids such as saliva have been found to correlate more closely with resistance to certain viruses than do serum antibodies or other immune parameters (Liew, Russell, Appleyard, Brand, & Beale, 1984; Murphy et al., 1982). A study by Callow (1985) introduced a specific cold-causing virus (coronavirus) into human volunteers and found that those who subsequently showed no clinical signs of infection had initial levels of sIgA that were nine times greater than those who became infected by the virus. Secretory IgA is also implicated in dental diseases (Taubman and Smith, 1993) and deficiencies in sIgA have been identified as one of the factors responsible for the frequent oral infections in patients with AIDS (Muller et al., 1992). Exceptional individuals who lack sIgA tend to suffer more respiratory illness than normal (Hanson, Bjökander & Oxelius, 1983), although often the sIgA deficit is somewhat compensated for by an increase in other immunoglobulins (Kuby 1994). The two commonly used measures of sIgA production in psychoneuroimmunological studies are sIgA concentration and sIgA secretion rate. Secretory IgA concentration is the amount of total IgA protein that is present in a certain volume of saliva (e.g., $\mu\text{g/ml}$) whereas sIgA secretion rate refers to the amount of IgA protein detected per unit of time (e.g., $\mu\text{g/min}$). As with cell-mediated immunity, research

indicates that sIgA levels of adults may be reduced in the presence of stress (see Valdimarsdottir & Stone, 1997, for a review; see Herbert & Cohen, 1993, for a meta-analysis). For example, Jemmott et al. (1983) reported that academic stress was associated with reductions in sIgA. Five saliva collections were made over an 11-month period: three during examination periods and two during low-stress periods. Salivary IgA secretion rate was lower during the three exam time points compared with the low-stress time points. To rule out the possible effects of seasonal variation in sIgA secretion, Jemmott and Magloire (1988) conducted a follow-up study using a shorter time interval. Saliva was collected five days prior to the participants' undergraduate exam, during the exam period, and two weeks after their exam. Both sIgA concentration and secretion rate were lower during the exam period compared to five days before and two weeks after the exam. It has been suggested that salivary flow may be reduced by anxiety, and that this reduction may explain the reduced sIgA secretion rate. This concern was addressed in a study by Graham, Bartholomeusz, Taboonpong, and La Brooy (1988). Graham et al. found that among 113 female registered nurses, nurses who reported frequent episodes of anxiety had significantly lower secretion rates of sIgA than nurses who reported only occasional episodes of anxiety. Additionally, although nurses who were frequently anxious did secrete less saliva on average than the occasionally anxious nurses the difference was not statistically significant.

Psychological techniques for immunoenhancement

Given the evidence that stress can effect the immune system, the possibility exists that by reducing stress one could ameliorate the negative immunological response. Probably the most promising and widely investigated approach to stress reduction has been through physiological response mediation. Armed with increased knowledge about the physiological indices of stress, a number of researchers have focused their efforts on investigating psychological techniques for both modulating the immunosuppressive effects of stress and increasing cellular and humoral immune components in healthy individuals (for a review, see Kiecolt-Glaser & Glaser, 1992). Several studies have indicated that both immunosuppression and immunostimulation can be achieved using behavioral conditioning techniques (e.g., see Ader & Cohen, 1982; Husband, King, & Brown, 1986; Russell et al., 1984). However, research has more typically involved the use of guided imagery, relaxation, and hypnosis.

Modulation of secretory IgA (sIgA) levels

Research suggests that psychological factors may play a part in increasing secretory IgA (e.g., see Dillon, Michoff, & Baker, 1985). The effect of mental imagery on immunoenhancement was investigated in a study by Rider et al. (1990). Forty-five college students were randomly assigned to three groups. The first group, the imagery group, participated in a short educational training session on the production of secretory IgA. A baseline measure of salivary sIgA was then taken, and the subjects listened to a 17-minute tape of imagery instructions, accompanied by specially composed

“entrainment” music in the background designed to enhance imagery. The second group, placebo controls, received no educational training, but just listened to the same music. The third, control, group were simply assessed for sIgA before and after 17 minutes of no activity. Both treatment groups were then given their tapes and instructed to listen to them at home on a bi-daily basis for six weeks. All three groups returned at three and six weeks for assessment of sIgA levels. For all three trials (after first session, at 3 weeks, and at 6 weeks) Groups 1 and 2 combined (treatment groups) yielded significantly greater increases in sIgA over Group 3 (control). Additionally, Group 1 (imagery) was significantly higher than Group 2 (music alone) in antibody production for at both the 3 and 6 week follow up assessment, indicating that there was an effect for the imagery instructions above and beyond the relaxation effect.

A more direct examination of the effects of relaxation on sIgA were reported in a study by Jasnoski & Kugler (1987) who contrasted effects of relaxation alone and relaxation with mental imagery of the immune system. Thirty college undergraduates who had scored high on a measure of absorption ability were randomly assigned to one of three conditions. The first group, the relaxation alone group, practiced progressive muscle relaxation and a focused breathing technique. The second group, the relaxation with imagery group, did the same relaxation training, but also used mental imagery of the immune system. Lastly, the third (control) group performed a vigilance task, discriminating between tones that were presented in variable inter-trial intervals, and thus serving as an alertness or mild arousal condition. Each treatment lasted for

approximately one hour. Before and after the session all subjects were assessed for salivary sIgA, along with some other measures including cortisol & catecholamine levels, and mood states. When compared to the vigilance task group, both relaxation groups showed significantly higher levels of sIgA post-treatment, though it is unclear whether this effect was due to increased sIgA (pre- to post-treatment) in the relaxation group, or decreased levels of sIgA in the vigilance task group. Unlike the Rider et al. (1990) study, however, no differences were reported between the relaxation alone and the relaxation with imagery groups.

Research by Green and Green (1987) supported the idea that even simple relaxation alone for just 20 minutes may be effective in raising sIgA concentrations. Fifty college students were assigned to relaxation groups (relaxation response, guided visualization, back massage) or control groups (lying quietly with eyes closed or touching-control group). Saliva samples collected before and immediately after each session indicated a significant increase in sIgA concentration from pre-to post- treatment for the relaxation groups, but no change for the control groups. Further support for this effect was provided by a follow up study (Green, Green, and Santoro, 1988) in which 24 subjects who took part in a supervised 20-minute relaxation session showed a significantly higher sIgA secretion rate following the session. These subjects then practiced relaxation techniques once a day for 3 weeks and, when compared with a control group practicing for the first time, showed a significantly greater increase in sIgA

concentrations during the session. These results suggest that relaxation techniques as a modulator of immune functioning have both short-term and long-term (practice) effects.

Humor has also been shown to have an effect on sIgA levels. Encouraged by Norman Cousins's popular account (1976) of how he treated a serious illness successfully by watching comic movies, Dillon, Minchoff, and Baker (1985) showed subjects a humorous videotape and a didactic control video in counterbalanced order. Salivary IgA concentration increased significantly after watching the humorous video (Richard Pryor Live) for 30 minutes, but no significant change occurred after watching the didactic video for the same amount of time. McClelland and Cheriff (1997) demonstrated a similar effect in a series of three studies that used humorous videotapes. Overall, participants who watched the humorous videotapes for at least 30 minutes showed an increase in sIgA concentrations significantly more often than students exposed to comparison films of equal duration. The effect was found for two different humor videos (W.C. Fields and Billy Crystal) and two different methods of assaying sIgA (radial immunodiffusion technique and enzyme-linked immunoabsorbent assay).

Hypnosis and immune functioning.

Hypnosis has long been suggested as an effective technique for altering physiological processes. Reviews of the literature (Bowers & Kelly, 1979; Goldberg, 1985; Hall, 1982; Kiecolt-Glaser, J. K., & Glaser, R., 1992) illustrate the diversity of physiological conditions that have been treated and investigated by hypnosis researchers and clinicians. Burns (Ewin, 1986), allergic reactions (Ikemi & Nakagawa, 1962), skin

temperature (Maslach, Marshall, & Zimbardo, 1972), blood pressure (Holroyd, Nuechterlein, Shapiro, & Ward, 1982) and warts (for a review, see DuBreuil & Spanos, 1993) have all proved mutable via hypnotic suggestions. Hypnosis was used in several of the earliest studies exploring the ability of a psychological intervention to affect immunological reactivity (Black, 1963a; Black, 1963b; Black, Humphrey, & Niven, 1963). However, in spite of the large amount of literature supporting the efficacy of hypnosis in treating a variety of immune-related disorders (Locke, 1985), there still remains a scarcity of solid experimental evidence demonstrating that the immune system is responsive to hypnotic suggestion. Additionally, many of the studies incorporating hypnosis to modulate immune functioning fail to control for the effects of relaxation, a technique capable of modulating the immune system (e.g., Green & Green, 1987). For example, Whitehouse et al. (1996) conducted a 19-week prospective study to investigate the effectiveness of a self-hypnosis/relaxation intervention in moderating immune system reactivity to examination stress. In this study, twenty-one first-year medical students were randomly selected for training in the use of self-hypnosis, while another fourteen subjects acted as controls without any training. Blood samples were taken for all subjects at orientation, late semester, examination period, and postsemester. During the examination period, significant increases in stress and fatigue were reported, with corresponding increases in B-lymphocyte counts and activated T-lymphocyte counts. Increases in PHA-induced and PWM-induced blastogenesis, and NK cell cytotoxicity also occurred at this time. Although subjects in the self-hypnosis group reported

significantly less distress and anxiety than the controls, the two groups did not show any differences in immune function. However, ratings relating to the level of relaxation achieved during the self-hypnosis exercises were positively associated with both the number of NK cells and NK activity. The findings of this study indicate that hypnosis may not directly modulate immune functioning, but the resultant relaxation levels achieved by some subjects using self-hypnosis may account for the reduction in the immunosuppressive effects of stress. It is unfortunate that the study omitted to include a relaxation-training group. The relative efficacy of self-hypnosis procedures in relation to simple relaxation exercises would have then been possible, a factor that would have important clinical ramifications. Studies that utilize measures of hypnotizability (e.g., Zachariae et al., 1994) as a subject variable help to untangle the relative efficacy of both procedures, but only by directly controlling for relaxation can hypnosis be acknowledged as the potent ingredient in studies of immunomodulation.

This concern was addressed in a study by Smith, Barabasz, Barabasz, and Warner (1995) who directly contrasted the immunomodulating effects of hypnosis with those of relaxation alone. Twenty-nine high hypnotizable subjects and twenty-six low hypnotizable subjects were randomly assigned to one of three groups: (1) hypnosis, (2) relaxation, and (3) waiting list control. All subjects were shown a brief video prior to treatment, which provided an overview of the human immune system. Subjects in the hypnosis group were given a standardized hypnotic induction, followed by a positive suggestion to “imagine your white blood cells attacking and destroying germ cells in your

body.” Following the hypnotic session, subjects were instructed to practice self-hypnosis twice daily during the week. Subjects in the relaxation group participated in 2 one-hour flotation Restricted Environmental Stimulation Therapy (REST) sessions, in which they floated effortlessly in a solution of Epsom salts in a fiberglass tank. The subjects in the control group were given the same suggestions as the hypnosis group, but did not receive a hypnotic induction. Blood samples were collected from all subjects before the first session and after the first and second session. Analyses of covariance with repeated measures were performed on B-cell, T-cell, helper T-cell, and suppressor T-cell counts, along with helper T-cells/suppressor T-cell (H/S) ratios. Results indicated significant immunomodulation for participants exposed to the hypnosis treatment based on B-cell and T-cell counts. Participants in the hypnosis treatment group showed significantly higher B-cell and T-cell counts than subjects exposed to the REST group and no treatment control group. This immune system modulation was specifically associated with hypnosis and not with a treatment known to produce significant relaxation effects (REST).

To date, only one study has investigated the potential for hypnotic suggestions to increase sIgA levels. Olness, Culbert, and Uden, (1989) used specific hypnotic suggestions to raise sIgA concentrations in children. Fifty-seven children ranging in age from 6 to 12 years were randomly assigned to three groups: two hypnosis groups and a control group. On their first visit, all subjects gave a saliva sample, viewed a short videotape that used puppets to explain basic immune system components, and then were

administered a standardized hypnotizability scale for children (The Stanford Hypnotic Clinical Scale for children; Morgan & Hilgard, 1979). Returning two weeks later, all subjects gave another saliva sample. Subjects in the first hypnosis group then listened to a self-hypnosis tape and were given a general suggestion that they might increase immune substances in their saliva. Subjects in the second hypnosis group listened to the same self-hypnosis tape but were given specific suggestions about increasing salivary immunoglobulins. Subjects in the control group were engaged in conversation during this time. A third saliva sample was obtained after this treatment session. Results showed a significant increase (pre self-hypnosis to post self-hypnosis) in salivary sIgA for the self-hypnosis group that received specific suggestions for alteration of salivary immunoglobulins. No corresponding increase was shown for either of the other two groups, indicating that this specific suggestion form of hypnosis may be useful in enhancement of sIgA concentrations. It is important to keep in mind however, that these results were based on a population of children. Baseline sIgA levels for children below seven years of age, between seven and fifteen years of age, and for adults have been shown to differ significantly (Kugler, Hess, & Haake, 1993).

These findings are consistent with those of Rider et al. (1990) in that both showed an effect for specific instructions (hypnotic suggestions and imagery instructions) above and beyond the effects of a basic relaxed state (hypnotic induction and “entrainment” music).

Anomalies

The concept that stress necessarily leads to a down-regulation of immune functioning has been challenged by a number of studies conducted during the 1990s. Although it is still widely agreed that chronic stress decreases immune functioning, there is growing evidence that acute stress can actually lead to an up-regulation of several measures of immunity in both laboratory and naturalistic settings. One of the first studies to directly assess the effects of acute stress on sIgA was conducted by Evans, Bristow, Hucklebridge, Clow, and Pang (1994). The authors were prompted by previous findings from their lab (Evans, Bristow, Hucklebridge, Clow and Walters, 1993) which indicated that although individuals appearing to be more stressed over a period of 2-weeks had lower levels of sIgA, the same individuals tended to have higher sIgA levels on their worst days (more undesirable events reported and worse mood state). The effects of an acute stressor, an assessed oral presentation to a group of fellow students, on sIgA were examined in Evans et al.'s (1994) follow-up study. Their results indicated that sIgA levels were higher on the day of assessment than a week before, and were highest of all immediately after assessment. These results were replicated in a subsequent study (Bristow, Hucklebridge, Clow, & Evans, 1997) that controlled for the potential influence of saliva flow rate. Salivary IgA effects were found to be independent of changes in saliva flow, which did not vary significantly across measurement occasions. As the authors speculated, could it be that chronic activation of mechanisms inducing short-term rises in sIgA leads ultimately to depressed secretory immunity?

Research by Carroll et al. (1996) demonstrated that an acute laboratory stressor was sufficient to raise sIgA levels. Twenty-eight male students were assessed for changes in sIgA following a 30-min computer game task, a task that has been shown previously to produce substantial cardiodynamic changes (Turner & Carroll, 1985). Salivary IgA secretion rates increased significantly by the end of the computer game task relative to a 6-min resting rate.

Subsequent research has replicated and extended these findings by comparing the effects of a mental arithmetic task to cold pressor (Willemsen et al, 1998), paced breathing (Ring et al., 1999) and cold pressor and exercise (Winzer et al., 1999). In each of these studies the same 8-min paced auditory serial arithmetic task (PASAT) was used as an acute mental stressor. The task requires participants to add two sequentially presented single digit numbers and to retain the latter of these two numbers in memory for subsequent addition to the next number presented. Participants then add each number that they hear to the immediately preceding number and write out the answer. Across all three studies, significant increases in sIgA levels were demonstrated immediately following the PASAT. As expected, corresponding increases in cardiovascular measures such as heart rate and systolic blood pressure took place during the arithmetic task, though in the one study that reported correlations between cardiovascular measures and sIgA increases (Willemsen et al.), none were shown to be statistically significant. The cold pressor task (immersion of hand in cold water) was shown to significantly increase sIgA in one study (Willemsen et al.) but this finding was not replicated by the subsequent

study (Winzer et al.). This discrepancy was addressed by the authors who suggested that the lack of consistency may have been due to methodological differences. Willemsen et al. collected saliva immediately after 4-min of hand immersion, whereas Winzer et al. collected saliva samples during the last 2 minutes of an 8-min period of hand immersion. Paced breathing (breathing at a consistent rate of 6 breaths per min) was not shown to increase sIgA (Ring et al.), however an 8-min submaximal ergometer exercise test resulted in a significant increase in sIgA. The impact of exercise on salivary S-IgA has been investigated in numerous studies (Baldwin, Craig, & Wilcox, 1999; Mackinnon, Chick, As, and Tomasi, 1989; Mackinnon, Ginn, and Seymour, 1993; Tharp & Barnes, 1990). It has been generally found that intense, exhaustive exercise has a suppressive effect on salivary S-IgA whereas moderately intense exercise has little impact on this same parameter (Mackinnon, 1994). The findings that exercise actually increases sIgA levels is at odds with much of this research, however a preliminary investigation on the effects of weight training on sIgA indicated that a single weight lifting workout lasting 50-min resulted in a 25% increase in sIgA secretion rate (McDowell et al., 1993).

The precise boundary between acute and chronic stress has yet to be defined. Although an 8-min mental arithmetic task can be readily categorized as an acute stressor, and even perhaps an assessed oral presentation fits within commonly accepted definitions, a study by Zeier, Brauchli, and Joller-Jemelka (1996) showed an increase in sIgA concentration and secretion rate for air traffic controllers following a 100-min workperiod at a radar workplace. This effect was observed in two different sessions three

months apart, and was obtained using two different sIgA assessment procedures, single radial immunodiffusion and kinetic nephelometry. A similar increase in sIgA was shown for soccer coaches during their team's soccer match (Kugler, Reintjes, Tewes, & Schedlowski, 1996). Salivary IgA concentrations increased at the beginning of the match, showed highest values during half time and at the end of the match, and normalized one hour later. In contrast, sIgA levels in control subjects remained constant over time.

Conclusion

Chronic stress has been identified as being a potential factor in the down-regulation of the immune system. Motivated by this, a number of researchers have investigated the potential for psychological techniques such as relaxation, hypnosis, humor, and imagery to beneficially increase immune functioning in healthy individuals and to attenuate the immunosuppressive effects of various stressors (for a review, see Kiecolt-Glaser & Glaser, 1992). Research by Olness et al. (1989), suggests that that specific hypnotic suggestions may have the potential to increase concentrations of salivary immunoglobulin A in children. However, research conducted during the past decade has made it increasingly clear that similar increases in sIgA may be produced via an acute mental stressor (Ring et al., 1999; Willemsen et al., 1998; Winzer et al., 1999). These latter studies have also demonstrated a corresponding increase in heart rate following the mental arithmetic task. Given these recent findings, it seems appropriate to reexamine the purported effects of hypnosis on sIgA and to evaluate whether these effects differ from those brought about by an acute mental stressor.

Hypotheses

The proposed study attempts to answer three questions. Firstly, can the findings that specific hypnotic suggestions increase sIgA levels (Olness et al., 1989) be replicated with an adult population of college students? Secondly, will a brief (8-min) mental stressor produce a similar increase in sIgA? Finally, if an increase in sIgA is seen for both hypnosis and the mental stressor, can the conditions be distinguished in terms of the extent of sIgA increases and in terms of heart rate responses to both tasks?

The following hypotheses will be tested:

H1: The hypnotic suggestions condition will result in an increase in sIgA levels above baseline.

H2: The mental arithmetic condition will result in an increase in sIgA levels above baseline.

H3: The two conditions will show different heart rate responses, with the mental arithmetic condition creating a rise in heart rate, and the hypnotic suggestions condition demonstrating a reduction in heart rate (from baseline levels).

CHAPTER II

METHODS

Participants

30 undergraduate students (14 males, 16 females) at the University of Tennessee, Knoxville participated in this study. Participants were recruited via class announcements from a pool of students who had recently been assessed for hypnotic susceptibility using a group administered scale, the Waterloo Stanford Group Scale of hypnotic susceptibility (WSGS; Bowers, 1998). Selection of participants was not dependent on the obtained hypnotic susceptibility scores. All participants were 18 years or older ($M = 22.93$, $SD = 6.97$). Five of the participants were cigarette smokers. At the beginning of the study, participants were again provided with an explanation of the study, and asked to read and sign an informed consent.

Mental Arithmetic and Hypnosis Tasks

The Paced Auditory Serial Arithmetic Task (PASAT)

The PASAT is an 8-minute task designed to act as an acute mental stressor. During the task, participants are required to add two sequentially presented single-digit numbers, from 1 to 9, while retaining the latter of the two numbers in memory for subsequent addition to the next number presented. Numbers are delivered via an audio tape player, and participants are instructed to write down their answers on a tabulated sheet of paper. The task consists of four 2-min series of 50, 60, 75 and 100 digits at

presentation rates of 2.4, 2.0, 1.6, and 1.2s, respectively. The tape used was a direct copy of the recording used in previous research (Ring et al., 1999, with permission). A complete list of the numbers presented in each 2-min series is provided in Appendix B.

Hypnosis Task

The hypnosis task lasted 16 minutes and was administered via a prerecorded audiotape in order to maintain consistency of task administration. The task began with a standardized hypnotic induction that incorporated suggestions of relaxation and focused breathing. As with the Olness et al. (1989) study, the induction was followed by specific suggestions for increasing immune components in the saliva. However, unlike the Olness et al. study, the induction was based on the Waterloo Stanford Group Scale (WSGS; Bowers, 1998) induction and the specific suggestions for altering immune components were modified for administration to adults, as follows:

“And as you sit there I want you to think back to the video that you saw about your immune system. I want you to think about the immune proteins that are secreted into your saliva. Every hour of every day these immune proteins help to keep you healthy and to protect your body. And I want you to focus now on these immune proteins in your saliva. I want you to imagine these immune proteins, picture them in your mind. Your mind and body are inseparably linked and your mind can control your body. As you picture these immune proteins, I want you to imagine that they are increasing in number. Perhaps you can picture them as small white objects,

like microscopic white pearls secreted from the lining of your mouth. Or maybe you see them in more detail as colored strands of protein – being passed from the tissue lining in your mouth into the saliva. You can picture these immune components in whatever way you choose, but however you picture the immune proteins, you will see them increase in number as they are secreted more and more from the lining of your mouth into your saliva. They are secreted more and more, faster and faster, pouring into the mouth. And as these immune proteins increase, you may also feel the saliva increasing in your mouth. You may notice that your mouth becomes more and more moist as the saliva and the immune proteins increase. Just focus on this for a moment as you picture the immune proteins being secreted into your saliva.. (pause). Your mind can control and direct your body, and you can direct your body to increase these immune proteins to make yourself healthier, more able to resist invading organisms. The immune proteins in your saliva protect your body and keep you healthy, and as you imagine these immune proteins increasing in your saliva, your body actually produces more and more immune proteins. You see the immune proteins in your saliva, protecting your body, and I just want you to focus on these images, these sensations, for a few moments.”

The entire script of hypnotic suggestions, including the induction and deinduction, are detailed in Appendix C.

Quantitative Measures

Hypnotic Susceptibility Measure

In order to test for a relationship between hypnotic susceptibility and any demonstrated changes in sIgA as a result of the hypnosis task, participants were assessed using a group-administered scale of hypnotic susceptibility, the Waterloo Stanford Group Scale of hypnotic susceptibility (WSGS; Bowers, 1998). The WSGC is a group adaptation of the individually administered Stanford Hypnotic Susceptibility Scale, Form C (SHSS:C; Weitzenhoffer & Hilgard, 1962). Like the SHSS:C, the WSGC samples a greater variety of suggested experiences (see Weitzenhoffer & Hilgard) than its predecessors. In particular, responses to the more difficult cognitive suggestions (e.g., age regression and positive and negative hallucinations in several sensory modalities) are more adequately tapped. The WSGS involves a hypnotic induction incorporating suggestions of relaxation, followed by twelve suggestions for the participant to experience various events. The twelve suggestions, in order, are: 1) Hand lowering, 2) Moving hands together, 3) Mosquito hallucination, 4) Taste hallucination, 5) Arm rigidity, 6) Dream about hypnosis, 7) Arm immobilization, 8) Age regression, 9) Music hallucination, 10) Negative visual hallucination, 11) Posthypnotic suggestion, and 12) Posthypnotic amnesia. Altogether, the WSGS takes just under an hour to administer, with an additional 10 minutes needed for participants to complete a response booklet in which

they answer questions about their experience. The response booklets are then scored by the researcher in order to obtain each participant's hypnotic susceptibility score. In order to reduce the time needed to administer the WSGS to under an hour, the present study used a modified version of the WSGS in which the age regression suggestion was not administered. Thus, the possible range of hypnotic susceptibility scores in this study was from 0 to 11.

Secretory IgA measures

During the study, four timed samples of saliva were obtained from each participant in order to determine sIgA concentration and secretion rate following each condition. The volume of the saliva samples from each subject was calculated from sample weight in order to determine saliva flow rates (volume per time: ml/min.). Saliva samples were centrifuged for ten minutes. Supernatants were aliquotted and stored in microcentrifuge tubes at -20°C for analyses. Saliva samples were analyzed for total sIgA concentration using the sandwich Enzyme Linked Immunosorbent Assay (ELISA). This procedure was conducted using a commercially available kit (Alpco Diagnostics, Windham, NH). Supernatant from samples were diluted 1:2000 in wash buffer. A 96-well precoated microtitre plate was then washed 5 times with 250 µl of wash buffer. 100µl of 5 standards (known sIgA concentrations) and samples were micropipetted into the wells of the microtitre plate. All samples were analyzed in duplicate. The plate was then incubated for 1 hour on a plate shaker at room temperature, and each well was then aspirated and washed 5 times with 250µl of wash buffer. 100µl of prediluted, peroxidase-

labelled antibody was then added to each well and the plate was again incubated for 1 hour on a plate shaker at room temperature. After incubation, each well was aspirated and washed 5 times with 250µl wash buffer. 100µl of TMB substrate solution was then added to each well, and the plate was incubated for 5 to 15 min at room temperature until the color differences were obvious. 50µl of stop solution was then added to each well. The color intensity was measured by a spectrophotometer using a wavelength 405nm. The average of absorbance values were used as the representative value. The concentration of IgA was determined by the standard curve formula (regression formula) for each plate. Additionally, salivary IgA concentrations were multiplied by saliva flow rates to determine salivary IgA secretion rates.

Post-experiment Questionnaire

In order to examine the participants' subjective experiences during the procedure, a questionnaire was administered at the end of the experiment. The questionnaire prompted for some basic demographic information, and included a number of Likert-type scales on which participants were asked to rate their experiences of relaxation, effortfulness, and engagement, both during the stress task and the hypnosis task. A copy of the questionnaire can be found in Appendix D.

Procedure

All participants were individually contacted to make an appointment with the experimenter. Participants were instructed to refrain from smoking and any consumption of food one-hour prior to the scheduled time, and to only drink water. Participants were

seen in groups of 2 or 3 during the mid afternoon at a time mutually agreeable to the participants and the experimenter. At each session, once all the group members were assembled, the experimenter explained that the study is investigating the effects of hypnosis and a mental arithmetic task on immune functioning, and that the study will involve suggestions under hypnosis as well as a mental arithmetic task. In accordance with previous research (Ring et al., 1999), the experimenter announced that once all participants have completed the study, the person with the best score on the mental arithmetic task will receive a \$25 gift certificate. The experimenter then addressed any questions or concerns that the participants had regarding the issue of hypnosis. After reading and signing the informed consent form, each participant was given a heart rate monitor to wear (Polar Accurex Plus™, Polar Electro Inc., Woodbury, NY). The heart monitor uses a transmitter belt that is worn against the skin using an elastic strap. Participants were asked to put on the transmitter themselves in a nearby restroom and return to the research lab when ready. Heart rate monitors were checked by the experimenter to determine whether the transmitter was properly fitted. Participants were seated and each participant was then handed a small cup of distilled water and a graduated cylinder on which the experimenter had written each participant's assigned identification number. The experimenter then explained the saliva collection procedure to the participants, as follows:

“In a few minutes I will be asking each of you to provide a saliva sample which we will collect for 4 minutes. I will take you step by step

through the procedure as we do it, but first I will explain it to you in case you have any questions. When we begin, I will first ask you to rinse your mouth out with some of the distilled water that you have each been given. Then I will ask you to swallow any distilled water that you have in your mouth, and swallow your saliva until you are aware of a feeling of dryness in your mouth. At that stage we will begin the saliva collection. All I want you to do is to take the cap off the container you've been given, place the opening of the plastic tube against the right corner of your mouth and, sitting straight, lower your head slightly and allow saliva to flow spontaneously into the tube [experimenter demonstrates position of tube to mouth]. Do not swallow your saliva – you may spit approximately once per minute during the collection.”

The experimenter then answered any of the participants' questions. Heart rate monitoring was then started and continued until the end of the session. Heart rate was automatically recorded every 5 seconds by the heart rate monitor for subsequent download to a computer for data analysis.

Participants were instructed to sit quietly while they watched a video on immune functioning (video produced by the experimenter, 3 minutes in length) that described the basic function and production of sIgA. Following the video, participants were asked to sit quietly for an 8-min rest period. During this 8-min period, participants watched a video of fish around a coral reef ("Coral Sea Dreaming", Small World Music, Inc.). The use of this

video has been suggested as a preferred methodology for the collection of baseline physiological data in that it controls for the effects of anticipation, recollection of the day's events, or anxiety that may affect participants' resting levels (Piferi, Kline, Younger, & Lawler, 2000). Immediately following the 8-min rest period the experimenter instructed participants to collect his or her saliva for a 4-min timed interval, taking the participants step by step again through the procedure as described above. At the end of the 4-minute interval, these baseline saliva samples were sealed and retrieved by the experimenter, then stored in an ice chest. Participants were then administered the mental arithmetic task and hypnosis task (detailed below) in a counterbalanced order. In between the two tasks, in keeping with previous research (Willemssen et al., 1998), a second 8-min rest period was conducted with participants once again instructed to sit quietly while they watched the coral reef video. To ascertain that sIgA levels do not remain significantly elevated post-hypnosis or PASAT, a second baseline measure of sIgA was taken at the end of this second rest period.

For the mental arithmetic condition, participants were given a clipboard with a single sheet of paper on which a table of blank boxes was printed. Participants were then given the following instructions:

“In just a moment I will be administering an 8-min arithmetic test that has been recorded on audiotape. The tape contains a series of single digit numbers. Your task is to remember the first number that is presented, then add the next number that is presented to it and write

down the number in the first top-left box. So if the first number is a 5 and the next number is an 8, you should write down 13. That's the easy part. From then on you must remember the last number that was presented and add it to the next number that is presented. So if you hear "five", then "eight", you write down 13, then you hear "six" you must add that to the last number that was presented ("eight"), and write down 14."

The experimenter then checked to make sure that all participants understood the task, and provided a practice by announcing four single digit numbers and asking all participants to call the answer out loud. Once the experimenter was satisfied that the task was understood, the participants were told the following additional information:

"Please fill out the boxes working your way down the first column then moving to the next column, etc. The numbers on the tape will gradually be presented at a faster rate. At some point you may lose track of the numbers, but it is important that you do not just 'give up' on the task. You must try your best to continue. If you lose track, just stop for a second and then start over with the next number that is presented. Try your best to stay focused on the task. At the end of the task, please do not talk about the procedure, we will be immediately collecting another saliva sample."

Following each task (PASAT and hypnosis), participants were asked to produce another saliva sample. As before, participants were handed a graduated cylinder and a 4-

minute timed saliva sample was taken. Saliva samples were then retrieved by the experimenter and stored in an ice chest for subsequent transportation. In total, 4 saliva samples (two rest/baseline samples, a post-hypnosis sample, and a post-PASAT sample) were collected from each participant. Following collection of the final saliva sample participants completed a one-page questionnaire (see Appendix D) that included some demographic data and a few questions about their experience during the session. All participants were then fully debriefed. Immediately following each experimental session all saliva samples were centrifuged and aliquoted into microcentrifuge tubes for later analysis. All samples were stored at -20°C until assayed

Data Reduction and Analysis

sIgA concentrations and secretion rates were obtained from the samples taken immediately after the initial baseline, the first task, the second baseline, and the second task. A repeated-measures MANOVA was applied separately to each task to examine the impact of each task on secretory activity (hypotheses 1 and 2). A repeated-measures MANOVA was also used to compare sIgA differences between the two tasks (PASAT and hypnosis).

Heart rate was averaged for each baseline period and each task period, resulting in four heart rate values for each participant. A repeated-measures ANOVA was used to compare participants' mean heart rates during the PASAT and hypnosis conditions (hypothesis 3). Two separate repeated-measures ANOVAs were also applied to each task (PASAT and hypnosis) separately to examine changes from their respective baselines.

CHAPTER III

RESULTS

Table 1 shows means and standard deviations for pre-task (baseline) and post-task sIgA measures and heart rate.

Salivary Measures

PASAT

Figure 1 shows a rise in sIgA concentration and secretion rate following the PASAT. Although both concentration and secretion rate increased as a result of the stress task, a repeated-measures MANOVA revealed that this increase was not significant $F(2,27) = .485, p > .05$ ($\eta^2 = .052$, power = .163).

Table 1. Means and Standard Deviations of sIgA and heart rate measures pre- and post-task.

	sIgA Concentration ($\mu\text{g/ml}$)		sIgA Secretion Rate ($\mu\text{g/min}$)		Heart Rate (bpm)	
	M	(SD)	M	(SD)	M	(SD)
Pre-PASAT baseline	304.52	(154.93)	173.14	(101.28)	75.00	(12.62)
PASAT	332.26	(190.82)	198.00	(96.23)	82.07	(12.51)
Pre-hypnosis baseline	300.35	(147.61)	154.63	(81.06)	74.20	(11.54)
Hypnosis	406.39	(187.23)	226.55	(101.41)	71.97	(11.70)

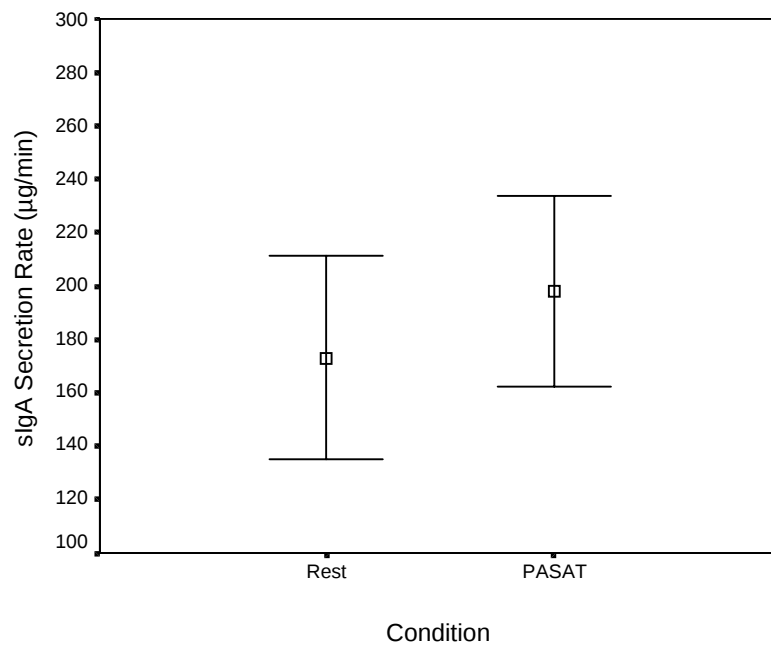
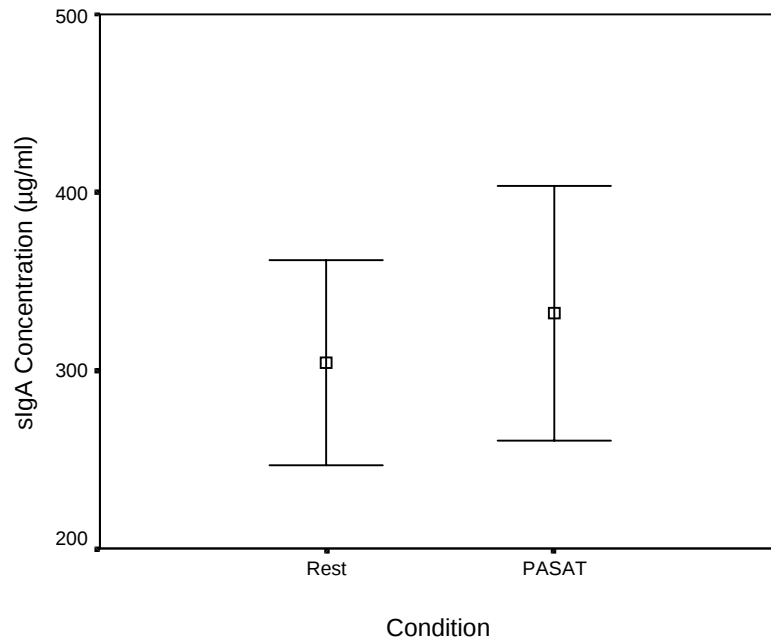


Figure 1. Mean sIgA concentration (µg/ml) and secretion rate (µg/min) from samples collected immediately following the PASAT and the pre-PASAT baseline.

Hypnosis

Figure 2 shows a rise in sIgA concentration and secretion rate following the hypnosis task. A repeated-measures MANOVA revealed that this increase was significant $F(2,27) = 13.9, p < .001$ ($\eta^2 = .507$, power = .996). Subsequent univariate repeated-measures ANOVAs applied separately to concentration and secretion rate revealed that both of these measures of sIgA increased significantly as a result of the hypnosis task (concentration, $F(1,28) = 18.20, p < .001, \eta^2 = .394$, power = .984; secretion rate, $F(1,28) = 27.07, p < .001, \eta^2 = .492$, power = .999).

PASAT vs. Hypnosis

A repeated-measures MANOVA was used to examine whether any significant difference existed between the post-PASAT and post-Hypnosis sIgA measures (concentration and secretion rate). There was a significant main effect for task type, with sIgA significantly higher post-hypnosis than post-PASAT $F(2,27) = 4.35, p = .023$ ($\eta^2 = .244$, power = .705). Subsequent univariate repeated-measures ANOVAs applied separately to concentration and secretion rate revealed that differences in sIgA were significant for concentration $F(1,28) = 8.03, p = .008$ ($\eta^2 = .223$, power = .781), but not for secretion rate $F(1,28) = 2.54, p > .05$ ($\eta^2 = .083$, power = .337).

Order Effects

Although there was no main effect for the stress task on sIgA, the order of task presentation (PASAT first or hypnosis first) demonstrated a significant interaction with the change in sIgA following the PASAT, $F(2, 27) = 5.38, p = .011$. For the hypnosis task,

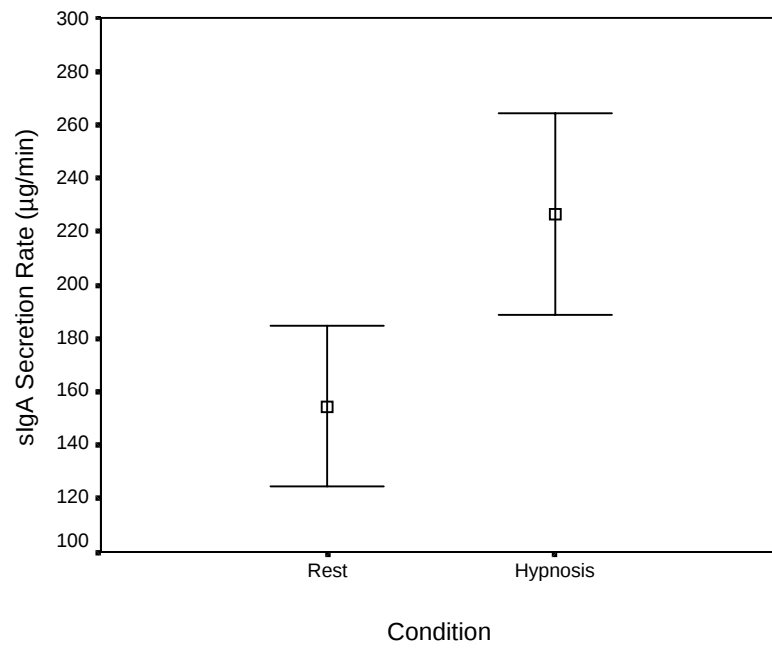
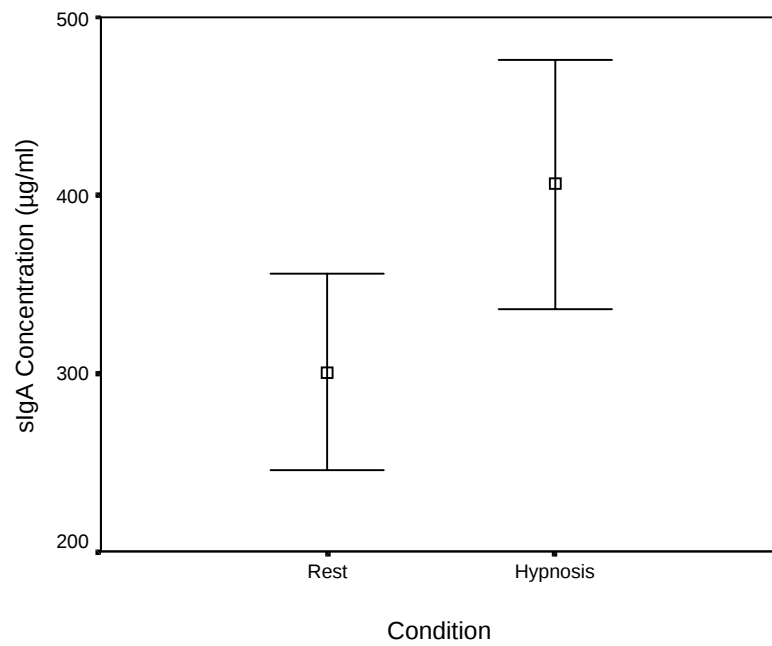


Figure 2. Mean sIgA concentration ($\mu\text{g/ml}$) and secretion rate ($\mu\text{g/min}$) from samples collected immediately following the hypnosis task and the pre-hypnosis baseline.

there was no interaction with order of task presentation $F(2,27) = .82, p > .05$, nor was there an order of task presentation main effect $F(2,27) = .80, p > .05$. When examining the post-PASAT vs. post-hypnosis differences, no significant interaction or order effects were found ($F(2,27) = 1.56, p > .05$, and $F(2,27) = .054, p > .05$, respectively).

Heart Rate Measures

PASAT

Figure 3 shows that during the PASAT heart rate was elevated from baseline levels. A repeated-measures ANOVA indicated that this increase was significant $F(1,28) = 31.83, p < .001$ ($\eta^2 = .532$, power = 1.000).

Hypnosis

Figure 3 shows that during the hypnosis task heart rate decreased from baseline levels. A repeated-measures ANOVA indicated that this decrease was significant $F(1,28) = 20.02, p < .001$ ($\eta^2 = .415$, power = .990).

PASAT vs. Hypnosis

Results of a repeated-measures ANOVA indicated that there was a significant difference between heart rates during the PASAT and hypnosis $F(1,28) = 52.03, p < .001$ ($\eta^2 = .663$, power = 1.000).

Order Effects

The order of task presentation (PASAT first or hypnosis first) had no significant effect on heart rate changes resulting from the PASAT, $F(1,28) = .06, p > .05$, or the hypnosis task, $F(1,28) = 1.26, p > .05$. Differences between the heart rate during the

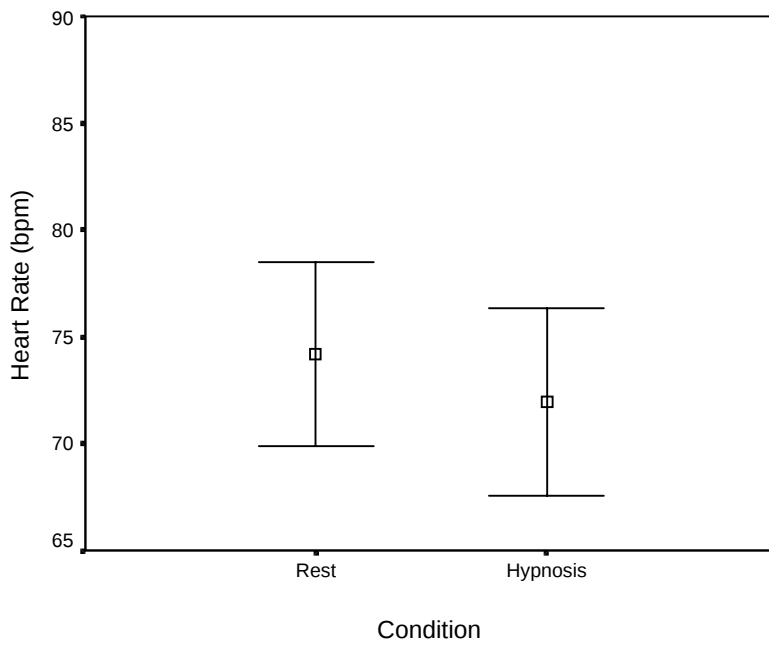
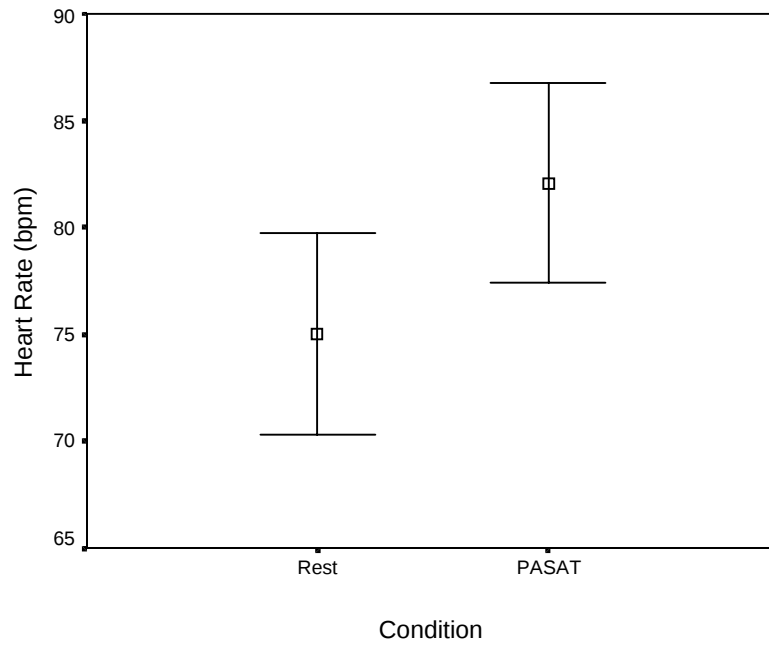


Figure 3. Mean heart rate levels (bpm) during the two tasks (PASAT and hypnosis) and their respective pre-task baselines.

PASAT and during hypnosis were not influenced by the order of task presentation, $F(1,28) = 3.44, p > .05$.

Supplemental Analyses

In addition to testing the main hypotheses of this study, supplemental analyses were performed to test a number of secondary hypotheses and investigate conspicuous patterns in the data.

The Relationship of Hypnotic Susceptibility to Changes in sIgA

A bivariate correlation between scores on the WSGS and change in sIgA following the hypnosis task was not significant for concentration $r(30) = .081, p > .05$, or secretion rate $r(30) = .263, p > .05$.

Subjective Ratings of the PASAT and Hypnosis Task

Rating of relaxation/stress level. In order to determine whether the PASAT had been effective as a psychological stressor, and that the hypnosis task had been relaxing, participants were asked to rate their experiences of the PASAT and hypnosis task on a Likert-type scale that ranged from -5 (extremely relaxing) through 0 (no change) to $+5$ (extremely stressful). The mean rating for the PASAT was $+2.9$ ($SD = 1.30$), the mean rating for the hypnosis task was -3.5 ($SD = 1.11$). A repeated-measures ANOVA revealed that the two tasks were rated significantly differently, $F(1,29) = 374.32, p < .001$. One sample t-tests revealed that the PASAT was a subjectively stressful experience, $t(29) = 12.26, p < .001$, whereas the hypnosis task was subjectively relaxing, $t(29) = -17.33, p < .001$.

Rating of effort/concentration required for the PASAT and hypnosis task.

For both tasks, participants were asked to rate how much effort or concentration was required to perform the task. A Likert-type scale ranging from 0 (no effort) to 5 (required lots of concentration) was used. A repeated-measures ANOVA revealed that hypnosis ($M = 1.13$, $SD = 1.17$) was rated as requiring significantly less effort or concentration than the PASAT ($M = 4.53$, $SD = .63$), $F(1,29) = 256.56$, $p < .001$.

Rating of task engagement for the PASAT and hypnosis task.

Participants were asked to rate how engaged they were in each task. A Likert-type scale ranging from 0 (engaged throughout task) to 5 (lost focus) was used. A repeated measures ANOVA revealed that participants were less engaged during the hypnosis task ($M = 1.77$, $SD = 1.48$) than during the PASAT ($M = 3.13$, $SD = 1.36$), $F(1,29) = 10.91$, $p = .003$.

Relationship Between Subjective Ratings and Changes in sIgA

To examine whether changes in sIgA were related to any of the subjective ratings of the PASAT and hypnosis task, a number of bivariate correlations were performed. No significant relationships (all $p > .05$) were found between either of the two sIgA measures (concentration and secretion rate) and the subjective measures (relaxation/stress, effort/concentration, and task engagement).

Differences Between Sexes

Table 2 shows means and standard deviations of a number of variables for both males and females. At the initial baseline, females showed higher resting heart rate ($M = 79.06$, $SD = 13.17$) than males ($M = 72.50$, $SD = 10.67$) but lower sIgA levels for both

Table 2. Sex differences between measures of heart rate, sIgA, and subjective experiences.

Note: Bolded values represent significant differences between males and females.

	Participant's sex			
	male (n=14)		female (n=16)	
	M	(SD)	M	(SD)
Initial Baseline				
sIgA concentration (µg/ml)	400.70	(127.75)	352.50	(103.73)
sIgA secretion rate (µg/min)	236.88	(101.03)	151.43	(56.82)
Heart rate (bpm)	72.50	(10.67)	79.06	(13.17)
Pre-PASAT measures				
sIgA concentration (µg/ml)	323.95	(176.49)	287.53	(136.92)
sIgA secretion rate (µg/min)	210.21	(124.85)	140.70	(62.59)
Heart rate (bpm)	71.93	(11.70)	77.69	(13.15)
PASAT				
sIgA concentration (µg/ml)	320.62	(181.92)	342.45	(203.66)
sIgA secretion rate (µg/min)	209.45	(111.00)	187.99	(83.65)
Heart rate (bpm)	80.95	(10.45)	83.05	(14.34)
Pre-hypnosis measures				
sIgA concentration (µg/ml)	295.52	(154.34)	304.58	(146.43)
sIgA secretion rate (µg/min)	162.70	(76.15)	147.56	(86.97)
Heart rate (bpm)	71.43	(10.02)	76.63	(12.54)
Hypnosis				
sIgA concentration (µg/ml)	402.53	(204.35)	409.77	(177.65)
sIgA secretion rate (µg/min)	243.90	(125.90)	211.37	(74.95)
Heart rate (bpm)	68.57	(9.64)	74.94	(12.80)
Questionnaire responses				
PASAT stress rating	2.36	(.93)	3.38	(1.41)
Hypnosis relaxation rating	-3.21	(1.12)	-3.75	(1.06)
PASAT effort/concentration rating	4.64	(.63)	4.44	(.63)
Hypnosis effort/concentration rating	1.21	(1.25)	1.06	(1.12)
Hypnosis engagement rating	1.93	(1.38)	1.63	(1.59)
PASAT engagement rating	2.57	(1.34)	3.63	(1.20)
Hypnosis saliva production rating	1.46	(1.05)	2.25	(1.61)
Other measures				
PASAT score (# items correct till error)	12.93	(16.27)	6.88	(6.06)
WSGS:C score (11 items)	3.71	(2.05)	6.06	(1.88)

concentration (females $M = 352.50$, $SD = 103.73$; males $M = 400.70$, $SD = 127.75$) and secretion rate (females $M = 151.43$, $SD = 56.82$; males $M = 236.87$, $SD = 101.03$). However, differences were only significant for the initial baseline measure of sIgA secretion rate, $t(28) = 2.90$, $p = .007$.

Immune measures following the PASAT and the hypnosis task showed no significant differences between males and females (PASAT sIgA concentration, $t(28) = -.31$, $p > .05$; PASAT sIgA secretion rate, $t(28) = .60$, $p > .05$; hypnosis sIgA concentration, $t(28) = -.10$, $p > .05$; hypnosis sIgA secretion rate, $t(28) = .87$, $p > .05$). Although females generally maintained a higher heart rate than males, no significant differences existed between males and females in average heart rate measures during the PASAT ($t(28) = -.45$, $p > .05$) or the hypnosis task ($t(28) = -1.52$, $p > .05$).

Although males, on average, scored higher on the PASAT than females, this difference was not significant, $t(28) = 1.38$, $p > .05$. However, the hypnotic susceptibility scores were significantly higher for females than males, $t(28) = -3.27$, $p = .003$. This difference between sexes is in keeping with prior research, and in the expected direction (e.g., Jackson, Channon-Little & Shannon, 1995; Kihlstrom, Diaz, McClellan, Ruskin, Pistole, & Shor, 1980; Weekes & Lynn, 1990; but see also McConkey, Barnier, Maccallum, & Bishop, 1996).

Baseline Differences

The second baseline measure was employed to monitor whether sIgA levels returned to initial baseline levels before the second task. Initial inspection of the data

revealed that the second baseline had decreased below the initial sIgA baselines for both concentration and secretion rate. A repeated-measures MANOVA using both sIgA measures revealed a significant difference in baselines $F(2,27) = 28.78, p < .001$, with values at the second baseline being below the first. Subsequent univariate repeated measures ANOVAs revealed that both concentration $F(1,28) = 40.21, p < .001$, and secretion rate $F(1,28) = 7.04, p = .013$, were significantly lower at the second baseline.

Given that some researchers argue against the use of the initial baseline measure in analyses of psychophysiological data (e.g., see Dobkin, Létourneau, & Breault, 1994), increases in sIgA as a result of the two tasks were compared against both the lower (second) baseline values and also an averaged baseline value (calculated from the mean of baseline 1 and baseline 2).

Recalculations based on second baseline only. A repeated-measures MANOVA revealed a significant effect for the PASAT $F(2,27) = 6.72, p = .002$. There was no main effect for order of task presentation $F(2,27) = .419, p > .05$, nor was there an interaction between order of task presentation and the PASAT $F(2,27) = 1.39, p > .05$. Univariate repeated-measures ANOVAs revealed that both sIgA concentration and sIgA secretion rate were significantly higher following the PASAT as compared to the second baseline measure ($F(1,28) = 13.92, p < .001$ and $F(1,28) = 10.86, p = .002$, respectively). A repeated-measures MANOVA revealed a significant effect for the hypnosis task $F(2,27) = 20.49, p < .001$. There was no main effect for order of task presentation $F(2,27) = .09, p > .05$, nor was there an interaction between order of task presentation and the hypnosis task

$F(2,27) = 1.58, p > .05$. Univariate repeated-measures ANOVAs revealed that both sIgA concentration and sIgA secretion rate were significantly higher following the hypnosis task as compared to the second baseline measure ($F(1,28) = 42.56, p < .001$ and $F(1,28) = 20.90, p < .001$, respectively).

Recalculations based on averaged baselines. A repeated-measures MANOVA revealed a significant effect for the PASAT $F(2,27) = 2.68, p = .044$. There was no main effect for order of task presentation $F(2,27) = .70, p > .05$, nor was there an interaction between order of task presentation and the PASAT $F(2,27) = .34, p > .05$. Univariate repeated-measures ANOVAs revealed that sIgA secretion rate was significantly higher following the PASAT as compared to the averaged baseline measure $F(1,28) = 3.85, p = .030$, but sIgA concentration was not $F(1,28) = 1.21, p > .05$. A repeated-measures MANOVA revealed a significant effect for the hypnosis task $F(2,27) = 10.99, p < .001$. There was no main effect for order of task presentation $F(2,27) < .001, p > .05$, nor was there an interaction between order of task presentation and the hypnosis task $F(2,27) = 1.62, p > .05$. Univariate repeated-measures ANOVAs revealed that both sIgA concentration and sIgA secretion rate were significantly higher following the hypnosis task as compared to the second baseline measure ($F(1,28) = 21.46, p < .001$ and $F(1,28) = 16.30, p < .001$, respectively).

CHAPTER IV

DISCUSSION

Replication and Extension of Previous Hypnosis Research

Over 10 years ago, Olness et al. (1989) reported the results of a study conducted with children that demonstrated significant increases in the concentration of salivary secretory immunoglobulin A following suggestions for immunoenhancement during hypnosis. The current study replicates and extends these findings by showing that the same technique can produce similar increases in sIgA in an adult population and that the increases occur for both sIgA concentration and sIgA secretion rate. Additionally, the significant increase in sIgA following the hypnosis task was demonstrated with two different methods of sIgA analysis (single radial immunodiffusion, Olness et al.; Enzyme-linked immunoabsorbent assay, current study)

Although previous research (Ring et al., 1999; Willemsen et al, 1998; Winzer et al., 1999) has demonstrated that an acute mental stress task (PASAT) can produce significant increases in sIgA, the current study indicated that the PASAT and hypnosis can be distinguished on the basis of both physiological (heart rate) and psychological (subjective rating) measures. This suggests that any task related changes in sIgA are brought about by different physiological mechanisms for the PASAT and the hypnosis task. The PASAT produced a trend for increased sIgA in the present investigation, but this increase was not significant for either sIgA concentration or sIgA secretion rate. SIgA concentration following the hypnosis task was significantly greater than following the

PASAT, suggesting that hypnosis is at least as powerful a technique as a mental stress task for increasing sIgA levels, without the corresponding increases in heart rate and subjective stress.

Longevity of Increases in sIgA Following Hypnotic Suggestions

The present study incorporated a second baseline measurement to verify that sIgA levels had returned to pre-task levels prior to the second task. For the participants who were administered the hypnosis task first, this second baseline acted as a measure of the extent to which sIgA increases endured. The results demonstrate that although the hypnosis task was a powerful technique for increasing sIgA, the effects were short-lived. Within an 8-min resting condition immediately following the hypnosis task, sIgA levels not only returned to initial baseline levels, but actually decreased below the original baseline. Because the second baseline measurement was followed by the PASAT, the longevity of these decreases can not be established from the present data set.

It is not clear that psychophysiological techniques such as hypnosis can have a lasting immunoenhancing effect. Prior research in this area has invariably involved a design consisting of a single pre-treatment baseline measure and a single post-treatment measure. Although researchers have shown significant effects for hypnosis (Olness et al., 1989), relaxation (Green & Green, 1987), imagery (Rider et al., 1990), music (McCraty, Atkinson, Rein, & Watkins, 1996), and humor (McClelland & Cheriff, 1997), none of the aforementioned studies included a follow up measure after the initial post-treatment measure. The researchers were thus unable to examine the longevity of the reported

immunoenhancement following treatment. Additionally, all of the aforementioned studies based their results on sIgA concentration, without assessing changes in sIgA secretion rate.

Failure to Replicate the Effects of the PASAT

It is unclear why the present investigation was unable to replicate the effects of mental stress on sIgA. Although a meta-analysis of stress and relaxation studies (Van Rood, Bogaards, Goulmy & van Houwelingen, 1993) showed inconsistent effects for stress studies using sIgA measures, the lack of overall significance may well have been due to the range of stress tasks employed. In the three prior studies that used the PASAT as a mental stressor, the researchers found an increase in either sIgA concentration (Willemsen et al., 1998) or sIgA secretion rate (Ring et al., 1999; Winzer et al., 1999), though none of the studies demonstrated a significant increase in both of the measures following the PASAT.

Methodological Differences

Sex differences. Two of the three prior studies (Willemsen et al., 1998; Ring et al., 1999) used male subjects exclusively. In the Winzer et al. (1999) study, an analysis of sex differences was not reported. Given that over half of the participants in the present investigation were female, one possibility is that our failure to replicate is due to sex differences. However, the current study failed to show any significant sex differences for the PASAT task, suggesting that this is not a factor.

Differences in the saliva collection technique. The method of saliva collection used in the prior studies differed from the current study. All prior studies employed Salivettes (Sarstedt Ltd.) which involved each participant placing under his or her tongue a cotton wool swab that was later centrifuged to remove the absorbed saliva. A study by Aufrecht et al. (1992) demonstrated that the sIgA measures are significantly effected by the method of saliva collection. The authors concluded that the spitting method (used in the current study) yields the most information for the assessment of salivary flow. Based on these conclusions, it seems unlikely that the current investigation's failure to replicate the effects of the PASAT was due to improper saliva collection techniques.

Differences in the saliva collection time. One further methodological discrepancy between the present study and previous PASAT research deserves some attention. In using the Salivette technique, all of the prior studies collected a 2-min timed saliva sample. The current research involved a 4-min timed saliva sample. This difference in collection time was not originally hypothesized to have any impact on the sIgA measures collected immediately following the PASAT. However, in light of the significant decrease in sIgA observed at the second baseline measure (8-min after the first task) it is possible that the effects of the PASAT on sIgA have a very short duration, and that the addition of 2-min to the collection time results in an average sample that is significantly less replete with sIgA (because the post-PASAT decline has already begun). If this were true, the increases seen after hypnosis may be due to either 1) comparatively higher sIgA levels immediately following the hypnosis task, or 2) a more enduring enhancement

following the hypnosis task. Unfortunately, no data are available to support or refute this hypothesis and little is known about the pattern of decline in sIgA levels immediately following an increase.

Issues of Baseline

In keeping with the previous sIgA research using the PASAT (Ring et al., 1999; Willemsen et al., 1998; Winzer et al., 1999) and hypnosis (Olness et al., 1989), our primary hypotheses were tested against the pre-task baseline measures. In the current study, the data demonstrated a significant difference between the first and second sIgA baseline (pre-task) measures, with the second baseline being significantly lower than the first baseline. Although there is insufficient data provided to test for significance, this trend is also noticeable for both sessions in the Willemsen et al. (1998) study. SIgA concentration following the second baseline is approximately 30% lower than the initial baseline. This trend may also extend to other psychophysiological measures. This trend can be seen for heart rate measures (e.g., Piferi et al., 2000), and preliminary data from research conducted in the Neuropsychology lab at the University of Tennessee, Knoxville, indicates that significant reductions in baseline levels post-task appear to occur for EEG measures such as Alpha waves (E. Angelakis, personal communication, October, 2000).

Despite the importance of baseline measures in determining statistical significance, psychophysiological researchers have yet to reach consensus on what is considered a valid and reliable assessment of baseline. Pollack (1991) has emphasized the

importance of the baseline period in cardiovascular reactivity studies and argued that inconsistencies in baseline procedures could be a potential reason for inconsistent results. Similarly, Obrist, Light, James, and Strogatz (1987) stated that the conditions under which baseline measurements are recorded might determine whether an expected relationship emerges. In a review of cardiovascular reactivity studies, Hastrup (1986) reported little consistency in the duration and methodology used to assess baseline levels.

One recommendation, which we followed in our design, is that researchers should employ a minimally demanding task to decrease the variability of participants' experience during baseline (Piferi et al., 2000). The purpose of this task (watching an aquatic video) is to allow participants to more readily reach a relaxed state during baseline assessment, and research has demonstrated that it is more effective in achieving this than sitting quietly (Piferi et al.). Despite the use of this baseline task, our second baseline measure was significantly lower than the first baseline measure for heart rate, sIgA concentration, and sIgA secretion rate. It is possible that the first baseline reflects a normal resting baseline, and that the second baseline measure is artificially lowered due to a "rebound effect" following the termination of the PASAT and hypnosis task. However, it is also possible that the first baseline was artificially elevated due to initial anxiety regarding the experimental setting or anticipation toward the upcoming tasks. Based on research investigating baseline measures, Dobkin et al. (1994) concluded that it is preferable not to use initial baseline measures in analyses of psychophysiological data. For this reason, we included supplemental analyses based on both the lower (second) baseline values

exclusively and also an averaged baseline value (calculated as the mean of baseline 1 and baseline 2). The results for the hypnosis task remained unchanged from our primary analysis: both sIgA concentration and sIgA secretion rate increased from baseline following the hypnosis task. The supplemental analysis of sIgA changes following the PASAT provided different results from our primary analysis. When comparing post-PASAT sIgA measures with the second baseline only, results indicated a significant increase in both sIgA concentration and sIgA secretion rate. When comparing post-PASAT sIgA measures with the averaged baseline, results indicated a significant increase in sIgA secretion rate, but no significant increase in sIgA concentration. Thus, although the pattern of results remains consistent, the significance of sIgA changes following the PASAT is dependent on which baseline measure is used as a comparison.

There are two arguments in favor of retaining the initial baseline measure in our analysis. The first relates to consistency of methodology. By retaining the initial baseline, our analysis remains consistent with the previous research on both the PASAT and the hypnosis task. The second argument is more conceptual, and relates to the initial question being addressed in psychophysiological research. When investigating the impact of the PASAT or hypnosis on sIgA, we are really asking whether these tasks cause an increase from the normal level, a level that can fluctuate depending on many factors. Whether a person is anxious about the upcoming experiment or not, we are ultimately most interested in the effect of the PASAT and hypnosis task above and beyond any normal fluctuations in sIgA. If we rely on pushing a person's physiological indices to a "basal"

level (Obrist, 1981; Pollack, 1991), we may be artificially extending the gap between normal levels and enhanced levels following a given task.

Mechanisms Underlying the Observed Increases in sIgA

Although stress-induced activation of the hypothalamic pituitary adrenal (HPA) axis influences immune function (Webster, Elenkov & Chrousos, 1997), there is a time lag before cortisol increases (Kirschbaum, Pirke, & Hellhammer, 1993) and a further delay before these cortisol-dependent immunological changes are observed (Munck & Guyre, 1991). Therefore the rapid changes observed for sIgA in response to the PASAT have to be mediated through another mechanism. One suggested candidate is the rapidly acting sympathetic nervous system. The sympathetic nervous system modulates immune function (Felten et al., 1998): noradrenergic sympathetic nerves innervate lymphoid organs, adrenoreceptors are present on lymphocytes, and adrenergic agonists influence antibody production. Additionally, Carpenter, Garrett, Hartley, and Proctor (1998) reported that sympathetic stimulation of submandibular glands in rats resulted in a six-fold increase in sIgA secretion. However, Winzer et al. (1999) demonstrated that administration of a selective adrenergic blockade (propranolol) to participants had no effect in dampening the increase in sIgA concentration following the PASAT. The authors concluded that although stress related increases in certain cellular components can be blocked with propranolol (Benschop et al., 1994) increases in sIgA concentration during mental stress were not beta-adrenergically mediated.

Carpenter et al. (1998) found that although the submandibular gland of rats is innervated by both sympathetic and parasympathetic nerves, stimulation of the sympathetic nerves resulted in a sIgA secretion rate approximately two times greater than the sIgA secretion rate achieved by parasympathetic nerve stimulation. However, the fact that both sympathetic and parasympathetic pathways exist to the sIgA secreting submandibular glands, may explain how two tasks that differ so much in terms of the associated autonomic responses (indexed by heart rate) can both result in an increase in sIgA. Based on this animal model, it is possible that the PASAT causes sympathetic activation of the submandibular glands, whereas the hypnosis task results in parasympathetic activation. One other finding from the animal research is suggestive. In anaesthetized rats, sIgA was found to accumulate in the submandibular gland if it were unstimulated. It was suggested that when the gland was stimulated after a period of non-stimulation, it expelled the accumulated material (sIgA). Results showed that as the length of the rest period was extended, a nearly linear increase in the amount of sIgA secreted into the saliva occurred, suggesting a steady accumulation of sIgA occurs within the gland with time. This may be a possible confound for the hypnosis task. If the task results in the simple accumulation of sIgA over time (16 minute task) because the glands are relatively unstimulated during hypnosis, then the stimulation that occurs following the hypnotic deinduction may be the only event causing the apparent increase in sIgA levels post-hypnosis. This explanation seems improbable given that Olness et al. (1989) failed to find an increase in sIgA following hypnosis without specific suggestions for

immunoenhancement, a condition that would be expected to fulfil the requirements suggested above. Direct assessment of this mechanism, involving the collection of sIgA at various intervals during hypnosis would aid in further evaluating this potential confound.

A study by Clow (as cited in Gruzelier, Clow, Evans, Lazar, & Walker, 1998) examined the influence of lateralized temporo-parietal-occipital (TPO) cortex transcranial magnetic stimulation (TMS) on sIgA. TMS works by generating a magnetic field that penetrates the skull and induces an electric current in the underlying cerebral cortex. A significant increase in sIgA was shown following left-sided stimulation that, unlike a corresponding increase with right-sided stimulation was not associated with sympathetic activation. Such studies might shed light on the possible dual mechanisms of sIgA production, again generating testable hypotheses for why two such dissimilar tasks can produce similar immune responses (increase in sIgA).

Clinical Significance

The current research is in agreement with the findings of Olness et al., (1989) who showed that hypnosis with specific suggestions for immunoenhancement results in significantly elevated levels of sIgA. Having established this effect, one must now address the clinical significance of such findings. The importance of sIgA as a first line of defense against upper respiratory illness has been established by a number of researchers. It is well known that individuals with a selective IgA-deficiency suffer a high incidence of infections, particularly upper respiratory tract infection (Hanson, Bjökander, Oxelius,

1983). Caries-prone persons have been found to have lower sIgA concentrations than caries resistant persons, (Gregory, Kim, Kindle, Hobbs, & Lloyd, 1992), and deficiencies in sIgA have been identified as one of the factors responsible for the frequent oral infections in patients with AIDS (Muller et al., 1992). In healthy individuals, reduced sIgA levels have been shown to be related to an increase in the incidence of subsequent upper respiratory tract infection (Jemmott & McClelland, 1989). However, given that a cause and effect between low levels of sIgA and illness has not been demonstrated, sIgA levels are best viewed as a risk factor for developing infection. Much of the research examining the association between sIgA and infection has surrounded the effects of exercise on immune functioning. For example, Mackinnon et al. (1993) showed that the development of symptoms by hockey and squash players was preceded within 2 days by reductions in sIgA of 22% and 23% respectively. In those players who remained healthy, sIgA either increased slightly or was unchanged. Studies of psychological stress have also demonstrated that higher levels of sIgA are associated with less frequent illness (e.g., McClelland, Alexander, & Marks, 1980; McClelland & Cheriff, 1997). However, the pattern is not always clear. For example, Graham et al. (1988) found no relation between sIgA secretion rate and upper respiratory infection in the past 12 months. Further research needs to establish the link between sIgA levels, both in terms of concentration and secretion rate, and upper respiratory illness.

Assuming that a causal link between lowered sIgA and illness can be substantiated, the second issue in establishing clinical significance is whether the acute

increases in sIgA following hypnosis can be prolonged. The current study indicates that the effects of specific immunoenhancing hypnotic suggestions are short lived. Indeed, not only had sIgA concentration and secretion rate returned to baseline within 8-min of sitting quietly, they were significantly below baseline. Such a drop might actually leave individuals more susceptible to infection shortly after the hypnosis intervention.

Unfortunately, given that the second baseline was followed by the second task, our data can not establish how long this decline sustains. Future research must demonstrate that psychological immunoenhancing techniques can provide an extended increase in sIgA, perhaps through ongoing training programs. One study investigating the effects of daily relaxation practice provides some support for this potential extended effect. Green et al. (1988) found that individuals had significantly higher baseline sIgA secretion rates after having practiced daily relaxation for 3 weeks. Individuals in a waiting list control group showed no significant increase in baseline levels. Since hypnosis has been shown as more effective than relaxation (Olness et al., 1989), perhaps practicing hypnosis with specific suggestions would aid in raising baseline levels. Even if this were established, one would need to check for reduced incidence of upper respiratory infections following increased baseline levels.

Conclusions

Research indicates that hypnosis with specific suggestions for immunoenhancement can significantly increase the levels of sIgA in the saliva. The current research suggests that this effect is as potent as increases resulting from a mental

stress task, and also demonstrates that the two tasks can be distinguished by both experiential (questionnaire) and physiological (heart rate) measures. However, based on the current investigation, the increases following hypnotic treatment are short-lived, and may even result in a temporary reduction in sIgA levels shortly afterwards. These findings are a small step towards substantiating the clinical significance of psychological techniques for immunoenhancement. Future research will need to establish the long-term effects of hypnosis in providing enhanced immunological protection from infection.

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APPENDICES

APPENDIX A INFORMED CONSENT

INFORMED CONSENT FORM

University of Tennessee
Human Subject Consent Form

Title of Research Proposal: Hypnosis, Mental Arithmetic, and Immune Functioning.

Principal Investigator: Grant Benham

Department: Psychology

Office and Phone: Austin Peay Building, Room 219, 974-4866

The general purpose of this study is to investigate the relationship of hypnosis, mental arithmetic, and immune functioning. The study is split in to two parts. During today's session you will be asked to watch a short videotape about the immune system and then we will be administering a mental arithmetic and hypnosis task each lasting between 10 and 15 minutes. You will be asked to provide four saliva samples during today's session, and the session will last about one hour

Once all participants have completed the first session, we will ask you to attend a second session where you will take part in a standardized hypnotic procedure. The PI, Grant Benham, has extensive experience with administering these measures of hypnotizability. Our participants have found this procedure to be both interesting and stimulating, and you will not be asked to do anything that will embarrass you. As with many experimental procedures, a few participants may feel some discomfort; we encourage you to express any uncomfortableness to the experimenter. We want you to enjoy and learn from your participation today.

Your first name and phone number will be part of our records, available only to the researcher (above) and Dr. Nash. Once we encode the scores, all identifying information will be destroyed. Other than consent forms, no information that would identify you will exist following our data entry. Consent forms will be stored for three years past the completion of the study at a UT location. Participation in this research is voluntary; you may terminate this experiment any time you wish without any penalty. Just tell the research assistant you wish to stop. The researcher will provide your instructor with an extra credit form indicating your involvement in extra credit research. You will be provided with a copy of this extra credit form for your records. We invite you to ask any questions about our project. We will answer them to the best of our ability.

I have read the above statement; I am 18 years of age or older; and I understand that I can terminate participation in this experiment any time I wish.

Name of participant

Signature of participant

Date

Signature of investigator

Date

APPENDIX B
MENTAL STRESSOR (PASAT) STIMULI

2.4 secs		2.0 secs			1.6 secs			1.2 secs			
4	5	5	2	7	8	9	4	8	9	6	2
1	5	4	4	3	3	9	9	6	4	3	5
8	9	5	1	4	7	3	9	5	9	4	9
3	7	6	7	8	7	8	8	3	6	4	3
2	5	7	1	3	9	1	2	4	1	2	1
6	4	3	9	1	5	3	2	6	5	4	7
7	6	3	2	8	4	2	6	6	2	9	6
8	3	6	2	6	1	5	4	5	9	4	2
3	1	8	6	7	1	3	1	2	1	1	1
2	7	9	6	8	6	5	4	5	6	7	8
6	3	1	2		2	7	7	9	8	6	4
7	3	5	4		4	4	8	3	2	8	6
9	2	3	6		2	6	7	1	9	4	7
4	2	7	3		5	6	5	3	5	2	9
6	4	2	3		4	4	8	2	7	3	8
4	4	9	5		3	8	6	7	5	6	6
3	9	2	1		6	5	9	8	8	8	5
9	5	8	5		7	7	1	7	8	5	3
2	7	1	8		8	1	3	2	7	8	2
7	1	9	5		2	2	5	4	1	1	6
9	8	8	9		3	9	4	1	4	3	7
8	8	7	4		6	2	7	8	3	7	4
1	6	1	8		1	1	2	1	7	9	2
1	9	6	9		5	7	3	9	2	5	9
5	5	4	7		8	6	9	7	3	6	2

APPENDIX C

HYPNOSIS SCRIPT

Hypnotic Induction with Suggestions for Relaxation

Now, please seat yourself comfortably and rest your hands in your lap. That's right. Rest your hands in your lap. Now look at your hands and find a spot on either hand and just focus on it. It doesn't matter what spot you choose; just select some spot to focus on. I will refer to the spot you have chosen as the target. That's right - hands relaxed ... look directly at the target.

I am about to help you to relax, and meanwhile I will give you some instructions that will help you to gradually enter a state of hypnosis. Please look steadily at the target and while staring at it, keep listening to my words. Just do your best to concentrate on the target -- pay close attention to my words, and let happen whatever you feel is going to take place. Just let yourself go. Pay close attention to what I tell you to think about; if your mind wanders, that will be okay; just bring your thoughts back to the target and my words.

Just let yourself relax. Keep looking at the target as steadily as you can, thinking only of it and my words. If your eyes drift away, don't let that bother you just focus again on the target. Pay attention to how the target changes, how the shadows play around it, how it is sometimes fuzzy, sometimes clear. Whatever you see is all right. Just let yourself experience whatever happens and keep staring at the target a little longer. After awhile, however, you will have stared long enough, and your eyes will feel very tired, and you will wish strongly that they were closed. Then they will close, as if by themselves. When this happens, just let it happen.

As I continue to talk, you will find that you will become more and more drowsy, but not all people respond at the same rate to what I have to say. Some people's eyes will close before others. When the time comes that your eyes have closed, just let them remain closed. You may find that I shall still give suggestions for your eyes to close. These suggestions will not bother you.

You will find that you can relax completely, but at the same time sit up comfortably in your chair with little effort. You will be able to shift your position to make yourself comfortable as needed without it disturbing you. For now, just relax more and more. As you think of relaxing, your muscles will actually begin to relax. Starting with your right foot, relax the muscles of your right leg ... Now the muscles of your left leg ... Just relax all over. Relax your right hand your forearm ... upper arm ... and shoulder ... That's it ... Now your left hand ... and forearm and upper arm ... and shoulder ... Relax your neck, and chest ... more and more relaxed ... completely relaxed ... completely relaxed.

As you become relaxed, your body will feel deeply at ease ... comfortably heavy. You will begin to have this pleasant feeling of heaviness and comfort in your legs and feet ... in your hands and arms ... throughout your body ... as though you were settling deep into the chair. Your body feels comfortable and heavy ... Your eyelids feel heavy too, heavy and tired. You are beginning to feel very relaxed and comfortable. You are breathing freely and deeply, freely and deeply. You are becoming more and more deeply and comfortably relaxed. Your eyelids are becoming heavier, more and more heavy and difficult to keep open.

Staring at the target so long has made your eyes very tired. Your eyes may hurt from staring and your eyelids feel very heavy. Soon you will no longer be able to keep your eyes open. Soon you will have stood the discomfort long enough; your eyes are tired from staring, and your eyelids will feel too tired to remain open. Perhaps your eyes are becoming moist from the strain.

You are becoming more and more relaxed and comfortable. The strain in your eyes is getting greater and greater. It would be a relief just to let your eyes close and to relax completely, to relax completely. The strain in your eyes will eventually be so great that you will welcome your eyes closing of themselves, of themselves.

Your eyes are tired and your eyelids feel very heavy. Your whole body feels heavy and relaxed. You feel a pleasant warm tingling throughout your body as you become more and more deeply relaxed ... deeper ... deeper ... more relaxed ... completely relaxed and drifting down into a warm pleasant state of relaxation. Keep your thoughts on what I am saying; listen to my voice. Your eyes are getting blurred from straining. You can hardly see the target, your eyes are so strained. The strain is getting greater, greater and greater, greater and greater. Your eyelids are heavy. Very heavy. Getting heavier and heavier, heavier and heavier. They are pushing down, down, down. Your eyelids seem weighted and heavy, pulled down by the weight ... so heavy Your eyes are blinking, blinking closing, closing

Your eyes may have closed by now, and if they have not, they would soon close of themselves. But there is no need to strain them more. You have concentrated well on the target, and have become very relaxed. Now we have come to the time when you may just let your eyes close. That's it, eyes closed now.

You now feel very relaxed, but you are going to become even more relaxed. It is easier to relax completely now that your eyes are closed. You will keep them closed until I tell you to open them or until I tell you to become alert ... You feel pleasantly, deeply relaxed and very comfortable as you continue to hear my voice. Just let your thoughts dwell on what I'm saying. You are going to become even more relaxed and comfortable. Soon you will be deeply hypnotized, but you will have no trouble hearing me. You will remain deeply hypnotized until I tell you to awaken later on. Soon I shall begin to count from one to ten. As I count, you will feel yourself going down further and further into a deeply relaxed, a deeply hypnotized state ... but you will be able to do all sorts of things I ask you to do without waking up... One ... you are going to become more deeply relaxed and hypnotized ... Two ... down, down, deeper and deeper... Three ... four ... more and more deeply hypnotized ... Five ... half way there, six ... seven ... you are sinking deeper and deeper into hypnosis. Nothing will disturb you ... Just let your thoughts focus on my voice and those things I tell you to think of. You are finding it easy just to listen to the things I tell you. Eight... always deeper ... although deeply hypnotized you can hear me clearly. You will always hear me distinctly no matter how deeply hypnotized you become.... Nine.... deeply hypnotized. Nothing will disturb you. You are going to experience many things that I can tell you to experience. Ten.... Deeply hypnotized now! You will wish to remain relaxed and hypnotized and to have the experiences I describe to you.

Now, just focus on your breathing for a moment or two. Notice when you breathe in the muscles in your chest expand and get tighter, and as you breathe out, the muscles loosen as you let go of the air. Every time you breathe out, enjoy relaxing along with those muscles that are naturally and automatically relaxing ... and let that relaxing and loosening move into the muscles of your stomach. Let the muscles of your stomach get loose and comfortable. With every breath you feel yourself becoming more and more relaxed, more and more comfortable. And I want you to focus now on this feeling of relaxation, of heaviness. And you may notice that your hands are heavy. Perhaps your hands and fingers feel warm and heavy as you continue to relax – more and more relaxed. Just focus on your breathing. And perhaps your mind may wander to a peaceful place, and you may experience images, and sounds, perhaps even pleasant smells, or perhaps you prefer to just focus on your breathing. Whatever you choose is alright, as you continue to feel comfortable and relaxed...

Specific Suggestions

And as you sit there I want you to think back to the video that you saw about your immune system. I want you to think about the immune proteins that are secreted into your saliva. Every hour of every day these immune proteins help to keep you healthy and to protect your body. And I want you to focus now on these immune proteins in your saliva. I want you to imagine these immune proteins, picture them in your mind. Your mind and body are inseparably linked and your mind can control your body. As you picture these immune proteins, I want you to imagine that they are increasing in number. Perhaps you can picture them as small white objects, like microscopic white pearls secreted from the lining of your mouth. Or maybe you see them in more detail as colored strands of protein – being passed from the tissue lining in your mouth into the saliva. You can picture these immune components in whatever way you choose, but however you picture the immune proteins, you will see them increase in number as they are secreted more and more from the lining of your mouth into your saliva. They are secreted more and more, faster and faster, pouring into the mouth. And as these immune proteins increase, you may also feel the saliva increasing in your mouth. You may notice that your mouth becomes more and more moist as the saliva and the immune proteins increase. Just focus on this for a moment as you picture the immune proteins being secreted into your saliva.. (pause). Your mind can control and direct your body, and you can direct your body to increase these immune proteins to make yourself healthier, more able to resist invading organisms. The immune proteins in your saliva protect your body and keep you healthy, and as you imagine these immune proteins increasing in your saliva, your body actually produces more and more immune proteins. You see the immune proteins in your saliva, protecting your body, and I just want you to focus on these images, these sensations, for a few moments. (pause for ~20 secs)

De-Induction for Both Groups

That's good. In a moment I shall begin counting backwards from ten to one. You will awaken gradually, but for most of the count you will remain in the pleasant, relaxed state that you are now in. By the time I reach "THREE" you will open your eyes, but you will not be fully aroused. When I get to "ONE", you will be fully alert, in your normal state of wakefulness, ready to enjoy the rest of the day.

Ready, now: 10...9...8...7....6...5, halfway there...4...THREE...2... ONE. Wake up! Wide awake! Any remaining drowsiness which you may feel will quickly pass. Please sit quietly until the experimenter instructs you further.

APPENDIX D QUESTIONNAIRE

Questionnaire

Name _____ SS# _____ - _____ - _____

Please answer the following questions:

Age: _____ yrs. Sex: M / F (Circle one) GPA _____

Height: _____ Weight: _____

Do you smoke? Y / N (Circle one)
If Yes, how many pack a week on average? _____ packs (to the nearest half pack)

⇒ Please rate your experience of the hypnosis and mental arithmetic procedures on the following scale where -5 represents an extremely relaxing experience and +5 represents an extremely stressful experience (circle the number that best represents your experience):

HYPNOSIS (circle one):

Extremely relaxing						No change						Extremely stressful
-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5		

MENTAL ARITHMETIC (circle one):

Extremely relaxing						No change						Extremely stressful
-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5		

⇒ For both the hypnosis and mental arithmetic task, please rate how much effort/concentration was required to perform the task.

Hypnosis (circle one)	Mental Arithmetic (circle one)
No effort	No effort
0 1 2 3 4 5	0 1 2 3 4 5
required lots of concentration	required lots of concentration

⇒ For both the hypnosis and mental arithmetic task, please rate how engaged you were in the task. Do you feel like you "gave up" at any point in the process?

Hypnosis (circle one)	Mental Arithmetic (circle one)
Engaged throughout task	Engaged throughout task
0 1 2 3 4 5	0 1 2 3 4 5
lost focus	lost focus

⇒ For the hypnosis task, please indicate whether you noticed any change in the amount of saliva being produced in your mouth.

Saliva Decreased						No change						Saliva Increased
-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5		

⇒ Did you notice any change in the taste in your mouth during hypnosis? Yes / No (circle one)

VITA

Grant Benham was born in Colchester, England on January 27th 1968. After completing his secondary education at the Colchester Royal Grammar School in June 1987, he enrolled in a Bachelor of Science (Hons.) program in Psychology at Plymouth University, England. During his undergraduate program, he spent a year at the University of Tennessee, Knoxville, as part of an international student exchange program. He graduated from Plymouth University in 1990 and spent the next 5 years working Austin, Texas, as Marketing Director for a large travel organization. In 1995 he returned to the University of Tennessee, Knoxville, to pursue a Ph.D. in Experimental Psychology. In January 1998, he accepted the position of Managing Editor for the International Journal of Clinical and Experimental Hypnosis, and continues to work in this capacity for the journal.

In June of 1999 Grant and his wife, Chelse, started up their own television and video production company, Silhouette Studios. The company has since produced a number of shows, including a documentary about women living with breast cancer, “The Healing Journey”. With Ph.D. in hand, Dr. Benham intends to dedicate time to producing high-quality psychology documentaries for the national television networks.