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A longitudinal investigation of the relationships amongst antibody response to influenza vaccination, affect, and stress in the elderly

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A LONGITUDINAL INVESTIGATION OF THE RELATIONSHIPS AMONGST ANTIBODY RESPONSE TO INFLUENZA VACCINATION, AFFECT, AND STRESS IN THE ELDERLY

By

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ABSTRACT OF THE DISSERTATION

A Longitudinal Investigation of the Relationships amongst Antibody Response to Influenza Vaccination, Affect, and Stress in the Elderly

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We examined the cross-sectional and longitudinal relationships amongst affect, stress exposure, and antibody (Ab) response to influenza inoculation in a healthy, elderly sample. We explored both efferent (CNS on immune activity) and afferent (immune activity on CNS function) pathways. Negative (NA) and positive (PA) affective states were examined in relation to Ab response, positing that high baseline NA (State, SNA, but not Trait, TNA) would predict reduced Ab response and that PA would predict enhanced response, and that the reduced Ab response in individuals displaying high baseline SNA would associate with decreases in NA. Moderator (for psychogenic and systemic stress) and mediator (systemic symptom reporting) tests were conducted to validate these relationships.

The 152 (97 female, 55 male; age M=72.49, SD=6.32) participants were residents of a retirement community, who met medical exclusionary criteria and completed all three annual assessments (in 1992, 1993, and 1996). Participants were inoculated with trivalent influenza vaccine, and Ab titer was assayed two weeks post-inoculation via hemagglutinin inhibition assay.

As hypothesized, TNA did not predict Ab response. Surprisingly, high baseline SNA predicted *enhanced* Ab response, which predicted decreased NA in a year- and strain-specific manner. Longitudinally, robust initial Ab response followed by steady decreases was

associated with combinations of no change in one dimension of NA and decreases in the other. High PA only showed transient associations with increased Ab response. Stress did not act as a moderator, though systemic symptom reporting did mediate the relationship between Ab response and change in NA in 1996.

Overall findings suggest a reconceptualization of initial NA. High levels of initial SNA did not predict impaired immunity, but instead robust response. Hence, instead of an aberrant affective state, high initial SNA likely indicates enhanced arousal, which returns to lower levels after threat ceases. This study also illustrates the complexities involved in investigating the relationship between health and immunity, and importantly sheds light on how varying temporal and antigenic variables may elicit differences. This study adds considerably to the body of work exploring the dynamic interplay between emotion, experience, aging, and physiological response to benign immune challenge.

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INTRODUCTION

The concept of mind-body connectivity is an ancient one, espoused in disciplines ranging from spirituality (e.g., the tenets of Buddhism of 200 B.C.E) to science and medicine (e.g., Greek physician Galen). However, in medicine the prevailing model was of a mind orchestrating physiology and corporal sensations being processed by a receptive mind. The concept of true bidirectional communication was not fully recognized until quite recently. The field of Psychoneuroimmunology (PNI) was bourn of the need to empirically investigate the mechanisms underlying mind-body interactions, and the pathways by which these interactions occurred. Since its inception in 1930, PNI has made great strides in elucidating both the efferent and afferent pathways between the central nervous system (CNS) and the immune system. To briefly illustrate some major lines of evidence for this interaction: hormones influence expression of Major Histocompatibility Complex (MHC) class (Muller and Ackenheil, 1998), immune products influence neurotransmitter activity (Muller and Ackenheil, 1998), cytokine receptors are found in the brain (Parnet et al., 2002), many psychiatric disorders parallel autoimmune development and pathology (Muller and Ackenheil, 1998), neuro-endocrine and immune products coexist in lymphoid, endocrine, and neural tissue (Besedovsky and DelRey, 1996) (see Figure 1 for a schematic representation of neuro-endocrine-immune interactions).

Efferent Pathways from CNS to Immune System

There are four primary routes by which the CNS efferently influences immune function: peripheral nervous system, sympathetic nervous system (via adrenergic mechanisms), parasympathetic nervous system (via cholinergic mechanisms), and neuroendocrine mechanisms (i.e., the hypothalamic-pituitary-adrenal [HPA] axis) (Sternberg, 2006). While the peripheral nervous system acts mainly in a localized fashion to promote inflammation at the beginning of pathogen invasion (though essential in the regulation of immunity, peripheral nervous system mechanisms will not be extensively discussed herein; for a review, see Sternberg 2006), the other routes act primarily in a regulatory capacity not only to facilitate clearance of pathogen but also to reign in the immune response itself. The autonomic nervous system components achieve this via direct innervation of immune organs, while the HPA axis does so systemically by stimulating production of anti-inflammatory glucocorticoids (Sternberg, 2006).

HPA Axis

Exposure to stress, be it psychological events or physiological insults (i.e., release of interleukin (IL)-1 subsequent to pathogen invasion), triggers the HPA axis via release of corticotropin-releasing hormone (CRH) from the hypothalamic paraventricular nucleus (Steinman, 2004). Corticotropin-releasing hormone then stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH), resulting in glucocorticoid synthesis and release from the adrenal cortex (Steinman, 2004). Glucocorticoids generally serve as a negative feedback mechanism to reign in an immune response, such that immune cells with highest affinity for the pathogen are selected for while reducing non-specific immune cells which could result in immunopathology, potentially providing enhanced efficacy of the

immune response (Besedovsky and del Rey, 2007). In a further regulatory capacity, glucocorticoids downregulate production of proinflammatory mediators (i.e., cytokines) to prevent development of life-threatening sepsis (Besedovsky and del Rey, 2007). Glucocorticoids also act to inhibit the HPA axis itself, in order to ensure complete clearance of the pathogen. Excessive glucocorticoid circulation has been associated with impaired ability to clear viral infections (Glaser and Kiecolt-Glaser, 2005; Glaser et al., 2005) and decreased antibody response subsequent to vaccination (Vedhara et al., 1999). Glucocorticoids can also influence antibody class switching between Immunoglobulin (Ig)M and IgG (Elliott and Sinclair, 1968), and there is an inverse relationship between glucocorticoids and splenic Ab production, further indicating the effect of endogenous glucocorticoids on B cell function (del Rey et al., 1984). Hence, a delicate balance of HPA axis activation must occur to optimize the immune response and return the organism to homeostasis. The HPA axis acts as the primary means by which hormones influence immunity. However, the sympathetic nervous system (SNS) also releases adrenaline from the adrenal medulla to regulate immune activity.

Sympathetic Nervous System

Adrenaline release from SNS activation systemically reduces circulating immune cells (i.e., monocytes, B and T cells, Natural Killer [NK] cells) through cell surface-expression of β-adrenergic receptors (Vedhara et al., 1999). Beta-adrenergic receptor stimulation also suppresses lymphocyte proliferation (Muller and Ackenheil, 1998), and β-adrenergic agonsists reduce IL-2 receptor expression on lymphocytes (Feldman et al., 1987). The SNS exerts immunomodulatory activity not only through adrenaline, but also via the neurotransmitter noradrenaline. Noradrenergic binding to β-adrenergic receptors results in

suppressed pro-inflammatory cytokine production mediated by second messengers (Vedhara et al., 1999). As with the HPA axis, the SNS can be stimulated through stress (particularly psychological). If of sufficient magnitude, activation of the SNS may release large amounts of noradrenaline, which acts immunosuppressively (Sternberg, 1989).

Parasympathetic Nervous System

Like the SNS and HPA axis, the parasympathetic nervous system (PNS) provides negative feedback subsequent to cytokine release to reign in inflammatory responses. It does so through both vagal afferents and the primary PNS neurotransmitter acetylcholine, Ach (Sternberg, 2006). The vagus detects presence of peripheral inflammation through paraganglia IL-1 receptors, triggering the release of Ach which then inhibits macrophage-induced pro-inflammatory cytokines (Sternberg, 2006). Acetylcholine receptors are found not only on macrophages, but also on T cells and B cells, indicating the diverse effects the PNS exerts on the immune system (Sternberg, 2006).

The paradigm behaviorally-conditioned immunosuppression is an elegant example of how the CNS directly impacts immune system functioning. Behaviorally-conditioned immunosuppression refers to the pairing of immunosuppressive treatment (the unconditioned stimulus, US) with a flavored beverage (the conditioned stimulus, CS), ultimately resulting in the CS alone eliciting immunosuppressive effects (Ader and Cohen, 1975). This effect reveals how effectively the immune system communicates with the CNS. While this effect may appear to be maladaptive, under certain circumstances suppression of the immune system may have beneficial implications. For example, if a non-toxic CS may be utilized to reduce the effective dosage of an immunosuppressant, this would have important

consequences for the treatment of autoimmune diseases. Indeed, in an animal model of lupus, lupus-prone mice can be trained to suppress clinical manifestations of the disease by consuming a saccharine solution once paired with the immunosuppressant cyclophosphamide (Ader and Cohen, 1982). Extending this, investigations of the conditionability of lipopolysaccharide (LPS)-induced endotoxin tolerance have been conducted. Using the conditioned taste aversion paradigm, investigators have found that after trained associations between a saccharine drinking solution (CS) and small doses of LPS (US), the saccharine solution alone was able to induce endotoxin tolerance, as evidenced by cessation of LPS-induced tumor necrosis factor (TNF)-α production (Oberbeck *et al.*, 2003).

Although the direct impact of CNS activation (e.g., via neurotransmitters, neuropeptides, hormones, neural efferents) had remained controversial for a considerable time, recent studies have illustrated quite convincingly the important role of CNS activation in a precisely regulated immune response. These efferent CNS influences facilitate the ability of the immune system to operate in a carefully orchestrated manner, maintaining homeostasis under pathological conditions.

Afferent Communication from the Immune System to the CNS

Less well-delineated are the mechanisms by which the immune system acts as an afferent sensory organ relaying physiological information to the CNS, particularly in reference to human behavior. As mentioned in the prior section, vagal afferents transporting IL-1 to the hypothalamus are often the first indicator to the CNS of peripheral immune activation (Goehler et al., 1999). Hypothalamic stimulation is often one of the first CNS effects of immune activation. For example, immunization causes sustained three- to four-fold elevations in glucocorticoids (Besedovsky and DelRey, 1996; Besedovsky and del Rey, 2007). Immunoglobulins have been shown to bind, via their Fc region, to ACTH-producing cells in the pituitary, illustrating the direct impact of antibodies on the HPA axis (Pouplard et al., 1976).

Cytokines produced in the periphery can also reach the CNS (via vagal afferents, active transport, circumventricular organs) to influence learning, behavior and emotion. The presence of cytokine receptors in the CNS also further supports this. The hippocampus and hypothalamus are the regions with the greatest expression of cytokine receptors (Rothwell et al., 1996; Mehler and Kessler, 1997), indicating the possible influence of cytokines in memory, learning, and neuroendocrine states. Interleukin-1 receptors are heavily expressed on neurons in the hippocampus (Besedovsky and DelRey, 1996) and hypothalamus, linking IL-1 to memory and neuroendocrine functions (Besedovsky and del Rey, 2007). Interleukin-1 also has an overall effect of tryptophan accumulation in the CNS, and reduces noradrenaline content (Besedovsky and del Rey, 2007), implicating this cytokine in emotion and arousal. Interleukin-2 receptors also show greater prominence in neurons, particularly in hippocampal neurons (Araujo et al., 1989), than in astrocytes or microglia, indicating the direct impact of IL-2 on neuronal function (Shimojo *et al.*, 1993).

Not only can the immune system exert its effects on the CNS through active transport of peripheral cytokines into the CNS across the blood-brain barrier (BBB), but also endogenous to the CNS itself are immunocompetent cells – astrocytes and microglia (Xiao and Link, 1998; Sredni-Kenigsbuch, 2002). Activated microglia secrete IL-1, IL-2, IL-6, IL-10, and a small amount of TNFα, while activated astrocytes produce IL-1, IL-6, and TNFα (Muller and Ackenheil, 1998; Sredni-Kenigsbuch, 2002). Interestingly, the cell type that produces IL-6 is dictated according to stimulation, with TNFα and IL-1 stimulating astrocytes (Sawada et al., 1992) and granulocyte-colony stimulating factor inducing microglia to secrete IL-6 (Muller and Ackenheil, 1998). Some reports even indicate that neurons themselves can produce IL-6 (Schobitz et al., 1992) and TNFα (Gendron et al., 1991; Breder et al., 1993). Studies of the sickness behavior have provided a wealth of knowledge on the impact of immune activation on behavior and will be briefly reviewed herein.

CNS Response to Immune Challenge: Sickness Behavior

When an organism is exposed to a pathogen, the result is a constellation of physiological and behavioral manifestations, broadly recognized as sickness behavior (Hart, 1988). Sickness behavior is often characterized by hypophagia, hyperalgesia, lethargy, psychomotor retardation, anhedonia, and cognitive impairments, and is considered to be an adaptive response allowing the organism to focus its resources toward recovery (Hart, 1988). This view has been further expanded to examine sickness behavior as a manifestation of adaptive motivational states (Dantzer, 2004). However, when the activation of these systems is sustained, the burden may result in an increased vulnerability toward developing other pathologies, such as depression (Anisman and Merali, 2003). Many of the symptom manifestations of sickness behavior parallel depression (i.e., lethargy, anhedonia, hypophagia) and anxiety (i.e., decreased locomotor activity and social interactions) (Anisman and Merali, 2003). As such, sickness behavior is one of the most useful and direct measures of the impact on the CNS and the behavioral sequelae of immune activation. Cytokines, soluble mediators of immunocompetent cellular proliferation and differentiation (Capuron et al., 2000), play a prominent role in the evolution of sickness behavior (Anisman et al., 2005). A number of animal and human models of sickness behavior have been developed to elucidate the effects of varying immunological challenge on CNS function.

Endotoxin/Lipopolysaccharide

Lipopolysaccharide is the pathogenic component produced by gram negative *Escherichia coli*. Challenge with LPS results in a plethora of immune system effects, particularly the development of sepsis, which is mediated primarily by proinflammatory cytokines such as TNFα, IL-1, and IL-6 (Besedovsky and DelRey, 1996; Oberbeck et al.,

2003; Dantzer, 2004). This antigen elicits many of the characteristic behavioral effects of sickness behavior, such as hypophagia, decreased locomotor activity, weakness (Dantzer, 2004). The anhedonic effects of LPS administration are one of the key symptoms of depression (Anisman and Merali, 2002), and are attenuated by antidepressant treatment (Yirmiya, 1996). Thus, exposure to this antigen is considered to represent an animal model of depression (Dunn et al., 2005). Yet, the afore-mentioned effect was only observed in rats and not in mice (Dunn *et al.*, 2005), indicating that genetic background may play a role in the cytokine effects on anhedonia. These behavioral effects of peripherally-administered LPS are mediated by neural afferents (Dantzer *et al.*, 1998).

Some work has also been conducted on humans to examine the cognitive and affective sequelae of endotoxin challenge. Though constrained by the ability to administer pathogenic doses in humans, limited work has been done investigating how low-dose *in vivo* endotoxin challenge impacts human emotion and behavior. At a dose of LPS high enough to induce cytokine production but not illness, endotoxin-induced cytokine activation has been shown to elicit cortisol-independent memory deficits and increased anxiety in healthy young men (Reichenberg *et al.*, 2001). The same dose of LPS also results in changes in declarative memory depending on degree of IL-6 production, with impaired declarative memory following a large increase in IL-6 and improved declarative memory subsequent to a small increase in this cytokine (Krabbe *et al.*, 2005), indicating that alterations in memory performance are contingent upon robustness of the proinflammatory cytokine response. Cohen and collaborators (2003) delved further into endotoxin-induced memory deficits by examining whether different types of memory may be affected by low-dose administration of LPS. Consistent with Krabbe and colleagues (2005), they found that endotoxin-induced cytokine production caused a reduction in declarative memory, however, they found an

unexpected *enhancement* in working memory (Cohen et al., 2003a). From an evolutionary perspective, it may be that forms of memory more salient to an organism's immediate survival (i.e., working memory) may be enhanced during immune activation so that limited resources can be focused on dealing with urgent stimuli.

Cytokines

Activated immune cells synthesize and release cytokines. As discussed in the prior sections, induction of cytokines by pathogens can act as potent stimulation of sickness behaviors. Direct cytokine administration acts similarly. Furthermore, cytokines have been shown to have a role in learning and memory (Dantzer, 2004). The presence of IL-1 receptors in the dentate gyrus of the hippocampus initiated investigations of the effects of proinflammatory cytokines on learning and memory (Dantzer, 2004). For the most part, IL-1 appears to impair performance on memory tasks such as the Morris water maze (Gibertini et al., 1995), contextual fear conditioning (Pugh et al., 2001), and Pavlovian conditioning (Aubert et al., 1995). Repeated systemic administration of the cytokine IL-2 also resulted in impairments in Morris water maze performance, also suggesting a role in working memory for this cytokine (Lacosta et al., 1999). Interleukin-1 administration is also frequently used as an animal model of depression, in that it induces many of the sickness behaviors indicative of depression (i.e., hypophagia, decreased locomotor activity, anhedonia) (Dunn *et al.*, 2005). Through its stimulatory impact on corticotropin-releasing factor, linked to depression and anxiety, IL-6 has also been utilized as a model of depression (Juttler et al., 2002).

Systemic administration of cytokines also has a direct effect on neurotransmitter levels (Anisman *et al.*, 2005). Interleukin-1β impacts serotonin (5-HT) turnover in the hippocampus, prefrontal cortex, and hypothalamus, as well as increases noradrenaline

turnover in key stress structures (Dunn et al., 1999; Brebner et al., 2000). Like IL-1β, TNFα administration in the paraventricular nucleus of the hypothalamus resulted in sensitization of noradrenaline activity (Hayley et al., 1999), although this finding may be due to TNFα's stimulation of IL-1 production (Dunn *et al.*, 2005). Although not as robust as the findings regarding IL-1 and TNFα, IL-2 administration also exhibits anhedonic effects (Anisman *et al.*, 2005), which may be mediated by its inhibitory influence on dopamine (Anisman et al., 1996). Moreover, the "depressogenic" effects of IL-1 and IL-6 administration can be attenuated by antidepressant treatment (Merali et al., 2003).

As with endotoxin challenge, ethical constraints prohibit the administration of large doses of cytokines to healthy humans. However, the advent of cytokine-based immunotherapy to treat a host of diseases such as cancer, autoimmune disorders, and transplant rejection shed further light on the psychological sequelae of immune dysregulation. Cytokines (i.e., interferon [IFN], IL-2, TNFα, IL-6), or agents that directly impact cytokines, are utilized in the treatment of immune-related disorders due to their potent immunomodulatory functions. Cytokines act as the primary signaling source between the immune system and CNS (Dantzer *et al.*, 1998), and as stated above are known to alter neurotransmitter levels (Anisman and Merali, 1999). It may be these actions that are responsible for the neuropsychiatric complications often associated with this type of treatment regimen (Anisman *et al.*, 2005). Decreasing IFNγ levels with the use of cyclosporin A has been associated with elevated levels of anxiety and depression in transplant patients (Kahan et al., 1987). Similarly, treatment of chronic Hepatitis C virus with IFNα resulted in increased levels of IL-6, IL-8, and IL-10, and the elevated levels of IL-6 were further associated with heightened depression and anxiety two to four weeks after initiation of treatment (Bonaccorso *et al.*, 2001). Interferon-alpha, IFNα, has direct influences on the

serotonergic system (i.e., increasing 5-HT transporter mRNA [(Morikawa et al., 1998)], decreasing brain levels of 5-HT (Kamata et al., 2000), inducing 5-HT catabolism (Bonaccorso *et al.*, 2001)); and subsequent studies revealed that the elevated depression scores associated with IFNα therapy were also negatively correlated with serum 5-HT levels (Bonaccorso et al., 2002; Capuron and Miller, 2004). Although it may seem that the somatic effects of treatment and/or illness may be responsible for the affective manifestations, the two effects can be dissociated in that the somatic symptoms arise very early and wane while the mood-related changes generally appear later (Capuron et al., 2002) and are the only symptoms responsive to antidepressant treatment (Musselman *et al.*, 2001). Yet, this depressogenic effect of IFNα treatment can be seen as early as three days post-treatment, and appears to act synergistically with IL-2 to exaggerate these early effects (Capuron *et al.*, 2000). Indeed, only when in conjunction with IL-2 are the anxiogenic effects of IFN observed so early, although the majority of this effect can be attributed to heightened somatic symptoms observed as side effects of treatment itself (Capuron *et al.*, 2000).

Psychological Sequelae of Immune-Related Disease States

That depression, anxiety and other affective disorders are often comorbid with immune-related disease states (i.e., rheumatoid arthritis, multiple sclerosis, cancer) indicates the magnitude of impact immune dysregulation has on CNS states (Marques-Deak et al., 2005). For example, rheumatoid arthritis has long been associated with major depressive disorder, and this association remains even when psychosocial and pain-related factors are taken into account (Marques-Deak *et al.*, 2005). Multiple sclerosis also evidences increased prevalence of depression (Minden and Schiffer, 1990), and the disease state precedes affective abnormalities (Foley *et al.*, 1992). However, it is not only overactivation of the

immune system that is associated with depressive symptomatology. Disease states, such as Human Immunodeficiency Virus (HIV) and infection, characterized by loss of immune function can also be linked to depression (Kopnisky et al., 2004). This points to the likelihood that dysregulation of the immune system, rather than static elevation or inhibition, is the mediator of affective pathology.

These experimental models of the effects of immune challenge illuminate how diversely immune products impact CNS states.

Bi-directional Communications between the CNS and Immune Systems

Having reviewed the mechanisms by which the CNS afferently impacts immune activation, and by which the immune system communicates with the CNS about physiological states, it is important to assert that these pathways do not occur in a vacuum. At all times, the two systems interact with one another in a dynamic manner. These complex interactions between the central nervous and immune systems are posited to occur via either "long loop" or "local" interactions (Besedovsky and del Rey, 2007). The long-loop interactions refer to activation of the immune system eliciting production of immune products which can then influence the function of distant neuro-endocrine structures (Besedovsky and del Rey, 2007). Local interactions are as they would seem, with the co-existence and co-expression of immune and neuroendocrine factors in the stimulated tissue (Besedovsky and del Rey, 2007). Of course, there is no absolute distinction between the two pathways, as there will invariably be a great deal of overlapping functionality (see Figure 2). Although most studies focus on *either* the efferent effects of the CNS on immune system functioning *or* afferent affective/cognitive/behavioral consequences of immune activation, the present series of studies will examine both seeking to better elucidate the true bi-directionality that occurs in an interactive, responsive organism. Using influenza vaccination as a practical, ecologically relevant model of immune challenge, this study will investigate both how affective states and stress impact antibody response to benign immune activation and how degree of immune activation will in turn influence affective states. Furthermore, it will do so both cross-sectionally and longitudinally to more fully explicate the immediate and long-term relationships amongst emotion, immunity, and health.

Vaccination and Human Health

Examining the psychological sequelae of immune activation as well as the psychosocial factors influencing immune responsiveness to vaccination provides an excellent, non-invasive *in vivo* source of evidence for immune-CNS interactions. The advent of vaccination in 1796 by Edward Jenner revolutionized human health. Since that time, vaccinations have aided in saving countless lives from common ailments, such as influenza. Indeed, influenza vaccination has been particularly meaningful for populations at greatest risk of disease-related mortality, vis a vis the immunocompromised (e.g., very young, ill, and elderly).

There are immune changes accompanying not only pathological aging processes, but also normal aging processes, which render vaccination crucial in the maintenance of health in older individuals. An *in vitro* comparison of immune function in young, healthy older, and individuals with Alzheimer's (DAT), found that in young versus healthy older individuals age was associated with decreased basophils (which are known to store anti-inflammatory histamine and activate IgE) and reduced proliferation to phytohemagglutinin, PHA (Song *et al.*, 1999). However, DAT relative to healthy age-matched controls was associated with decreased large unsustained cells, increased monocytes and surprisingly increased lymphocyte proliferation to PHA (Song *et al.*, 1999). This illustrates distinctions in immune parameter changes between young and normal aging processes, as well as those associated with DAT. A four-year longitudinal study of the relationship between depression and immunity in a sample of elderly adults living in a retirement community revealed that age differences were seen for lymphocytes, CD4+DR+ T-cells, CD8+ T-cells, & CD8+DR+ T-cells (Fortes *et al.*, 2003). Independent of these differences, people with at least seven depressive symptoms had lower percentages of CD4+DR+ T-cells and CD8+DR+ T-cells,

indeed for every unit increase in depression there was a 3.72% and 4.11% decrease in these cell types, respectively (Fortes *et al.*, 2003). This impaired cellular immunity may lead to increased susceptibility to infectious agents, and hence increased mortality. Conversely, rumination has been found to be associated with elevated numbers of leukocytes, lymphocytes, and CD19+ B lymphocytes (Thomsen *et al.*, 2004). These associations were not found in a young sample, providing evidence that psychological states may have a greater impact on immunity in the elderly than in young populations (Thomsen *et al.*, 2004). These physiological alterations in immunity illuminate the importance of vaccination in a healthy elderly sample.

While influenza and pneumonia are the fifth-leading cause of death in the over-65 population, only 68% receive influenza inoculation (Thompson et al., 2004). The three most frequent reasons why individuals desist from vaccination were prior adverse reaction, belief in unlikelihood of contracting flu, and fear of side effects, the most important of which was the first, as it predicted a 15 times greater likelihood of repeated vaccination (Tabbarah *et al.*, 2005). Given the necessity of influenza vaccination due to altered immunity and the impact of psychological states on immune profiles, elucidating factors which may contribute to vaccine efficacy and willingness to receive vaccination is crucial.

Negative Affect and Response to Vaccination

The role of negative affect, NA, often characterized by depressed and/or anxious mood, has received much attention in relation to vaccine responsiveness, both symptomatic and immunological. Negative affect has been defined as the "general dimension of subjective distress and unpleasurable engagement that subsumes a variety of aversive mood states" (Watson et al., 1988), p. 1063). Testing the symptom-perception hypothesis, which asserts that individuals high in NA direct greater attention to somatic sensations and that they misattribute emotional activation to physical symptomatology, Diefenbach et al (1996) examined the role of negative affect in both cross-sectional and longitudinal symptom (local and systemic) reports to influenza vaccination in healthy older individuals. Cross-sectionally, individuals with high depression reported greater systemic symptoms, consistent with the hypothesis and the literature in general (Diefenbach et al., 1996). As expected, local symptom reports were not associated with NA, and did increase after active injection, reflecting accurate local symptom reports. Contrary to expectations, individuals reported a decrease in systemic symptoms two weeks after active inoculation, and furthermore this decrease was unrelated to NA (Diefenbach et al., 1996). Yet, there was a trend for individuals with high levels of both anxiety and depression to return to baseline levels of symptom reports 72 hours after inoculation, whereas all other groups continued to decline. Age of participants may have been a factor contributing to lack of associations, as elderly are more experienced with somatic activation and are thus likely to be more objective and accurate with somatic evaluation, termed the "commonsense framework" (Diefenbach et al., 1996). Also, the types of immune activation applied here do not induce robust systemic symptoms, which would be necessary for increased reporting. As an extension of their prior study, Leventhal and colleague (1996) sought to determine whether NA could act as a

predictor of somatic symptoms six months after assessment in two cohorts of healthy elderly participants. During the first wave, depressed affect predicted greater symptom reports six months after assessment, while anxious affect did not (Leventhal et al., 1996). The second wave showed even stronger effects. Also, state and trait were distinguishable in that state measures consistently showed significant associations, while trait NA did not. This supports the notion that state affect is a much better predictor of somatic complaint than is trait (Leventhal et al., 1996). Mora and colleagues (2002) extended this work to investigate how trait negative affect and self-assessed health relate to acute and chronic symptoms reports, as well as reported illness episodes and medical care-seeking in elderly white individuals. Furthermore, they sought to test the validity of the symptom perception hypothesis, which states that high NA predicts increased vigilance to the internal state manifests as increased symptom reporting, in relation to the commonsense framework, which suggests that these associations emerge from a reasonable self-awareness (Mora et al., 2002). While trait NA was positively and self-assessed health negatively associated with chronic symptom reports (primarily in women, which may be due to the somatic distress of osteoarthritis), the data did not support the symptom perception hypothesis (Mora *et al.*, 2002). The symptom reports accurately reflected individuals' somatic states, which lends credence to the commonsense framework, suggesting that elderly individuals' long experience with health changes makes them experts in their own somatic states. A placebo-controlled study of the impact of depression and anxiety on systemic symptom reporting subsequent to influenza inoculation showed that 59% of those with high anxiety reported systemic side effects (versus 34% controls) and 73% of those with high depression did the same (relative to 35% of controls), and both high anxiety and depression was associated with reduced quality of life (Allsup and Gosney, 2002). However, the prevalence of definite anxiety and depression were quite low in

this sample, 4% and 1.2% respectively, and thus caution must be used when assessing generalizability (Allsup and Gosney, 2002).

It is possible that the modest associations between negative affect and symptom reporting in healthy older adults is mediated by attenuated physiological response to pathogens. The current investigation seeks to clarify this by examining the antibody response to influenza vaccination in relation to negative affect and systemic symptom reporting. Trait and state affect may represent distinct pathways between emotion and health, with trait representing an efferent pathway and state both an efferent baseline reactivity to immediate circumstances and an afferent response (i.e., sickness behavior) to immune activation and stress. Although trait NA may act as a predisposing efferent factor for state reactivity, the stable nature of trait affect makes it likely that the organisms' physiological processes have adapted to potential deleterious effects thereby making trait NA a poor predictor of physiological variability. Also given trait NA's questionable relatedness to systemic symptom reports, we expect no association between trait NA and Ab response, bringing us to the first hypothesis.

H1: Trait negative affect (NA) will have no impact on Ab response to influenza inoculation.

Unlike Trait NA, State NA should more accurately reflect stress reactivity bound to a contextual event (i.e., inoculation), and as such should associate with alterations in Ab response to inoculation. The psychological stress of inoculation may enhance NA, particularly anxious affect at baseline, and the noradrenergic and glucocorticoid effects of this heightened stressful state may associate with decreased Ab response. The decreased Ab response in individuals with high NA may then result in an alleviation of NA at a follow-up time point, from which Hypothesis 2 is derived.

<u>H2:</u> State changes (from baseline to two weeks post-inoculation) in NA will associate with differential Ab response to influenza vaccination. High baseline State NA will associate with decreased Ab response. This attenuated Ab response will then lead to reduced NA at follow-up.

Furthermore, since the psychologically and physiologically stressful effects of inoculation will be most intense the first year of inoculation, it is expected that the relationships between state NA and Ab response will be less pronounced over the long-term:

<u>H2a:</u> The relationship between NA and Ab response will be most evident the first year of strain exposure, but will remain to a lesser degree over the long-term.

Glaser and colleagues have conducted extensive investigations into the relationships amongst NA, stress, and immunological vaccine responsiveness. A seminal study by this group examined the effects of chronic stress (e.g., caring for a spouse with dementia) on the immune response to influenza vaccination in the elderly (Kiecolt-Glaser et al., 1996). In spite of similar baseline Ab titers, caregivers showed less responsiveness to vaccination than did controls. This was especially evident in the older subgroup. Furthermore, caregivers also produced less IL-1β and IL-2 than controls (they differed neither in IL-6, nor in percentages of lymphocytes and monocytes). As expected, care-givers had more depressive symptoms than controls, though this did not interact with Ab or cytokine measures, suggesting dissociation between stress, depression, and immune dysregulation (Kiecolt-Glaser *et al.*, 1996). Glaser and group (1998) compared both humoral and cellular immune response to influenza vaccination in two cohorts of elderly individuals. Two consecutive flu seasons were used to assess immune response in current caregivers of spouses with Alzheimer's Disease,

former caregivers, and controls for the first season (Glaser et al., 1998). Prior vaccination was not controlled for, and more caregivers had received prior inoculations, yet they showed poorer baseline immune response than controls as well as impaired cellular and humoral response at two weeks (when baseline differences were accounted for). Current and former caregivers did not differ from one another. To control for variation in vaccine history, in the second cohort, only those who had received inoculation the prior year were included. This time, baseline Ab levels did not differ (Glaser et al., 1998). Similar to the prior study, caregivers significantly differed in responsiveness to controls, with only 32% of former and 38% of current caregivers mounting a four-fold increase from baseline compared to 59% of controls (Glaser et al., 1998). Only responders were followed the subsequent three and six months, which revealed that former relative to current caregivers showed a less robust T cell response. The data from each study reveals that caregiving stress (regardless of current or former status) impairs humoral and cellular immune response to influenza vaccination relative to age, sex, socioeconomic status, and health-matched controls (Glaser et al., 1998). Furthermore, it shows that the stress of care-giving has persistent immune consequences that do not cease upon alleviation of care-giving responsibility. The same group later examined whether mild depressive symptoms were associated with IL-6 response to flu vaccination in healthy elderly individuals (Glaser et al., 2003). They found a significant time X depressive symptoms interaction (which persisted even when health factors impacting IL-6 were accounted for), with elevated IL-6 at two weeks associated with higher depressive symptoms (Glaser *et al.*, 2003). Although those with prior vaccination history did not differ from those without, this may have been an artifact of different strain compositions across years. As it was not specified whether assays were conducted for each strain, this may have accounted for a lack of Ab effects. Indeed, Vedhara and colleagues (1999) found that

caregivers exposed to influenza were less likely to show an adequate Ab response to vaccination, and had less Ab production to the *Nanchang* strain, but not to other strains. This impaired Ab response was also inversely correlated to cortisol. Thus, it appears that HPA activation mediates the stress-immune suppression relationship in elderly caregivers of dementia patients in a strain-specific manner (Vedhara *et al.*, 1999). Miller and collaborators (2004) have found similar strain-specific effects in a young sample. High stress groups (compared to low and medium) showed a blunted Ab response only to the *New Caledonia* strain (Miller *et al.*, 2004). It is clear from these studies that exogenous (non-inoculation based) stress has a profound interactive effect with NA on immunological response to inoculation. Hence, the present study will examine whether presence of stressors will moderate the relationship between NA and Ab response. Stressors will be examined across two dimensions: psychogenic (i.e., stressful life events) and systemic (i.e., presence of a chronic disease state).

H3: Existence of stressors (psychogenic and/or systemic) may act as moderators in the relationship between State NA and Ab response.

This approach is particularly relevant to the population under study, as the likelihood of both stressful life events (i.e., death of a loved one) and chronic illness increases in an elderly sample. Additionally, presence of a disease state may drive down the immune response due to constant physiological demands reducing functional capacity. As such, it is a necessary piece of the PNI puzzle to identify whether these stressors influence the relationship between NA and immune response. As an extension of this hypothesis, and a further clarification of how individuals with NA somatize, the impact of NA and psychological and systemic stress will be investigated in relation to systemic symptom reports. Systemic symptoms will be restricted to those related to immune activation subsequent to inoculation

to control for possible confounds between symptoms associated with chronic disease states, though some overlap may inevitably occur.

H3a: Individuals with high NA and high stress will show enhanced systemic symptom reporting.

Although superficially contrary to the common sense framework, which asserts that individuals experienced with somatic activation accurately assess somatic states, we speculate that this heightened systemic symptom reporting will be unrelated to Ab response. Given the hypothesized associations between high NA, stress and chronic disease, and Ab response, it could be expected that those high in NA with comorbid chronic disease states and low Ab response would actually be more sensitive to somatic changes. The exaggerated NA would direct attention to the soma, with chronic illness experience informing somatic cues of physiological activation in spite of reduced Ab response. Also consistent with the common sense framework, individuals who display a robust Ab response will report higher levels of systemic symptoms, hence hypothesis 3b.

H3b: Systemic symptom reporting alone will be unrelated to Ab response.

Positive Affect, Health, and Immunity

While the detrimental effect of negative affect on health has been the prevailing concentration in investigating the relationship between emotion and health, a paradigm shift seems to be emerging that changes focus from pathology to prophylaxis. Seligman's pioneering approach to human mental health, broadly termed Positive Psychology, examines how an optimistic, happy emotional state contributes to human health. Positive Affect, PA, is not merely a diminished negative affectivity but instead represents a distinct construct (Watson, 1988a). Still in its infancy, this perspective has been largely neglected in PNI until quite recently. It is feasible that PA may promote better health via a number of potentially interacting pathways: more positive health practices (Watson, 1988b), enhanced regulation of emotional circuits influencing immunity (Stone et al., 1987), more adaptive stress coping style (Fredrickson, 2001). Higher PA has been shown to predict fewer negative health symptoms in a healthy collegiate sample, even when cigarette smoking, alcohol consumption, and NA were all controlled. Also, health worsened for those low in PA, but slightly improved for those with high PA (Pettit et al., 2001). Conclusions must be made with caution; however, as the likelihood of symptomatology in this group would be low, especially as no controlled immune challenge was given. Cohen and collaborators have begun to investigate the relationship between PA and immune challenge. Using rhinovirus challenge, they found that PA was inversely associated, in a dose-dependent manner, with rate of clinical cold, whether quantified as objective or subjective illness (Cohen et al., 2003b). Although PA was significantly associated with better health practices (i.e., sleep quality, diet, and exercise) and endocrine measures (i.e., decreased noradrenaline, adrenaline, and cortisol), none of these acted as mediators, suggesting PA has a direct influence on cold illness (Cohen et al., 2003b). Negative affect showed no relationships with PA, cold susceptibility or

verifiable illness, though individuals high in NA did show increased vague systemic symptom reports (Cohen et al., 2003b).

H4: Positive affect, PA, will be associated with enhanced Ab response to influenza vaccination.

The following hypotheses summarize the predictions of the current investigation:

H1: Trait negative affect, NA, will have no impact on Ab response to influenza inoculation.

H2: State changes in NA will associate with differential Ab response to influenza vaccination. High baseline State NA will associate with decreased Ab response. This attenuated Ab response will then lead to reduced NA at follow-up.

H2a: The relationship between State NA and Ab response will be most evident the first year of strain exposure, but will remain to a lesser degree over the long-term.

H3: Existence of stressors (psychogenic and/or systemic) may act as moderators in the relationship between NA and Ab response.

H3a: Individuals with high NA and high stress will show enhanced systemic symptom reporting.

H3b: Systemic symptom reporting alone will be unrelated to Ab response.

H4: Positive affect, PA, will be associated with enhanced Ab response to influenza vaccination.

The present study adds substantially to the existing literature by following immunological, health, affective, and experiential information in healthy, elderly individuals throughout the span of five years. Further, instead of focusing on a sole explanatory factor in the relationship between mind and health, it provides a multifactorial perspective on the dynamic interplay amongst affective, experiential, and physiological variables. This approach is more consistent with how an organism exists in the natural world, thus making it a more pragmatic model than standard reductionist approaches. The current series of studies also provides a new perspective on the relationship between affect and health in exploring the impact of positive affect on immunological response to influenza vaccination. It also has many potential intervention applications. For example, through enhancing certain affective characteristics while reducing others, and understanding the conditions under which the tendencies most robustly operate, vaccination outcomes can be enhanced. Moreover, knowing the populations which would benefit the most from such interventions can lead to enhanced targeting of strategies. To examine the dynamic interplay between affective tendencies (both negative and positive), stress, and physiological response to immune challenge enables a much broader and more ecologically relevant perspective on the complex relationship between emotion and human health.

METHODS

1. Study I: A longitudinal (five-year) investigation of the relationships amongst negative affect, chronic illness, stress, systemic symptom reporting, and antibody response to influenza vaccination in the elderly.

1.1 Materials and Methods

*1.1.1 Participants***.** The participants of this study were residents of a retirement community recruited from a larger-scale (N=851) nine-year longitudinal study of physical health and psychosocial well-being (Rutgers Aging and Health Study, RAH). Of the original participants, 152 participants (97 female, 55 male; age range: 59-90 years, M=72.49, SD=6.32) from two separate immunological cohorts (1991, N=41, 27%; 1992, N=111, 73%) met medical exclusionary criteria (refer to Appendix A) and completed all three annual assessments spanning the years 1992, 1993, and 1996. Annual assessments consisted of both in-person (for baseline inoculation and two-week post-inoculation sessions) and telephone (for summer assessment six months prior to inoculation) interviews reporting information about lifetime medical history, recent and current illness episodes, health care utilization, life events, social networks, and emotional status.

1.1.2 Psychometric Assessments. In the summer months preceding each year of the study, participants completed a comprehensive psychometric evaluation. Along with demographic, overall health status, psychosocial, and stress information, the evaluation consisted of an interview assessing the cognitive, mood, and somatic components of depression and anxiety.

Anxiety and Depression. Items from each of the Depression and Anxiety subscales of the Beck Cognition Checklist (Beck et al., 1987) measured the cognitive components of depression and anxiety, respectively. Participants were asked to respond on a Likert scale (1 to 5, with 1= never to 5= always) to items such as "When you're with a friend how often do

you think 'I don't deserve to be loved'?" and "How often do you think 'I'm going to have an accident'?" for depressed and anxious thoughts, respectively. The somatic aspects of depression and anxiety were assessed by the Centers for Epidemiological Studies, Depression subscale (CES-D, Radloff 1977) and Krantz anxiety inventory (Contrada, Hill, Krantz, Durel & Wright, 1986), respectively. For the CES-D, participants rated on a Likert scale ranging from 1 to 4 (1=none of the time or rarely, to 4= most or all of the time) how often in the past two weeks they had experienced a host of symptoms (i.e., restless sleep, poor appetite). Somatic anxiety was assessed with participants rating frequency (on a Likert scale of 1=never, to 5=always) with which they experienced somatic sensations associated with anxiety, such as increased heart beat or jitteriness. Questions were phrased as "When anxious or nervous, how often do you feel _____?" Mood items were also queried using a Likert scale (1=never, to 5=always) with mood adjectives inserted in the following sentence structure: "How _____ are you usually?" for trait assessment, and "How _____ were you in the past 24 hours?" for state assessment. For state inquiry, items were phrased using "the past 24 hours" to decrease the likelihood of biased responses.

Principle components factor analysis was used to extract one component representing depression and one representing anxiety. Upon extraction of the factor score, separation into tertiles yielded the following groups for depression and anxiety: high, moderate, and low.

Symptomatology. To examine whether systemic symptom reporting consistent with immunological activation mediated the relationship between NA and Ab response, a systemic symptom score was created. Only symptoms that could be attributed to vaccine-related immunological activation were utilized (e.g., headache, throat symptoms, muscle

aches, fatigue, general sickness, and fever). Participants rated not only presence of symptoms, but also degree of symptomatology on a Likert scale ranging from 0 to 6, indicating complete lack of symptoms to severe and chronic symptoms, respectively. Items were summed to give a systemic symptom score for each time point.

Stress. Number of stressful life events (including only those of maximal impact, i.e., death and martial discord/divorce) were summed as an objective measure of stress exposure to yield a psychological stress score. Also, since it was not only valuable to examine the role of systemic stress, but also of immunological significance to consider the impact of chronic illness, presence of chronic disease states was quantified and entered as an independent variable. A dichotomous variable specifying whether participants had ever (during the entire span of the study) experienced chronic illness or not was created.

1.1.3 Immunological Assays. Phlebotomists drew 27-ml venous blood samples from each participant immediately before inoculation on the day of inoculation, and again at two weeks post-inoculation to assess IgG response to inoculation. All inoculations were administered by a trained nurse. A portion of each blood sample was placed into a seven ml preservative-free vacuum tube. The whole blood samples were centrifuged, after clotting at ambient temperature, and the serum portion remove and aliqouted into to samples which were then frozen and stored at -70 degrees C. Upon study completion, one set of aliquots was shipped on dry ice to the University of Wisconsin, Geriatric Research Laboratory, Milwaukee Clinical Campus. Hemagglutinin Inhibition Assay (HAIA) was used to measure IgG antibodies to each of the influenza, FLU, strains (provided by Wyeth, Marietta, PA) (see Table 1 for list of viral components in the annual trivalent inoculation), and was performed according to Centers for Disease Control specifications.

Chicken red blood cells (cRBCs) were used in the assay. Sera were treated with 0.1 milliliter, ml, of 0.1% trypsin and incubated in a 56 degree C water bath for 30 minutes. After ambient cooling for 5-10 minutes, 0.5 ml KIO_4 was added and another 30 minute incubation occurred, followed by addition of 0.1 ml glycerol (in 4% NaCl) and overnight storage at 4 degrees C in 0.2 ml 0.01 M PBS. In a 96-well V-bottom microtiter plate, 25 microliter (µl) of PBS were added to all test wells and 50 µl of sample serum were added to the first wells. All antibody titers were run with serially diluted samples from 1:10 to 1:10,240, and calculated based upon dilution of the antigen. Twenty-five µl of FLU antigen (diluted to the concentration previously determined by antigen titration) were added to each test well. Gentle tapping mixed the plates, which were incubated at ambient temperature for 30 minutes. After 50 µl of 0.5% cRBCs were added to test and control wells, plates were again mixed and incubated for 30 minutes. Upon complete settling of all cRBCs to the bottom of wells, plates were tilted at a 45 degree angle and the wells read for agglutination/inhibition of agglutination or "streaming." Titers were reported as the reciprocal of the highest sample dilution showing complete agglutination of the antigen according to the following formula: $Z * Do/8 = Dt$ (Z=reciprocal of highest dilution showing complete agglutination; Do= original dilution of antigen to begin titration; Dt= dilution factor to give HAU per unit volume [25 µl]). To ensure accuracy, a cRBC control serum and a back titration were included on each plate. The laboratories performing immunological assays were blind to the participants' name, age, sex, and condition.

1.2 Procedure

Initial immunological participation occurred in two waves (1991 and 1992), using an AB-BA design in which participants were randomly assigned to either placebo-inoculation or

inoculation-placebo groups. Each of the three years 1992, 1993, and 1996, participants underwent a series of interviews followed by influenza vaccination and subsequent immunological assays (while inoculation did occur in 1994 and 1995, immunological assays were not conducted and hence these years were excluded). All participant visits were conducted between 0800 and 1200. Participants were interviewed in person at a centrally-located meeting room in the retirement community in the summer months preceding immunological assays to collect initial affective measures, overall health assessment, and demographic and psychological information. Six months later, at the end of October through early November, inoculations, immunological assays and follow-up interviews were conducted.

At the first winter appointment, written consent was obtained and participants were administered a baseline interview, blood pressure and temperature readings were taken, blood was drawn, and inoculation with either placebo (saline) or active inoculants (trivalent FLU, tetanus toxoid [TET], and keyhole limpet hemocyanin [KLH]) was delivered. The TET and FLU inoculations were delivered intramuscularly (one in the deltoid of each arm), and the KLH was delivered subcutaneously in the deltoid of the non-dominant arm (the placebo, 0.5 ml each of saline, condition likewise consisted of two intramuscular and one subcutaneous administration). (Although TET and KLH were also administered, only FLU response will be discussed herein.) Two weeks later participants returned for the same procedure, receiving the alternate inoculation at this time. Venous blood was drawn at the first visit and two weeks and four weeks (only for those participants who received placebo at the first visit) later to assess immunological response to inoculation. As there was no response to placebo, only inoculation immunological profiles will be discussed. The double-

blind, placebo-controlled study was approved by the Human Subjects Internal Review Board at Rutgers University and the University of Medicine and Dentistry of New Jersey.

2. Study II: A longitudinal (five-year) investigation of the relationship between positive affect and antibody response to influenza vaccination in the elderly.

2.1 Materials and Methods

*2.1.1 Participants***.** The same study participants detailed in Section 1.1.1 were used to assess the relationship between positive affect and Ab response to influenza vaccination.

2.1.2 Psychometric Assessments. Positive affect, PA, was quantified along two dimensions: optimism and mood. Optimism was measured via the Mehrabian Optimism-Pessimism scale (Mehrabian, 2000). Mood items were drawn from the same scale as discussed in Section 1.1.2, but only included items relating to pleasant (happy, cheerful, content, pleased) and energetic affect (enthusiastic). Both measures were administered in the summer months preceding inoculation. Scores for each year were summed yielding continuous variables, from which the two PA dimensions were categorized into three groups each (Low, Moderate, and High) from tertiles.

2.1.3 Hemagglutinin Inhibition Assay. Refer to Section 1.1.3 for details of the HAIA.

2.2 Procedure

The procedure was identical to that of Section 1.2.

RESULTS

Statistical Analyses and Data Cleaning. Antibody titers were log (base-10) transformed to better approximate normal distributions, and quantified as percentage change at two weeks from baseline. Furthermore, vaccine response is conventionally recognized as 4-fold antibody increase, and as such individuals whose influenza titers increased by this amount or more were considered to be "responders." While it was plausible that the 1991 and 1992 cohorts would show different baseline levels of Ab due to prior exposure, analyses did not reveal a group difference in baseline Ab titer ($p>0.15$). As such, all Ab results are reported pooled across cohorts. Within subjects regressions yielding slopes were performed to examine changes in affect and Ab production. Longitudinal hypotheses were tested using repeated-measures general linear models and multiple linear regression models, allowing for incorporation of the within-subjects repeated antibody response. For regression, standard controls, including sex, age, and baseline Ab levels, were entered as the first step, followed by appropriate psychological variables, and a third step was included when interactions were tested. Affect groups (High, Moderate, or Low) were the between-subjects independent variables, and the dependent measures of antibody response were repeated across time (the years 1992, 1993, and 1996). Given the prevalence of influenza strain-specific effects, separate regressions were carried out for each of the strains.

To further clarify the relationship between NA and Ab response, tests were also conducted to determine whether psychogenic and/or systemic stress, as well as systemic symptomatology, acted as moderators/mediators. Bivariate data were analyzed with χ^2. All data was analyzed using SPSS 15.0 for Windows, with α level set at 0.05 to indicate statistical significance. Greenhouse-Geisser corrections were applied when the data did not meet

assumptions of sphericity, and Wilks Lambda was used when data did not meet assumptions of equality of covariance.

1. Hypothesis I: Trait negative affect (NA), measured in the summer months preceding inoculation, will have no impact on Ab response to influenza vaccination.

As Trait affect represents a stable personality attribute, to which the organisms' physiology adapts over a lifetime, there are no expected alterations of Ab response (either cross-sectionally or longitudinally) according to Trait NA category. However, in as much as high Trait NA may represent an enhanced predisposition toward stress reactivity, the psychological and physiological stress of inoculation may result in short-term (only cross-sectional) associations with Ab response.

1.1 Associations between Negative Affect and Ab Response to Influenza Inoculation

Separate analyses were run to assess whether the two components of NA, depression and anxiety, were associated with Ab response to the individual strains. Both cross-sectional and longitudinal assessments were made.

1.1.1 Cross-sectional Associations between Trait NA and Ab Response

Preliminary correlations showed no association between Trait NA and Ab response the first or last years of the study, although Trait depressed and anxious NA were significantly positively correlated (see Tables 2 and 3). However, in 1993, Trait anxious NA did positively correlate with Ab change to *Panama,* though this correlation was modest (see Table 4). Across all years, Ab responses to each of the strains were significantly positively correlated with one another (see Tables 2, 3, and 4).

Univariate ANOVA was used to clarify the relationship between Trait NA and Ab response to *Panama* in 1993. No significant effects were observed of Trait anxious NA group on Ab response to *Panama*, either in multivariate, $F(2,144)=1.038$, $p=0.357$, or univariate tests, $F(2,144)=1.663$, $p=0.193$, indicating this was a spurious effect. As hypothesized, Trait NA was unrelated to cross-sectional examinations of Ab response.

1.1.2 Longitudinal Associations between Trait NA and Ab Response

To examine whether trait NA bears a relationship with long-term Ab response, first it was important to ensure that it represents a stable attribute. Repeated measures ANOVA confirmed stability across years for Trait depressed NA, $F(2,150)=0.059$, $p=0.942$, and Trait anxious NA, $F(2,150)=0.176$, $p=0.839$. Trait NA had no long-term effects on Ab response to *Beijing89, Beijing92, Taiwan* or, *Texas* strains ($p>0.05$). However, there were significant univariate effects for Trait anxiety and *Panama* response, $F(2,144)=3.157$, $p=0.046$. Individuals with Low Trait anxiety showed a consistent Ab response to *Panama* across all years, while those with High Trait Anxiety showed a steady decline, and Moderate Trait Anxiety showed a steady response the first two years with a subsequent decline in 1996 similar to those with High anxiety (see Figure 3).

Given this unexpected finding, further examinations of potential confounding extraneous factors accounting for this long-term relationship between Trait anxious affect and *Panama* response were sought. As we speculate that baseline State NA is likely to bear a relationship with Ab changes, correlations between Trait anxious affect, baseline state anxious affect, and *Panama* response were investigated. Although Trait anxiety and state anxiety were indeed significantly positively correlated ($p<0.001$), changes in State anxiety added as a covariate did not alter results of the repeated measures analysis, indicating this

factor was not attributable to the relationship between Trait anxiety and *Panama* response. Similarly, presence of chronic illness and psychological stress history did not act as covariates. Hence, it appears that Trait anxious affect does exert a long-term impact on Ab response profile, if only to the *Panama* strain.

1.2 Summary

As hypothesized, Trait NA showed no cross-sectional relationships with Ab response. Likewise, there were no associations between Trait NA and long-term Ab response to *Beijing89, Beijing92, Taiwan,* or *Texas*. However, a relationship between Trait NA and long-term Ab response to *Panama* did emerge. Moderate Trait anxiety showed enhanced Ab response to *Panama* the first year, followed by a steady decline subsequent years, while Low Trait anxiety showed a low, stable response across years (High levels of Trait anxiety did not vary significantly from the other two groups). Although potential confounding factors were investigated, the relationship remained, indicating that Trait anxiety exerts a long-term effect on response to *Panama.*

2. Hypothesis 2: State changes (from baseline to two weeks post-inoculation) in NA will associate with differential Ab response to influenza inoculation, with High baseline State NA associating with decreased Ab response. Decreased Ab response will then predict decreases in State NA at follow-up.

Unlike Trait NA, State NA should more accurately reflect stress reactivity bound to a contextual event (i.e., inoculation), and as such should associate with alterations in Ab response to inoculation. The psychological stress of inoculation may enhance NA, particularly anxious affect, at baseline and the noradrenergic and HPA effects of this

heightened stressful state will associate with decreased Ab response. The decreased Ab response in individuals with high NA will then result in an alleviation of NA at follow-up (the 2-week time point) in those with high baseline NA. Thus, we expect to see declines in State NA in individuals with poor Ab response.

2.1.1 Cross-sectional Associations between Changes in State NA and Ab response

Two separate models will be investigated herein. Model I seeks to explain degree of Ab response (slope from baseline to 2-weeks post-inoculation) dependent upon baseline NA. Model II seeks to identify how State NA varies according to magnitude of Ab response. Hence, two separate regression models will be conducted to examine these distinct efferent and afferent pathways, respectively, linking affect and immunity.

2.1.1a Model I: Impact of Baseline State NA on Ab Response

Multiple linear regression was used to investigate whether baseline State NA impacts Ab response at two weeks post-inoculation. Age and baseline Ab titer were entered as the first step, followed by baseline NA (depression and anxiety). Change in Ab response over two weeks (slope) was used as the independent measure. As stated above, separate regressions were implemented for each strain. In 1992, there was only a significant effect of baseline level of depression on Ab response to *Panama* (ΔR^2=0.029), F(4,147)=7.865, p<0.001. However, in 1993 depression had no effect but baseline anxiety showed significant associations with Ab response to *Beijing92* (ΔR^2=0.022; F(4,147)=15.962, p<0.001), *Taiwan* (ΔR^2=0.024; F(4,147)=12.878, p<0.001), and *Texas* (ΔR^2=0.024; F(4,147)=8.734, p<0.001). Baseline anxiety in conjunction with depression acted as a predictor for *Taiwan* (ΔR^2=0.030), F(4,147)=2.578, p<0.040, response during the last year of the study. Contrary to

expectations, *low* baseline depression and anxiety predicted poor Ab response. High baseline anxiety only equated with poorer Ab response to *Taiwan* in 1996, as hypothesized.

2.1.1b Model II: Impact of Ab Response on NA

In this model, we sought to understand the impact of immune activation on follow-up State NA, and as such multiple linear regression was employed. Age was again entered as the first step, excluding baseline Ab titer, and including instead baseline NA. The second step included both Ab titer at week 2 and Ab slope. The independent variable was change in NA (depression and anxiety, separately). As before, separate regressions were carried out for each strain. During the first year of the study, elevated Ab titer at two weeks predicted decreased anxiety for *Beijing92* (ΔR^2=0.042; F(4,147)=11.948, p<0.001)*, Taiwan* (ΔR^2=0.019; F(4,147)=8.988, p<0.001)*,* and *Texas* (ΔR^2=0.020; F(4,147)=10.587, p<0.001). Interestingly, the effects of *Taiwan* titer and change dissociated from one another, with decreases in response (slope) predicting decreases in anxiety. Enhanced *Taiwan* titer also predicted decreases in depression, and there was a trend for *Panama* titer to predict decreases in depression, as well. In 1993, there were no observable associations between Ab responsiveness and changes in NA, but associations again emerged in 1996. *Beijing89* change acted as a negative predictor of depression change (ΔR^2=0.039), F(4,147)=10.163, p<0.001, while *Beijing92* titer was a positive predictor of depression change (ΔR^2=0.030), F(4,147)=11.199, p<0.001.

Contrary to hypotheses, elevated Ab titer at two weeks post-inoculation predicted decreased state anxiety and depression, particularly the first year of the study. All associations disappeared the subsequent year, but in 1996 changes in Ab response then predicted alterations in state depression in a similar manner as in 1992. It is also important to

note that the two measures of Ab response (slope and titer value) had paradoxical associations with NA, illuminating the importance of considering how this dependent measure is quantified.

2.1.2 Longitudinal Associations between State NA and Ab Response

As the initial stressful nature of the inoculations may wane with repeated experience, it is thus important to examine how affective states and Ab response covary across time. No correlations were observed between change in baseline NA and Ab response slope across years. However, there were significant negative correlations between change in both measures of NA at 2 weeks and *Taiwan* Ab slope (see Table 5), indicating that declines in state NA associate with enhanced long-term Ab response to *Taiwan*. Repeated measures ANOVA was used to more fully examine this relationship. For this purpose, individuals were grouped into one of three categories (No change, Decrease, or Increase), depending on the direction of State NA slope they exhibited across years. Repeated measures ANOVA confirmed a lack of effects for changes in baseline state NA on long-term Ab responsiveness ($p>0.20$). Follow-up state NA also had no relationship with Ab response to *Beijing89* (Dep: $F(2,144)=1.966$, $p=0.144$; Anx: $F(2,144)=0.132$, $p=0.876$) or *Texas* (Dep: $F(2,144)=1.862$, $p=0.159$; Anx: $F(2,144)=0.391$, $p=0.677$) strains.

However, interesting multivariate and univariate effects, and interactions were discerned for *Beijing92, Panama,* and *Taiwan.* Significant multivariate year ($F(2,143)=29.296$, $p<0.001$), year*Dep ($F(4,286)=5.251$, $p<0.001$) and year*Dep*Anx ($F(6,286)=5.355$, $p<0.001$) effects were found for *Beijing92* Ab response. The Depression change categories showed distinct Ab response profiles across years (see Figure 4). Individuals displaying no change (N/C) in follow-up Depression showed a very robust Ab response to *Beijing92* in 1992

followed by diminished but stable response the following years. Those whose follow-up Depression increased (Inc Dep) over the study showed blunted Ab response the first year, but then a peak the following year, with a return to initial levels in 1996. Stable long-term Ab response was associated with decreasing Depression (Dec Dep). While there were no significant multivariate effects of follow-up Anxiety, $F(4,286)=0.400$, $p=0.808$, there were significant there were significant univariate effects for follow-up Anxiety category, $F(2,144)=3.351$, $p=0.038$. Individuals with decreasing levels of follow-up Anxiety (Dec Anx) had significantly greater Ab response to *Beijing92* than those with increasing follow-up Anxiety (Inc Anx) (see Figure 5). The picture becomes more complex when the interaction between year, Depression, and Anxiety is observed (see Figure 6). It is apparent that lack of State NA change is associated with long-term Ab response profiles distinct from other NA combinations, in that individuals with no change in one domain of NA combined with deceases in the other show the most robust Ab response the first year followed by a dramatic decline subsequent years, while all other affective permutations demonstrate a similar pattern of stability or slight increase after the first year of inoculation. This interactive effect was also observed for response to *Panama*, $F(6,286)=2.146$, $p=0.048$, though to a much lesser degree and only for the decreased Depression-N/C Anxiety group (see Figure 7). Follow-up Depression category had significant univariate effects on *Panama* response, $F(2,144)=3.410$, $p=0.036$, with decreased Depression showing a more robust Ab response compared to increased Depression (see Figure 8). As with *Panama* response, although the interaction between year, Depression, and Anxiety was the only significant multivariate effect for *Taiwan* response, $F(6,286)=2.404$, $p=0.028$, both follow-up Anxiety and Depression showed univariate effects ($F(2,144)=3.685$, $p=0.027$ and $F(2,144)=2.898$, $p=0.058$, respectively). While stable follow-up Anxiety was associated with enhanced Ab

response relative to increased Anxiety (see Figure 9), decreases in Depression showed enhanced Ab response compared to those showing increases (see Figure 10).

2.2 Summary

Cross-sectionally, two models were proposed to examine the relationship between state NA and Ab response to inoculation. Model I examined how baseline State NA would act efferently to predict Ab response two weeks post-immunization, while Model II investigated the afferent impact of Ab response on changes in state affect two weeks post-inoculation. Unexpectedly, high baseline levels of NA were associated with *enhanced* Ab response in a year- and strain-dependent fashion. The only finding which supported the hypothesis was that high baseline State anxiety equated with poorer Ab response to *Taiwan* in 1996. While significant associations were observed for Model I, most of the models accounted for less than 50% of the variability in the Ab response (the most robust model was observed for *Beijing92* response in 1993, accounting for 55% of the variability in Ab response), hence other factors are exerting an influence on Ab response either independently of or in conjunction with NA, which will be investigated in Hypothesis 3. Again with Model II the anticipated associations were, for the most part, not observed. While it was expected that lower Ab titer/less robust response (slope) would predict decreases in NA, the opposite relationship emerged. Strain- and year-specific effects again emerged, and furthermore the two means of quantifying Ab response dissociated from one another in their impact on changes in state NA. While slope exhibited the predicted direction of effects (positive associations), it was also a less consistent predictor than titer, which showed the unexpected associations with State NA. Also, since the most consistent effects were observed the first year, it is likely that the novelty of initial exposure produced the most robust relationships

between Ab response and state NA. As with Model I, the factors in Model II accounted for maximally 50% variability in NA change, and as such, this indicates that other factors influenced alterations in state NA during the time of immune activation. Hypothesis 3 will address some of these potential mediating factors.

Another aspect of Hypothesis 2 investigated how changes in state NA covary with Ab response over the course of the entire study, not just within each year. As such, longitudinal tests were conducted relating long-term state NA slope (decrease, increase, or no change across years) with Ab response in a repeated measures design. While changes in baseline state NA had no effect on Ab response to any of the strains, contrary to expectations, long-term changes in follow-up (two weeks post-immunization) NA were observed in relation with response to *Beijing92, Panama,* and *Taiwan*. A multitude of effects (multivariate, univariate, and interaction) were observed for Ab response to *Beijing92*. Decreasing levels of follow-up Anxiety associated with much greater Ab response to *Beijing92* than did those with increasing levels of Anxiety. Depression change category interacted with year, evidencing distinct immunological profiles. Very robust Ab response to *Beijing92* the first year of the study, followed by a dramatic decrease in subsequent years was associated with no change in follow-up Depression. Stable Ab response across years coincided with decreases in follow-up Depression; while increases in follow-up Depression were associated with low Ab response the first year, with a peak the following year and returning to low levels the last year of the study. Interactions between changes in Depression, Anxiety, and year were also observed, with no change in one NA dimension combining with decreases in the other associating with robust Ab response the first year followed by decreases subsequent years. This interaction was observed across *Beijing92, Panama,* and *Taiwan* strains. However, for *Panama* and *Taiwan* this effect was observed only for those with

decreases in Depression and no change in Anxiety, which appeared to be mainly driven by Depression category for the former and Anxiety category for the latter.

Contrary to the hypothesis that baseline alterations in NA would be the best predictor of Ab response, it was follow-up NA that showed the most consistent relationships with Ab response. Establishing directionality is challenging, as variations in Ab response may actually be the driving force for long-term changes state NA. In this case, the most robust Ab response associates with either no change or decreases in NA in conjunction with subsequent decreases in robustness of Ab response. This may be explained in that it would be expected that Ab response would be the most pronounced the first year of antigenic challenge, and as such relate to higher levels of NA, which would decline as immune responsiveness declines. The longitudinal results help reconcile some of the inconsistencies found in cross-sectional investigations, as there is a dynamic relationship between changes in state NA, Ab strain, and time that is masked when examining a single point in time.

<u>Hypothesis 3:</u> Existence of stressors (psychogenic and/or systemic) may act as moderators in the relationship between NA and Ab response.

The presence of a chronic disease state may inform how an individual reacts to a systemic stressor, particularly one of a known immunological origin. It is predicted that individuals with high NA and a chronic disease will be more reactive to stressors, and hence show the greatest downregulation of Ab response. Additionally, presence of the disease state itself may drive down the immune response due to constant physiological demands reducing functional capacity. Conversely, experience with chronic disease may protect an individual from the psychologically stressful nature of immunological activation due to extensive prior

experience with somatic symptomatology, thereby protecting against decreased Ab production.

3.1 Tests of Psychogenic and Systemic Stress as Moderators

To examine whether psychogenic and systemic stress operated as pathways between NA and Ab response, moderator tests were executed. As such, it was expected that psychogenic and systemic stress would correlate with NA and account for variations in Ab response. Furthermore, when the moderators are controlled for, associations between NA and Ab response should be substantially reduced (Baron and Kenny, 1986).

3.1.1 Psychogenic Stress as Moderator

No significant correlations existed between stressful life events and NA (see Tables 6 through 8), indicating psychogenic stress does not act a moderator for NA and Ab response.

3.1.2 Systemic Stress as Moderator

Positive correlations between existence of chronic illness in 1992 and both baseline and follow-up State anxiety, and follow-up depression were indeed found, indicating that systemic stress potentially acted as a moderator for NA and Ab response (see Table 9). However, this relationship did not persist in 1993 or 1996, with all significant correlations between chronic disease and NA disappearing (see Table 10 and Table 11, respectively). This provides evidence that any moderating effects of systemic stress on the relationship between NA and Ab response are transient, at best.

Since there were significant correlations between systemic stress and three measures of State NA (with a trend also in baseline State depression) in 1992, chronic illness was

included in regression models (Model I) to determine whether it acted as a moderator or even as an independent predictor of Ab response in 1992. Systemic stress acted as neither a moderator nor an independent factor in the relationship with Ab response, as it did not account for any further variability in the dependent measure nor did it change the degree to which baseline depression influenced Ab response to *Panama* in 1992.

<u>Hypothesis 3a:</u> Individuals with high NA and high stress will show enhanced systemic symptom (those symptoms associated with immune activation) reporting. This enhanced systemic symptom reporting will act as a covariate in the relationships between Ab response and changes in NA (Model II).

There were significant positive correlations between systemic stress and systemic symptom reporting, and systemic symptom reporting and three out of four measures of changes in State NA the first year of the study (see Table 12). The following year, significant correlations remained between systemic stress and the NA measures, however, the correlation with systemic symptom reporting ceased to exist (see Table 13). The correlation between systemic symptoms and systemic stress re-emerged in 1996, and systemic symptoms also positively correlated with stress history and 2-week NA (see Table 14). Across all years, stress history did not correlate with NA or systemic stress, making psychogenic stress an unlikely moderator.

Again, to examine whether systemic symptom reporting acted as a mediator, this measure was entered into regressions (Model II) to identify whether it reduced the association between Ab response and follow-up NA. Systemic symptom reporting did not substantially reduce the association between Ab response and follow-up NA in 1992, though

it did act as an independent factor to strengthen the model. Hence, although not a mediator, systemic symptoms acted as a facilitator in the relationship between Ab response and changes in NA. However, in the final year of the study, systemic symptom reporting did reduce the associations between *Beijing89* response and depression (from ΔR^2 of 0.039 to 0.021) and *Beijing92* response and depression (from ΔR^2 of 0.030 to 0.011). Thus, systemic symptom reporting likely mediated the associations between Ab response and change in depression in 1996.

Hypothesis 3b: Systemic symptom reporting alone will be unrelated to Ab response.

Although superficially contrary to the common sense framework, which asserts that individuals experienced with somatic activation accurately assess somatic states, we speculate that this heightened systemic symptom reporting will be unrelated to Ab response. Given the associations between high NA, stress, and Ab response it is expected that those high in NA with comorbid chronic disease states and low Ab response will actually be more sensitive to somatic changes. Also consistent with the common sense framework, individuals who display a robust Ab response will report higher levels of systemic symptoms.

As hypothesized, preliminary correlations revealed no significant associations between systemic symptom reporting and Ab response across all years (see Tables 15 through 17). To ensure that non-linear relationships between systemic symptom reports and Ab response were not being overlooked, individuals were categorized as being Non-reporters, Low reporters or High reporters and ANOVA was used to test for significant effects. There were no significant relationships between systemic symptom reporting frequency and Ab response in 1993 ($p>0.15$) and 1996 ($p>0.20$). In 1992, only *Beijing89*

response barely associated with systemic symptom report status, $F(2,148)=3.063$, $p=0.050$. Individuals who were Low symptom reporters showed enhanced Ab response relative to Non-reporters and High reporters (see Figure 11). Overall, as hypothesized, systemic symptom reporting was unrelated to actual Ab response.

3.2 Summary

Hypothesis 3 sought to address the unexplained variance in Ab response and State NA changes by examining a number of potential moderating and mediating factors in the relationship between State NA and Ab response. Neither stress domain – psychogenic or systemic – acted as a moderator in the relationship between State NA and Ab response (Model I). Systemic symptom reporting, however, acted as both an independent factor and a mediator (Model II), depending upon the temporal reference. Systemic symptom reporting strengthened the relationship between Ab response and changes in State NA in 1992, and acted as a mediator between Ab response to both *Beijing* strains and depression in 1996. Yet further investigations showed that systemic symptom reporting was unrelated to actual immune activation. Thus, it appears that while baseline State NA acted as a good predictor of Ab response independent of the potential exogenous explanatory factors postulated, the afferent relationship between immune activation and alterations appears sensitive to somatization.

<u>Hypothesis 4:</u> Positive affect, PA, will be associated with enhanced Ab response to influenza inoculation.

Although supporting evidence is scarce, some work indicates that PA associates with more robust immunity. Hence, it is hypothesized that high PA will predict greater Ab response to inoculation.

4.1 Associations between Positive Affect and Ab Response to Influenza Inoculation

Separate analyses were run to assess whether the two components of PA, optimism and mood, were associated with Ab response to the individual strains. As with Trait NA, both cross-sectional and longitudinal assessments were made.

4.1.1 Cross-sectional Associations between Trait PA and Ab Response

Positive mood and optimism showed significant positive associations with one another throughout the study ($p<0.001$). While PA showed no associations with Ab response to any strain in the first and last years of the study (see Tables 18 and 19, respectively), 1993 Ab response did show associations with Positive mood. Positive mood was significantly positively associated with Ab slope to the *Beijing89* and *Beijing92* strains, and showed a trend toward significance to *Taiwan* (see Table 20). Thus, as Positive mood increases, so does Ab responsiveness to these strains in 1993. Again, although positive mood and optimism were highly correlated, optimism showed no such association with Ab response, indicating a dissociation of the effect of the two measures of PA on Ab response.

Univariate ANOVA was used to further clarify the relationship between PA, particularly Positive mood and Ab response in 1993, and to identify whether any interactive effects between the two PA measures would be unmasked. When Positive mood was

categorized into groups, associations did not remain for four of the strains in 1993 (*Beijing89*: $F(2,143)=1.794$, $p=0.170$; *Beijing92:* $F(2,143)=1.380$, $p=0.255$; *Taiwan:* $F(2,143)=0.810$, $p=0.447$). Positive mood did show a significant association with Ab response to *Texas*, $F(2,143)=3.470$, $p=0.034$, with Moderate Positive mood showing the greatest Ab response. A significant interaction effect between Optimism and Positive mood on *Taiwan* response in 1992 was also uncovered, $F(4,143)=2.701$, $p=0.033$. It appears that having a High level of either Optimism or Positive mood results in an enhanced Ab response to *Taiwan* in 1992, and High levels of both aspects of PA shows one of the most robust responses with the least variability (see Figure 12). Also, Positive mood shows a trend toward associating with *Taiwan* responsiveness in 1996, $F(2,143)=2.434$, $p=0.091$, with Low Positive mood showing reduced Ab response (see Figure 13). Together these results, while not overwhelmingly compelling, appear to reveal that Positive Affect may have an enhancing effect on Ab response, again, in a strain-dependent manner.

4.1.2 Longitudinal Associations between PA and Ab Response

Level of Optimism remained stable across years, $F(2,150)=0.426$, $p=0.631$, and repeated measures ANOVA revealed no long-term associations between Optimism and Ab response to any of the strains ($p>0.50$). Positive mood was not a stable attribute, however, as it gradually but significantly declined over the course of the study, $F(2,150)=17.429$, $p<0.001$ (see Figure 14). As such, Positive mood was across years converted to a slope, and generalized linear regression was used to examine the relationship between Positive mood and Ab response over the span of the study. Unfortunately, Positive mood showed no long-term relationship with Ab response to any of the strains ($p>0.25$). To ensure that an interaction between the two measures was not accounting for this lack of effect, when

Optimism was added to the model, results were not altered. Overall, while Positive mood may have some weak immediate associations with Ab response to some strains, neither measure of PA appears to have long-term immunological consequences.

4.2 Summary

It was posited that heightened PA would have an enhancing effect on Ab response. As hypothesized, increases in positive mood (though not optimism) tended to associate with enhanced Ab response in a strain-specific manner in 1993. No associations were observed in 1992 or 1996. Positive affect showed no long-term impact on Ab response in longitudinal investigations. Hence, although there appears to be a transient enhancing effect of positive mood on immunity, the long-term effects of PA are not compelling. As PA was a Trait construct (due to lack of PA assessments during immunization), effects may have been more evident by measuring PA as a state construct (as with NA).

DISCUSSION

This series of investigations sought to add to the existing literature by delineating the dynamic cross-sectional and long-term interactions between affective states, stress exposure, and immunity subsequent to immunological challenge in a healthy, elderly sample. In attempting to create a more holistic perspective of this dynamic situation, we examined both efferent pathways from the CNS to the immune system, as well as afferent pathways from immune activation to affective state. Also, we examined whether Trait versus State affective qualities would differentially equate with immunity.

Trait NA and Immunity

The first hypothesis speculated that Trait negative affect (depression and anxiety), as a stable personality attribute, would be unrelated to either cross-sectional or longitudinal Ab response to influenza inoculation. This hypothesis was largely supported, in that no cross-sectional associations were observed, and only one influenza strain associated longitudinally with Trait NA. Given the unexpectedness of the finding that degree of Trait anxiety was associated with varying patterns of long-term Ab response to the *Panama* strain, we sought to identify potential extraneous variables. Even after controlling for the effects of stress (both psychogenic and systemic) and State anxiety, the relationship between Trait anxiety and *Panama* responsiveness remained. While this does not preclude that existence of another, unaccounted for, variable is mediating this relationship, it does appear to indicate that Trait anxiety (but not depression) may have antigen-dependent influences on Ab response to inoculation. Overall, that Trait NA does not act as a good predictor of Ab responsiveness is consistent with prior research demonstrating that Trait, relative to State, NA is a poor

predictor of somatic complaint consequent to benign immune activation (Leventhal et al., 1996).

Dynamic interplay between State NA and Immunity

Unlike Trait NA, it was posited that State NA, as a more direct index of stress reactivity, would have a dynamic relationship with immune activation to influenza inoculation. Specifically, we formulated that baseline State NA would act in an efferent manner to influence Ab response, in that high levels of baseline State NA would reflect a heightened stress response to inoculation, and this would in turn lead to reductions in Ab response (possibly via an HPA-driven mechanism). The attenuated Ab response associated with high baseline NA would afferently elicit fewer overt symptoms and physiological signals of immune activation, hence alleviating follow-up NA in those with high levels of baseline NA. As such, low Ab response would be positively associated with decreases in NA from baseline to follow-up, whereas robust Ab response would be associated with elevations in State NA. Contrary to expectations, high baseline depression and anxiety, at times independently and at other times in concert with one another, predicted *enhanced* Ab response in a year- and strain-specific manner. Only poorer Ab response to *Taiwan* in 1996 consequent to high baseline anxiety supported the hypothesis. These unexpected findings suggest that reconceptualizations of the "value judgments" of initial NA are in order. High levels of initial NA did not predict impaired immunity, but instead robust response. It is possible that although baseline State NA was high, it may have diminished rapidly after the stress of inoculation, hence reflecting not a pathological affective response but a rational reaction to the situation, thus having no adverse impact on immunity.

While it was expected that lower follow-up Ab titer/less robust response (slope) would predict a reduction in NA, the opposite relationship generally emerged. Contrary to hypotheses, elevated Ab titer at two weeks post-inoculation predicted decreased state anxiety and depression, particularly the first year of the study. As before, and consistent with the commonsense framework, initial affective reactivity may have been high, but once the immediate stressor had passed individuals soon returned to a lower state of NA, hence the relationship observed. All associations disappeared the subsequent year, but in 1996 changes in Ab response then predicted alterations in State depression in a similar manner as in 1992. Strain- and year-specific effects again emerged, and furthermore the two means of quantifying Ab response dissociated from one another in their impact on changes in state NA. While slope exhibited the predicted direction of effects (positive associations) for follow-up State anxiety subsequent to *Beijing92* Ab response, it was also a less consistent predictor than titer, which showed the unexpected negative associations with follow-up State NA. The slope measure is more theoretically sound, in that the baseline Ab titer is taken into account in the dependent measure itself (though accounting for baseline Ab titer did not alter results). And since the two-week follow-up time point is sub-optimal in an elderly sample, who generally show a four-week delay of peak Ab response (Levine et al., 1987), the slope findings may have been stronger and more consistent if the four-week time point was assayed. Since the two measures of Ab response (slope and titer value) had paradoxical associations with NA (in spite of baseline titer being accounted for), this illuminates the importance of considering how this dependent measure is quantified when making cross-study comparisons. Also, as the most consistent effects were observed the first year, it is likely that the novelty of initial exposure produced the most robust relationships between Ab response and follow-up State NA. In both models of the relationship between State NA and

Ab response, the posited factors accounted for maximally 50% variability in the dependent measure (either Ab response or State NA change), and as such, this indicates that other factors influenced both variability of Ab response and alterations in state NA during the time of immune activation.

Stress and Systemic Symptom Reporting as Potential Moderators/Mediators

Given the degree of unexplained variance in the dependent measures of Ab response and State NA change subsequent to immune activation, we sought to identify possible moderators and/or mediators. Since stress is know to influence both affect (Leventhal et al., 1998) and immune function (Kiecolt-Glaser et al., 1996), it was posited that stress would likely act as a moderator. Stress was conceptualized under two domains: psychogenic (stressful life events) and systemic (existence of a chronic, non-immunological illness). This strategy was employed in order to tap both the emotional and physiological aspects of stress, and as such to examine its potential efferent and afferent consequences. Systemic symptom reporting was also included as a potential mediator in the afferent relationship between Ab response and follow-up State NA, as it was likely that the degree to which participants experience inoculation-based symptoms would influence their subjective NA state. Surprisingly, neither stress domain, psychological nor systemic, acted as a moderator in the efferent relationship between baseline State NA and Ab response. Systemic symptom reporting, however, acted as both an independent factor and a mediator in the relationship between Ab response and follow-up State NA, depending upon the temporal reference. Systemic symptom reporting strengthened the relationship between Ab response and changes in State NA in 1992, and acted as a mediator between Ab response to both *Beijing* strains and depression in 1996. Yet further investigations showed that systemic symptom

reporting was unrelated to actual immune activation. Thus, it appears that while baseline State NA acted as a sound predictor of Ab response independent of the potential exogenous explanatory factors postulated, the afferent relationship between immune activation and emotional response appears sensitive to somatization.

Long-Term Associations between State NA and Ab Response

Longitudinal analyses revealed a complex interactive picture of the relationship between follow-up State NA tendency and Ab response profile, and provided a broader context in which to place the cross-sectional results. There is a dynamic relationship, depending on State NA reactivity, strain, and time that is masked in cross-sectional investigations, indicating the danger of making sweeping generalities from a single sampling point. The most dramatic findings were related to *Beijing92, Taiwan,* and *Panama* strain responses and the interaction between both dimensions of follow-up State NA. The Ab response profile of robust initial response followed by steady decreases over the years was associated with combinations of no change in one dimension of NA and decreases in the other. Initially high levels of follow-up NA also contributed to the subsequent declines over the years. This pattern was most consistent in those with no change in anxiety and decreases in depression. Interestingly, none of the individuals who showed "no change" in follow-up State NA over the years exhibited low levels of either depression or anxiety, hence they existed in a static condition of moderate or high levels of NA, and inflexible affective styles have been associated with poorer health outcomes (Rozanski and Kubzansky, 2005). Given how large the Ab response was that first year, it likely represents not an efficient response but a potentially maladaptively excessive one.

Positive Affect and Immunity

Though supported by far fewer studies, it was posited that higher levels of PA would have an enhancing effect on Ab response. As hypothesized, increases in positive mood (though not optimism) tended to cross-sectionally associate with enhanced Ab response in a strain-specific manner during the second year of the study. Positive affect showed no long-term impact on Ab response in longitudinal investigations. Hence, although there appears to be a transient enhancing effect of positive mood on immunity, the long-term effects of PA are not compelling. As PA was a Trait construct, effects may have been more evident by measuring PA as a State construct (as was observed with NA, and which has been found in prior studies, Cohen et al 2003b).

The Strain Effect

Far more difficult to explain and justify with supporting literature are the profoundly different findings dependent upon influenza strain. Though other investigators have also found similar strain-specific effects (Vedhara et al., 1999; Miller et al., 2004; Pressman et al., 2005), the precise explanation for them has been elusive. It is known that influenza A viruses have greater genetic variability and are associated with greater mortality (Mak *et al.*, 2006). Hence, the differences in virus type may account for some of the variability in our findings. As such, certain individuals may be genetically predisposed toward inoculation responsiveness, depending on strain mutations. Furthermore, certain strains are phylogentically "older" and hence individuals, particularly elderly individuals with a long history of possible exposure, have increased likelihood of prior encounters with these strains. Lastly, some strains may bear closer antigenic resemblance to one another than others (for example, *Beijing* and *Harbin* are antigenically indistinguishable (Li et al., 2008)), thus resulting

in equivalent Ab response. Importantly, in this study design antibodies for each strain were assayed every year even if they were not an inoculation component at that time. Hence, *Beijing89* and *Taiwan* Ab responses subsequent to 1991 reflected a memory response, non-inoculation exposure, and/or epitope similarity to another strain. *Texas* was the only strain consistently present in the inoculant. While the experimental ideal would have been to deliver novel antigen at each annual inoculation time point, even if this were possible antigenic overlap and inability to accurately assess prior exposure history would still render interpretation questionable.

Limitations

There are certain considerations that must be made when interpreting the findings in this study. The nature of the population from which we drew our sample posed some challenges. Due to recidivism and the morbidity expectant in an elderly population, our sample size was restricted. However, there was only a participant loss of approximately 3% each year, which is a low rate considering the extensive nature of the study. The nature of the study sample also posed drawbacks and strengths. For the most part, this sample represented a fairly homogenous group in terms of socioeconomic status, education, age, and ethnicity. While this may limit the generalizability of findings to the elderly population at large, it also controls for the potential extraneous effects of these variables. Also, although it was not ideal to include participants with comorbid chronic disease states, excluding would have prevented obtaining the sample size necessary to detect effects. Moreover, examining how the presence of an additional (non-immunological) systemic stressor interacted with both the systemic challenge of inoculation and NA to modulate immune activity was an important element of this scientific query. Surprisingly, neither dimension of stress (systemic

or psychogenic) acted as a moderator in the relationships amongst NA and immune activity. It is possible, and indeed likely, that other psychosocial factor (i.e., quality of life, activity level, social support networks, and/or loneliness) may have acted as moderators in these relationships. Though beyond the scope of the present endeavor, future studies should incorporate these factors.

Methodologically, while the HAIA is not as sensitive as other measures (i.e., ELISA), it provided the enhanced specificity necessary when considering epitope similarity and strain-specific effects, which were extensively found in the present study. More importantly than technique, the follow-up time point for examining Ab response was sub-optimal for this sample, as older individuals often show delay of peak response until four weeks post-inoculation (Levine et al., 1987). Stronger and additional findings may have been elicited had the four-week follow-up time point been used. Furthermore, subjective characterization of an "appropriate" antibody response is highly debatable. While the four-fold increase in titer from baseline is the accepted minimum to be considered "responsive," the opposite end of what constitutes a deleteriously high level of antibody response is subject to great debate. As elderly individuals generally show diminished immunity, one may extrapolate that any amount of increase over the median would be advantageous. However, it is unclear as to whether there exists an antibody response that is excessively high. This may indeed be the case in younger population samples, as the potential for cross-reactivity between strains and exaggerated antibody response has the potential to result in autoimmune consequences; however, this likelihood is negligible in elderly samples. Also, unfortunately cortisol was not measured. Measures of cortisol would have been a meaningful index of neuroendocrine activity and may have been a more objective measure of stress reactivity than self-reports, especially as it has been shown that changes in glucocorticoids can inhibit immunoglobulin

production (Besedovsky and del Rey, 2007). Thus, while it cannot be ruled out that the pathway between State NA and altered Ab response is mediated by cortisol, Miller and colleagues (2004) found that cortisol did not act as a mediator between stress and Ab response in a young sample.

Conclusions

Now we arrive at the crucial question: What does this all practically mean? Essentially, the practical implications for these diverse findings suggest that certain healthy elderly individuals, with certain affective and immunological tendencies exhibit certain emotional and physiological responses to certain stimuli at certain points in time. While this may be an unsatisfyingly vague statement from a reductionist perspective, it accurately reflects not only the findings of the present study, but also what occurs in the natural environment. No two individuals exposed to the same stimulus will react, either emotionally or physiologically, in an identical manner. While the presence of pathogen is necessary to induce immune activation, it is not necessarily sufficient to produce physical manifestations of illness, and factors such as genetic susceptibility, environmental circumstances, threat perception and coping mechanisms are just a few factors that combine to determine disruption/maintenance of homeostasis subsequent to pathogen exposure (Ader, 1980). Though patterns may emerge (such as the tendency for elevated Ab response to predict decreases in follow-up State NA), there are a constellation of factors at play, some to a greater and some to a lesser degree, which dictate individual differences. It is precisely this individual variability (in exposure, experience, affective tendency, coping abilities, support structures) which forms the essence of psychosomatic research endeavors. As Robert Ader so eloquently stated in his 1980 Presidential address to the American Psychosomatic Society,

"Despite the most sophisticated strategies designed to achieve uniformity, variability remains one of the most ubiquitous results of all natural and contrived biological experiments" (p. 307). For the purposes of interpretation and intervention strategy implementation, it would have been ideal to find consistent, systematic relationships amongst NA, PA and Ab response to influenza inoculation supporting the hypotheses. However, what this study illustrates quite elegantly is the complexities involved in investigating the relationship between health and immunity, and importantly sheds light on how varying temporal, antigenic, and quantification strategies may elicit substantially differing findings. As such, this study adds considerably to the body of work exploring the dynamic interplay between emotion, experience, aging, and physiological response to benign immune challenge.

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TABLE CAPTIONS

Table 1. Annual influenza vaccination viral components.

Table 2. Correlations between Trait NA and Ab response slope in 1992. Significant correlations highlighted in yellow.

Table 3. Correlations between Trait NA and Ab response slope in 1993. Significant correlations highlighted in yellow.

Table 4. Correlations between Trait NA and Ab response slope in 1996. Significant correlations highlighted in yellow.

Table 5. Correlations between long-term changes in follow-up State NA and Ab response. Significant correlations highlighted in yellow.

Table 6. Correlations between State NA and psychological stress (life events) in 1992. Significant correlations highlighted in yellow.

Table 7. Correlations between State NA and psychological stress (lie events) in 1993. Significant correlations highlighted in yellow.

Table 8. Correlations between State NA and psychological stress (life events) in 1996. Significant correlations highlighted in yellow.

Table 9. Correlations between State NA and systemic stress (chronic illness) in 1992. Significant correlations highlighted in yellow.

Table 10. Correlations between State NA and systemic stress (chronic illness) in 1993. Significant correlations highlighted in yellow.

Table 11. Correlations between State NA and systemic stress (chronic illness) in 1996. Significant correlations highlighted in yellow.

Table 12. Correlations between State NA (both score at follow-up and slope of change from baseline), stress, and systemic symptom reports in 1992. Significant correlations highlighted in yellow. Trends toward significance highlighted in blue.

Table 13. Correlations between State NA (both score at follow-up and slope of change from baseline), stress, and systemic symptom reports in 1993. Significant correlations highlighted in yellow. Trends toward significance highlighted in blue.

Table 14. Correlations between State NA (both score at follow-up and slope of change from baseline), stress, and systemic symptom reports in 1996. Significant correlations highlighted in yellow. Trends toward significance highlighted in blue.

Table 15. Correlations between systemic symptoms and Ab response slope in 1992. Significant correlations highlighted in yellow. Trends toward significance highlighted in blue.

Table 16. No correlations existed between systemic symptoms and Ab response slope in 1993.

Table 17. No correlations existed between systemic symptoms and Ab response slope in 1996.

Table 18. Correlations between PA and Ab response in 1992. Significant correlations highlighted in yellow.

Table 19. Correlations between PA and Ab response in 1996. Significant correlations highlighted in yellow.

Table 20. Correlations between PA and Ab response in 1993. Significant correlations highlighted in yellow. Trends toward significance highlighted in blue.

TABLES

Table 1. Annual influenza vaccination viral components.

1991	1992	1993	1994	1995	1996
B/Beijing 89	B/Beijing 92	B/Beijing 92	B/Shandong	B/Harbin	B/Harbin
A/Panama (H1N1)	A/Panama (H1N1)	A/Panama (H1N1)	A/Panama (H1N1)	A/Johannesburg (H1N1)	A/Nanchang 95 (H3N2)
A/Taiwan (H1N1)	A/Texas (H3N2)	A/Texas (H3N2)	A/Texas (H3N2)	A/Texas (H3N2)	A/Texas (H3N2)

Table 2. Correlations between Trait NA and Ab response slope in 1992.

Correlations

		92 Trait dep	92 Trait anx	Be89_92_ change_slope	Be92_92_ change_slope	Pan92_change_ slope	Tai92_change_ slope
92 Trait dep	Pearson Correlation	1	.560	-.012	.061	.082	.026
	Sig. (2-tailed)		.000	.882	.455	.313	.751
	N	152	152	152	152	152	152
92 Trait anx	Pearson Correlation	.560	1	-.016	.141	.104	.002
	Sig. (2-tailed)	.000		.848	.083	.204	.985
	N	152	152	152	152	152	152
Be89_92_change_slope	Pearson Correlation	-.012	-.016	1	.226	.224	.266
	Sig. (2-tailed)	.882	.848		.005	.005	.001
	N	152	152	152	152	152	152
Be92_92_change_slope	Pearson Correlation	.061	.141	.226	1	.460	.397
	Sig. (2-tailed)	.455	.083	.005		.000	.000
	N	152	152	152	152	152	152
Pan92_change_slope	Pearson Correlation	.082	.104	.224	.460	1	.487
	Sig. (2-tailed)	.313	.204	.005	.000		.000
	N	152	152	152	152	152	152
Tai92_change_slope	Pearson Correlation	.026	.002	.266	.397	.487	1
	Sig. (2-tailed)	.751	.985	.001	.000	.000	
	N	152	152	152	152	152	152
Tex92_change_slope	Pearson Correlation	.055	.026	.343	.483	.381	.366
	Sig. (2-tailed)	.497	.751	.000	.000	.000	.000
	N	152	152	152	152	152	152

Table 3. Correlations between Trait NA and Ab response slope in 1993.

Correlations

		96 Trait dep	96 Trait anx	Be89_96_ change_slope	Be92_96_ change_slope	Pan96_change_ slope	Tai96_change_ slope
96 Trait dep	Pearson Correlation	1	.730	.021	-.106	-.053	-.063
	Sig. (2-tailed)		.000	.802	.192	.513	.441
	N	152	152	152	152	152	152
96 Trait anx	Pearson Correlation	.730	1	.032	-.113	-.033	-.075
	Sig. (2-tailed)	.000		.699	.165	.689	.361
	N	152	152	152	152	152	152
Be89_96_change_slope	Pearson Correlation	.021	.032	1	.394	.164	.258
	Sig. (2-tailed)	.802	.699		.000	.044	.001
	N	152	152	152	152	152	152
Be92_96_change_slope	Pearson Correlation	-.106	-.113	.394	1	.068	.072
	Sig. (2-tailed)	.192	.165	.000		.403	.381
	N	152	152	152	152	152	152
Pan96_change_slope	Pearson Correlation	-.053	-.033	.164	.068	1	.385
	Sig. (2-tailed)	.513	.689	.044	.403		.000
	N	152	152	152	152	152	152
Tai96_change_slope	Pearson Correlation	-.063	-.075	.258	.072	.385	1
	Sig. (2-tailed)	.441	.361	.001	.381	.000	
	N	152	152	152	152	152	152
Tex96_change_slope	Pearson Correlation	.007	-.031	.309	.273	.163	.253
	Sig. (2-tailed)	.934	.703	.000	.001	.044	.002
	N	152	152	152	152	152	152

Table 4. Correlations between Trait NA and Ab response slope in 1996.

Correlations

		93 Trait dep	93 Trait anx	Be89_93_ change_slope	Be92_93_ change_slope	Pan93_change_ slope	Tai93_change_ slope
93 Trait dep	Pearson Correlation	1	.596	-.046	-.066	.091	.022
	Sig. (2-tailed)		.000	.573	.419	.266	.792
	N	152	152	152	152	152	152
93 Trait anx	Pearson Correlation	.596	1	.007	.041	.169	.087
	Sig. (2-tailed)	.000		.928	.615	.037	.289
	N	152	152	152	152	152	152
Be89_93_change_slope	Pearson Correlation	-.046	.007	1	.593	.187	.436
	Sig. (2-tailed)	.573	.928		.000	.021	.000
	N	152	152	152	152	152	152
Be92_93_change_slope	Pearson Correlation	-.066	.041	.593	1	.305	.511
	Sig. (2-tailed)	.419	.615	.000		.000	.000
	N	152	152	152	152	152	152
Pan93_change_slope	Pearson Correlation	.091	.169	.187	.305	1	.310
	Sig. (2-tailed)	.266	.037	.021	.000		.000
	N	152	152	152	152	152	152
Tai93_change_slope	Pearson Correlation	.022	.087	.436	.511	.310	1
	Sig. (2-tailed)	.792	.289	.000	.000	.000	
	N	152	152	152	152	152	152
Tex93_change_slope	Pearson Correlation	.013	.155	.332	.534	.447	.269
	Sig. (2-tailed)	.870	.057	.000	.000	.000	.001
	N	152	152	152	152	152	152

Table 5. Correlations between long-term changes in State NA and Ab response.

Correlations

		Dep2wk_ slope	Anx2wk_ slope
Dep2wk_slope	Pearson Correlation	1	.663
	Sig. (2-tailed)		.000
	N	152	152
Anx2wk_slope	Pearson Correlation	.663	1
	Sig. (2-tailed)	.000	
	N	152	152
be89_slope	Pearson Correlation	-.117	-.038
	Sig. (2-tailed)	.152	.639
	N	152	152
be92_slope	Pearson Correlation	-.049	-.086
	Sig. (2-tailed)	.552	.292
	N	152	152
pan_slope	Pearson Correlation	-.052	-.041
	Sig. (2-tailed)	.521	.619
	N	152	152
tai_slope	Pearson Correlation	-.310	-.175
	Sig. (2-tailed)	.000	.031
	N	152	152
tex_slope	Pearson Correlation	-.060	-.056
	Sig. (2-tailed)	.466	.493
	N	152	152

Table 6. Correlations between State NA and psychological stress in 1992.

Correlations

Variables	Statistics	(92 BL dep)	(92 2wk dep)	(92 BL anx)	(92 2wk anx)
(92 BL dep)	Pearson Correlation	1	.668	.752	.516
	Sig. (2-tailed)		.000	.000	.000
	N	152	152	152	152
(92 2wk dep)	Pearson Correlation	.668	1	.557	.696
	Sig. (2-tailed)	.000		.000	.000
	N	152	152	152	152
(92 BL anx)	Pearson Correlation	.752	.557	1	.582
	Sig. (2-tailed)	.000	.000		.000
	N	152	152	152	152
(92 2wk anx)	Pearson Correlation	.516	.696	.582	1
	Sig. (2-tailed)	.000	.000	.000	
	N	152	152	152	152
Stressor History Sum92	Pearson Correlation	.027	-.057	-.013	.048
	Sig. (2-tailed)	.741	.496	.880	.563
	N	147	147	147	147

Table 7. Correlations between State NA and psychological stress in 1993.

Correlations

		(93 BL dep)	(93 2wk dep)	(93 BL anx)	(93 2wk anx)
(93 BL dep)	Pearson Correlation	1	.576	.732	.518
	Sig. (2-tailed)		.000	.000	.000
	N	152	152	152	152
(93 2wk dep)	Pearson Correlation	.576	1	.553	.842
	Sig. (2-tailed)	.000		.000	.000
	N	152	152	152	152
(93 BL anx)	Pearson Correlation	.732	.553	1	.625
	Sig. (2-tailed)	.000	.000		.000
	N	152	152	152	152
(93 2wk anx)	Pearson Correlation	.518	.842	.625	1
	Sig. (2-tailed)	.000	.000	.000	
	N	152	152	152	152
Stressor History Sum93	Pearson Correlation	.029	.051	.021	.069
	Sig. (2-tailed)	.726	.538	.796	.400
	N	151	151	151	151

Table 8. Correlations between State NA and psychological stress in 1996.

Correlations

		(96 BL dep)	(96 2wk dep)	(96 BL anx)	(96 2wk anx)
(96 BL dep)	Pearson Correlation	1	.693	.775	.643
	Sig. (2-tailed)		.000	.000	.000
	N	152	152	152	152
(96 2wk dep)	Pearson Correlation	.693	1	.545	.774
	Sig. (2-tailed)	.000		.000	.000
	N	152	152	152	152
(96 BL anx)	Pearson Correlation	.775	.545	1	.692
	Sig. (2-tailed)	.000	.000		.000
	N	152	152	152	152
(96 2wk anx)	Pearson Correlation	.643	.774	.692	1
	Sig. (2-tailed)	.000	.000	.000	
	N	152	152	152	152
Stressor History Sum96	Pearson Correlation	.093	.025	.109	.045
	Sig. (2-tailed)	.255	.759	.182	.580
	N	152	152	152	152

Table 9. Correlations between State NA and systemic stress in 1992.

Correlations

Variables	Statistics	(92 BL dep)	(92 2wk dep)	(92 BL anx)	(92 2wk anx)
(92 BL dep)	Pearson Correlation	1	.668	.752	.516
	Sig. (2-tailed)		.000	.000	.000
	N	152	152	152	152
(92 2wk dep)	Pearson Correlation	.668	1	.557	.696
	Sig. (2-tailed)	.000		.000	.000
	N	152	152	152	152
(92 BL anx)	Pearson Correlation	.752	.557	1	.582
	Sig. (2-tailed)	.000	.000		.000
	N	152	152	152	152
(92 2wk anx)	Pearson Correlation	.516	.696	.582	1
	Sig. (2-tailed)	.000	.000	.000	
	N	152	152	152	152
chronic illness Sum92	Pearson Correlation	.132	.171	.178	.251
	Sig. (2-tailed)	.104	.035	.028	.002
	N	152	152	152	152

Table 10. Correlations between State NA and systemic stress in 1993.

Correlations

Variables	Statistics	(93 BL dep)	(93 2wk dep)	(93 BL anx)	(93 2wk anx)
(93 BL dep)	Pearson Correlation	1	.576	.732	.518
	Sig. (2-tailed)		.000	.000	.000
	N	152	152	152	152
(93 2wk dep)	Pearson Correlation	.576	1	.553	.842
	Sig. (2-tailed)	.000		.000	.000
	N	152	152	152	152
(93 BL anx)	Pearson Correlation	.732	.553	1	.625
	Sig. (2-tailed)	.000	.000		.000
	N	152	152	152	152
(93 2wk anx)	Pearson Correlation	.518	.842	.625	1
	Sig. (2-tailed)	.000	.000	.000	
	N	152	152	152	152
chronic illness Sum93	Pearson Correlation	.033	-.096	.025	-.077
	Sig. (2-tailed)	.689	.241	.759	.343
	N	152	152	152	152

Table 11. Correlations between State NA and systemic stress in 1996.

Correlations

Variables	Statistics	(96 BL dep)	(96 2wk dep)	(96 BL anx)	(96 2wk anx)
(96 BL dep)	Pearson Correlation	1	.693	.775	.643
	Sig. (2-tailed)		.000	.000	.000
	N	152	152	152	152
(96 2wk dep)	Pearson Correlation	.693	1	.545	.774
	Sig. (2-tailed)	.000		.000	.000
	N	152	152	152	152
(96 BL anx)	Pearson Correlation	.775	.545	1	.692
	Sig. (2-tailed)	.000	.000		.000
	N	152	152	152	152
(96 2wk anx)	Pearson Correlation	.643	.774	.692	1
	Sig. (2-tailed)	.000	.000	.000	
	N	152	152	152	152
chronic illness Sum96	Pearson Correlation	.109	.066	.140	.060
	Sig. (2-tailed)	.181	.416	.085	.461
	N	152	152	152	152

Table 12. Correlations between State NA, stress, and systemic symptom reports in 1992.

Correlations

		dep92_slope	anx92_slope	92 2wk dep	92 2wk anx	Stressor History 92	chronic illness 92
dep92_slope	Pearson Correlation	1	.503	.417	.228	-.105	.050
	Sig. (2-tailed)		.000	.000	.005	.207	.544
	N	152	152	152	152	147	152
anx92_slope	Pearson Correlation	.503	1	.157	.464	.067	.082
	Sig. (2-tailed)	.000		.053	.000	.421	.317
	N	152	152	152	152	147	152
92 2wk dep	Pearson Correlation	.417	.157	1	.696	-.057	.171
	Sig. (2-tailed)	.000	.053		.000	.496	.035
	N	152	152	152	152	147	152
92 2wk anx	Pearson Correlation	.228	.464	.696	1	.048	.251
	Sig. (2-tailed)	.005	.000	.000		.563	.002
	N	152	152	152	152	147	152
Stressor History 92	Pearson Correlation	-.105	.067	-.057	.048	1	-.041
	Sig. (2-tailed)	.207	.421	.496	.563		.618
	N	147	147	147	147	147	147
chronic illness 92	Pearson Correlation	.050	.082	.171	.251	-.041	1
	Sig. (2-tailed)	.544	.317	.035	.002	.618	
	N	152	152	152	152	147	152
Systemic symptoms 92 2 weeks	Pearson Correlation	.280	.128	.498	.352	-.025	.161
	Sig. (2-tailed)	.000	.116	.000	.000	.763	.048
	N	151	151	151	151	146	151

Table 13. Correlations between State NA, stress, and systemic symptom reports in 1993.

Correlations

		dep93_slope	anx93_slope	93 2wk dep	93 2wk anx	Stressor History 93	chronic illness 93
dep93_slope	Pearson Correlation	1	.633	.460	.352	.024	-.139
	Sig. (2-tailed)		.000	.000	.000	.774	.087
	N	152	152	152	152	151	152
anx93_slope	Pearson Correlation	.633	1	.347	.447	.056	-.119
	Sig. (2-tailed)	.000		.000	.000	.494	.145
	N	152	152	152	152	151	152
93 2wk dep	Pearson Correlation	.460	.347	1	.842	.051	-.096
	Sig. (2-tailed)	.000	.000		.000	.538	.241
	N	152	152	152	152	151	152
93 2wk anx	Pearson Correlation	.352	.447	.842	1	.069	-.077
	Sig. (2-tailed)	.000	.000	.000		.400	.343
	N	152	152	152	152	151	152
Stressor History 93	Pearson Correlation	.024	.056	.051	.069	1	.063
	Sig. (2-tailed)	.774	.494	.538	.400		.445
	N	151	151	151	151	151	151
chronic illness 93	Pearson Correlation	-.139	-.119	-.096	-.077	.063	1
	Sig. (2-tailed)	.087	.145	.241	.343	.445	
	N	152	152	152	152	151	152
Systemic symptoms 93 2 weeks	Pearson Correlation	.159	-.005	.410	.304	.098	.013
	Sig. (2-tailed)	.050	.948	.000	.000	.233	.871
	N	152	152	152	152	151	152

Table 14. Correlations between State NA, stress, and systemic symptom reports in 1996.

Correlations

		dep96_slope	anx96_slope	96 2wk dep	96 2wk anx	Stressor History 96	chronic illness 96
dep96_slope	Pearson Correlation	1	.588	.396	.170	-.086	-.054
	Sig. (2-tailed)		.000	.000	.036	.292	.509
	N	152	152	152	152	152	152
anx96_slope	Pearson Correlation	.588	1	.289	.389	-.081	-.102
	Sig. (2-tailed)	.000		.000	.000	.319	.209
	N	152	152	152	152	152	152
96 2wk dep	Pearson Correlation	.396	.289	1	.774	.025	.066
	Sig. (2-tailed)	.000	.000		.000	.759	.416
	N	152	152	152	152	152	152
96 2wk anx	Pearson Correlation	.170	.389	.774	1	.045	.060
	Sig. (2-tailed)	.036	.000	.000		.580	.461
	N	152	152	152	152	152	152
Stressor History 96	Pearson Correlation	-.086	-.081	.025	.045	1	.003
	Sig. (2-tailed)	.292	.319	.759	.580		.972
	N	152	152	152	152	152	152
chronic illness 96	Pearson Correlation	-.054	-.102	.066	.060	.003	1
	Sig. (2-tailed)	.509	.209	.416	.461	.972	
	N	152	152	152	152	152	152
Systemic symptoms 96 2 weeks	Pearson Correlation	.067	-.023	.346	.238	.220	.262
	Sig. (2-tailed)	.413	.778	.000	.003	.007	.001
	N	151	151	151	151	151	151

Table 15. Correlations between systemic symptoms and Ab response slope in 1992.

Correlations

		Systemic symptoms 92 2 weeks
Systemic symptoms 92 2 weeks	Pearson Correlation	1
	N	151
Be89_92_slope	Pearson Correlation	-.091
	Sig. (2-tailed)	.268
	N	151
Be92_92_slope	Pearson Correlation	-.046
	Sig. (2-tailed)	.576
	N	151
Pan92_slope	Pearson Correlation	.000
	Sig. (2-tailed)	.997
	N	151
Tai92_slope	Pearson Correlation	-.028
	Sig. (2-tailed)	.734
	N	151
Tex92_slope	Pearson Correlation	-.144
	Sig. (2-tailed)	.077
	N	151

Table 16. No correlations existed between systemic symptoms and Ab response slope in 1993.

Correlations

		Systemic symptoms 93 2 weeks
Systemic symptoms 93 2 weeks	Pearson Correlation	1
	N	152
Be89_93_slope	Pearson Correlation	.008
	Sig. (2-tailed)	.918
	N	152
Be92_93_slope	Pearson Correlation	-.054
	Sig. (2-tailed)	.510
	N	152
Pan93_slope	Pearson Correlation	.120
	Sig. (2-tailed)	.141
	N	152
Tai93_slope	Pearson Correlation	-.009
	Sig. (2-tailed)	.912
	N	152
Tex93_slope	Pearson Correlation	.032
	Sig. (2-tailed)	.692
	N	152

Table 17. No correlations existed between systemic symptoms and Ab response slope in 1996.

Correlations

		Systemic symptoms 96 2 weeks
Systemic symptoms 96 2 weeks	Pearson Correlation	1
	N	151
Be89_96_slope	Pearson Correlation	.058
	Sig. (2-tailed)	.478
	N	151
Be92_96_slope	Pearson Correlation	.087
	Sig. (2-tailed)	.287
	N	151
Pan96_slope	Pearson Correlation	-.010
	Sig. (2-tailed)	.907
	N	151
Tai96_slope	Pearson Correlation	.102
	Sig. (2-tailed)	.211
	N	151
Tex96_slope	Pearson Correlation	.018
	Sig. (2-tailed)	.825
	N	151

Table 18. Correlations between PA and Ab response in 1992.

Correlations

		optimism 92	positive mood 92
optimism 92	Pearson Correlation	1	.507
	Sig. (2-tailed)		.000
	N	152	152
positive affect 92	Pearson Correlation	.507	1
	Sig. (2-tailed)	.000	
	N	152	152
Be89_92_slope	Pearson Correlation	.116	.041
	Sig. (2-tailed)	.154	.616
	N	152	152
Be92_92_slope	Pearson Correlation	-.034	.031
	Sig. (2-tailed)	.677	.708
	N	152	152
Pan92_slope	Pearson Correlation	-.017	.045
	Sig. (2-tailed)	.834	.582
	N	152	152
Tai92_slope	Pearson Correlation	-.025	.071
	Sig. (2-tailed)	.760	.386
	N	152	152
Tex92_slope	Pearson Correlation	.047	.000
	Sig. (2-tailed)	.568	.996
	N	152	152

Table 19. Correlations between PA and Ab response in 1996.

Correlations

		optimism 96	positive mood 96
optimism 96	Pearson Correlation	1	.415
	Sig. (2-tailed)		.000
	N	152	152
positive affect 96	Pearson Correlation	.415	1
	Sig. (2-tailed)	.000	
	N	152	152
Be89_96_slope	Pearson Correlation	.123	-.057
	Sig. (2-tailed)	.133	.485
	N	152	152
Be92_96_slope	Pearson Correlation	.119	.072
	Sig. (2-tailed)	.145	.375
	N	152	152
Pan96_slope	Pearson Correlation	-.036	-.059
	Sig. (2-tailed)	.663	.472
	N	152	152
Tai96_slope	Pearson Correlation	.044	.082
	Sig. (2-tailed)	.586	.316
	N	152	152
Tex96_slope	Pearson Correlation	.034	-.010
	Sig. (2-tailed)	.673	.900
	N	152	152

Table 20. Correlations between PA and Ab response in 1993.

Correlations

		optimism 93	positive mood 93
optimism 93	Pearson Correlation	1	.569
	Sig. (2-tailed)		.000
	N	152	152
positive affect 93	Pearson Correlation	.569	1
	Sig. (2-tailed)	.000	
	N	152	152
Be89_93_slope	Pearson Correlation	.119	.257
	Sig. (2-tailed)	.143	.001
	N	152	152
Be92_93_slope	Pearson Correlation	.049	.173
	Sig. (2-tailed)	.548	.033
	N	152	152
Pan93_slope	Pearson Correlation	-.029	-.048
	Sig. (2-tailed)	.721	.554
	N	152	152
Tai93_slope	Pearson Correlation	.095	.146
	Sig. (2-tailed)	.245	.073
	N	152	152
Tex93_slope	Pearson Correlation	-.050	.024
	Sig. (2-tailed)	.538	.766
	N	152	152

FIGURE LEGENDS

Figure 1. Schematic representation of the communications between neuro-endocrine and immune systems. Communication between the CNS and immune system occur via multiple routes: vagal afferents, HPA axis, the peripheral nervous system, and both sympathetic and parasympathetic branches of the autonomic nervous system. From Sternberg 2006.

Figure 2. Graphical depiction of both long-loop and local interactions between the immune and neuro-endocrine systems. The box depicting the immune system represents not only classical immunological organs (i.e., spleen) but also any peripheral tissue in which immune responses may take place. Neural and endocrine systems are depicted together due to their high degree of interconnectivity. When immune activation at a distant location impacts neuro-endocrine functions, this is referred to as "long-loop" interactions, whereas co-expression of immune and neuro-endocrine factors site-specifically refers to "local" interactions (circles within boxes). Branching out arrows symbolize the organismic consequences of immune, nervous, and endocrine interactions. From Besedovsky and del Rey, 1996.

Figure 3. Trait anxiety groups show distinct long-term immunological profiles to *Panama*. Error bars reflect SEM. X-axis: year; Y-axis: *Panama* Ab response slope; lines represent Trait Anxiety categories.

Figure 4. Distinct *Beijing92* Ab response profiles were dependent upon follow-up state Depression. Error bars reflect SEM. X-axis: year; Y-axis: *Beijing92* Ab response slope; lines represent Depression categories quantified as whether participants increased, decreased, or showed no change in follow-up depression across years.

Figure 5. There was a relationship between follow-up Anxiety change category and Ab response to *Beijing92*. Error bars reflect SEM. X-axis: Anxiety categories quantified as whether participants increased, decreased, or showed no change in follow-up anxiety across years; Y-axis: *Beijing92* Ab response slope.

Figure 6. Interactive effects between follow-up NA response category and long-term Ab response to *Beijing92*. Note: No individuals showed an absence of change in both dimensions of NA. Error bars reflect SEM. X-axis: year; Y-axis: *Beijing92* Ab response slope; lines represent combinations of follow-up State NA (depression and anxiety) categories quantified as whether participants increased, decreased, or showed no change in follow-up depression across years.

Figure 7. Interactive effects of follow-up NA response category on long-term Ab response to *Panama*. Note: No individuals showed an absence of change in both dimensions of NA. Error bars reflect SEM. X-axis: year; Y-axis: *Panama* Ab response slope; lines represent combinations of follow-up State NA (depression and anxiety) categories quantified as whether participants increased, decreased, or showed no change in follow-up depression across years.

Figure 8. Follow-up Depression category and Ab response to *Panama*. Error bars reflect SEM. X-axis: Depression categories quantified as whether participants increased, decreased, or showed no change in follow-up depression across years; Y-axis: *Panama* Ab response slope.

Figure 9. There was a relationship between follow-up Anxiety change category and Ab response to *Taiwain*. Error bars reflect SEM. X-axis: Anxiety categories quantified as whether participants increased, decreased, or showed no change in follow-up anxiety across years; Y-axis: *Taiwan* Ab response slope.

Figure 10. There was a relationship between follow-up Depression change category and Ab response to *Taiwan*. Error bars reflect SEM. X-axis: Depression categories quantified as whether participants increased, decreased, or showed no change in follow-up depression across years; Y-axis: *Taiwan* Ab response slope.

Figure 11. Low systemic symptom reporters showed enhanced Ab response to *Beijing89* relative to other groups in 1992. Error bars reflect SEM. X-axis: Systemic symptom reporting category quantified as whether participants reported no (Non-reporter), few (Low reporter), or many (High reporter) systemic symptoms two weeks after inoculation; Y-axis: 1992 *Beijing89* Ab response slope.

Figure 12. Relationships between PA and *Taiwan* Ab slope in 1993. Error bars reflect SEM. X-axis: PA group categories (combinations of Positive mood and Optimism: Low, Moderate, and High); Y-axis: 1993 *Taiwan* Ab response slope.

Figure 13. Low Positive mood tends to show reduced Ab response to *Taiwan* in 1996. Error bars reflect SEM. X-axis: Positive mood category (Low, Moderate, and High); Y-axis: 1996 *Taiwan* Ab response slope.

Figure 14. Positive mood decreased significantly over the five years of the study. Error bars reflect SEM. X-axis: year; Y-axis: Positive mood sum score.

FIGURES

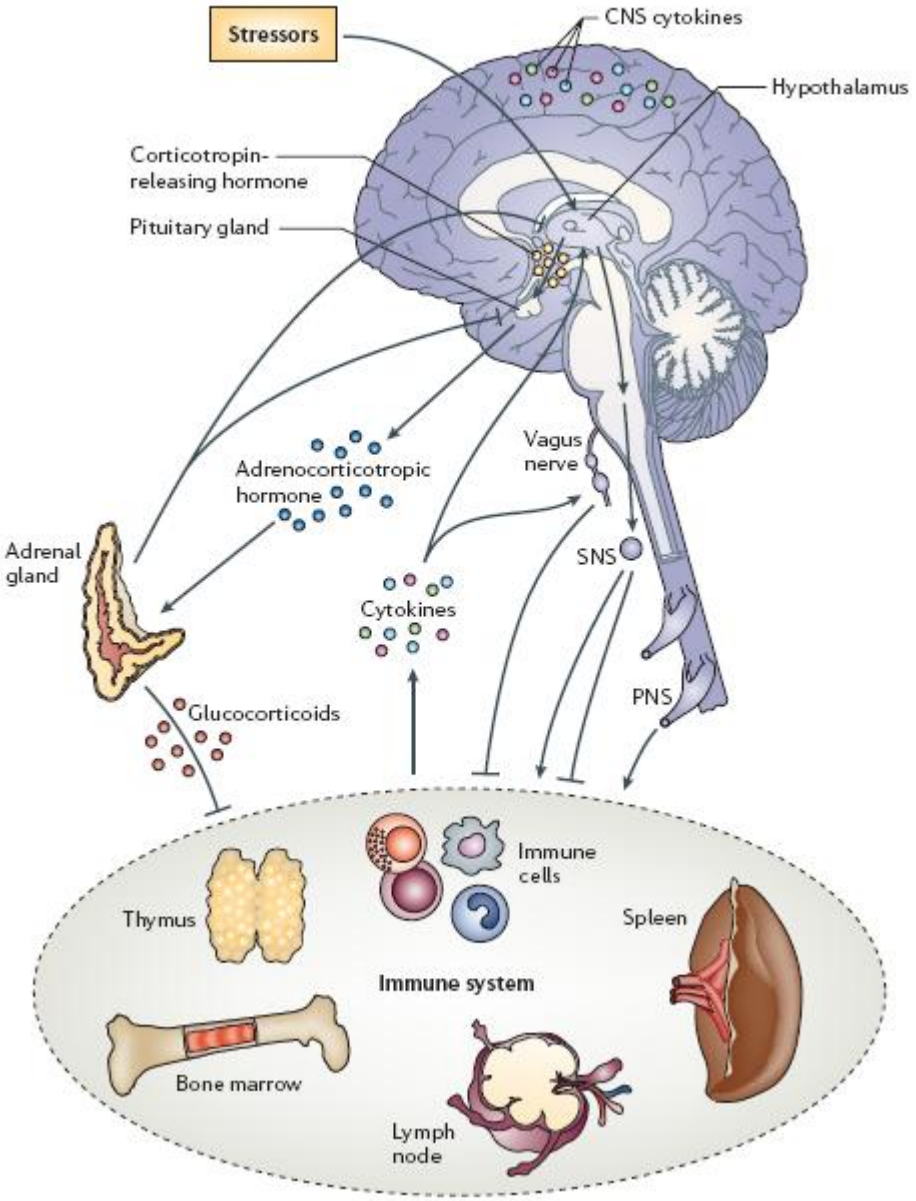


Figure 1.

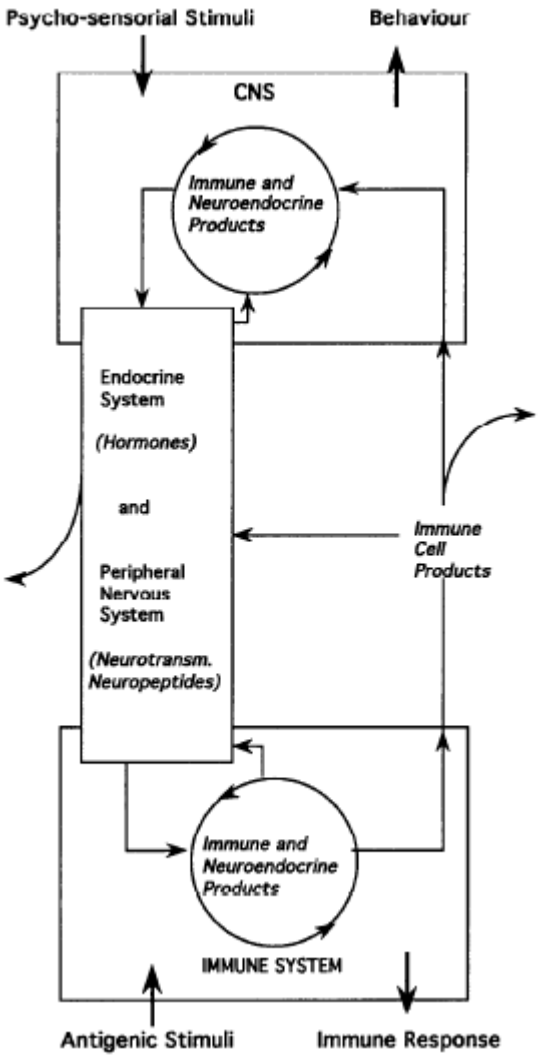

Psycho-sensorial Stimuli
Behaviour
CNS
Immune and Neuroendocrine Products
Endocrine System
(Hormones)
and
Peripheral Nervous System
(Neurotransm. Neuropeptides)
Immune Cell Products
Immune and Neuroendocrine Products
IMMUNE SYSTEM
Antigenic Stimuli
Immune Response

Figure 2.

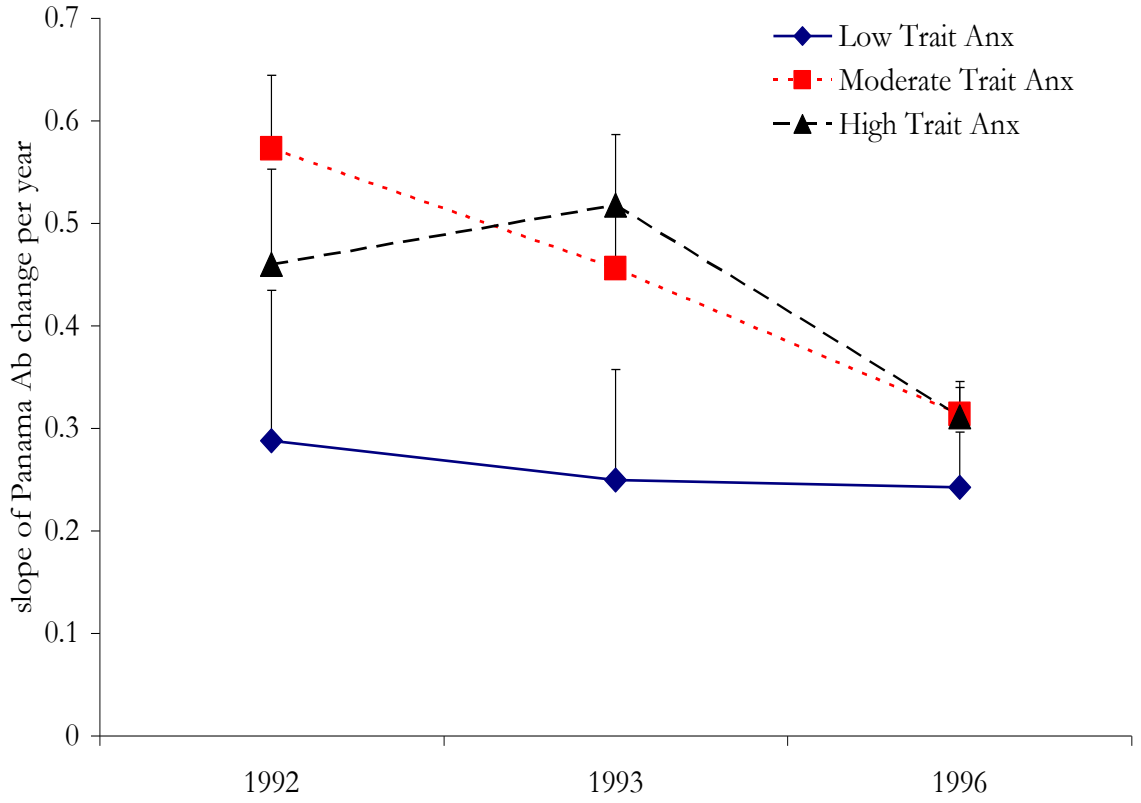
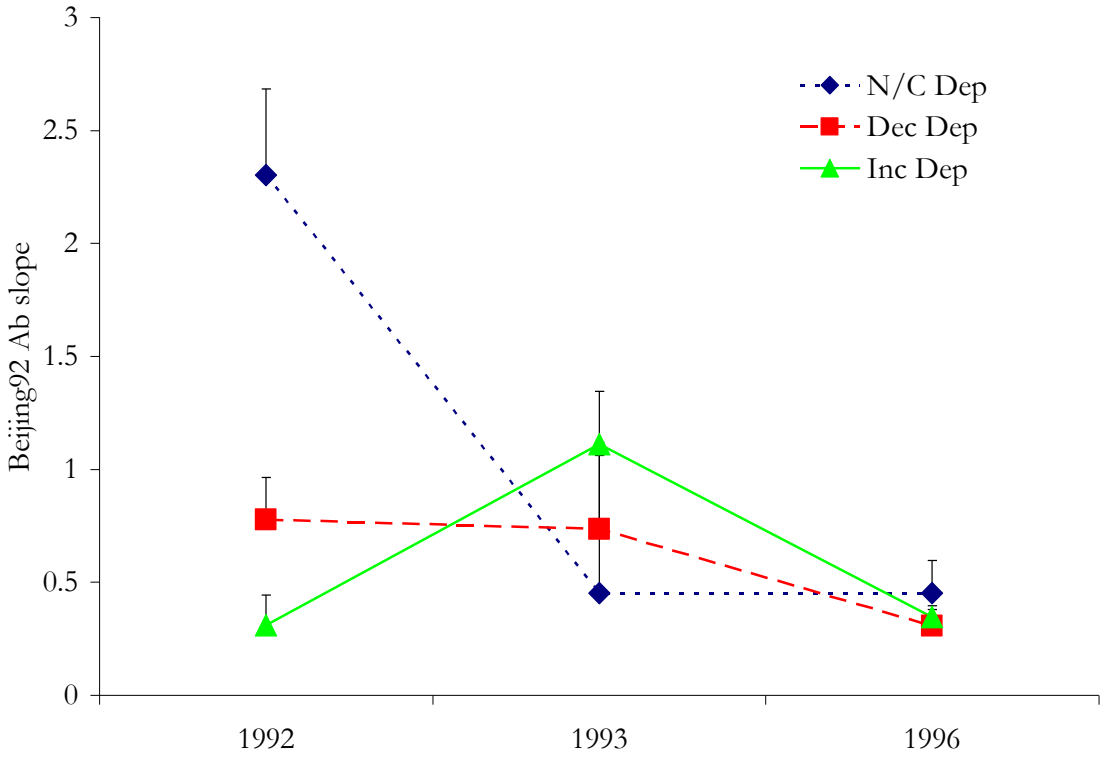

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
slope of Panama Ab change per year
1992
1993
1996
Low Trait Anx
Moderate Trait Anx
High Trait Anx

Figure 3.



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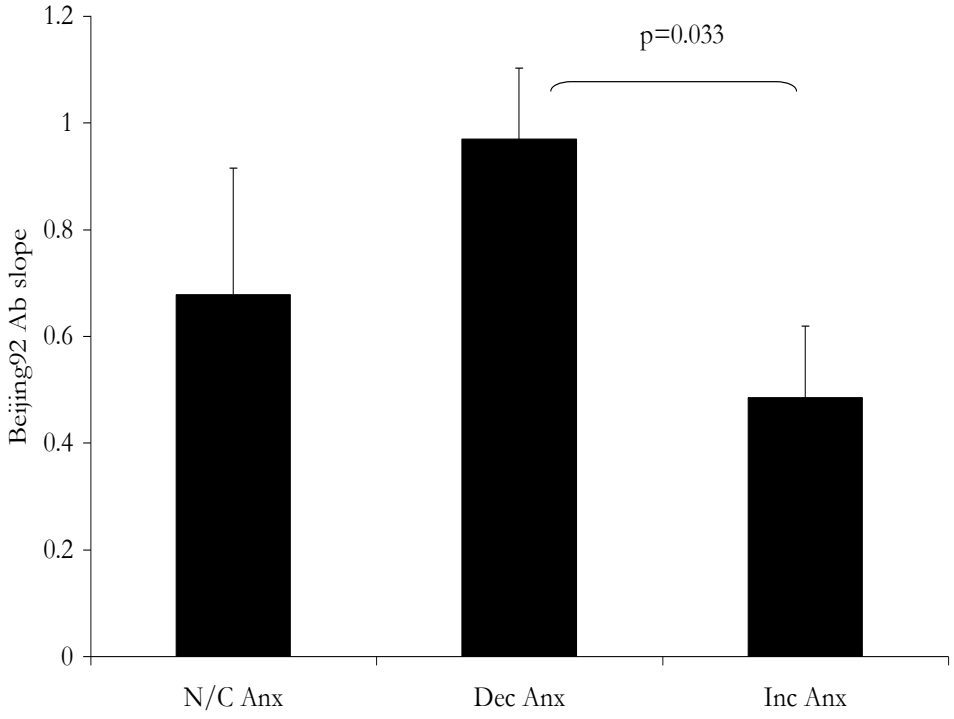


Figure 5.

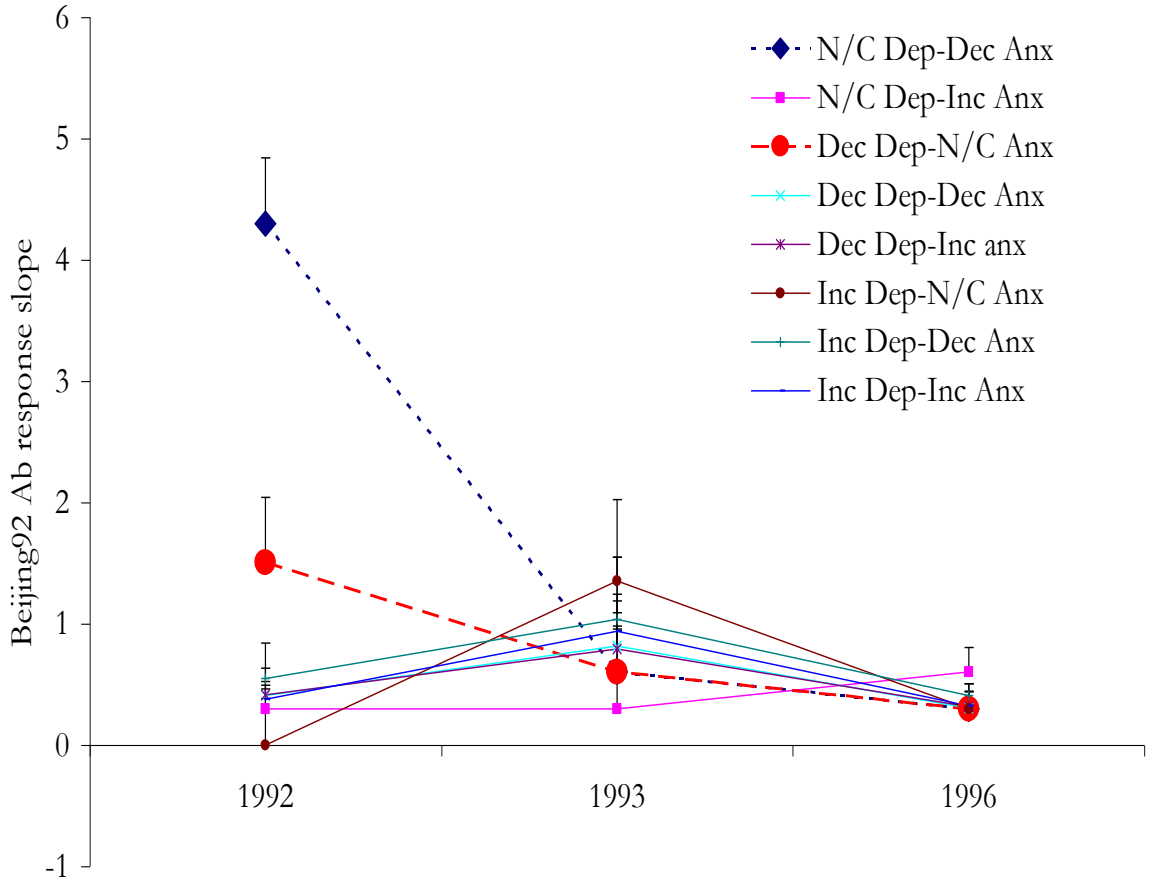
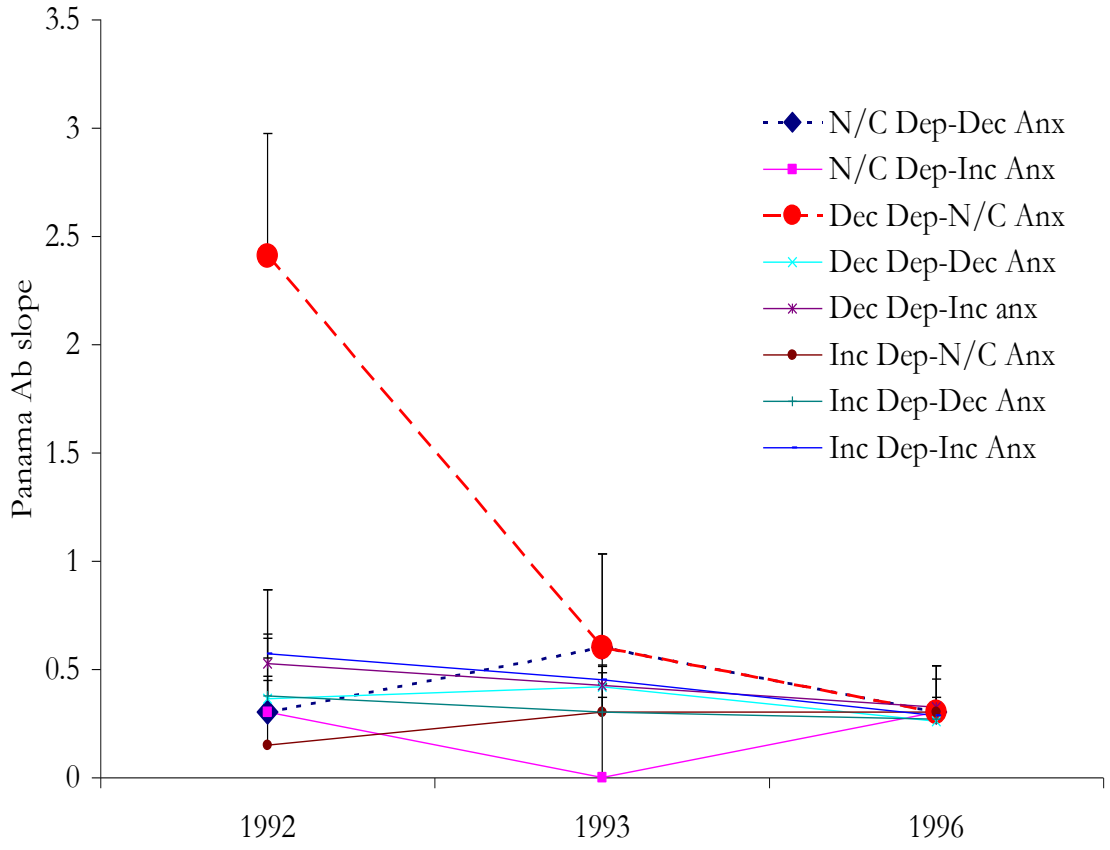

6
5
4
3
2
1
0
-1
Beijing92 Ab response slope
1992
1993
1996
N/C Dep-Dec Anx
N/C Dep-Inc Anx
Dec Dep-N/C Anx
Dec Dep-Dec Anx
Dec Dep-Inc anx
Inc Dep-N/C Anx
Inc Dep-Dec Anx
Inc Dep-Inc Anx

Figure 6.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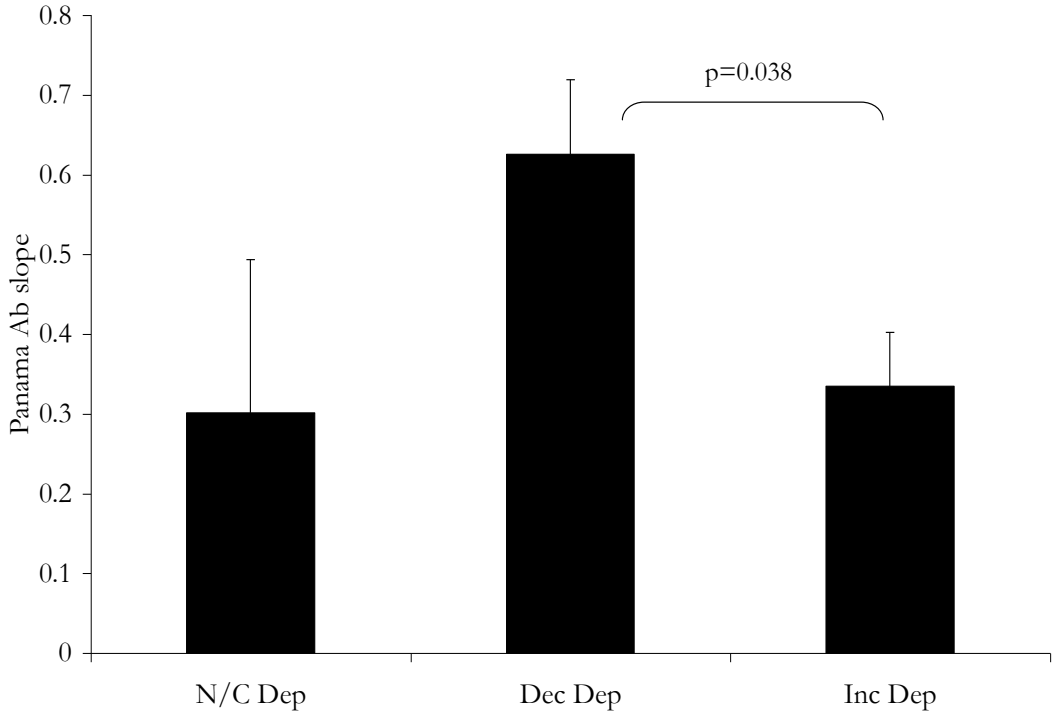
3
2.5
2
1.5
1
0.5
0
Panama Ab slope
1992
1993
1996
N/C Dep-Dec Anx
N/C Dep-Inc Anx
Dec Dep-N/C Anx
Dec Dep-Dec Anx
Dec Dep-Inc anx
Inc Dep-N/C Anx
Inc Dep-Dec Anx
Inc Dep-Inc Anx

Figure 7.



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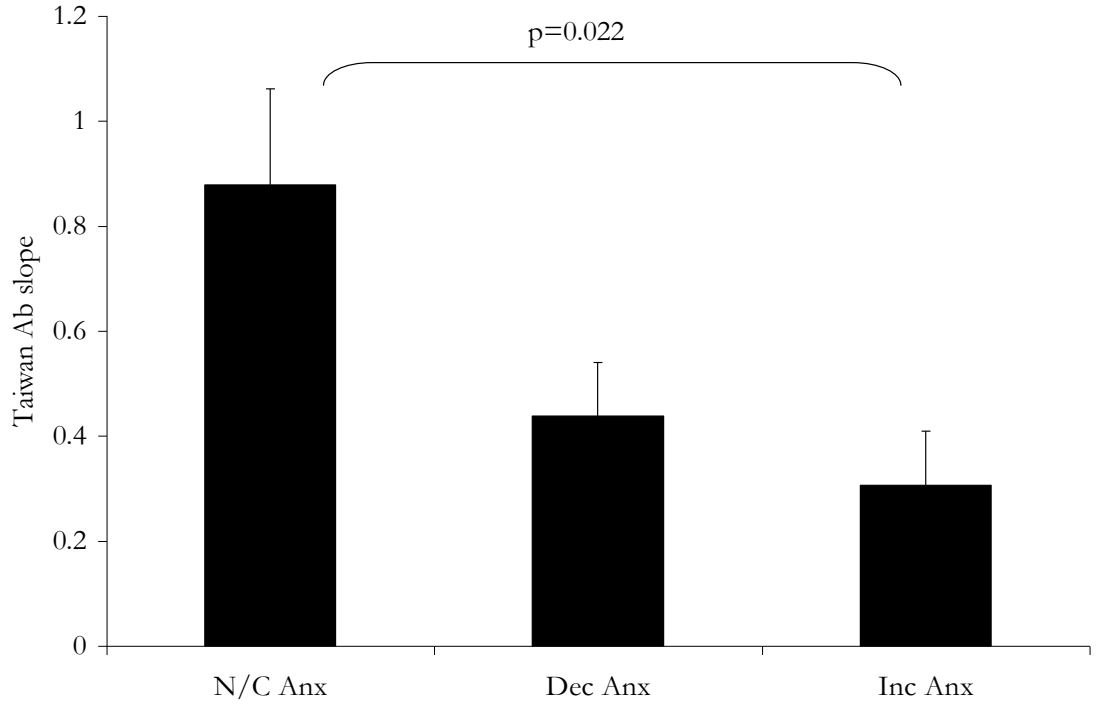

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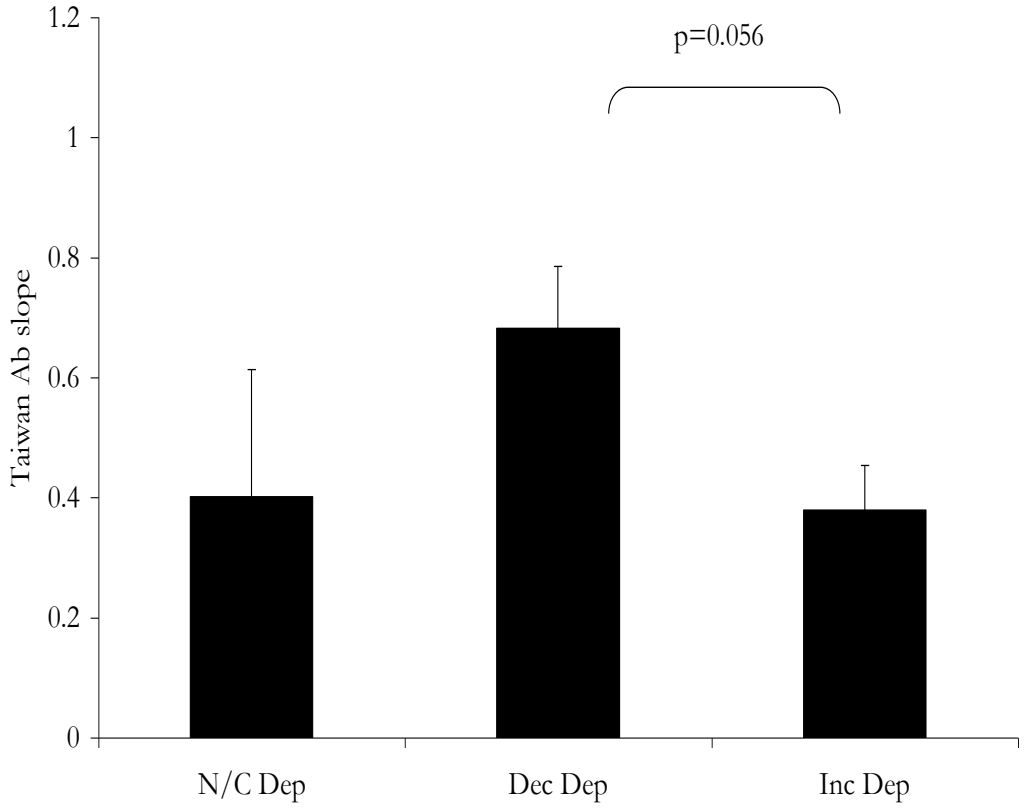

Figure 10.

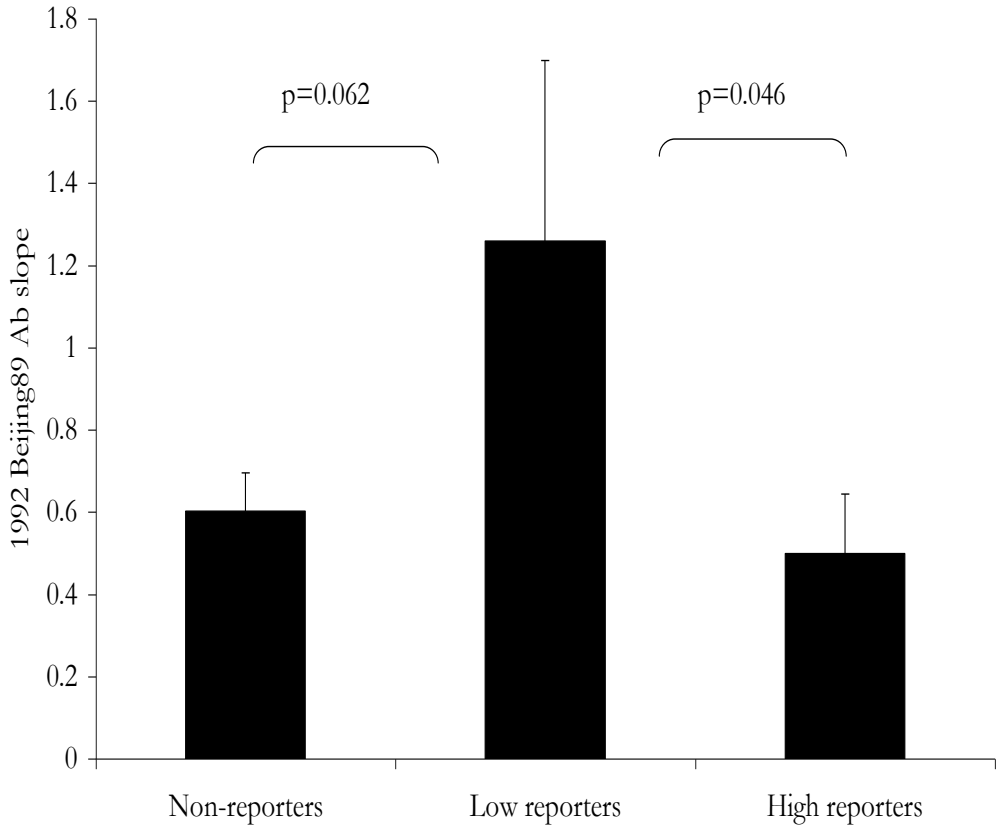
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1992 Beijing89 Ab slope
p=0.062
p=0.046
Non-reporters
Low reporters
High reporters

Figure 11.

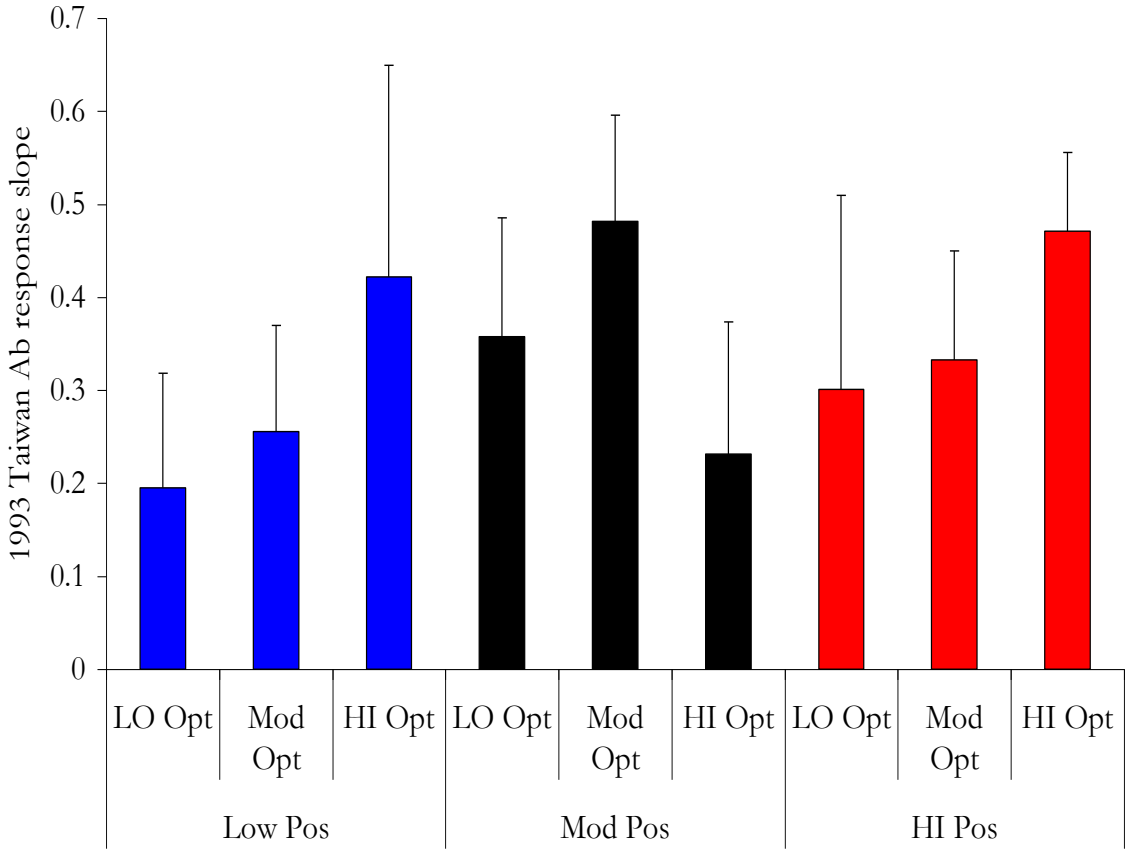
1993 Taiwan Ab response slope
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
LO Opt
Mod Opt
HI Opt
LO Opt
Mod Opt
HI Opt
LO Opt
Mod Opt
HI Opt
Low Pos
Mod Pos
HI Pos

Figure 12.

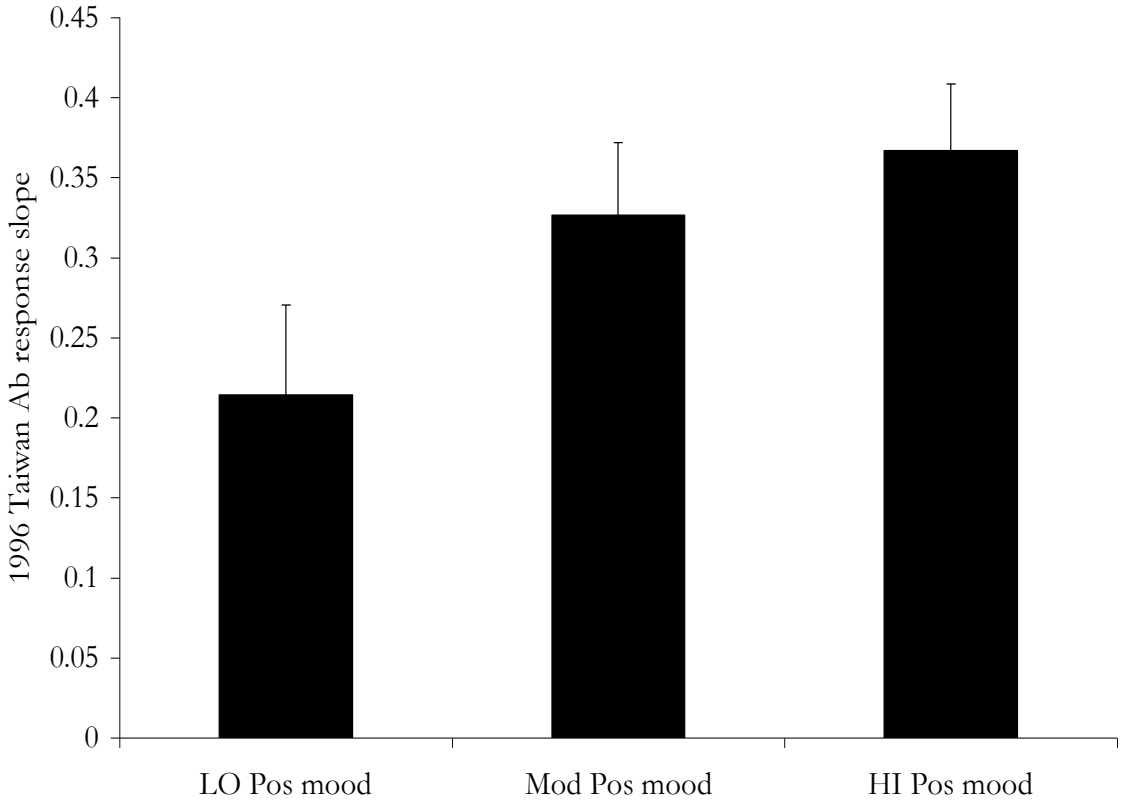
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1996 Taiwan Ab response slope
LO Pos mood
Mod Pos mood
HI Pos mood

Figure 13.

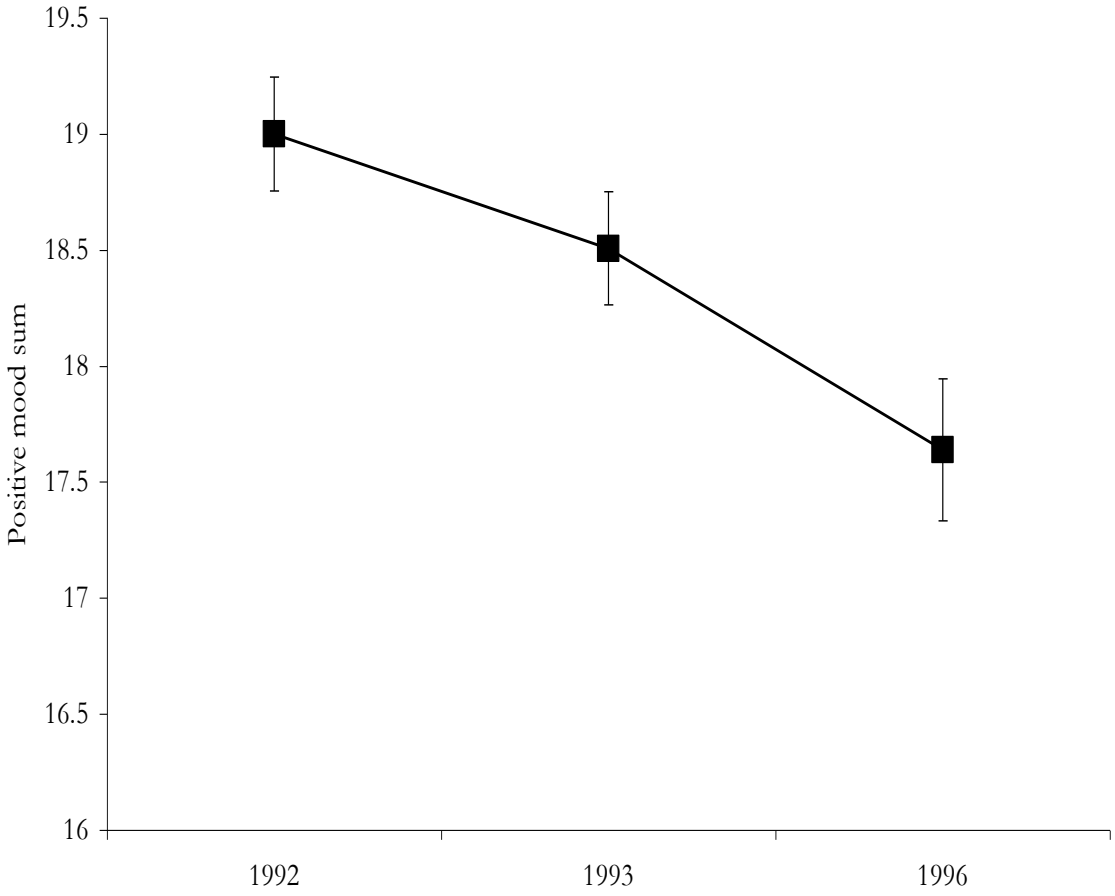

Figure 14.

APPENDIX A: MEDICAL EXCLUSIONARY CRITERIA

1. DISEASES:
   - ♦ Malignancy (solid tumor):
     <1 year without evidence of active disease
     <1 year since completion of treatment
     received chemotherapeutic treatment at any time
   - ♦ Leukemia
   - ♦ Lymphoma
   - ♦ Myeloma
   - ♦ Lupus erythematosus
   - ♦ Rheumatoid arthritis
   - ♦ Polymyalgia rheumatica
   - ♦ Temporal arteritis
   - ♦ Sjorgren's disease
   - ♦ Myasthenia gravis
   - ♦ Multiple sclerosis
   - ♦ Amyotrophic lateral sclerosis
   - ♦ Vasculitis
   - ♦ Graves disease
   - ♦ Gaucher's disease
   - ♦ Chrohn's disease
   - ♦ Ulcerative colitis
   - ♦ Chronic fatigue immunodeficiency syndrome
   - ♦ Hypogammaglobulinemia
   - ♦ Hepatitis: <5 years without evidence of active disease
   - ♦ Cirrhosis: <5 years without evidence of active disease

2. MEDICATIONS:
   - ♦ Antineoplastics
   - ♦ Tamoxifen
   - ♦ DES
   - ♦ Steroids (oral)
   - ♦ Aspirin/aspirin compounds: unable to abstain 2 weeks prior to and 4 weeks during study
   - ♦ NSAIDs: unable to abstain 2 weeks prior to and 4 weeks during study
   - ♦ Beta-blockers
   - ♦ Lithium

CURRICULUM VITAE

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Rutgers, the State University of NJ	PhD	Oct 2008
Rutgers, the State University of NJ	MS	May 2006
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PUBLICATIONS:

Hash-Converse, J.M. & Kusnecov, A.W. (2008). Immunological influences on brain and behavior: The immune response as a stressor. In Contrada, R.J., & Baum, A.S. (Eds.) The Handbook of Stress Science: Psychology, Biology, and Health. (in preparation)

Kohman, R.A., Hash-Converse, J.M., & Kusnecov, A.W. (2008). Effects of systemic challenge with bacterial toxins on behaviors relevant to mood, anxiety and cognition. In Siegel, A. & Zalcman, S.S. (Eds.). The Neuroimmunological Basis of Behavior and Mental Disorders.

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Seibert, P.S., Reedy, D.P., Hash, J., Webb, A., Stridh-Igo, P., Basom, J., & Zimmerman, C.G. (2002). Brain injury: Quality of life's greatest challenge. *Brain Injury, 16,* 837-848.