BEHAVIORAL CHARACTERIZATION OF ACUTE PHASE OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS INFECTION

A Senior Honors Thesis

by

JESSICA MARY HARRISON

Submitted to the Office of Honors
Programs & Academic Scholarships
Texas A&M University
In Partial Fulfillment of the Designation of

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

April 2004

Major: Psychology

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Approved as to style and content by:

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(Fellows Advisor)	(Executive Director)

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ABSTRACT

Behavioral Characterization of the Acute Phase of Theiler's Murine Encephalomyelitis Virus Infection. (May 2004)

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Theiler's Murine Encephalomyelitis virus (TMEV) infection induces a bi-phasic disease with distinct acute and chronic infection patterns. This study characterizes the behavioral markers of disease during the acute phase utilizing behavioral tests to quantify sickness syndrome. Allodynia was exhibited in infected mice on day 1 post infection (pi), reflected by a decrease in von Frey scores from baseline measures. Sucrose preference was decreased on day 0 to 3 pi in infected animals relative to mock-infected vehicle animals and both groups decreased preference relative to uninfected animals. Body temperature and body weight measures did not conclusively differentiate between infected and mock-infected vehicle

animals. Infected animals experienced fever on day 1 and 3 pi, while mock-infected vehicle animals displayed hypothermia on the same days, with temperatures taken at 1 and 3 pm. Infection did not significantly affect fear conditioning nesting, open field locomotor activity, and social interaction. Summarily, mock-infected vehicle animals showed mild sickness behaviors in comparison to the control-uninfected animals, but the infected mice exhibited the highest magnitude of sickness responses. Chronic phase data will be collected in these mice to characterize behavioral manifestations of the demyelinating autoimmune process.

DEDICATION

This work is dedicated to my parents, Gary and Ethel Harrison.

Love,

Jess

ACKNOWLEDGMENTS

Foremost I thank Dr. Mary Meagher for her superior mentorship, leadership, and scientific insight. Under any other tutelage, this endeavor would have been a harrowing experience. I truly appreciate her scientific and professional guidance.

Additionally the assistance of Robin Johnson, Amy Sieve, Thomas Prentice, and Patrick Bridegam has been essential to complete this project. From the initial preparation to the final kills, they have each taught me and helped me fill in the gaps where my experience lacked.

Many thanks to Dr. Jane Welsh for her help in scoring the chronic phase disease and for advice about the study.

Thanks to Dr. Colin Young for providing the mice for the study.

Finally, without the work of my research assistants there is no way that I would have been able to complete this project. From taking 9 am body temperatures to running digiscan, they were dependable and willing to be there when I could not. In a project with multiple measures at various time points, they helped me to maintain a semblance of a life and to complete my class work.

Many thanks to everyone in the PNI group whose advice and kind words have meant so much. It is a privilege to work alongside so many talented scientists this early in one's career.

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INTRODUCTION

Psychoneuroimmunology

Psychoneuroimmunolgy (PNI) is a relatively new area of research that invites the collaboration of diverse fields such as psychology, immunology, neuroscience, and microbiology. PNI examines the interaction of behavior, immunology, and the brain from multiple aspects and models. Activation of the stress response through immunological, psychological, or environmental insult is studied independently or in conjunction with illness. The temporal relationship of stress and illness influences the organism's ability to reconcile environmental or pathological challenges as well as orchestrate the mediating immune processes within the body. Although multiple models of stress and disease are utilized, data consistently support the interaction of stress and immunity (Solomon, 1969; Maier et al, 1994; Oleszak et al, 2004). There is not a simple explanation, however, for the relationship between stress and immune function exists. A key goal is to delineate the bi-directional communication between the immune system and the brain, and how this communication affects behavioral manifestations of sickness.

Within PNI research, many animal models of disease are used to understand how the human immune system reacts to stress and

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This thesis follows the style and format of *American Psychological Association* publication manual.

Within PNI research, many animal models of disease are used to understand how the human immune system reacts to stress and physiological effects. The intent of the present study is to provide a thorough behavioral characterization of an animal model of multiple sclerosis, TMEV infection. My project will focus on the early acute phase of infection but extend into the chronic demyelinating phase of disease. Meticulous behavioral and physiological characterization quantifies phenomena that are not readily apparent by observation. Specifically, it translates microscopic internal processes into macro-scale behaviors.

Theiler's Murine Encephalomyelitis Virus Infection

TMEV induces a biphasesic disease in susceptible strains of mice. The acute phase constitutes the first month of infection and is a gray matter disease similar to polio. The later disease occurs 1-2 months post-infection and is characterized by primary inflammatory demyelination, which is remarkably similar to MS (Lipton, 1975). TMEV can serve as a model of Multiple Sclerosis (MS) during the chronic phase, as it reflects a viral etiology for the autoimmune attack of the central nervous system and the chronic-progressive form of the disease (Welsh et al, 1990). In addition, the acute phase offers an apt model of sickness syndrome and immune response to environmental and physiological manipulations (Campbell et al,

2001; McGavern et al, 2000). Although often described as asymptomatic, the present study shows appreciable behavioral manifestations of illness.

TMEV infection increases the expression of pro-inflammatory cytokines and chemokines in the central nervous system (CNS) (Olezak et al, 2004). Cytokines are polypeptide hormones associated with immune and inflammatory response. Considerable research identifies cytokine release both peripherally and centrally as responsible or concurrent with the onset of sickness syndrome, the subject of which will be expanded upon shortly (Connor & Leonard, 1997; Doinarello, 1994; Maier & Watkins, 1998; Pollack et al, 2003; Pugh et al, 2001). Although the intent of this study is not to measure cytokine activity with respect to sickness syndrome in TMEV, it is a future objective in elucidating the mechanisms and effects of Theiler's virus.

TMEV has several strains that differ in virulence, with the BeAn strain moderate in strength among these. A large body of TMEV research utilizes the BeAn strain, as does the present study, due to its disease course and symptoms. Detailed analyses of behavioral and physiological disease severity indices are frequently neglected in TMEV research. The current study characterizes the disease course of infected and non-infected animals by producing a multivariate picture infection. Finally histological evidence of disease will be analyzed to accompany behavioral evidence.

Sickness Syndrome

"Sickness Syndrome" is defined as a series of adaptive behavioral and physiological responses orchestrated by the central nervous system (CNS) following immune challenge. Traditionally, these responses have been seen as direct effects of infection; however, they are actually protective responses initiated by the brain to conserve energy, reallocate resources to fight infection, and promote recuperation during an immune challenge.

Sickness syndrome includes anhedonia, allodynia, depressed social activity, decreases in physical activity and exploratory behavior, and impairments in learning and memory. Anhedonia, or loss of pleasure seeking behavior, can be measured using food or taste preference, sexual activity, or enriched environment choice. All underscore a failure to pursue a gratifying activity, despite its availability. Another construct, allodynia, is defined as a pain response to a non-noxious stimulus. This reflects the depressed sensitivity threshold during sickness. Sickness is also associated with selective impairments in learning and memory, notably impairments in working memory tasks. All symptoms are familiar to anyone who has been sick. Each aspect can be measured to characterize sickness behavior and various precursors to its initiation (Maes, 1994; Maier & Watkins, 1998; Pollack et al, 2000). Physiological adaptations include thermoregulation, anorexia, and slow wave sleep disturbance.

Cytokines play a central role as messengers between immune cells and the CNS in order to initiate the sickness syndrome. Proinflammatory cytokines are released as macrophages encounter pathogens in the periphery. Several models of cytokine transport into the brain have been proposed; however, cytokines produced within the CNS have been strongly implicated as responsible factors in initiating sickness syndrome. Regardless of origin or method of transport, vast research correlates their levels with sickness syndrome. Cytokines have been shown to be both necessary and sufficient in immune to brain communication that preempts sickness behavior. Blocking cytokines, especially IL-1, thwarts the onset of sickness behaviors, while administration of IL-1 initiates them. Cytokine levels prior and subsequent to sickness syndrome may be predictive and explanatory in nature Studies reveal method and time of cytokine intervention influence the observed effects (Connor & Leonard, 1997; Dinarello et al, 1991; Maier & Watkins, 1998; Pollack et al, 2002; Pugh et al, 2000). Prior research indicates in our lab finds the cytokines IL-1, IL-6, IL-12, and IFN-γ are peaking on day 2 and 7 pi of TMEV. Behavioral measures of sickness were taken with these time-points in mind, due to the relationship between cytokines and sickness syndrome. Observed effects cannot be directly linked to certain cytokines or cytokine and chemokine activity per se, however it does suggest a connection.

Understanding cytokine-mediated sickness syndrome presents treatment possibilities for depression and chronic disease symptoms, as well as greater understanding of the etiology deficits. The similitude between sickness syndrome and depression has not been overlooked. Behavioral, physiological and even immune changes catalogued in depressed human populations map directly onto animal models of sickness syndrome. Not surprisingly, researchers have sought to delineate this relationship and uncover the role that inflammatory immune activation plays in forms of depression. Utilizing various means of investigation including: cytokine administration and antagonist studies, anti-depressant treatments to counteract sickness syndrome, and other cytokine modulating interventions, the complex mechanisms have been elucidated to an extent. Lippopolysaccharide (LPS) is the most commonly used model for immune system activation. It is a foreign bacterial pathogen that produces a well-characterized immune cascade and sickness syndrome. Investigations have also examined experimental autoimmune encephalomyelitis (EAE) and the role of IL-1 in mediating sickness (Connor & Leonard, 1997 Pollack et al, 2002; Maes, 1994; Pugh et al, 1998). From this literature, characterization of sickness syndrome in TMEV, which unlike other immune challenges is a natural viral pathogen in mice, will be a step towards examining these cytokine-mediated adaptations in a naturalistic model.

Additionally the present work compilies sickness measures from multiple studies to obtain a multi-dimensional description of sickness syndrome.

Fear Conditioning

Contextual fear conditioning is an example of the learning and memory impairment arising from immune challenge. Fear conditioning is a form of Pavlovian conditioning in which the organism learns that a previously neutral stimulus predicts the occurrence of a biologically relevant stimulus. In Pavlov's studies, he paired a biologically relevant unconditional stimulus (US), such as food, that elicits the unconditional response (UR) of salivation, with a neutral conditional stimulus (CS) such as a bell that initially does not elicit a response. After multiple pairings of the bell/CS with the food/US, Pavlov's dogs exhibited a conditioned response (CR) to the bell CS alone. Fear conditioning is a subtype of this classical learning paradigm, which the organism is evolutionary predisposed to master very quickly. It involves a single pairing a neutral stimulus (CS) with an aversive unconditioned stimulus (US). After one or two pairings of the CS and US, the rodent will exhibit a conditioned fear response (CR) when the CS is presented alone. A commonly used US is foot shock, which is predicted by the presentation of a tone CS in a standard cue conditioning study. After one or two pairings, the animal will learn that the tone predicts the presentation of the shock. Subsequently, the presentation of the tone CS

alone evokes a fear response, which in mice constitutes freezing and suspension of all activity apart from breathing (Holmes et al, 2001; Pugh et al, 2000; Wiltgen et al, 2001). In addition to learning a CR of freezing, the rodent also freezes when returned to the training context 24 h later because it has learned that this specific context is where it received shock. Thus, the context itself serves as a configurable CS that predicts the occurrence of the US. This is known as contextual fear conditioning. In both cases conditioned freezing is assessed by measuring the percentage of time spent freezing when the rodent is returned to the same context or presented with the same tone in a modified context. Prior studies indicate that immune challenge disrupts contextual fear condition, but has no effect on cue conditioning.

Fear Conditioning and TMEV

To date, immune challenges have been shown to elicit cytokine activation that disrupts contextual fear conditioning but not cued conditioning (Pugh et al, 1998; 2000; 2001; Ferguson et al, 2003). This suggests that hippocampally-mediated learning and memory capacities are selectively impaired by immune mediated cytokine activation. The present study intends to examine whether contextual fear conditioning is disrupted during acute TMEV infection. We anticipate that impairment will be observed because prior histological and immunological studies indicate

TMEV increases cytokine expression in brain and infection-induced inflammation within the hippocampus.

Whether this inflammation inhibits memory formation, specifically context memory formation, will be ascertained through the paradigm of fear conditioning. As previously stated the ability to recall the tone cue, which is preferentially mediated via the amygdala, will verify the functional integrity of this system. However, context-learning deficits offer behavioral indication that neural infection and inflammation in the hippocampus translates impaired configural learning.

Applications of the Behavioral Characterization of TMEV

Objectively and accurately cataloging the behavioral manifestations of a disease process is an invaluable aspect of PNI research for several reasons. It permits scientists to examine the implications of cellular and molecular phenomena on the global functioning and health of the organism. Internal immunological processes are of limited clinical relevance if they are not related to observable behavior and functional deficits. Behavioral deficits in depression, chronic pain, and chronic disease could be the result of cytokine and immune activity in some cases, rather than disease specific symptoms (Connor & Leonard, 1997; Maes, 1994; Pollack et al, 2000).

Furthermore, variations in behavior can be subtle due to multiple mechanistic origins. Effects usually appear as an attenuation or

exacerbation, not a complete degradation. Therefore, a meticulous analysis of baseline behavioral abilities facilitates the identification of fluctuations, especially when more variables are introduced in more complex designs.

Other Goals of this Investigation

Similar and prior research has been done using other models of immune challenge (LPS, EAE, gp120). The present study will utilize body temperatures, social interaction, sucrose preference, body weight, von Frey, fear conditioning, and activity monitoring as further behavioral and physiological measures of infection. This protocol integrates TMEV research, which lacks behavioral and physiological description, with measures of sickness syndrome used in these other models (Anagnostaras et al, 2003; Campbell et al, 2001; Holmes et al, 2001; McGavern et al, 1999, 2000; Pollack et al, 2000, 2003).

Finally, this study will provide extensive information on the particular strain of virus that is used in our laboratory. It is responsible for piloting the behavioral measures listed above to discern the most informative ones for complex designs involving disease and stress. A succinct and basic investigation, it will serve to clarify previous and future work in the field.

Mice were assigned to one of three conditions: infected, mock-infected vehicle, or uninfected. These groups permitted examination of

infection, the process of infection, and baseline behavioral and physiological characterization of this strain of mice.

METHODS AND MATERIALS

Mice

SJL mice were obtained at postnatal day (pnd) 19 from in-house breeding facilities. They were separated according to sex, then weighed and assigned to cages on the following day. Mice were housed 2-3 per cage and maintained on a 12-h light/dark cycle, with lights on at 0600 h and lights off at 1800 h. They were acclimated to the environment for a week before infection and experimental manipulation on pnd 28. Food and water were available ad libitum. Room temperature was maintained 72-74°F and recorded daily.

Theiler's Virus Infection

On pnd 28 mice were anesthetized with isofluorine and injected intracranially into the right parietal cortex with 20 µl of 5.0×10^5 p.f.u of BeAn strain of Theiler's virus. Vehicle animals were injected with 20 µl of PBS solution following the same procedure.

Fear Conditioning

Fear conditioning was utilized to assess the functional integrity of memory systems during the acute phase of infection when cytokines and inflammation are elevated in the neural memory structures. The procedures were conducted based upon prior work demonstrating that immune

challenge disrupts contextual fear conditioning (Wiltgen, Sanders, Behne & Fanselow,, 2001; Pugh et al., 1999, 2000). Conditioning chambers were housed away from the animal colony; therefore, mice were transported in opaque containers during habituation, conditioning, and testing procedures. On the day 6 pi the mice were habituated to the transportation for a 30 min period. On day 7 pi the mice were conditioned between 13.00 and 16.00 h in Coulbourn Instruments chambers and shockers. Activity was recorded with an infrared video camera and thermal activity monitors built into the chambers. Clear plexi-glass was added to the chambers to prevent the mice from climbing the walls. Data was collected with the Graphic State program.

Conditioning consisted of 2 tone-shock pairings in the novel context. Prior to the first shock, the mice were permitted to explore the environment for 180 s, as this time-period was reported to equalize sex differences in memory consolidation (Wiltgen et al., 2000). A 30 s tone preceded the shock that was concurrent with the last 2 s of the tone (2976 Hz, 74 dB). A 180 s interval preceded the second 30 s cue and shock. The mice were removed 30 s after the last tone and shock had ended.

Context conditioning was measured the following day at the same time of day using the same room and cage environment. The animals were returned to the cages and their activity was measured for a 20 min period. The same opaque containers were used for transport and subjects were

returned to their home cages following the session. The order of testing was consistent with the order of conditioning the prior day.

Cue conditioning was measured approximately 2 h following the context testing. The fear chambers were dramatically altered by replacing the clear plexi-glass walls with black plexi-glass walls and a partition that divided the chamber into a triangular area of half the size. Orange extract was dabbed onto the partitioning wall of each chamber and lights were dimmed in the room. Freezing was measured for 7 min before the animals were removed and returned to the home cage.

Memory of the context or cue was quantified by the amount of time the animals assumed a defensive response, which in mice is freezing or total suspension of movement.

Freezing was analyzed by digital data recorded using heat-imagery technology. This was analyzed using Graphic State software. Video recordings were also scored, with movement or non-movement assessed every 3 s.

Open Field Locomotor Activity:

There is reason to believe that general fatigue and sickness syndrome will affect open field locomotor activity and provides a sensitive measure of sickness behavior in rodents. In the present study, 6 Accuscan open field chambers (Omnitech), equipped with two banks of photocells on

each wall, were used to measure horizontal and vertical locomotor prior to and during infection. These open field boxes are interfaced with a digital multiplexor (Coulbourn E61-58). Testing was conducted in the dark between 15.00 and 19.00 h. White noise was continually present to mask extraneous disturbances. Total activity, a composite of horizontal and vertical activity measures, was sampled 30 / 1 min every min during a 30 min session. During testing, mice were transported to the Digiscan room in clear containers (19 x 12 cm) large enough to house all cage mates, usually 2 mice. The day before the first data collection session, mice were habituated to the room and chambers for 1 h.

Von Frey

Prior research indicates that pain sensitivity is increased following immune challenge. The von Frey procedure provides a measure of pain reactivity to a non-noxious mechanical stimulus applied to the hind-paw. Von Frey i was used to test for mechanical allodynia, which is a pain response to a non-noxious stimulus. Each mouse was placed in a clear plastic container (12.5 cm diameter) on top of an elevated screen (17.8 cm above counter-top), which allowed full access to the ventral aspect of the hind-paws. Prior to testing, the mice were habituated to the experimental context room and apparatus on 2 consecutive days for 10 min per day. On the day of behavioral testing, mice were placed in the experimental context

for 10 min followed by von Frey testing. A logarithmic series of 12 ascending calibrated microfilament von Frey hairs (Stoelting, Wood Dale, IL) were applied randomly to the left and right hind-paws to determine the threshold stiffness in Newtons (N) required for the paw withdrawal. Each trial started with a von Frey force of 1.65 mN delivered to one of the hind-paws for approximately 1 s, and then the other hindpaw. If there was no withdrawal response, the next higher force was delivered. This procedure was performed until a hind-paw withdrawal response was made. Log stiffness of the hairs is defined as a log grams=10,000. The 12 stimuli had the following log stiffness values. The value in grams range between 1.65 g to 4.93 g. Each test session lasted approximately 45 min and occurred at baseline on day –1 and on days 1, 2, 3 and 5 pi. Prior studies suggest there are no differences in mechanical thresholds between left and right hind-paws. Consequently, data obtained from the left and right hind-paws were averaged. In instances when mice failed to respond to the strongest stimulus 6.65 g, the upper cut-off value would be assigned; however, responses were always elicited prior to this cut-off. Monofilaments with greater stimulus intensities lifted the paw and were deemed unreliable. Responses that occurred to the weakest stimulus 0.407 g were assigned the lower cut-off value for that time-point.

Sucrose Preference

One manifestation of sickness behavior is anhedonia, or absence of pleasure seeking behavior. Normally, mice prefer sweet tasting water solutions instead of plain water. However, sickness has been shown to reduce sucrose preference in rodents (Pollack et al, 2000). Mice were given both water and 2% sucrose bottles from day -1 to 7 pi, and intake was measured every 24 h. Preference was determined as a percentage of sucrose versus total fluid intake. Intake on day -1 pi was used as the baseline measure. Bottles remained in the cages at all times and consumption was determined by dividing between the number of mice housed in the cage. To prevent place preference, location of the bottles was reversed daily when the bottles were weighed and refilled at approximately 13.00 h.

Food and Body Weight

Food and body weights were taken daily from day –1 to day 3 pi between 11.00 and 14.00 h. Subsequently weights were taken on a weekly basis during the same time.

Nesting Behavior

Nests were scored daily according to a published scale (Annisman et al., 1998). Complexity and organization were rated as follows: 1= no

apparent organization, no height; 2= some organization, no height; 3= good organization, low height; 4= great organization and height. Nests were also weighed after scoring had taken place.

Body Temperature

Body temperature was taken from day -1 to 3 pi at 09.00, 11.00, 13.00, and 15.00 h. Temperature was measured with a laser body heat thermometer 6 in from the abdomen of each subject 3 times for each time point and the values were averaged. Room temperature was also recorded to account for any variations across groups.

Social Interaction (SIXN)

Mice were videotaped and observed for 3 min while in the presence of a conspecific juvenile that was uninfected and labeled a donor mouse. Interaction was quantified by the origin of initiation and the number of times either the donor or the subject initiated interaction. The procedure was conducted on day 3 pi (Pollack et al, 1999).

Statistical Analysis

Analyses of variance (ANOVA) were used to evaluate difference across the infected, vehicle and uninfected animals. Duncan's New Multiple

Range Test was used as a further means of analysis when the uninfected condition was included. Results were considered significant if $p < .05$.

Overall Study Design

The entire study was divided into an initial investigation occurring during fall 2003 and a replication during the spring of 2004. Due to the challenge of adapting measures to mice and TMEV infection, it was necessary to amend some protocols used in the fall prior for the spring study. Data was not included in analyses if protocol changes were made between replications.

RESULTS

Body Weight

Figure 1 depicts the effects of infection, mock-infected vehicle, and control conditions on body weight expressed as a change from baseline body weight averaged over day 0 to 2 pi. ANOVA indicated a significant effect of infection, $F(2,34)=4.4$, $p<.02$ and day pi $F(2,34)=7.3$, $p<.001$ as a change from baseline. Infected and vehicle mice decreased body weight; this patter is significantly different from the weight gain exhibited in the uninfected condition. There was no interaction of day pi and condition, however. All groups were included in this analysis.

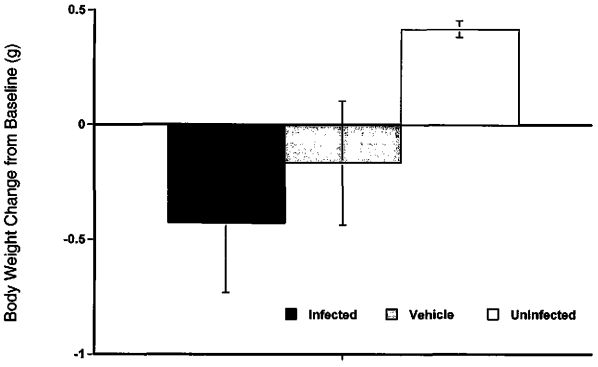

Figure 1. Body Weight Change from Baseline on day 0-2 pi.

The infected and mock-infected vehicle groups lost weight over the first 2 days pi, where as the uninfected controls gained weight, $F(2,34)=4.4$, $p>.02$. Results are expressed in mean weight loss from baseline weight ±*SEM*.

Social Interaction

There was not a significant difference between the infected and mock-infected vehicle mice on this measure, and only mock-infected

the quantity of subject-initiated interactions was displayed in the infected group; however, this trend was not statistically significant, $F(1,11)=.9$, $p<.4$.

Open Field Locomotor Activity

There was no significant difference between infected, mock-infected vehicle, and uninfected groups on activity monitoring during the first day 0-3 pi on the measures of center time ($F(2,26)=1.0$, $p<.4$), vertical activity ($F(2,23)=2.0$, $p<.2$), horizontal activity ($F(2,37)=1.1$, $p<.4$) and center distance ($F(2,25)=.1$, $p<.9$).

Von Frey

As shown in Figure 2, the infected animals exhibited greater mechanical sensitivity on the von Frey test than the mock-infected vehicle animals when tested 5 h pi on day 0. An ANOVA confirmed there was a significant main effect of infection condition, $F(1,16) =12.0$, $p<.003$. This indicates that TMEV infection induces mechanical allodynia beyond the effects of mock-infection. Measures are represented as a change from baseline. Baseline threshold sensitivity revealed a significant difference at between mock-infected vehicle and infected groups, $F(1,16)= 8.0$, $p<.01$. Only infected and vehicle animals were included in this analysis.

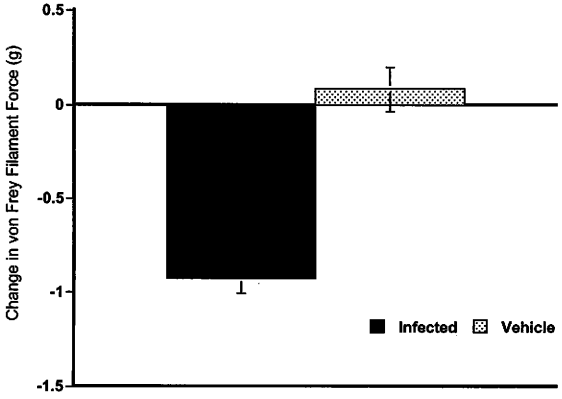

Figure 2: Von Frey Filament Size Change from Baseline

Von Frey filament size decreased significantly in the infected group relative to the mock-infected vehicle group on day 0 pi, $F(1,16)=12.0$, $p<.003$. Effects are presented as a change from the baseline sensitivity level, $\pm SEM$.

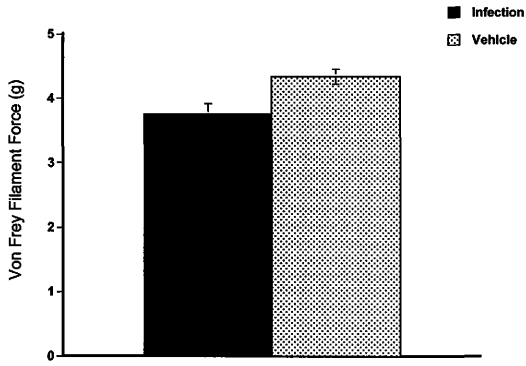

Figure 3: Von Frey Filament Baseline Differences

Von Frey filament size differed significantly at baseline between the infected group relative to the mock-infected vehicle group on day 0 pi, $F(1,16)=8.0$, $p<.01$. Effects are presented as baseline group means of the von Frey sensitivity level, $\pm SEM$.

Body Temperature

Figure 3 reflects the body temperature differences on days 1 and 3 pi expressed as a change from baseline body temperature. Body temperatures were significantly elevated in the infected mice relative to the

mock-infected vehicle mice at 1 and 3 pm on day 1 and 3 pi. On day 1 pi at 1 and 3 pm mock-infected vehicle animals experienced hypothermia, while infected animals had a slight fever, $F(1,22)= 4.7$, $p<.04$. Again on day 3 pi at 1 and 3 pm, the groups significantly differed in the same directions with $F(1,22)=4.9$, $p<.04$.

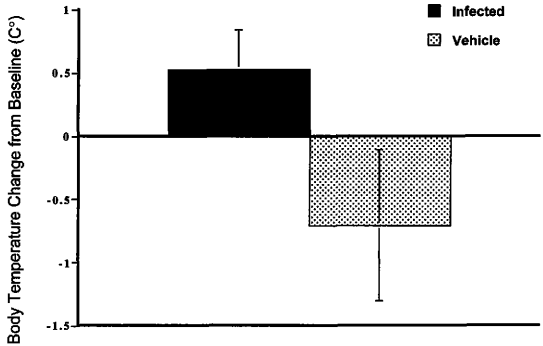

Figure 4. Body Temperature Change from Baseline on day 1 pi.

Body temperatures were collected one day prior to and during the first 3 days of infection. Graphed effects are displayed as an average change from

baseline to day 1 pi, with temperature recorded at the same time pre-infection ±*SEM*, $F(1,22)=4.7$, $p<.04$.

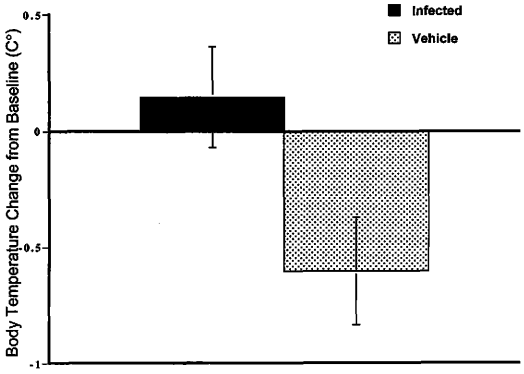

Figure 5. Body Temperature Change from Baseline on day 3 pi. Infected mice exhibited mild fever and mock-infected vehicle mice showed hypothermia. Body temperatures were significantly different between

groups on day 3 pi at 1 and 3 pm, $F(1,22)=4.9$, $p<.04$. Effects are depicted as a change from baseline temperatures at the same time points, $\pm SEM$.

Sucrose Preference

Figure 4 depicts the effect of infection on average sucrose preference on days 0 -3 pi. There was a significant difference between all conditions, with infected and mock-infected vehicle subjects decreasing consumption relative to the uninfected condition, $F(2,40)=36.4$, $p>.0001$. Between groups differences were verified by post hoc means comparisons. Changes are from baseline consumption levels prior to infection; therefore negative values reflect a decrease in preference. The uninfected condition significantly increased sucrose preference from baseline levels. Both mock-infected vehicle and infected conditions failed to preferentially increase sucrose consumption over time, suggesting that the process of infection alone induced a non-specific immune response and CNS mediated sickness behavior.

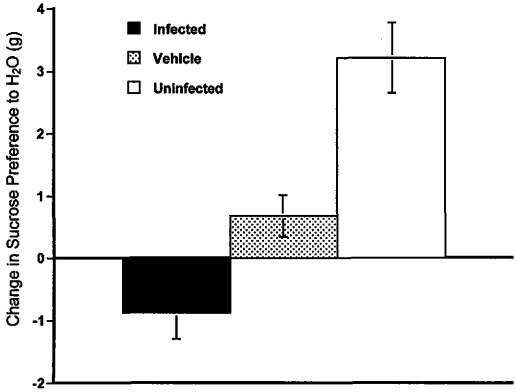

Figure 6. Mean Sucrose Preference Averaged across days 0-3 pi. Sucrose preference was decreased significantly in the infected and mock-infected vehicle groups relative to the uninfected groups on day 0-3 pi $F(2,40)=36.4$, $p<.001$. Post hoc means comparisons revealed significant attenuation of infected preference from mock-infected vehicle preference level. Bars reflect average sucrose intake over pure water expressed as a change from baseline on day 0-3 pi. ± *SEM*.

Fear Conditioning

Fear conditioning was scored by manually and digitally. Neither method reveled significant results, $F(2,25)=.8$, $p<.5$ and $F(3,36)=1.7$, $p<.2$ respectively.

Nesting

Nesting scores $F(1,5)=.8$, $p<.4$ and weights $F(1,9)=2.5$, $p<.2$ failed to yield significant differences between conditions on individual dpi or over the first three days of infection. Due handling the nest and scoring procedures, it is possible that a subtle effect was washed out by the process of data collection.

DISCUSSION

This investigation delineates the behavioral and physiological effects of TMEV infection during the acute phase. Theiler's virus is a bi-phasic disease with the acute phase constituting the first 4 weeks of infection, by prior descriptions, with onset of illness at approximately day 12 pi. There is a paucity of knowledge regarding the specific behavioral and physiological adaptations or deficits during the acute phase, largely owing to its traditional asymptomatic description (Theiler, 1930; Lipton, 1975). The present study is the first to provide a detailed characterization of the behavioral and physiological manifestations of early Theiler's virus infection.

Our results indicate that TMEV infection induces allodynia and anhedonia; both are exacerbated in infected mice beyond the deficits rendered by vehicle mock-infection. However, body weight attrition and body temperature fluctuations do not statistically differentiate between the infected and mock-infected vehicle animals. This finding attests to the cytokine activation following the trauma of anesthesia and the process of infection. Trauma, even placing a rat in a novel environment, can induce a fever (Maier & Watkins, 1998). Therefore, the lack of significance between the groups does not necessarily diminish the behavioral or physiological effects of infection. Rather it calls attention to the significant ramifications of mock-infection and other potential cytokine-activating events. Social interaction showed a trend toward infection specific attenuation, but failed to

reach statistical significance, $F(1,11)=0.890$, $p>0.05$. Finally, nesting behavior, fear conditioning, and locomotor activity revealed no significant differences between conditions.

Cytokine activation sensitizes pathways that relate the pain sensation to an organism's brain. Research examining pain response to non-noxious stimuli, referred to as allodynia, and pain response to noxious stimuli, or hyperalgesia, links these conditions with inflammatory cell activation and cytokine release. Hyperalgesia and allodynia were found in rats that had gp120, the human immunodeficiency protein, microinjected into their spinal cords (Pugh et al,2000). Cytokine activation by this viral protein is responsible for the observed hyperalgesia and allodynia. Therefore, plans are underway to test for the hyperalgesic response in TMEV infected mice. Both responses indicate cyotkine modulation of pain thresholds following immune challenge. Allodynia during acute phase TMEV infection was established using the von Frey filament sensitivity test. Results were commensurate with the magnitude of expected cytokine activation in the infected and mock-infected vehicle conditions.

Cytokine-mediated pain as a symptom of sickness syndrome is relevant to human immune activation, as most individuals who have experienced illness can attest. The presence of allodynia in TMEV replicates pain reports in MS patients that are not attributable to a physical origin. More fundamentally, it correlates a measurable immune system

component with behavioral impairment. Pain reports in MS patients could be tied to the autoimmune attack or immune cell activation that cause MS lesions and inflammations. Identifying such symptoms as resulting from cytokine activity could offer potential therapies for such conditions in MS and other diseases.

Prior studies using IL-1 antagonists demonstrate its necessity in the onset of sickness syndrome. IL-1 production could take place within the brain as well as the peripheral, however the origin and specific cytokine responsible for sickness syndrome is not the focus of this study. It is, however, essential to acknowledge the possible role IL-1 and other cytokines play in these behavioral and physiological observations. The effect on TMEV sickness behaviors of specific cytokine and chemokine antagonists is a future endeavor, with primary interest on IL-1, IL-6 and TNF-α.

Anhedonia, or the lack of pleasure seeking behavior, was also significantly affected by TMEV infection. Sucrose intake was significantly depressed in infected animals relative to the mock-infected vehicle and non-infected conditions. This demonstrates that infected animals lost preference for the sweet water during the first 3 days of infection and exhibited an immune mediated state of anhedonia. EAE behavioral syndrome reported infection specific anhedonia, as did gp120 and other sickness syndrome investigations (Maier & Watkins, 1998; Watkins et al,

Pollack et al, 2000). Our findings further establish the existence of immune and cytokine mediated anhedonia. Additionally, the degree of impairment following mock-infection alone and its relationship to the uninfected and infected conditions was established.

Apart from the results that separate infected and mock-infected mice, some measures failed to differentiate between the two conditions. The residual damage caused by the anesthesia and intracerebral injections obscured findings of body temperature variances and body weight. Cytokine and chemokines are released peripherally by macrophages presented with pathogens; however, IL-1 mRNA, IL-1, and its receptors have been identified within the brain and could be activated during trauma such as sub-cortical injection (Connor & Leonard, 1997; Maier & Watkins, 1998). Although cytokine assays will not be conducted for this project, plans for this research are under way and will delineate the differential impacts of mock and viral infection.

Body temperature data presented ambiguity between the infected and mock-infected vehicle conditions due to the effects of the infection process and anesthesia. The trauma of the surgery rendered body temperature variations that did not allow examination of a unique effect of TMEV infection alone. Day 0 pi was not significantly different between groups, as was reported in the gp120 model where infected groups displayed hypothermia shortly after infection. Instead, days 1 and 3 pi were

significantly different between groups. Fundamental differences such as collection time points and disease models may account for this discrepancy. The gp120 infection process occurs via a previously implanted cannula and thereby removes the effects of surgery and anesthesia encountered in the present study. Furthermore, gp120 is a human viral protein; it is quite different from TMEV that is a natural murine virus. As well, alternative methods of temperature collection might eliminate potential sources of error that accompany manual collection and variations in body surface temperature.

Anorexia is a hallmark of sickness syndrome. However, it failed to be a discriminating factor between infected and vehicle conditions in the present study. Again, this may be a result of the non-specific immune activation arising from the infection process. Acute neurological trauma induces an inflammatory cascade that includes the cytokines and chemokines previously implicated in sickness syndrome. Sickness behaviors are not specific to a pathogen induced immune activation; they can arise from acute trauma, such as surgery. Attenuation of the infection driven weight loss is apparent in the vehicle group, however not statistically significant. When the pathogen, here TMEV, is introduced within the brain, immune cells must communicate its presence throughout the body. Therefore, both central and peripheral responses are evoked in order to prepare the organism to mount a defense against the pathogen. Due to the

compounded effects of systemic and central cytokine production, the infected group reflects the cumulative impact of disease and physical trauma. Again, cytokine data collected both peripherally and inside the brain could discern if the observed pattern of attenuation is a result of the magnitude of cytokine levels between groups. Future studies will address this issue and delineate any relationships between the magnitude of cytokine release and observed behavioral and physiological measures.

Summarily these data indicate that acute phase behavioral symptoms result from the cumulative effects of TMEV infection and the infection process. TMEV infected mice displayed significant differences on von Frey and sucrose preference measures. This evidences that allodynia and anhedonia are viable facets of acute sickness syndrome in the TMEV infection model. An alternative process of infection could alleviate trauma hindering significant differences between infected and mock-infected vehicle groups on body temperature, social interaction, and body weight. Changes in the protocol for fear conditioning and nesting behavior could refine these measures' ability to distinguish between conditions. Future work may address specific cytokine and chemokine activity during the acute phase and the relationship these levels have with the observed sickness syndrome. Moreover, plans to administer increased viral titers exist in order to plot relationships between viral strength, behavioral changes, and physiological deviations accompanying severity of infection. Chronic phase

data is being collected and baseline sickness characterization of this phase should further portray the infection pattern and its impact on behavior and physiological measures throughout infection.

Sickness syndrome is applicable to any immune activation resulting in functional deficits. It is a universal observation that with illness, a degradation of energy stores, mental acuity, and general well-being inherently ensues. Due to the link between behavior and potential remediation with anti-inflammatory treatments, research in this area offers treatment via cytokine levels. The present study verifies that sickness syndrome varies according to the specific infection and suggests that cytokine levels are responsible for the observations. TMEV is an intriguing line of work using a naturalistic viral pathogen to induce measurable sickness syndrome and future studies will seek to flesh out this complex relationship.

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Objective

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Education

Texas A&M University, College Station, TX
Bachelor of Science degree in Psychology with a minor in Spanish in May 2004. University and Fellows Honors Designations from the Honors College.

La Universidad de Complutense
Took classes in Latin American studies for a semester in Madrid, Spain.

Research

Conducted project on the *Behavioral Characterization of Theiler's Murine Encephalomyelitis Virus.*

Presented Poster for Student Research Week at Texas A&M, 2004. Won 1st Place Poster Award.

Presented Poster at Texas A&M Neurodegerative Conference and 3rd Place poster award, 2004.

Attended the Society for Neuroscience Conference as an undergraduate researcher in 2003. Prepared and presented a poster summarizing research at our laboratory. Received a travel grant from Texas A&M faculty of Neuroscience to attend.

Trainee member of Psychoneuroimmunology Research Society.

Awards

Faculty of Neuroscience Travel Grant recipient, 2003

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Academic Achievement Scholarship, 2002