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13. ABSTRACT *(Maximum 200 Words)*

Troops deployed in the Persian Gulf War were exposed to an unusually diverse mix of odorous chemicals at the same time as they were exposed to physiological and psychological stressors B a scenario that research in animal models suggests will lead to the development of specific conditioned responses. The goal of this research is to investigate the extent to which people can acquire stress reactions as conditioned responses to odors and exhibit health symptoms as a result of such conditioning episodes. Thus, the paradigm investigated in this project can serve as a model system for examining and understanding the persistent symptom constellations found in GWS and other stress-mediated syndromes. Due to delays in the approval of the protocol for human research participation, the brief period of time during which we have been able to test human research participants has limited the conclusions that can be drawn at this juncture. However, preliminary results suggest that odors paired with a stressful situation subsequently elicit negative responses when experienced alone. This finding, if supported, has considerable relevance to understanding symptoms that occur in deployment situations, typified by exposure to stressors experienced in the presence of novel odors.

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INTRODUCTION:

The overall goal of the project is to investigate the hypothesis that the symptom constellation of Gulf War Syndrome (GWS) and other stress-mediated illnesses stemming from military deployment can be understood as conditioned responses to chemical odors encountered under stressful conditions (Bouton, Barlow & Mineka 2001). The specific goals of Year 1 were to develop a protocol that could successfully induce a moderate level of stress in the laboratory (Pilot studies), to investigate whether an odor could be conditioned to a psychological stressor in a conditioning session such that re-exposure to that odor alone would subsequently elicit stress and somatic responses (Study 1), and to explore whether odors control responding because they serve as a discriminative stimulus for the occurrence of an adverse (or positive) outcome (Study 1A).

BODY:

Testing of human research participants in the studies listed in the approved SOW for Year 1 did not commence until August 27, 2002 when the Monell Center received approval for testing human participants in the protocol from the US Army HSRRB. The protocol was first approved by the local Institutional Review Board of record (University of Pennsylvania) in October, 2001 and immediately submitted to the US Army HSRRB for their review and approval. However, concerns about the potential risks to research participants from the stress manipulation and exposure to standard commercial fragrance materials and questions about the goals of the research kept the HSRRB from approving the protocol until a favorable review by the Surgeon General's psychology consultant in July 2002 was accepted. Since the HSRRB approval (on August 14, 2002) was received by our institution on August 26, 2002, testing has been conducted on an extended work schedule in an attempt to compensate for the period of time during which we were unable to begin these studies. To date the proposed Year 1, "pilot" study has been completed and "Study 1" has been initiated.

Work Accomplished During IRB Review: During the period from November 2001 through August 2002, extensive pilot tests of the various experimental manipulations were conducted in order to refine the data collection efforts once they were permitted to begin. The environmental testing chambers were upgraded with a two-way sound system and multiple closed-circuit

cameras in order to be able to observe the research participant from multiple angles during the test. Additionally, a new software system for controlling the data acquisition was purchased and the technical staff of the laboratory learned to program the computers for on-line data collection.

The selection of odors for the initial studies was determined through extensive pilot tests to verify their 'novelty' (to preclude stress-associations from being revived during the period between the conditioning and the extinction) and they were equated for intensity as well. Odorants selected are (1) (unpleasant) a 50% v/v mixture of galbanum and hinoki, both of which are commercial fragrance ingredients in Asia, but are judged as highly unfamiliar and unpleasant by most Westerners and (2) (neutral-pleasant) a commercial fragrance mixture known as TEA (proprietary formula- KAO Corporation) (hedonically neutral), which was judged as unfamiliar, but neutral by our Philadelphia respondents.

<u>YEAR 1: PILOT STUDY</u>: The intention of the pilot study was to determine whether the 'Trier Social Stress Test' (TSST) (Kirschbaum, Pirke & Hellhammer, 1993) is an effective elicitor of stress as measured by subjective stress, anxiety and annoyance ratings; in combination with objective physiological measures such as heart rate. Ten research participants (6 females, 4 males) participated in this study and each was tested on the proposed modified TSST procedure. The subjective ratings were obtained 5 times throughout the procedure: when the subject first arrived, immediately after the speech preparation period, during the math task, immediately after the math task and 5 minutes after the math task. Analysis of the stress, anxiety and annoyance ratings revealed an overall significant increase from baseline during the arithmetic task ($p< 0.05$). Post hoc analysis has shown the annoyance factor to be the main effect over time ($p< 0.05$). The objective end points were measured at three times during the procedure: when the subject first arrived, immediately after the preparation period, and immediately after the math task. Analysis of heart rate revealed a significant increase from baseline to the post-preparation period ($p< 0.01$). Heart rate remained significantly elevated throughout the procedure ($p< 0.05$) compared to baseline. Thus, both subjective and objective measures support that the TSST is effective in eliciting stress responses in participants. Therefore, we determined that no further elaborations of the stress manipulation were necessary in order to produce the required level of 'moderate' stress and that the modified TSST would be more than adequate for the main

experiments. Salivary cortisol samples were collected at various times during the procedure for comparison with the subjective stress ratings. However, due to the costs involved in analyzing small batches of samples, we have elected to have these analyzed with the first set of samples from Study 1.

YEAR 1 STUDY 1: The objective of this study was to investigate whether an odor can be conditioned to a psychological stressor such that a subsequent re-exposure to this odor alone could elicit the same stress responses.

Research Participants: Thus far eleven research participants (6 females, 5 males) have been tested on each of two visits, each visit lasting approximately two hours in duration. Participants were assigned to the groups as listed in the design table below.

Table 1. Design of Study 1

Group	Conditioning Phase	Test Phase
1 (Congruent)	CS_b + 20 min.stressor CS_a + 20 min. relaxation	CS_a - HR/Resp/Startle/Cog. CS_b - HR/Resp/Startle/Cog.
2 (Incongruent)	CS_a + 20 min.stressor CS_b + 20 min. relaxation	CS_a - HR/Resp/Startle/Cog. CS_b - HR/Resp/Startle/Cog.
3 (Control)	+ 20min. stressor - 20 min. relaxation	CS_a - HR/Resp/Startle/Cog. CS_b - HR/Resp/Startle/Cog

Design: Groups 1 and 2 will be exposed to each of two odors (CS_a and CS_b) that vary in their sensory and hedonic properties. As determined in earlier studies, CS_a will be a neutral odor and CS_b will be an unpleasant odor. As shown in the table below, for Group 1, the odors will be congruently paired with the US (unpleasant odor-stressor or neutral odor-relaxation); for Group 2, they will be incongruently paired. A control condition, Group 3, will be exposed to the US (stressor vs. relaxation) but without an odor, in order to evaluate the strength of conditioning that occurs to the context (room) alone. During the conditioning phase and the test phase, we will monitor heart rate and respiration rate of each participant, as measures of autonomic arousal; we will also evaluate subjective symptom reports and mood. The test phase will utilize these

measures as well as several additional dependent measures, including a test of cognitive function (short-term and general memory performance) and startle evocability. In both conditioning and test phases we will collect salivary samples 8 times in order to measure cortisol levels. Sixteen research participants will be tested in each group, yielding a total of 48 research participants.

Procedure: The study was introduced to the participant as a study about the influence of odors on cognitive performance and attention. Table 2 describes a timetable and schedule of dependent measures. During Session 1 (the conditioning session), research participants filled out personality questionnaires for half an hour, to allow for serum cortisol levels and any anticipatory stress related to participating in a study to decrease to a comfortable baseline level. Thereupon, the participant entered the environmental chamber, where electrodes were connected to the subject's body for 10 minutes of baseline biomonitoring of autonomic endpoints. After 10 minutes elapsed, , the subject was administered a modified version of the Trier Social Stress Task (TSST). The TSST is a mental stress provocation task consisting of a 10 minute preparation/anticipation phase and a 10 minute performance-under-stress phase (Kirschbaum, Pirke, & Hellhammer, 1993). The participant was given 10 minutes to prepare 5-minute oral presentation that would be videotaped and judged by a panel of judges. This instruction coincided with the dispersion of a detectable concentration of the Conditioning Odor, which was either hinoki/galbanum ($CS_{a)}$ or TEA ($CS_{b)}$. After 10 minutes of preparation, the experimenter announced the end of preparation and the start of the speech via intercom, and the (sham) videotape was started. Following the completion of the speech, the participant was given a mental arithmetic task to perform for 5 additional minutes, during which the experimenter prompted the subject via intercom. After completion of the TSST/Stress-Conditioning phase, the subject was given a 10 minute rest period while the chamber odor was purged. They were then brought back to the chamber for the second half of the conditioning phase, consisting of a different odor paired with relaxation instructions.

Two days later, research participants returned for Session 2 (the test session). During this session, all research participants were exposed to the two odors for 30 minutes each in the chamber (in counter-balanced order) during which they completed cognitive tasks, sensory ratings while various physiological endpoints were measured.

Measures:

Table 2: Study 1, Sessions 1 and 2: Timetable of events and endpoints

Prechamber		Chamber	Session 1: Odor a/b + Stressor Session 2: Odor a/b + Test			Both sessions: Odor b/a + Relaxation		
	T-40	T-10	T0	T+10	T+20	T+30	T+40	T+50
Questionnaires	X							
Cortisol	Y	Y	Y	Y	Y	Y	Y	Y
VAS	Y	Y	Y	Y	Y	Y	Y	Y
Symptom		Y			Y			Y
Mood		Y			Y			Y
Intensity ratings		Y*						
HR/Resp/EDA		Y**						
Startle			Z	Z	Z	Z	Z	Z
Memory			Z**			Z**		

Note: The symbols X, Y, Z denote when the given measures were collected: X was measured only during Session 1, Y during both Session 1 and 2, and Z only during Session 2.

* Odor intensity ratings were collected every 5 minutes throughout the stay in the chamber

** These measures were collected continuously throughout exposure

VAS = Visual Analog Scales, HR= Heart Rate, Resp = Respiratory Rate, EDA=Electrodermal Activity, Startle= Startle Evocability

The following endpoints were measured *at both conditioning and test sessions*:

Stress: Salivary samples for cortisol assessments were obtained upon arrival (Baseline 1: T-40), just prior to entering the chamber (Baseline 2:T-10), 10 minutes after entering chamber (Baseline

3:T0), 10 minutes into the preparation for the TSST (T+10), 10 minutes into the performance phase of the TSST (T+20), and 10, 20, and 30 minutes into the relaxation phase (T+30, T+40, and T+50). Subjective ratings of perceived stress and anxiety were rated on Visual Analogue Scales at the same time-points when saliva samples were obtained. The one change we instituted in this procedure that became apparent during pilot tests was the addition of a swish and spit with mineral water immediately before each saliva sample in order to overcome the effects of 'dry mouth' and to obtain sufficient saliva for analysis.

Odor, irritation and annoyance intensity ratings: While in the chamber, the subject rated the intensity of the odor, sensory irritation and annoyance on a computer version of the Labeled Magnitude Scale every five minutes.

Mood State: Current mood states were assessed, using the Profile of Mood States, just prior to entering the chamber and after the CS+ and CS-conditions.

Health symptoms: Health symptoms were rated on a laptop just prior to entering the chamber, and after the CS+ and CS-conditions.

Autonomic arousal: Respiratory rate, heart rate and electrodermal activity were continuously monitored throughout baseline, the stressor task and the relaxation phase, using the Lablinc V system (Coulbourn, Allentown, PA).

The following endpoints were measured *only during the test phase*:

Startle evocability: To evaluate whether differential autonomic reactivity occurs to the CS+ and the CS-, we measured startle evocability to an auditory stimulus (95-dBA burst of white noise lasting for 100 ms).

Cognitive Function: To evaluate the degree to which conditioned stress can impair cognitive function, we administered the California Verbal Learning Test (CVLT) as a measure of learning and memory compared with the subject's own assessment of their performance on these tests. The CVLT was obtained twice during the test session: once during each phase (stress vs. relaxing odor).

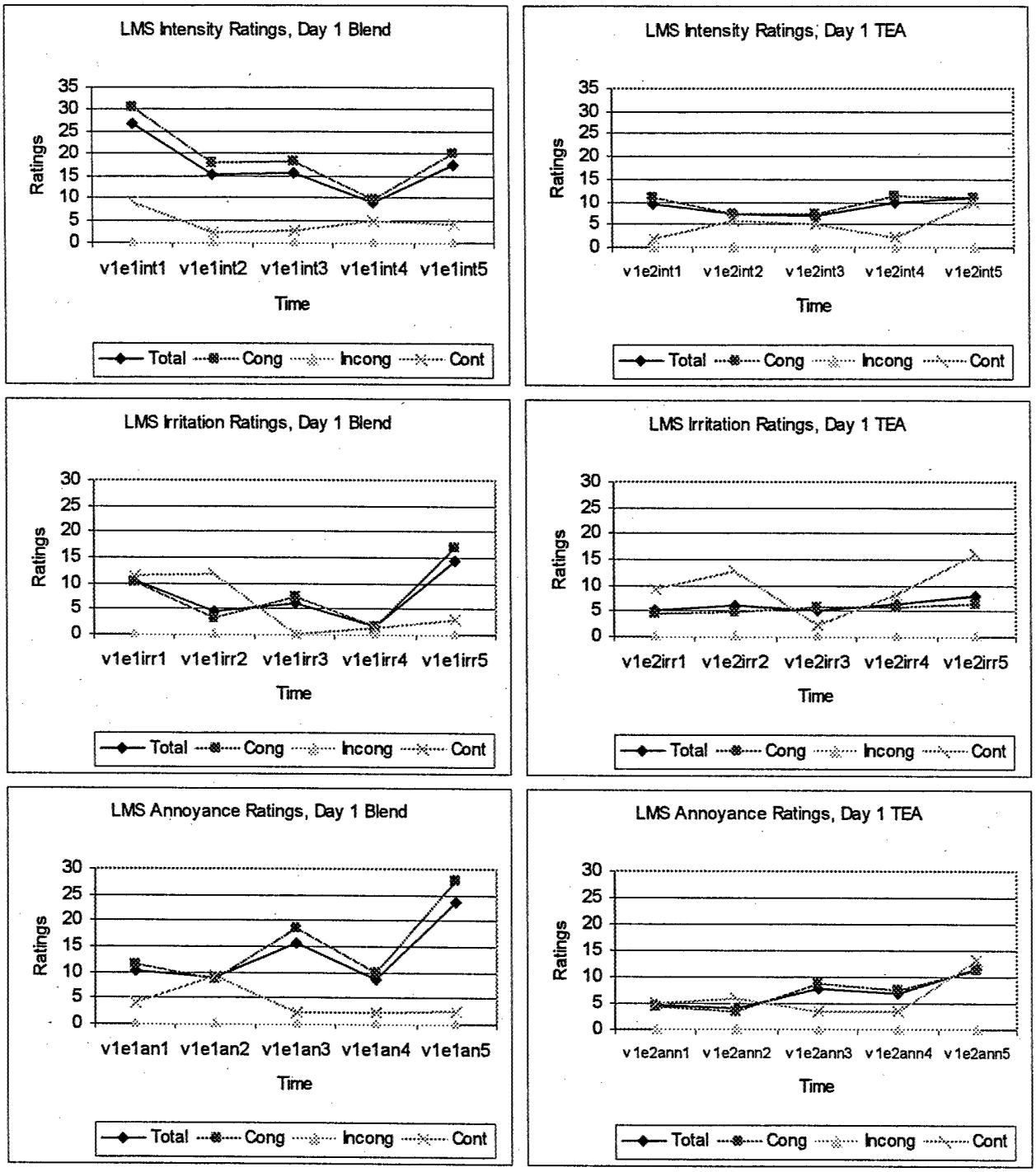
Results: To date, research participants have been tested in the proposed "congruent odor / stressor" and "no odor" conditions. During session 1, the subjective ratings for intensity,

irritation and annoyance are markedly higher for the stressor condition (+ negative odor) than for the relaxation condition (+ positive odor) for visit one (see Figure 1 a-c). Additionally, on the second visit subjective ratings of intensity, irritation and annoyance were again noticeably higher for the negative odor exposure than for the positive odor exposure (see Figure 2 a-c). Visit two consisted of an exposure to both odors (positive and negative),which were paired with the completion of the modified CVLT(California Verbal Learning Task) (Vasterley, Brailey, Consntans & Suker, 1998). Overall, performance on the CVLT was worse for the negative/stress odor when compared to the positive/relaxation odor.

Salivary cortisol samples were collected periodically during both visits and will be analyzed as an objective measure of stress to contrast with the self-rated levels of stress that were also collected (Kirschbaum & Hellhammer, 1989). Our statistical consultant, Dr. Jeffrey Wang, is currently evaluating the data collected thus far in order to suggest some additional analyses that might be performed.

Figure 1 (a-c) Intensity ratings of odor, irritation and annoyance during conditioning sessions

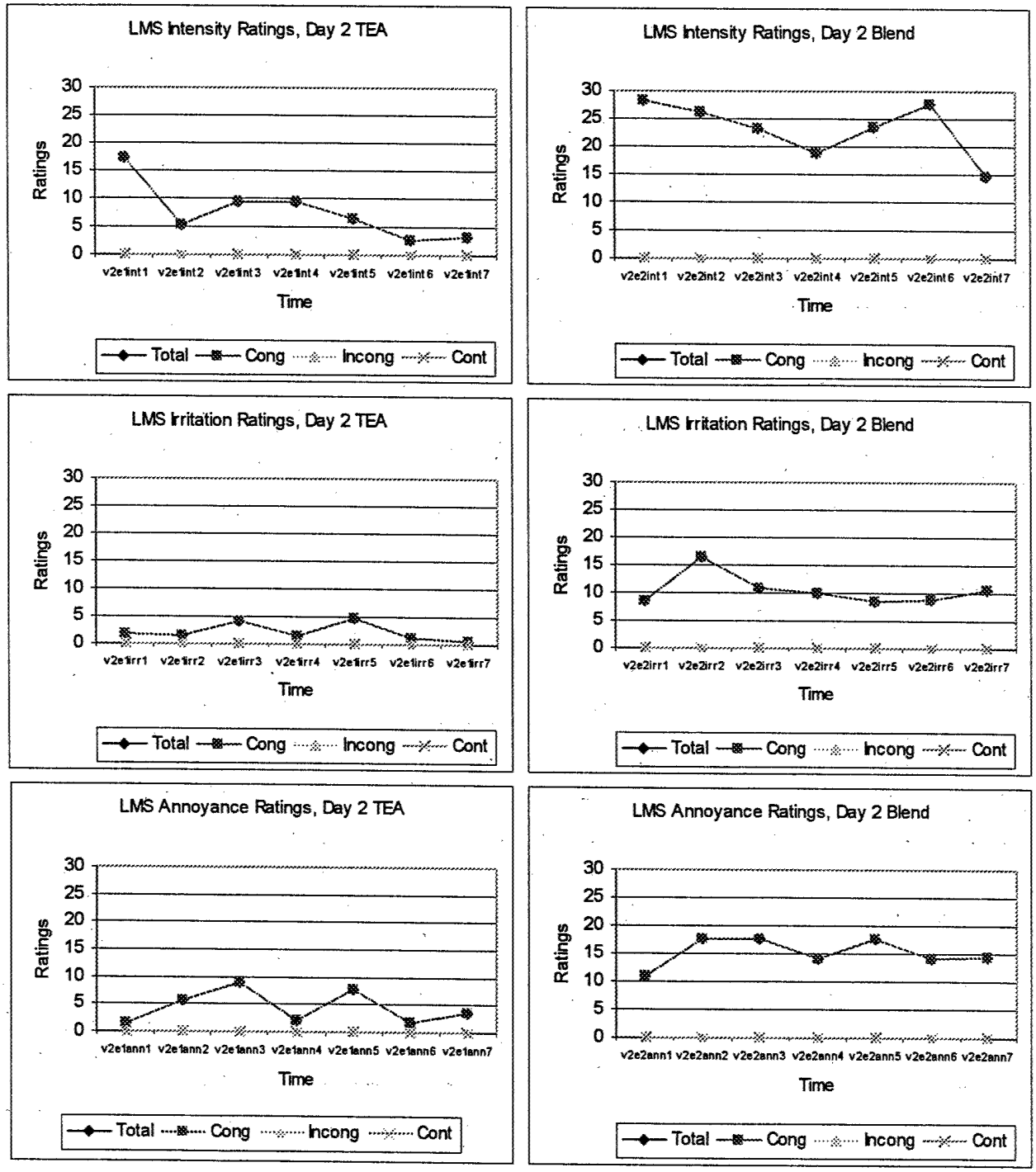
Unpleasant Odor *Neutral Odor*



Note: No research participants have yet been tested in Incongruent Condition, hence the zero values on graph.

Figure 2 (a-c) Intensity ratings of odor, irritation and annoyance during test session.

Unpleasant Odor *Neutral Odor*



Adjusted Timetable

Our inability to commence human testing until late August of 2002 obviously impacted on the proposed timetable for Year 1 goals. However, with 25% of the research participants tested in Study 1, we anticipate that completion will occur in mid-November, whereupon we will immediately begin data collection in Study 1A. As Study 1A (as well as Studies 2-3) uses the same types of dependent measures and data collection tools that are being used in Study 1, we anticipate no difficulty with a smooth transition between the two studies. Based on our timetable for Study1, we anticipate completion of Study 1A by early January. Thus, by mid-January, we anticipate being able to return to the original schedule of proposed studies to be completed during Year 2.

KEY RESEARCH ACCOMPLISHMENTS:

- Determined the ability of the modified Trier Stress Test to elicit the required moderate level of stress that was evidenced on multiple indices, including: self-reported stress and anxiety, increase in autonomic arousal (Heart rate, EDA).

REPORTABLE OUTCOMES:

None to date, due to the unforeseeable delay in receiving HSRRB approval for commencing human testing.

CONCLUSIONS:

Unfortunately, the brief period of time during which we have been able to test human research participants has limited the conclusions that can be drawn at this juncture. However, preliminary results suggest that odors that are paired with a stressful situation subsequently elicit a negative response to the odor alone. This response can be seen in self-reported annoyance to the odor, self-reported stress ratings during odor exposure, increases in heart rate and performance on a cognitive learning and memory task. Of course, these results are based on only 25% of the population to be studied, hence, they must be regarded with appropriate cautions. If supported, however, this finding ha relevance to understanding the often diffuse symptom complex that individuals report following their exposure to situations where stressors are initially experienced in the presence of novel odors (e.g., many different types of

deployment situations). Thus, the paradigm investigated in this project can serve as a useful laboratory-based model system for examining and understanding the persistent symptom constellations found in GWS and other stress-mediated syndromes.

The Gulf War exemplified a trend of increasing threat posed by chemical warfare and biological weapons, accompanied by improved access through media, internet and other resources, to information about the nature and hazard potential of these agents. The combination of actual threat of exposure to dangerous agents and their odors, and the knowledge about the hazard potential and health effects of exposures, introduces a new factor to modern warfare that needs to be acknowledged and understood. This factor is the increased likelihood of a syndrome of health symptoms brought on by potential exposure to probably hazardous odors, their feared effects, and their stress potential. The prospect for GWS –like illness extends beyond the Persian Gulf War, and is likely to intensify.

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APPENDICES: None