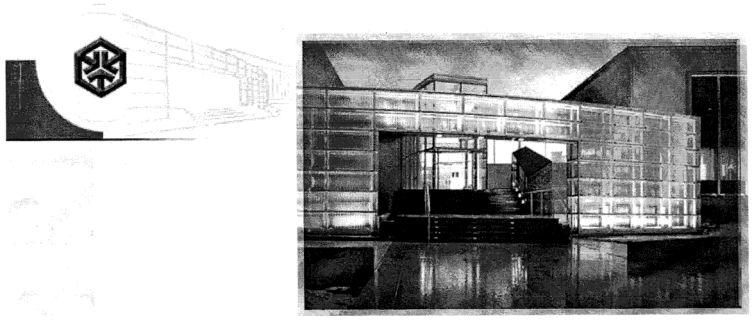
Appendix A

Presentation 1 – Antonio Sastre



Physiological and Genetic Aspects of Autonomic Dysfunction in Gulf War Veterans

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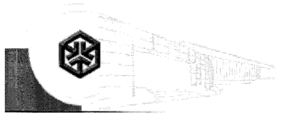
Presentation to the U.S. Department of Veterans Affairs
Research Advisory Committee on Gulf War Veterans' Illnesses
June 16, 2003

Introduction and Terminology

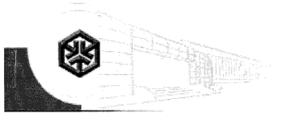
- **Physiological and Genetic Aspects of Autonomic Dysfunction in Gulf War Veterans**
  - Physiological: referring to the objective, quantitative measure of function of organ systems of the body
  - Genetic: dealing with inherited components of DNA which are eventually transcribed into proteins
  - Autonomic: the involuntary, autonomic nervous system (ANS), as distinct from the conscious or somatic (voluntary) nervous system
  - Dysfunction: meaning a failure to function within the range considered consistent with health



Overview of the Project

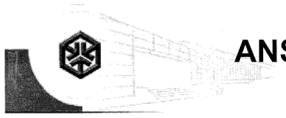
Two integrated studies (total of 387 veterans) provided evidence that:

- Veterans suffering from Gulf War Illness (GWI) differ from controls on a broad range of objective physiological measures of ANS function.
- In addition to case/control differences, ANS measures also differ significantly by BChE genotype.
- Genotype interacts with case status: ANS responses are different in GWI cases vs. controls depending on genotype.
- Genotype interacts with exposures: Veterans with variant BChE genotypes appear to be at substantially increased risk for GWI, but only if they experienced certain exposures.



General Introduction

- The ability of the body to respond to environmental and internal perturbations depends on non-conscious input and feedback from the autonomic nervous system (ANS).
- Perturbations include:
  - Gravity
  - Temperature
  - Exercise
  - Stressors (internal and external, including exposures)
- The ANS is composed of the sympathetic (mostly adrenergic) and parasympathetic (cholinergic) branches.
- These branches have generally opposing actions in target tissues, but often with an asymmetric distribution: the parasympathetic is much more selective.



ANS Feedback and Integration

- The ANS receives feedback information from multiple sensors - intra-arterial pressure sensors, pulmonary stretch receptors, skin thermal sensors, etc.
- Feedback information is sent via *afferent* nerves to pontine and medullary centers, where it is integrated and in some cases modulated by descending input.
- *Efferent* output then goes via cranial, vagus or sacral nerves, or through the intermediolateral columns of the spinal cord, the sympathetic ganglia, and the sympathetic nerves to the target organs.

Everyday Example of ANS Integration

- When rising from a supine (horizontal) or sitting position to standing, we take for granted that we are ***not*** going to faint.
- The following complex sequence of events occurs ***without any conscious awareness***:
  - Gravity immediately pulls blood down to our feet
  - Lowered blood pressure is sensed in aortic and carotid baroreceptors and info conveyed to pons and medulla
  - Increased sympathetic firing to resistance arterioles increases mean arterial pressure, and to lower leg veins to help increase venous return
  - Increased efferent sympathetic firing to SA node increases heart rate; increased firing to ventricles increases force of contraction; both combine to increase cardiac output

Why is ANS Function Important?

- Autonomic function, such as that described for standing up, is involved in multiple essential body processes.
- We take those body processes, and their autonomic regulation, for granted. Examples:
  - Pupillary adjustment to changes in light intensity
  - Processing and digestion of food
  - Sugar and fat metabolism
  - Thermoregulation
  - Blood pressure control
  - Aspects of reproductive function
- We only become aware of the importance of autonomic processes when there is some degree of autonomic dysfunction.

Complexity of What Can Go Wrong (cf. Kapoor, JAMA, 5/7/2003)

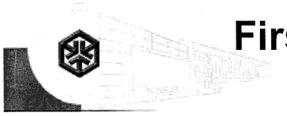
- Neurally-mediated syncope (fainting) induced by tilt-testing, can occur by at least 3 kinds of distinct mechanisms, which must be differentiated:
  - Neurally-mediated sudden hypotension, bradycardia, or both when the volunteer is kept upright;
  - Postural orthostatic tachycardia syndrome: tachycardia upon volunteer being brought upright that persists through procedure;
  - Dysautonomia: gradual decrease in blood pressure with little or no change in heart rate during the procedure.

Optimization of Tilt Testing Protocols

- There is no standardized approach for tilt-testing across medical centers, whether in time, tilt angle, or use of vasoactive agents.
- Highly-aggressive protocols have lower specificity and may be at increased risk for false-positives.
- Our approach, after an exhaustive examination of the clinical and experimental literature, was a protocol that maximized our ability to obtain useful data and preserve specificity while eliminating discomfort for the volunteers.

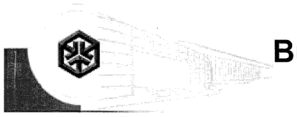
Syndromes and Illnesses

- A number of 'post-war syndromes' have been described.
- The unexplained symptoms and conditions reported by Gulf War Veterans (GWV) have been labeled 'Gulf War Syndrome' by media reports.
  - *Problem*: Review panels have concluded that there is no single, unique syndrome linked to Gulf War service.
  - *But*: Investigators using different study designs describe a fairly consistent set of symptom types and illness categories that occur at higher rates in GWV.
- Hence, we will refer to these symptom types and illness categories as Gulf War Illness (GWI).



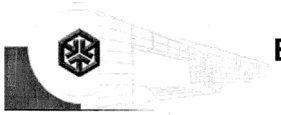
First Component of Our Hypotheses

- We hypothesized that sufferers of Gulf War Illness (GWI) i.e., cases, would differ in **ANS reactivity** from suitable controls.



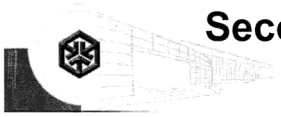
Butyrylcholinesterase (1)

- Butyrylcholinesterase (BChE) is a soluble, circulating and tissue enzyme that hydrolyzes acetylcholine (ACh) and several other drug classes; it is believed to serve as a 'scavenger' for those drug classes.
- BChE exhibits considerable genetic variation in humans.
- BChE's genetic variation is of importance in clinical medicine; carriers of some mutations will exhibit considerable morbidity upon exposure to paralytic drugs used during surgery.



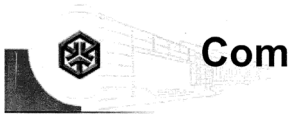
Butyrylcholinesterase (2)

- The long-held belief that BChE does not play a role in normal cholinergic physiology is under re-examination as a result of Lockridge's results with the AChE(-/-) double knock-out mouse.
- Soreq and others have conjectured non-enzymatic roles for BChE in mammals and other higher vertebrates.
- At the time we began our study there were preliminary data suggesting a link between carriers of low-velocity BChE genetic variants and GWI.



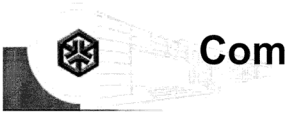
Second Component of Our Hypotheses

- We hypothesized that our data would support the conjectured link between **carriers of low-velocity BChE genetic variants** and **GWI**.
- We hypothesized that ANS reactivity would also differ depending on the **genotype of BChE.**
- We further hypothesized that **case status and BChE genotype would interact**, as revealed in response to ANS stressors.



Complementary Studies (1)

- To test the various aspects of our hypotheses, we designed two complementary, interdependent studies:
- *Study 1* was designed to
  - Obtain BChE genetic data, demographics, exposure and epidemiologic data on a substantial number of cases and controls
  - Test whether a link exists between carriers of low-velocity BChE genetic variants and GWI, using a rigorous case definition
  - Identify a number carriers of low-velocity BChE genetic variants who could be invited to participate in physiologic tests in *Study 2*

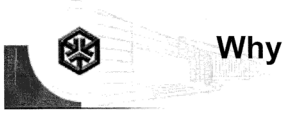


Complementary Studies (2)

- *Study 2* was designed to:
  - Examine GWV cases and controls, as well as non-deployed (PGW-era) veteran controls
    - who served with only one of two Army units
    - deployed 8/1990 – 7/1991
    - enlisted personnel only
  - Examine BChE variant carriers from *Study 1,* and determine genetics of new volunteers
  - Examine ANS normal function and reactivity through a multi-faceted *battery* of tests

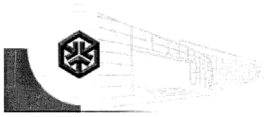
Why a ***Battery*** of ANS Tests (1) ?

- The ANS consists of numerous interacting feedback loops with distinct neuroanatomical pathways, integrative and regulatory centers, several neurotransmitters, and dozens of physical triggers and regulators.
- No one test, or small number of tests, can capture all the functions of the ANS.
- It is too easy to obtain false negative or misleading results in ANS studies through an unduly restrictive choice of tests.
- Most medical and research centers use a battery of tests customized for specific purposes.



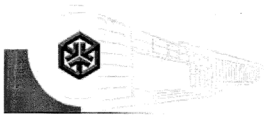
Why a ***Battery*** of ANS Tests (2) ?

- For example, based on Valsalva and temperature tests only, Sharief et al (*Neurology* ***59***:1518 [2002]) reported "…no objective abnormalities of autonomic nervous system…in symptomatic veterans." (p.1524)
- If we had only performed Valsalva maneuvers, our data would have shown no difference between case and control veterans.
- As will be seen below, a conclusion of no difference in autonomic function between cases and controls for our volunteers would have been ***erroneous***.



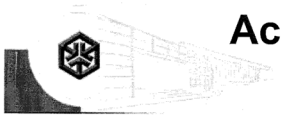
Is a Battery of Tests a 'Fishing Expedition'?

- Concerns can be legitimately be raised about multiple tests leading to spurious results.
- Due to the recognized complexity of the ANS, especially with respect to cardiovascular reflexes, ANS test batteries have been developed and guidelines issued in Consensus Statements by the American Autonomic Society and the American Academy of Neurology.
- When examining and analyzing the data, one looks for ***consistency*** and ***coherence*** of findings across tests, corrects statistically for multiple comparisons (when present), and maintains healthy skepticism.



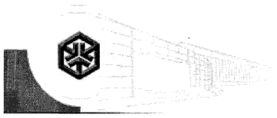
The Research Team

- All work was performed jointly with:
  - Drs. Mary R. Cook (**co-PI** at MRI; overall design and Physiology) and Mary M. Gerkovich (Statistics at MRI)
  - Dr. Lea Steele (Epidemiology; Kansas Commission on Veterans Affairs, Kansas Health Institute)
  - Dr. Oksana Lockridge (Mol. Bio. and Biochem; Eppley Institute, University of Nebraska, Omaha)
- And with the highly-skilled assistance of:
  - Dr. J. Hackman
  - Mrs. D. D. Dozier, Mr. S. Hoffman, Mrs. R. C. Peterson, Mrs. K. Whitson



Acknowledgements and Disclaimers

- All protocols were approved by the Midwest Research Institute's Institutional Review Board and by the Surgeon General of the Army's Human Subjects Research Review Board.
- This research was supported by the U.S. Army Medical Research and Materiel Command under contract USAMRAA DAMD17-00C-0018. Opinions, interpretations, conclusions and recommendations are those of the investigators and do not necessarily reflect an official position or opinion of the U.S. Army.



Study Design: ANS *Study 1*

- **Population-based**: *Random* sample of Gulf veterans living in Kansas City area (from Kansas Gulf veterans database, DOD-provided list of Missouri Gulf veterans)

  Case/Control Design: Cases met Kansas "Gulf War Illness" case definition; CDC definition also assessed

- **Final sample**: 304 Gulf War veterans: 144 cases, 160 controls

- Participants screened by telephone interview to determine study eligibility, filled out questionnaire, came to study site to provide blood sample

Study Design: ANS *Study 1*

Eligibility Criteria: both Cases and Controls:

- **Lived in Kansas City area**
- **Deployed to Persian Gulf** any time between August 1990 and July 1991
- **Never diagnosed** by physician for: diabetes, heart disease, stroke, lupus, multiple sclerosis, cancer, liver disease, conditions associated with psychosis
- **Not hospitalized** since the Gulf War for: alcohol or drug dependence, PTSD

Case Definition: Gulf War Illness (GWI)

Kansas GWI Definition

- Empirically derived from 1998 study of 2,030 Kansas Gulf War-era veterans
- Based on symptoms reported from 6 highly correlated domains, in pattern that differentiates Gulf War veterans from similar veterans who did not serve in the Gulf War
- Requires multiple or moderately severe symptoms in at least 3 of 6 domains:
  - » ***Neuro/cognitive/mood symptoms***
  - » ***Fatigue/sleep problems***
  - » ***Pain symptoms***
  - » ***Gastrointestinal symptoms***
  - » ***Respiratory symptoms***
  - » ***Skin problems***

Study 1 Methods - Biochemistry and Molecular Biology of BChE

- Phenotyping by velocity with benzoylcholine as substrate, followed by
- Inhibition profile with dibucaine, sodium fluoride, and RO 2-0683 (dimethylcarbamate of [2-hydroxy-5-phenylbenzyl]-trimethylammonium bromide)
- For ambiguous phenotypes, genotyping is performed:
  - Amplification of genomic DNA
  - Four primers for 2 PCR amplifications are created
  - A amplification creates a Mae III restriction site when the K-variant ACA codon (Thr 539) is present
  - B amplification creates a Bgl I restriction site when the GCA codon (Ala 539) is present

Study 1 Methods - Biochemistry and Molecular Biology of BChE (2)

- Not necessary to genotype samples that phenotype as heterozygous for the A variant (Asp 70->Gly) because dibucaine inhibition is extremely accurate.
- Samples that phenotype as heterozygous for the F variant were sequenced to determine which of the three reported mutations were responsible for F resistance.
- One sample was found whose inhibition values were a novel set; a single mutation was found in one allele. Codon 70 had C in place of G, changing Asp 70 (GAT) to His (CAT), nucleotide 208G->C. No other mutations were found in the coding region. The presence of the mutation was confirmed by repeating the PCR and sequencing in both directions.
- The k_{cat} value for benzoylcholine was determined by measuring Vmax and titrating the active sites with chlorpyrifos oxon.

ANS *Study 1* Results: Genotype

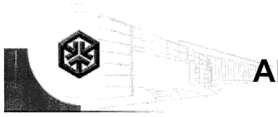
Association of GWI with BChE Genotype

BChE Genotype	% Cases (n=144)	% Controls (n=160)
U/U	62 %	62 %
U/K	28 %	29 %
K/K	5 %	4 %
U/AK	3 %	3 %
U/A	1 %	1 %
A/F	0 %	1 %
AK/F	1 %	0 %

ANS *Study 1* Results: Genotype

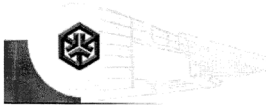
Association of GWI with Variant Status

BChE Genotype	% Cases (n=144)	% Controls (n=160)	OR (95% C.I.)
U/U and U/K	90%	91%	1.0
All other variants	10%	9%	1.12 (0.52 - 2.44)



ANS *Study 1* Results: Conclusions

1. **Overall, *no* independent association was found between BChE genotype and Gulf War illness**



ANS *Study 1* Results: Locations/Experiences in Theater

- "Exposure" information was obtained from questions asked about specific locations and experiences (as opposed to exposures) in theater
- For example, we asked: ***"Did you come into direct contact with destroyed enemy vehicles?"***
- We did **not** ask: ***"Were you exposed to depleted uranium?"***

ANS *Study 1* Results: Locations

Association of GWI with Locations in Theater

	% Cases (n=144)	% Controls (n=160)	OR (95% C.I.)
Kuwait	64 %	39 %	**2.80** (1.75 -- 4.49)
Eastern Saudi Arabia	89 %	76 %	**2.59** (1.35 – 4.96)
Iraq	46 %	32 %	**1.80** (1.12 – 2.89)
Bahrain	57 %	55 %	1.09 (0.69 – 1.73)
Northern Saudi Arabia	22 %	23 %	0.94 (0.54 – 1.64)
Western Saudi Arabia	10 %	12 %	0.81 (0.39 – 1.68)
At sea in the Persian Gulf	9 %	18%	**0.44** (0.21 – 0.90)

ANS *Study 1* Results: Exposures

Association of GWI with Experiences in Theater

	% Cases (n=144)	% Controls (n=160)	OR (95% C.I.)
Wore uniform treated with pesticides	27 %	9 %	**3.72** (1,91 – 7.21)
Took NAPP pills (Pyridostigmine Br; PB)	72 %	44 %	**3.21** (1.91 – 7.21)
Used pesticide cream/spray on skin	57 %	31 %	**2.89** (1.80 – 4.64)
Saw Iraqis/civilians badly wounded or killed	65 %	40 %	**2.71** (1.70 – 4.31)
Contact w/destroyed enemy vehicles	60 %	36 %	**2.63** (1.65 – 4.18)
Saw/contact with dead animals	54 %	34 %	**2.20** (1.38 – 3.51)
Received 1 or more shots in arm in theater	73 %	58 %	**2.00** (1.21 – 3.29)
Directly involved in ground combat	32 %	25 %	1.42 (0.86 – 2.36)
Saw living area sprayed/fogged with pesticides	22 %	17 %	1.33 (0.74 – 2.37)
Saw U.S. troops badly wounded or killed	39 %	33 %	1.31 (0.82 – 2.11)
Heard chemical alarms sound	59 %	53 %	1.31 (0.83 – 2.07)

ANS *Study 1* Results: Conclusions

1. Overall, *no* independent association of BChE was found between BChE genotype and Gulf War illness

2. **Some locations and exposures in theater *were* associated with increased GWI risk**

ANS *Study 1* Results: Interaction of Genotype with Exposures

- BChE genotype, alone, was not associated with an increased risk of GWI.
- But illness risk was higher for veterans who carried variant BChE genes who also reported several specific exposures in theater.
- For our analyses, non-variants were genotypes U/U and U/K; all others were considered "variants."

ANS *Study 1* Results: Interaction of Genotype with Exposures

Association of GWI with Exposures

	NonVariant Subjects Only OR (95% C.I.)
Directly involved in ground combat	1.18 (0.69 – 2.02)
Saw Iraqis/civilians badly wounded or killed	2.34 (1.44 – 3.80)
Contact with POWs	2.26 (1.39 - 3.67)
Frequently had < 4 hours sleep in 24 hours	1.89 (1.15 – 3.08)
Received 1 or more shots in arm in theater	1.71 (1.02 – 2.86)
Saw/contact with dead animals	1.75 (1.07 – 2.85)
Took NAPP pills (PB)	2.68 (1.62 – 4.44)

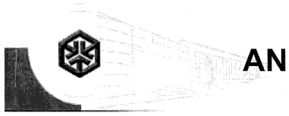
[NOTE: Associations in non-variant veterans are weaker than in the study population as a whole]

ANS *Study 1* Results

Interaction of Genotype with Exposures

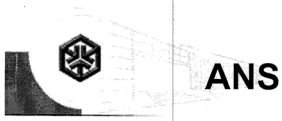
	Non-Variant Subjects Only (n= 276) OR (95% C.I.)	BChE Variant Subjects Only (n = 28) OR (95% C.I.)
Directly involved in ground combat	1.18 (0.69 – 2.02)	8.00 (1.28 – 50.04)
Saw Iraqis/civilians badly wounded or killed	2.34 (1.44 – 3.80)	15.00 (2.26 – 99.64)
Contact with POWs	2.26 (1.39 - 3.67)	15.00 (2.26 – 99.64)
Frequently had < 4 hours sleep in 24 hours	1.89 (1.15 – 3.08)	18.33 (2.52 -133.26)
Received 1 or more shots in arm in theater	1.71 (1.02 – 2.86)	23.40* (1.15 -475.56)
Saw/contact with dead animals	1.75 (1.07 – 2.85)	36.00 (4.33 -299.02)
Took NAPP pills (PB)	2.68 (1.62 – 4.44)	40.00 (3.58 -447.04)

*No unexposed cases: OR estimate added .5 to 0 cell



ANS *Study 1* Results: Conclusions

1. Overall, *no* independent association of BChE genotype with Gulf War illness
2. Some locations and exposures in theater *were* associated with increased GWI risk
3. **For those with BChE variant genotypes, some exposures appear to be linked to a large increase in risk of illness.**



ANS *Study 1*: Remaining Analyses

1. Identify exposure "clusters," e.g. multiple exposures occurring in the same veterans, and possible links with locations in theater.
2. Assess confounding due to co-occurrence of multiple exposures with one another, to determine independent associations of exposures with illness and with BChE genotype.

Study Design: ANS *Study 2*

- **Case/Control Design**: Cases met Kansas "Gulf War Illness" case definition; controls did not
- **Study 2 case/control sample**:
  - » 49 Gulf War veteran cases
  - » 19 Gulf War veteran controls
  - » 23 non-deployed (PGW-era) veteran controls
- **Variant Sample**:
  - » 23 BChE variant subjects from Study 1
- Participants screened by telephone interview to determine study eligibility, filled out questionnaire, came to study site for 3.5-hour battery of physiological testing, and provided blood samples

Study Design: ANS *Study 2*

Study 2 veterans much more homogenous re: locations and experiences in theater than Study 1 veterans

Eligibility Criteria: Study 2 Case/Control Sample

- Deployed some time between August 1990-July 1991 (PGW subjects)
- Served with one of two units (PGW subjects)
- Enlisted only
- Army only
- 10% female
- Never diagnosed by physician for: diabetes, heart disease, stroke, lupus, multiple sclerosis, cancer, liver disease, conditions associated with psychosis
- Not hospitalized since the Gulf War for: alcohol or drug dependence, PTSD

Biochemistry and Molecular Biology Methods

- Same as Study 1

Study Design - *Study 2* ANS Battery - Direct Measures

- Endpoints that were measured and digitized continuously at 256 Hz:
  - ECG (Lead II)
  - Non-invasive radial arterial tonometry (Colin)
  - Respiration (strain gauge)
- Endpoints measured at the end of the test session digitized at 1,024 Hz:
  - Quantitative electromyography from the *orbicularis oculi* for amplitude of the startle reflex and its inhibition by a pre-pulse (PPI)

Study Design - *Study 2* ANS Battery - Derived Measures (1)

- From Lead II ECG:
  - Mean Heart Rate (MHR)
  - Fourier Spectral Heart Rate Variability (S-HRV)
    - Total Power
    - Absolute and Percent Low Frequency Power
    - Absolute and Percent High Frequency Power
    - Low to High Frequency Power Ratio
  - Time-Domain Heart Rate Variability (TD-HRV)
    - SDNN
    - rMSSD
    - SDSD
    - %NN

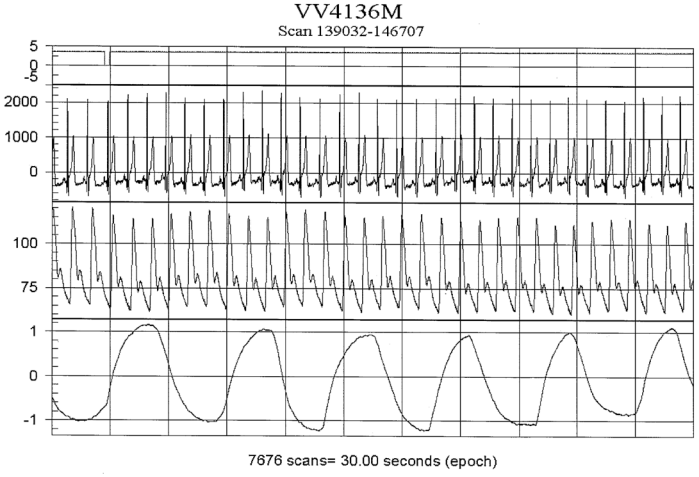
Study Design - *Study 2* ANS Battery - Derived Measures (2)

- From Non-invasive radial arterial tonometry:
  - Mean Blood Pressure (MBP)
  - Mean Systolic Pressure (SP)
  - Mean Diastolic Pressure (DP)
  - Peak Systolic Pressure for a given challenge
  - Trough Diastolic Pressure for a given challenge
- From ECG and tonometry combined:
  - Estimate of pulse transit time
  - Changes in pulse transit time with challenges

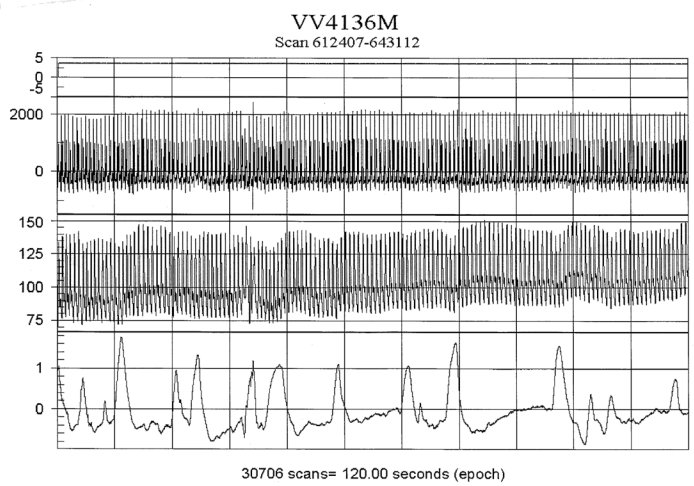
ANS Stressors

- Stressors (separated by appropriate baselines and recovery periods):
  - Deep breathing
  - Paced breathing
  - Hand grip
  - Mental Arithmetic
  - Valsalva maneuver (3x)
  - Stressful event: recall and tell
  - Tilt-up (80°, 20 min)
  - Tilt-down

Example of Real-Time Beat-to-Beat Data: Baseline



Example of Real-Time Beat-to-Beat Data: Mental Arithmetic



It is Important to Demonstrate Robust Responses from Baseline

- Elements of test batteries need to exhibit robust responses to the stressor from the baselines.
- The more robust the response, the more likely that a difference between cases and controls, *if there is one*, can be detected reliably.
- We observed robust and significant responses from baseline to seven of our eight stressors.
- Examples:

Physiological Effects (Mean, SD) of the Autonomic Reactivity Battery (ARB)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Hand Grip	Mean HR	.0001	68.6(9.7)	70.3(9.8)	75.2(10.7)
	SD HR	.0004	2.6(1.6)	2.9(1.2)	2.8(1.4)
	Mean BP	.0001	91.5(10.9)	98.2(10.2)	107.7(14.7)
	SBP	.0001	127.2(13.5)	134(13.5)	143.5(18.2)
	DBP	.0001	72.8(10.1)	78.9(9.4)	86.7(13.2)

Physiological Effects of the ARB (2)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Arithmetic	Mean HR	.0001	67.3(9.8)	73.2(10.2)	
	SD HR	.0001	2.9(1.4)	3.6(1.4)	
	Mean BP	.0001	91.5(9.6)	98.5(11.2)	
	SBP	.0001	125.7(12.8)	134.2(14.8)	
	DBP	.0001	73.3(9.1)	79.4(10.1)	

Physiological Effects of the ARB (3)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Valsalva	Mean HR	.0001	68.7(9.7)	72.2(9.8)	70.9(9.6)
	SD HR	.0001	2.5(1.4)	9.0(4.4)	9.0(4.5)
	Mean BP	.0001	91.7(11.1)	100.2(9.3)	98.7(8.9)
	SBP	.0001	127.5(13.6)	136.7(13.8)	134.4(12.5
	DBP	.0001	72.9(10.2)	83(8.3)	81.5(8.3)

Physiological Effects of the ARB (4)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Recall of stress	Mean HR	.0001	67.3(9.9)	73.2(10.5)	
	Mean BP	.0001	94.6(9.6)	98.1(10.3)	
	SBP	.0001	129.9(13.1)	134.3(13.5)	
	DBP	.0004	76(8.8)	78.6(8.9)	
	Power	.0001	28.7(12.4)	34.9(13.4)	
	ABS LF	.0001	11.2(5.2)	13.1(5.4)	

Physiological Effects of the ARB (5)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Initial Up-Tilt	Mean HR	.0001	67.2(10.1)	81.7(10.9)	
	DBP	.0001	78.2(9.2)	83.9(9.2)	
	Power	.0001	35.7(14.6)	44.7(15.9)	
	ABS LF	.0001	14.1(7)	20.9(9.4)	
	ABS HF	.0006	13.3(7.4)	10.7(5.9)	
	L/H ratio	.0001	1.22(.5)	2.15(.8)	

Physiological Effects of the ARB (6)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Initial Up-Tilt	%LF	.0001	39.2(7.7)	45.7(8.8)	
	%HF	.0001	36(9.7)	23.4(7.3)	
	SDNN	.0009	50.4(23.3)	57.1(23.7)	
	RMSSD	.0001	39.7(29.6)	23.5(16)	
	SDSD	.0001	39.8(29.6)	23.5(16.0)	
	%NN	.0001	15.2(18.1)	5.3(9.4)	

Selected ANS Results

- Some of the most striking results on case-control differences in ANS reactivity were observed in responses to four of the stressors:
  - Stressful event recall and telling
  - Orthostatic up-tilt (80°, initial and 20 min)
  - Orthostatic down-tilt (initial and 10 min)
  - Prepulse Inhibition and Startle reflex
- Examples:

Case/Control Differences in Physiological Response to the ARB (Example 1)

Task	Variable	p<	Cases	Deployed Controls	Nondeployed Controls
Initial Up-Tilt	SDNN	.02	49(25.1)	56.8(19.7)	61.4(21.7)*
	RMSSD	.008	27.5(22.7)	28.7(15.9)	43.1(32.4)*
	Abs LF	.007	15.8(8.9)	20.7(9.6)	18.4(7.7)
	%NN	.007	8.1(14.0)	8.8(10.9)	16.2(19.1)*

*Pairwise comparison showed that the marked Group differed from the Case Group at $p < .05$

Case/Control Differences in Physiological Response to the ARB (Example 2)

Task	Variable	p<	Cases	Deployed Controls	Nondeployed Controls
Prepulse Inhibition	Startle	.003	330(370)	611(464)*	639(552)*
	Prepulse	.002	12.1(31.3)	22.7(40.3)	65.8(104.6)*
	PPI score	.012	6.7(24.2)	3.4(6.3)	15.4(30.3)*

*Pairwise comparison showed that the marked Group differed from the Case Group at $p < .05$

Effects of BChE Genetic Status

- We examined the role of BChE variants in the responses to the ANS battery independent of case-control status.
- We also examined any possible interaction between case-control status and BChE variant status.
- Examples:

Variant/Nonvariant Differences in Physiological Responses to the ARB

Task	Variable	p <	Variant	Nonvariant
Baseline	Mean HR	.02	64.2(7.6)	69.1(9.6)
	ABS LF	.006	13.2(4.9)	10.4(5.0)
	L/H ratio	.04	1.4(.7)	1.1(.4)
	%LF	.004	42.3(8.2)	38(6.2)
	SDNN	.008	53.6(29.4)	39.9(25.5)
Breathing	Mean HR	.02	63.5(7.1)	68.4(9.1)

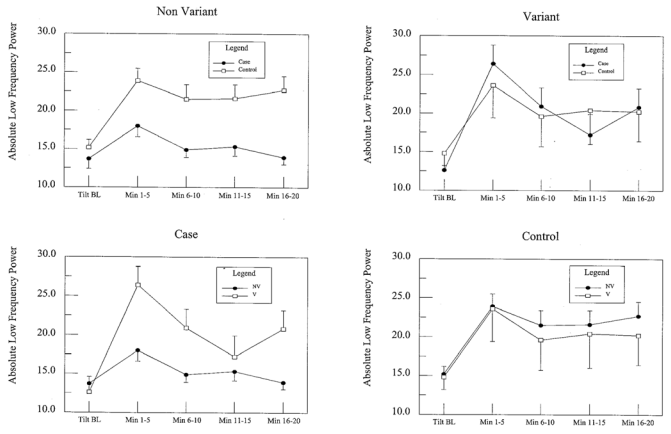
Variant/Nonvariant Differences in Physiological Responses to the ARB (2)

Task	Variable	p <	Variant	Nonvariant
Hand Grip	Mean HR	.02	66.4(8.7)	71.6(10.4)
Arithmetic	Mean HR	.04	66.4(10.1)	70.6(10.3)
Valsalva	Mean HR	.008	65.7(7.3)	71(9.7)
Stressful event recall	Mean HR	.02	65.3(8.9)	70.6(10.4)
	Power	.04	36.7(14.7)	31.5(13.2)
	ABS LF	.05	13.9(5.4)	12(5.3)
	SDNN	.0006	73.7(35)	54.2(28)
	RMSSD	.02	56(49.7)	38(34.8)
	SDSD	.02	56.1(49.8)	38.1(34.9)

Variant/Nonvariant Differences in Physiological Responses to the ARB (3)

Task	Variable	p <	Variant	Nonvariant
Initial Up-Tilt	Mean HR	.02	69.8(12.7)	74.8(12.7)
Initial Down-Tilt	Mean HR	.03	64.4(8.7)	68.6(9.8)
Recovery	Mean HR	.02	61.6(9.1)	66.2(9.9)
	DBP	.05	70.6(10.2)	74.2(9.9)
Prepulse Inhibition	Startle	.007	738(599)	475(469)
	PPI Score	.04	11.8(19.9)	5.9(20.4)

ANS Response and Variant Status Interaction



Summary of ANS *Study 2* Results

Association of GWI with ANS Physiologic Measures

Test	Response Measures that Differed between Cases and Controls
Baseline	Mean BP, L/H ratio, %HF, rMSSD, SDSD
Deep Breathing	Mean BP, DBP
Mental Arithmetic	DBP
Recall of stressful event	Mean HR, Mean BP,DBP,%HF, rMSSD,SDSD,%NN
Initial up-tilt	Mean BP, DBP, Power, ABS LF, ABS HF, L/H ratio, %HF, SDNN, rMSSD, SDSD, %NN
Up-Tilt @ 20 min	Mean BP, SBP, DBP, Power, ABS LF, %LF, SDNN, %NN
Initial down-tilt	Mean HR, ABS LF, SDNN, rMSSD, SDSD, %NN
10 min. down	Mean HR, DBP, Abs LF, SDNN, rMSSD, SDSD, %NN
Pre-pulse inhibition	Initial startle, auditory pulse, PPI score

ANS *Study 2* Results

Association of GWI with ANS Physiologic Measures

- ***No*** significant associations between case status and:
  - –Hand grip
  - –Valsalva maneuver

ANS *Study 2* Results: Conclusions (1)

1. Compared to controls, veterans with GWI show significant autonomic dysfunction, reflected in a broad range of objective measures.
2. GWI-associated physiological differences are found in both baseline (resting state) measures and in response to autonomic challenge.
3. Veterans with variant BChE genotypes differ autonomically from those with non-variant BChE genotypes, without regard to their GWI case status.

ANS *Study 2* Results: Conclusions (2)

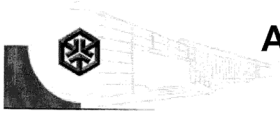
4. There are significant interactions between genotype and case status. That is, ANS responses in GWI cases vs. controls also depend on BChE genotype.
5. In general terms, autonomic reactivity appears to be blunted in GWI cases compared to healthy veterans, but there are significant exceptions to this generalization.
6. Additional analyses will combine deployed and non-deployed control groups (when appropriate) to further evaluate associations between GWI and ANS dysfunction.

ANS *Study 2:* Important Research Implication

- The results from Study 2 will allow us to design targeted follow-up studies capable of identifying specific components of the ANS that may be involved in the dysfunctions we have identified.
- Specifically, it will be possible to use ***ethical*** designs, with ***FDA-approved procedures*** and ***pharmaceuticals*** to examine:
  - Sympathetic efferents
  - Parasympathetic efferents
  - Ganglionic efferents
  - ANS Sensory **a**fferents

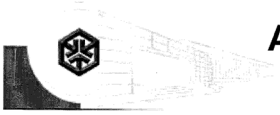
ANS Study Implications: Take-Home Points (1)

1. **Objective physiological tests indicate that Gulf War Illness is associated with autonomic dysfunction**.
   - Similar differences in heart rate variability measures have been found, in other studies, linked to eventual higher cardiac and overall mortality rates.
2. **Autonomic dysfunction is also associated with BChE genotype, regardless of case/control status.**
3. **Case/Control Status and BChE variant status interact.**



ANS Study Implications: Take-Home Points (2)

4. **Veterans with variant BChE genotypes appear to have a substantially increased risk of GWI in association with certain exposures.**
   - In the absence of these exposures, carriers of BChE variants were not at increased risk for GWI
   - Further study of genetic variants of other key enzymes, such as Serum Paraoxonase 1, is needed to help clarify the complex interactions between environmental exposures, physiologic ranges and genetic polymorphisms.



ANS Study Implications: Take-Home Points (3)

5. **Methodology is extremely important**. The clear results from our study depended on:
   a. **Interdisciplinary expertise.** Study designed and executed by a team that included experts in autonomic physiology, psychophysiology, molecular biology, and epidemiology.
   b. **Test battery.** Comprehensive and well-executed battery of physiological tests. One ANS measure (e.g. Valsalva test in prior study) doesn't tell the whole ANS story.
   c. **Case definition**. Use of a pre-determined and functional GWI case definition (e.g. CDC case definition gave results similar in direction, but with weaker associations)
   d. **Well-defined sample, veteran participation**. Sufficient numbers of veterans proactively contacted from defined **population-based** samples.