



**IMMEDIATE AND DELAYED DRUG THERAPY EFFECTS ON LOW DOSE
SARIN EXPOSED MICE MYOCARDIAL PERFORMANCE**

THESIS

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Abstract

Recent studies have shown that a single asymptomatic dose exposure to the nerve agent sarin in mice leads to long term cardiac dysfunction. This study looked at immediate and delayed treatment therapies post sarin exposure on cardiac function. Heart function and structure were studied using electrocardiography (EKG) and histological/immunochemical techniques (staining with hematoxylin/eosin and brain natriuretic peptide, BNP). Male C57BL/6J mice were injected with sarin (0.4LD50) followed immediately with atropine/2PAMCL (standard nerve agent treatment) or at 9 wks with β adrenergic agonist/antagonist. Measurements were made ~ 3 months post sarin. Mice given atropine/2PAMCL showed significantly higher cardiomyocyte size and BNP levels than controls ($p = 0.001$). EKGs showed T-wave anomalies. These results are indicators of cardiac insult. The second treatment groups received isoproterenol or propranolol (β agonist and antagonist, respectively, sc infusion for 2 wks) after onset of cardiac remodeling. Isoproterenol increased heart weight ($p = 0.0018$) and myocyte size and caused inverted T-waves. Propranolol treated mice showed only T-wave depression with no change in heart size. Results suggest 1) standard atropine/2PAMCL treatment does not prevent sarin-induced cardiac dysfunction and in fact may cause pathologies and 2) β agonist stimulation exacerbates the cardiac response to sarin suggesting possible interactions between sarin and stress exposure.

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Joshua T. Miller

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IMMEDIATE AND DELAYED DRUG THERAPY EFFECTS ON LOW DOSE SARIN EXPOSED MICE MYOCARDIAL PERFORMANCE

I. Introduction

Historical Use and Future Concerns of Sarin

Sarin is a highly lethal neurological toxin which was first created in 1938. It is a colorless and odorless liquid which rapidly evaporates into the gaseous state. The Germans weaponized and stockpiled sarin during WWII, along with other chemical agents, but never employed them. Since WWII, there have been multiple confirmed and suspected uses of chemical warfare agents (CWA) in conflicts around the world; Yemen Civil War (1963-1967), Soviet's in Afghanistan (1979-1989), Iran-Iraq War (1980-1988), Iraq against the Kurds (1988), and Libya against Chad (1987) (Salem, et al. 2008). In all of these military actions nerve agents were among those chemical agents suspected of being employed, additionally the perpetrators of the chemical agent use were all national governmental organizations. In the mid 90's chemical agent attacks no-longer became the sole domain of nation states. In 1994 and 1995 a cult group known as Aum Shinrikyo, employed sarin against Japanese government and civilian targets leading to wide spread civilian casualties (Committee on Gulf War and Health: Updated Literature Review of Sarin 2004, Ferguson 2009). In 1998 Bin Laden stated it was a religious duty to acquire Weapons of Mass Destruction (WMD) in defense of Muslims. In 2003, Shaykh nasir bin Hamid al-Fahd, a young cleric wrote a religious paper entitled "A

Treatise on the Legal Status of Using Weapons of Mass Destruction”, further defending the “right” of terrorists to acquire and use WMDs (Ferguson 2009). As a result, increasing efforts have been undertaken to be able to better protect and respond to both military and civilian casualties from a nerve agent attack. However, treatment and detection capabilities are still based on symptomatic dose levels, which, leaves gaps in our ability to properly identify and treat casualties to low level asymptomatic exposure to nerve agent.

Sarin and nerve agents have again become a topic for research in the last two decades following the unintentional exposure of U.S. Soldiers in 1991 to low levels of nerve agent following the destruction of the Khamisiyah munitions site in Iraq (see Figure 1).

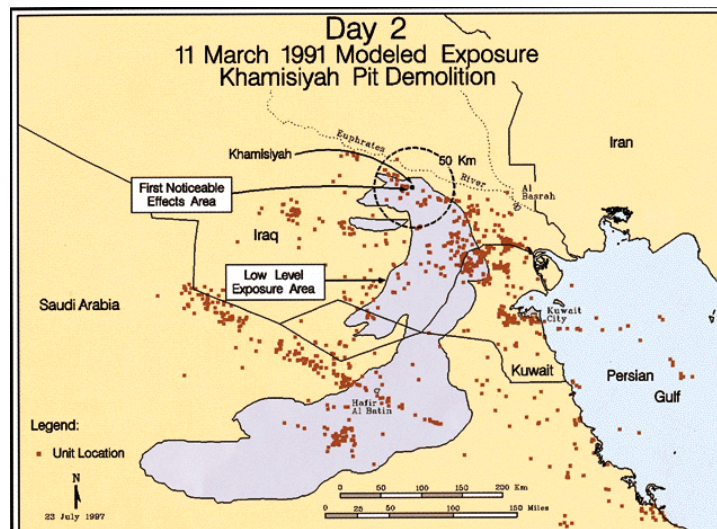


Figure 1. Plume Models for the Demolition of Bunkers at Khamisiyah, 12 March 1991
(Persian Gulf War Illnesses Task Force 1997)

According to the Committee on Gulf War and Health estimates, nearly 90,000 Soldiers may have been exposed to sarin and/or cyclosarin following the demolitions at the Khamisiyah site (Committee on Gulf War and Health: Updated Literature Review of Sarin 2004). Due to this potential exposure to sarin and other nerve agents, they were implicated as possible etiological agents in those suffering from Gulf War Illness (GWI). These chemical agents were therefore recommended by a Presidential Advisory Committee to be the subject of extensive investigation by agencies that treat those veterans (Couzin 2004, McDonough and Romano 2008)

Problem Statement

Past research and medical information focused on understanding symptomatic large dose exposure effects from nerve agents, little to no information is available concerning asymptomatic low dose and late-onset effects from nerve agent exposure. With the possibility of personnel exposures at these low levels occurring near the attack site, a better understanding is needed on possible health effects of low dose exposure levels. Additional understanding of these possible health effects could also lead to changes in current mission oriented protective posture (MOPP) guidance for military personnel. This is due to the fact that current guidance only considers doses which present clinical signs of nerve agent exposure as relevant. Also, current detectors are unable to detect agents below symptomatic levels. Further understanding of late-onset symptoms and effects on personnel could also lead to changes in long term treatment of nerve agent exposure victims.

Recent studies by Morris and Horenziak focused on the long term cardio-toxic effects following a single asymptomatic dose (Morris, Key and Farah 2007, Horenziak

2010). Initial findings by Morris *et al.* linked autonomic changes with the heart in mice, after exposure to low doses of nerve agent, which showed increases in heart rate variability, (Morris, et al. 2007). In the recent study by Horezniak, data indicates delayed onset of morphology and reduced cardiac function of mice exposed to low doses of the nerve agent, sarin. It was also shown that, even with normal cardiac function during basal conditions, cardiac function showed reduced levels of performance when stressed (Horezniak 2010).

A Review on the Chemistry and Toxicology of Sarin

Sarin, chemical name isopropyl methylphosphonofluoridate (military designation – GB), belongs to a class of chemical compounds known as organophosphates. Organophosphates are chemical toxins that inhibit acetyl cholinesterase (AChE) and are widely used as commercial pesticides and CWA. Sarin's toxic effect is the result of its ability to phosphorylate the serine hydroxyl residue in the active pocket of AChE, forming either a phosphoric or phosphonic acid ester which is extremely stable. This reaction irreversibly deactivates or “inhibits” the enzyme (Abou-Donia 2003). In sarin's case the irreversible binding or “aging” is a process which can occur that permanently binds the sarin to AChE thus preventing reactivation of the enzyme (CDC 2007). As AChE becomes inhibited acetylcholine (ACh), a cholinergic neurotransmitter, builds up at receptor sites causing overstimulation. Overstimulation occurs at the central and peripheral cholinergic sites in smooth muscles, skeletal muscles, most exocrine glands, the central nervous system (CNS), and the cardiac system (e.g. central and ganglionic afferents), (Horezniak 2010).

Stimulation of muscarinic receptors at the smooth muscle level causes miosis, bronchioconstriction, vasodilation, increased peristalsis, and reduced heart rate at smooth muscle level. At exocrine glands, it causes secretions in the lung, nasal, oral, and sweat glands. Clinical effects due to nicotinic receptor overstimulation at skeletal muscles are fasciculation, twitching, flaccid paralysis and stimulation of autonomic ganglia leading to tachycardia and hypertension. The CNS has both muscarinic and nicotinic receptors and overstimulation leads to seizures and can cause death; whereas, a mild exposure leads to variety of symptoms which include forgetfulness, bad dreams, insomnia, and the inability to concentrate (USAMRID 2007, Horenziak 2010).

Sarin affects the cardiovascular system in a complex manner; effects are due to actions on both ganglion and postganglionic consequences of accumulated ACh on the heart and blood vessels. The overall acute effect on the heart depends on the relative prevalence of sympathetic or parasympathetic effects. Cholinergic effects from ACh suppress vagal tone resulting in a decreased refractory period and conduction time at sinoatrial (SA) and atrioventricular (AV) nodes. An excess of ACh also affects the muscarinic ACh M2 receptors in heart leading to negative chronotropic and ionotropic effects. Thus, ACh creates contradicting excitatory effects on parasympathetic ganglionic cells in the face of the inhibitory effects of the sympathetic ganglia. The build-up of ACh elicits similar excitatory, followed by inhibitory, effects at the medullary vasomotor and cardiac centers. In severe ACh accumulation, hypoxia resulting from broncho-constriction also affects the cardiovascular system by reinforcing sympathetic tone, while ACh induced epinephrine release from adrenals leads to increased heart rate.

As a result, sarin induces bradycardia, tachycardia, and arrhythmias in the heart that can lead to spontaneous cardiac failure (Taylor 2001, Horezniak 2010).

To counteract nerve agent exposure, the medical and military community uses a two or three drug combination to treat affected persons. Each drug has a specific affect on the patient which when used in combination can counter the acute effects of nerve agent exposure. The drugs are atropine, an anticholinergic compound, pralidoxime chloride (2PAMCL), an oxime, and diazepam, an anticonvulsant. Atropine/2PAMCL or MARK I will be used throughout the paper to denote the use of the two drug antidote sequence atropine plus pralidoxime chloride. Atropine (the primary drug treatment for nerve agent exposure) is used to block excess Ach at peripheral muscarinic sites which results in a decrease in secretions and an increase in heart rate. 2PAMCL is used to reactivate affected Ache and acts by binding to the nerve agent that is attached to the Ache and then breaks the nerve agent-enzyme bond, restoring the Ache to full functionality. This reaction is only possible if the nerve agent has not “aged”, if aging has occurred the Ache cannot be restored to functionality and Ache levels can only be increased by new production in the body, typically at the rate of about 1% a day. Diazepam is only used in cases of extreme nerve agent exposure to prevent brain injury caused by convulsions and seizures (USAMRID 2007).

Research Objectives/Hypothesis

The research objectives for this study are two-fold. First, is to evaluate the effectiveness of immediate treatment after exposure with atropine/2PAMCL to prevent cardiac remodeling. Second, is to study the effects of β receptor agonists and antagonists on cardiac performance and morphology, with no prior treatment, after sarin induced

cardiac remodeling has occurred. These studies will help to create a better understanding of the long term effects expected to manifest following a low-dose sarin exposure with the use of the MARKI antidote kit in troops or the general population.

Additional Specific Aims are:

- Determine the time course of cardio toxic actions of sarin in mice. Using an asymptomatic dose of sarin, follow the EHCO and ECG changes up to 8 weeks post exposure in mice with and without nerve agent antidote treatment (atropine/2-PAMCL). This will help to test the hypothesis that prompt treatment with nerve agent antidote will prevent long cardiac remodeling in exposure victims.
- Determine the effect of sarin combined with a hypertrophic stimulus, isoproterenol. The hypothesis is that there will be an exacerbation of the cardio toxic effects of sarin.
- Determine the effects of the blockade of β -adrenergic function and inhibition of central sympathetic outflow on the cardio toxic effects of sarin. The hypothesis is that treatment with a β -blocker will help reverse the cardiac remodeling induced by an acute low dose exposure to sarin.

Experimental Design

Male mice (C57BL6 strain) were injected on two consecutive days by WSU personnel and broken into six groups of 10 mice each. The groups were; control, control+atropine/2PAMCL, 0.4LD₅₀ sarin, 0.4LD₅₀ sarin+atropine/2PAMCL, groups five and six were given 0.4LD₅₀ of sarin and then further broken down into three groups which were given saline, isoproterenol, and propranolol respectively.

Table 1. Study Groups

	Sarin Injection	Recovery	Week 3	Week 7	Week 9	Week 11
Control	x	x	x,y	x,y,z		
Sarin	x	x	x,y	x,y,z		
Control+Atropine/2PAMCL	x	x		x,y,z		
Sarin+Atropine/2PAMCL	x	x		x,y,z		
Sarin+Saline	x	x		x,y	x,y	x,y,z
Sarin+Isoproterenol	x	x		x,y	x,y	x,y,z
Sarin+Propranolol	x	x		x,y	x,y	x,y,z

x - Body Weight Measurements Taken

y - ECG Measurements Taken

z - Sacrifice

Each group was studied using both *in vivo* (ECG analysis, echocardiography (ECHO)) and histological techniques (brain natriuretic peptide (BNP), hematoxylin and eosin (H&E), and Masson Trichrome) to determine if there were any changes in cardiac function or any cardiac remodeling occurring. At various points post exposure a short-acting β -1 specific agonist (dobutamine) was used to study cardiac performance during elevated cardiac output conditions. Various ECHO and ECG measurements were taken to measure left ventricular structure and cardiac performance before and after a dobutamine stress test. At the end of each study period the mice were sacrificed and relevant tissues and organs were taken for follow-on histological analysis.

II. Literature Review

Introduction

Recent studies by Morris Lab at Wright State University (WSU) have examined cardiac morphology and other changes to the hearts of mice after acute asymptomatic doses of sarin (Morris, Key and Farah 2007, Horezniak 2010). This study looked at effects of atropine/2PAMCL antidote treatment and various β adrenergic drug treatments on mice, after asymptomatic dose exposure levels to sarin.

Animal Considerations

Mice, unlike humans have higher levels of carboxyl esterase which has been shown too rapidly and degrade sarin. These elevated levels of carboxyl esterase change the normal dose response curve in mice compared to that of humans (Li, et al. 2005, Jokanovic, et al. 1996). This natural protective nature of carboxyl esterase requires that the mice be dosed on consecutive days. The first dose is used to consume the endogenous carboxyl esterase in the mice which then allows the second dose to follow a dose response curve that more accurately reflects that seen in humans. To verify that the desired level of acetyl cholinesterase inhibition has been achieved in the mice, serum cholinesterase assays are performed by Dr. Lucot following a protocol used by Sidell *et al* (Sidell and Borak 1992).

Choice of Anesthesia and Expected Effects

Conducting non-invasive observation techniques to measure cardiac performance requires taking into account affects on the mice due to the techniques used and the data that is to be collected. There have been well documented differences between conscious and unconscious mice in ECHO and ECG which are used to measure cardiac

performance. Studies which used ECHOs have shown a decrease in heart rate variability, fractional shorting, and ejection fraction in unconscious mice (Tan, et al. 2003, Stein, et al. 2005, Roth, et al. 2002). Additionally, ECG recordings have shown increases in QT and PR intervals and decreases in heart rate (Chavesa, et al. 2003).

Despite the potential drawbacks with using sedatives, it has been shown that they help to reduce stress in the mice, which, should lead to significantly reduced variabilities seen in ECHOs when compared to conscious mice; this is due to differences in stress created in the mice from the restraints or the handling training the mice received (Tan, et al. 2003). Other research by, Kramer *et al.* has shown that using restraints on mice leads to increases in body temperature, blood pressure, heart rate, plasma levels, and variable responses to pharmaceutical treatments (Kramer, et al. 2001). Additionally, issues related to high heart rates from stress and transducer placement due to small movements in conscious mice have led to failures in obtaining quality ECHO recordings (Roth, et al. 2002, Tan, et al. 2003). Issues associated with taking ECG recordings in conscious mice include being able to only take short time interval readings and the development of movement artifacts in the recordings. To prevent issues with lead placement on the mice the ECG probes are typically implanted in the mice or require restraints, both of which lead to increases in time and cost verses using anesthesia (Chavesa, et al. 2003).

Isoflurane (ISF) is the agent of choice for animal research and has been shown in multiple research papers to have the least affects on the heart compared to other anesthesia during ECHO and ECG analysis (Tan, et al. 2003, Stein, et al. 2005, Roth, et al. 2002, Chavesa, et al. 2003). Another benefit of ISF is that it has a more rapid onset time and provides more reproducible results than other which make it preferable for short

lived experiments (Stein, et al. 2005). Due to inherent advantages of ISF over conscious mice and its extensive successful use at WSU's Live Animal Research (LAR) lab in previous sarin related research it was chosen for use in this experiment in order to maintain procedural consistency. All mice were treated with ISF in the same manner to conduct ECHO and ECG analysis, as such, there is expected to be little to no effect on the procedural outcomes.

Body Temperature Regulation Concerns

Research has shown that mice are unable to regulate their body temperature when they are sedated; this can lead to significant fluctuations in heart rate and cardiac function (Hartley, et al. 1995, Hartley, et al. 2002). Changes in performance can occur with changes as little as 4⁰C (Swoap, et al. 2004). To minimize issues with changes in body temperature, the room was temperature controlled at (22⁰C ± 1⁰C); additionally, thermal pads (Brain Tree Scientific, Delta Phase Isothermal Pad for 37⁰C (model # 39DP)) were used to maintain body temperature at 32⁰C ± 2⁰C during the *in vivo* monitoring process.

Dobutamine Stress Testing

Cardiac function can change depending upon whether the subject's heart is experiencing normal (unstressed) or increased (stressed) cardiac output conditions. It is therefore important to test cardiac function in both states to evaluate overall cardiac function. Dobutamine is a drug that is commonly used in cardiac stress testing to study cardiac function in a stressed state. It acts on the β_1 adrenergic site and is a preferred drug for this type of testing due to its strong but short lived actions on the heart (Anderson, et al. 2008, Daly, et al. 1997, Mertes, et al. 1993, Shaheen, et al. 1998). The duration of the tachycardia lasts for approximately 25 minutes and creates an oxygen

supply-demand mismatch in the heart by increasing myocardial oxygen demand (Weissman, et al. 1996, Wiesmann, et al. 2001). Due to the preferential nature of dobutamine to bind to the β_1 -adrenoreceptors, using it to conduct the stress test can provide information on the overall function of the receptor cascade by using ECG and ECHO analysis to observe the changes (Anderson, et al 2008, Weissman, et al. 1996). An important consideration in using dobutamine for the stress testing is that in low doses it has not been shown to have any long lasting effects on the heart. However, if it is administered continuously over a period of time (seven days) it has been shown to increase fibrosis and lead to hypertrophy in the heart (Anderson, et al. 2008). The mice were given dobutamine during weeks; 3, 7, 9, and 11 as part of the stress test in doses of 1mg/kg body weight (approximately 30 μ g/mouse). The low dose levels and time interval in-between injections should prevent any long-term damage due to the dobutamine stimulation of the hearts.

Noninvasive In Vivo Measurements

Echocardiograms

Echocardiography is a well known and widely used noninvasive procedure to measure and determine aspects of overall cardiac function in humans and animals. The most widely used imaging techniques are the 2D, M-mode, and Doppler. Using these modes it is possible to measure heart rate, wall thickness, left ventricular area, and the myocardial performance index (Horezniak 2010). While ECHO was performed during each stage of the experiment, equipment data recording failures prevented proper analysis of the data and thus precluded its use in this study.

Electrocardiograms (ECG)

Electrocardiography has been widely used in murine research for over 70 years to look at electrical impulses of the (Hartley, et al. 2002, Wehrens, et al. 2000). The characteristic readout of ECGs is due to the successive electric impulse propagation throughout the heart. The beginning of the heart beat occurs at the sinus node then rapidly moves through the atria, which is indicated by the P wave. The following PR interval represents the movement of the impulse to the AV node. The ventricular contraction is shown via the QRS complex that immediately follows the PR interval. Atria repolarization occurs during ventricular contraction, but does not show due to the height and intensity of the QRS complex. The following ST segment represents ventricular relaxation and is then followed by the T-wave which represents ventricular repolarization. The initial impulse then dissipates, which is shown by the return to the iso-electric line (see Figure 2), (Ramos 2003, Geiter 2007, Horenziak 2010).

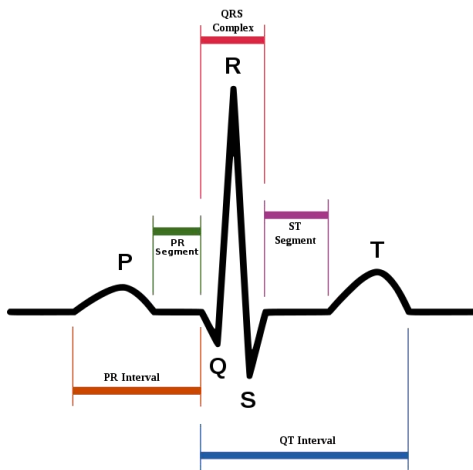


Figure 2. Typical Human ECG
(This image is licensed for unrestricted redistribution (Atkielski 2007)).

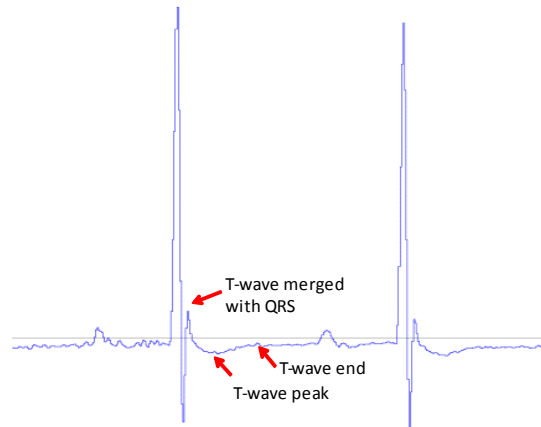


Figure 3. Typical Mouse ECG Illustrating the Difference in the T-wave when Compared to the Human ECG

The ECG of mice has one distinct difference from that of humans. In mice the T-wave is merged with the end of the QRS complex which results in noticeable lack of a ST segment (see Figure 3), (Wehrens, et al. 2000, Mitchell, et al. 1998, London 2001). The T wave in mice begins above the iso-electric line and then falls below it and is followed by a gradual return back to the iso-electric line (Mitchell, et al. 1998, Horenziak 2010, Wehrens, et al. 2000, London 2001). The T-wave is typically the first place on an ECG that changes from its normal pattern when issues occur in cardiac performance. Cardiac insults can result in T-wave inversions, ST depression, elongation of the QT interval, or even a lack of a T-wave (Geiter 2006). By analyzing changes to and anomalies on the ECG, any number of dysfunctions in the cardiovascular system can be observed and therefore treated.

In Vitro/Histological Markers

Utility of BNP Measurements

In the recent study by conducted by Horenziak, brain natriuretic peptide (BNP) levels were significantly increased in sarin treated mice. This peptide was first observed in pig brains (hence the name) but is only expressed in the ventricles of humans and mice (Brain natriuretic peptide 2010). The presence of increased BNP levels has been shown to occur after the heart undergoes a serious insult and hypertrophy has occurred (He, et al. 2001). Brain natriuretic peptide is created and stored in ventricular cells and increases in response to cardiac stress and are a sign of heart failure, hypertrophy, or any number of stresses to the heart (Goetze, et al. 2009, Yasuno, et al. 2009). The different natriuretic peptides have also been shown to have protective effects against hypertrophy *in vitro* when released from the cells (London 2006). Brain natriuretic peptide exerts its

natriuretic, diuretic, and vasorelaxant effects through activation of its common receptor Natriuretic Peptide Receptor A (NPRA) (Kawakami, et al. 2010, London 2006). Another important aspect of BNP is that it is expressed in fetal ventricles but is not present in significant amounts in adult mice ventricles (Scott, et al. 2009). These characteristics of BNP mean that it can serve as an excellent marker of cardiac insult to the murine heart, as such its presence or lack thereof should provide an indication of whether atropine/2PAMCL treatment, post sarin exposure can protect the heart.

Hematoxylin and Eosin (H&E) Staining

Hematoxylin and eosin staining is a commonly used technique in preparation of histology slides. Hematoxylin and eosin stains nuclei purple to blue and the rest of the cell pink to red (Eroschenko 2008). As such, the H&E stain provides important information about the nuclei and muscle fiber arrangement and cell size when heart sections are examined under a microscope (Horezniak 2010). For this study the H&E stained slides were used to obtain an estimate of average cell size by taking a ratio of nuclei to cell area.

Drug Therapies

Atropine+2PAMCL

Atropine plus 2PAMCL is the standard treatment for nerve agent poisoning by both the military and emergency medical personnel. It is administered via the Mark-I kit which has 2mg of Atropine and 600mg of 2PAMCL (Meridian Medical Technologies, Inc. 2003). After exposure to a nerve agent the Mark I kit can be administered up to three times prior to treatment by medical personnel (USAMRID 2007). The number of Kits used depends upon the severity of the poisoning; current protocol is not to use the kits if

the person is asymptomatic (USAMRID 2007, New York State Department of Health Bureau of Emergency Medical Services 2003).

Atropine acts as a competitive receptor antagonist, blocking the effects of ACh at the muscarinic sites preventing it from lowering the heart rate to dangerous levels after nerve agent exposure (USAMRID 2007, Brown and Taylor 2001). The second half of the Mark I kit, 2PAMCL, acts as a cholinesterase reactivator by rehydrolyzing the enzyme allowing it to resume hydroxylation of the ACh, thus deactivating it as neurotransmitter (Parkinson 2003, Hoffman and Taylor, Neurotransmission: The Autonomic and Somatic Motor Nervous Systems 2001). The intent of this research is to determine whether a small dose of the atropine/2PAMCL nerve agent treatment kit can have a protective effect on the cardiac tissue post exposure to sarin.

Propranolol

Propranolol is a Class II antiarrhythmic drug which acts as a β -adrenergic receptor blockade (Ramos, et al. 2003). Major benefits of propranolol are reduced heart rate, decrease in myocardial oxygen demand, and reduction in arterial blood pressure (Kerins, et al. 2001). Recent studies have shown that the antihypertrophic effects of propranolol are independent of its β -adrenergic blocking ability. These effects in rats were found when doses of 40 and 80 mg/kg per day were given over a 17 day period (Marano, et al. 2002).

Isoproterenol

Isoproterenol is a non selective β -adrenergic agonist which also has a low affinity for α -adrenergic receptors. As a catecholamine, effects of isoproterenol on the heart lead to increased heart rate, increased myocardial oxygen demand, and possible electrolyte

alterations (Ramos, et al. 2003, Hoffman 2001). Cardiac researchers have been using isoproterenol for around fifty years as a means to induce hypertrophy in small animals (Anderson, et al. 2008). By treating the sarin treated mice with isoproterenol and with a β -blocker (propranolol) it is hoped that insight into mechanisms by which sarin acts on heart can be found.

III. Methodology

Animal Experimental Protocol

Sixty-two C57BL/6J male mice were used in two studies (41 in the atropine study and 21 in the β adrenergic drug therapy study). They were individually housed at 22°C on a 12:12 light-dark cycle at the WSU LAR. Forty-two mice were treated with sarin nerve agent on two consecutive days. The other 20 mice were treated with saline and acted as the control mice. *In-vivo* cardiac performance measurements were taken along with weight during weeks three, seven, nine, and 11 post sarin exposure.

Compared to humans, mice have a higher level of blood cholinesterase which interacts with and consumes nerve agent, preventing the agent from acting on the nervous system and organs. To ensure proper dose response, mice were treated with sarin twice; the first dose metabolically consumes the cholinesterase in the blood followed by a second injection 24hrs later which exerted the inhibitory response that was studied. Twenty-four hours after the final injection the blood cholinesterase levels were measured using a standard assay developed at WSU (Horenziak 2010). Following the last dose of sarin, 10 mice from the control group and 10 mice from the sarin group were given an atropine/2PAMCL treatment of 10 and 25 mg/kg respectively.

Two separate studies were conducted; the first (referred to as the Atropine Study) looked at antidote treatment effects following low dose exposure to sarin, which consisted of control, control+atropine/2PAMCL, 0.4LD₅₀ sarin, and 0.4LD₅₀ sarin+atropine/2PAMCL groups. The control and sarin groups from the first study had 8 and 7 mice surgically implanted with ECG telemetry probes from Data Science

International (model TA-F10) to record continuous ECG data after the week three *in vivo* analysis. The probes were set to record for 15 minutes every hour for 24hrs.

The second (referred to as the β adrenergic drug therapy study) looked at various drug treatments on sarin exposed mice, which consisted of 0.4LD₅₀ sarin+saline, 0.4LD₅₀ sarin+isoproterenol, and 0.4LD₅₀ sarin+propranolol groups. These mice were evaluated at week nine and then micro-osmotic pumps (model 1007D from Alzet®) were implanted to administer doses of saline (20mg/kg/day), isoproterenol (30mg/kg/day), and propranolol(20mg/kg/day) over a two week period. At the end of the two weeks (week 11) the mice were evaluated *in vivo* and then sacrificed.

Tissue Collection and Histology Sectioning

In the seventh week the atropine study mice were sacrificed and in week 11 the β adrenergic drug therapy study mice were sacrificed via decapitation and tissue samples were collected and preserved. After the mice were decapitated the heart was collected, weighed, and then placed into a phosphate buffered saline (PBS) solution. Each heart had its apex removed and the heart was placed in a 4% paraformaldehyde (PFA) solution and stored at 4°C for 48 hours. Following soaking for 48 hours in the PFA the tissues were removed and fixed in 20% sucrose, 4% PFA solution. They hearts were then stored for another 24 hours at 4°C and then transferred to a -80°C freezer where they were stored until they were sent to AML Laboratories for heart sectioning. Each mouse was sectioned into five slides with two heart sections per slide. One of the slides from each mouse was stained with Hematoxylin and Eosin (H&E) by AML. Additionally, AML stained one slide from each group with masson's trichrome which allows for the study of collagen fibers.

Hematoxylin and Eosin Analysis Methods

Hematoxylin and eosin staining is a common staining technique used to highlight nuclei from cell tissue. In this experiment H&E stained slides were analyzed to compare overall cell size. All H&E staining was conducted by the AML personnel at the same time as the heart sectioning. They were used to conduct cell size analysis via nucleus to pixel area ratio. The H&E stained slides were analyzed using a slightly modified version of a protocol that was used by Horenziak. The slides were viewed under a Leica Microsystems® DMR microscope at 20X Magnification (see Figure 4).

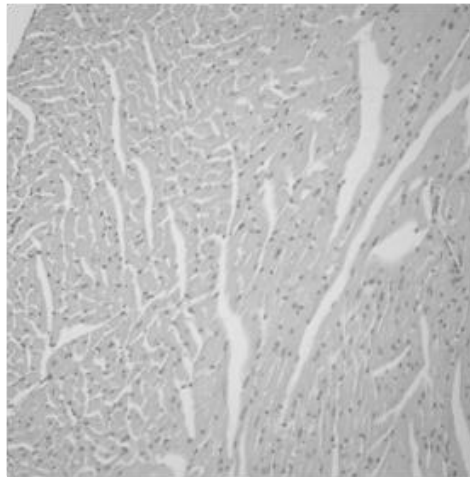


Figure 4. H&E Stained Slide at 20X Magnification

Pictures of the whole heart section were taken with an Optronics® QuantFIRE® XI/MacroFIRE® 2.3A camera attached to the microscope. Corresponding software was used to manipulate the image to obtain the greatest contrast from the stain. Once the settings for the image were set they were not changed. It was found during research

conducted by Horenziak that taking the images in a gray scale allowed for better contrast and analysis later on.

The first step was to take a picture of the white background in an area near the heart sections, followed by a black background taken with the light source turned off. These images were then used to correct the background later during analysis. Each heart had three photos taken of the left ventricle at random locations that did not overlap.

To conduct the image analysis a Molecular Devices'® software package called Metamorph® (version 7.6) was used. The *Background and Shading Correction* function was used to correct uneven intensity on all images due to staining irregularities. This required taking both the white and black background images along with the image being analyzed and running it through the *Background and Shading Correction* function. The new “corrected” image became the image that was used to conduct all analysis. Using a control mouse the *Color/Intensity threshold selection tool* was used to determine the threshold levels that allowed distinction between the background and cell tissue which where, 0-252 (see Figure 5).



Figure 5. Threshold Selection of Total Cell Area

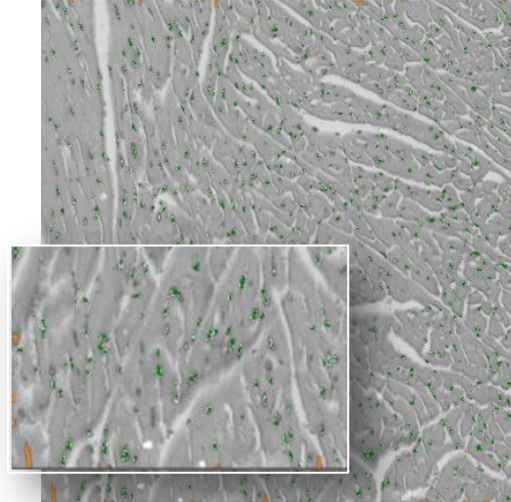


Figure 6. Threshold and IMA Selection of Nuclei

Next a range of threshold values and *Area/Shape* filter parameters from the Integrated Morphometry Analysis (IMA) tool kit were determined to allow for the counting of nuclei (see Figure 6). The *Area* filter was set to only select highlighted areas consisting of areas from 50-800 pixels total. The *Shape* filter was also set to select highlighted areas which conformed to a circular shape match of .250 to 1.00. These values were based on measurements of nuclei in a control mouse. The threshold values for the nuclei varied between slides due to inconsistencies in staining intensity and nuclei locations. This required adjustment of the threshold values mouse by mouse to ensure proper selection of nuclei. Finally, the total number of objects was exported to Excel™ using the *Object* tab in IMA tool kit. Next, the total area of the cell tissue was determined using the values stated above and then exported to Excel™ in the same manner as the nuclei data. The average cell size was computed as the number of pixels per nuclei. The values were averaged and compared using statistical techniques described later.

Brain Natriuretic Peptide Methods

Brain Natriuretic Peptide Staining Methods

Brain natriuretic peptides are commonly released after a cardiac insult and its presence is a good indicator of heart damage. Unstained slides from AML with fixed heart tissue were first de-paraffined by soaking the slides in xylene for five minutes twice. This was followed by a rehydration sequence which took the slides from 100% ethyl alcohol down to 70% over a 24 minute period. The slides were then rinsed in 0.01M phosphate buffered saline solution (PBS), twice. Then the slides were placed in a 0.15% hydrogen peroxide solution for 15 minutes and washed in PBS (standard washing was conducted three times). Following this the slides were blocked using normal goat serum for one hour prior to the addition of the primary antibody solution. The primary antibody solution consisted of rabbit/anti-rat antibodies and was obtained from Bachem AG® and was diluted to a ratio of 1/500, antibody to blocking solution. The slides were maintained at room temperature for one hour and transferred to a 4°C storage container overnight. The following morning the slides were washed in PBS and then the second antibody was placed on the slide tissue for 35 minutes. The second antibody was a goat/anti-rabbit solution obtained from Vectastain™ and was diluted to a ration of 1/500, antibody to blocking solution. The slides were then washed in PBS and the reagent A+B solution from the Elite® Vectastain™ ABC stain kit (rabbit IgG) was added to the tissue for one hour. The slides were then washed using a nickel buffer solution three times and then stained for ten minutes. The staining solution was a 3-3'-Diaminobenzidine tetrahydrochloride (DAB) tablet crushed and dissolved in the nickel buffer solution. The slides were then washed in PBS and dehydrated in ethyl alcohol of increasing

concentrations from 70% to 100%. Finally they were cover slipped and allowed to dry before being analyzed using a microscope.

Brain Natriuretic Peptide Analysis Methods

The BNP stained slides were analyzed using a slightly modified version of a protocol that was used by Horenziak. The slides were viewed under the same microscope and pictures taken with the same camera as the H&E tissue. The same software was used to manipulate the image to obtain the greatest contrast from the stain, but unlike the H&E these images were taken in color. Again, once the settings for the image and light settings were set they were not changed. Background images in light and dark settings were taken in the same manner as the H&E slides. Each heart section was completely photographed, which required approximately four to six screen shots each.

The image correction process was the same as the H&E tissues and like them the “corrected” image was then analyzed. To conduct the image analysis a Molecular Devices’[®] software package called Metamorph[®] (version 7.6) was used. The *Background and Shading Correction* function was used to correct all images to correct uneven intensity of the images due to staining irregularities. This required taking both the white and black background images along with the image being analyzed and running it through the *Background and Shading Correction* function. The new “corrected” image became the image that was used to conduct all analysis.

Once all the images for each heart section were corrected a selection tool was used to trace sections from each image which when combined with the other sections selected, outlined the whole heart section. The selection of all the cell tissue and BNP stained tissue was accomplished using the *Threshold Analysis Tool*. The threshold values

used to select the BNP for each slide were slightly different due to variations of the stain concentrations for each slide. The threshold values for the tissue were conducted in the same manner as that for the BNP stained tissue but were set to include all heart tissue.

Using the *regional statistics* tab, the total number of pixels in the traced region for both BNP and total cell area were calculated for each picture. This information was recorded and then combined with all picture sections in Excel™ to determine the total BNP and tissue pixel areas for the complete heart section. A ratio of BNP to total cell area was then calculated to normalize the data for statistical comparison.

Electrocardiography Methods

Data Collection Procedure

Electrocardiography is an excellent non-invasive method to observe cardiac performance. It was conducted on every mouse that underwent ECHOs during weeks three, seven, nine, and 10. The mice were first weighed using a Denver Instruments® XP-500 electronic scale in the surgical suite. After the mice were weighed they were placed in an anesthesia chamber and anesthetized using a forced flow 2% ISF in pure oxygen mixture, the anesthesia was administered via a Surgivet Anesco® Isotec 4™ anesthesia machine (see Figure 7). Once the mice were knocked out, they were moved to observation table and placed on the isothermal pad and subjected to a constant flow of 1% ISF for the duration of the examination. The front two legs of the mice were spread and taped to the pad to help immobilize the mice. To achieve proper contact for the two ECG leads the mice had their chest hair removed with Nair™ and cleaned with an isopropyl alcohol pad (see Figure 8).



Figure 7. Mouse in Anesthesia Chamber



Figure 8. Application of Hair Remover to Mouse

Electrocardiography data recordings were taken, before and after, dobutamine was administered to the mice (via subcutaneous injection see Figure 9), and between the pre and post dobutamine ECHO imaging.

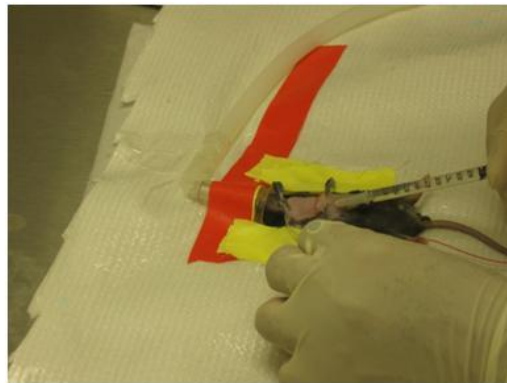


Figure 9. Injection of Dobutamine to Study Mouse

Recordings pre and post dobutamine injection lasted approximately three to four minutes each. The electrodes were attached using Schwartz micro-serrefine clips. The first lead was placed below the neck line in the dorsal thoracic region near the front right leg; the second lead was placed in the lower left abdominal area of the mouse (see Figure 10).



Figure 10. Placement of ECG Leads on Mouse

The advantage of using the isothermal pad was that it did not interfere with the ECG recording process like the electric heating pads used in the past. After the leads were placed the probe was laid on the DSI PhysioTel® receiver to allow maximum reception.

The ECG readings were recorded using DSI's Dataquest A.R.T.™ (version 4.0) software. Prior to recording, the probes calibration information from the manufacturer was inputted into the software and set to record in ECG mode and the sampling frequency was set to 5000 kilohertz. The ECG recordings were taken for approximately six to seven minutes. After the first two minutes the mice were injected with dobutamine (1mg/kg) to create a stressed state in the heart. The ECG readouts were monitored to observe the onset of the dobutamine induced stress and once achieved the recordings continued for a few more minutes.

Electrocardiography Analysis Method

All ECG recordings were analyzed using DSI's Ponemah™ software program (version 4.90), and a modified version of the protocol developed by Horenziak. The A.R.T. files were uploaded and converted into the software program. Prior to beginning

data analysis, under the *Ponemah*TM Platform tab, data recording parameters were set to record, all derived data and an analysis template blank were opened. Additionally, the scale was set to -0.5mV and 1.0mV to allow for the best view of the ECG recording.

Once the parameters was set up, the ECG recording *analysis attributes* values had to be adjusted because the predetermined QT interval for murine analysis was to long (it was lowered from 100 to 70 milliseconds). After changing the QT interval the entire ECG tracing was analyzed. Due to the limits of the software and the variability in the recording values not all P and T-waves were marked. In order to mark the missing P and T-waves the software's *template analysis* toolkit was used by taking a representative wave and adding the missing markers and reanalyzing the ECG tracing for any wave sections that were an 80% match (see Figures 11 and 12 for defined points on the ECG readout).

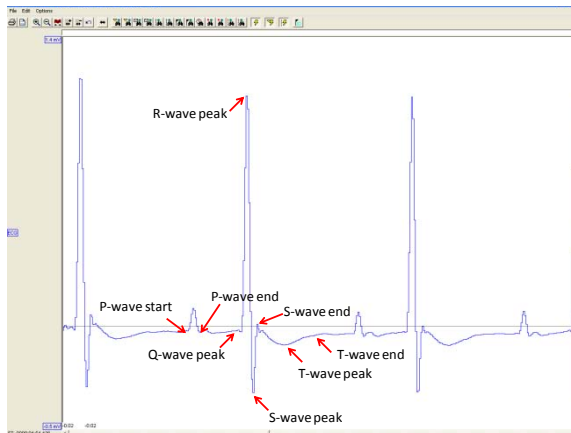


Figure 11. Significant Points on a Mouse ECG

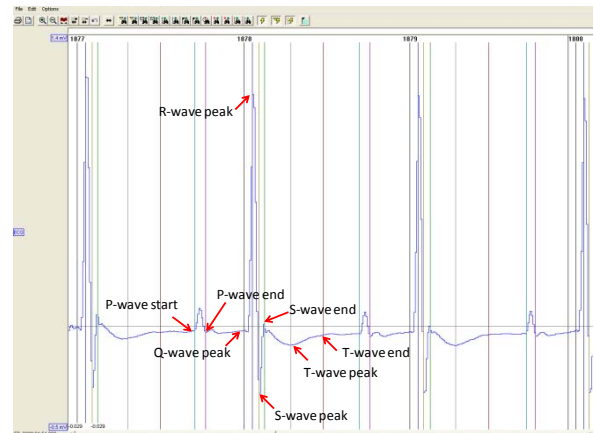


Figure 12. Example of Marker Placement by PonemahTM Software

The wave analysis using templates was done until the entire tracing was marked properly.

The subjective nature of placing the missing markers required that standardization of

wave placements be adhered to. The placement of the P-wave was defined as the small inverted u shaped wave prior to the QRS complex. Due to differences between T-waves in humans and mice the T-wave markings had to be further defined. The peak of the T-wave was defined as the maximum deflection from the iso-electric line after the end of the S-wave (see Figure 11). However, in stressed conditions and some pre stressed drug treated mice the peak of the T-wave was defined as the last saddle point prior to the tracing returning to the iso-electric line due to an inversion of the T-wave. The end of the T-wave was defined in all cases as the point where the tracing returned to the iso-electric line. If the T-wave had not returned to the iso-electric line prior the start of the P-wave (thus merging with the P-wave) the end of T-wave was defined as the point just prior to the start of the P-wave (see Figure 13).

Once the tracing was marked it was reviewed to find a uniform 30 second interval pre and post dobutamine. These 30 second intervals were then reviewed beat-by-beat to remove any beats that could not be marked properly (caused mainly by movement or breathing (see Figure 14). These marks were removed and any beats that could be marked and were missed were marked at this time. After this the *Derived data* was then converted and saved as an Excel™ file.

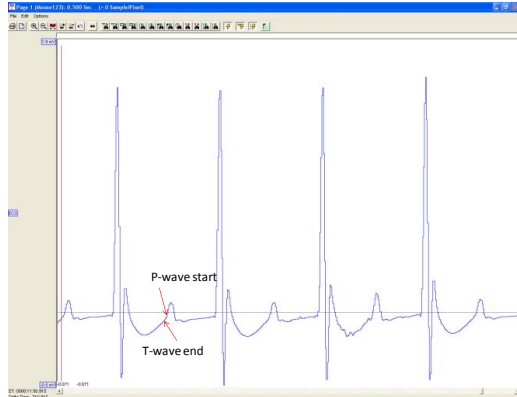


Figure 13. Example of ST Segment Merging with the P wave

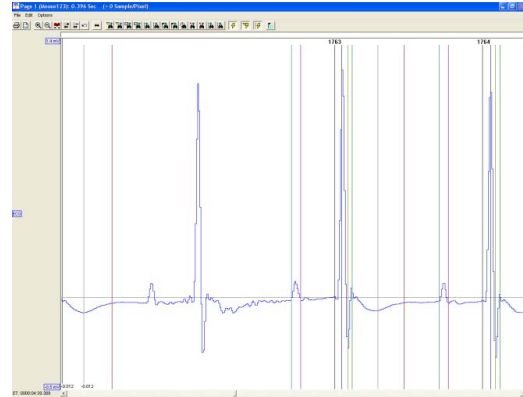


Figure 14. Example of ECG Degradation Due to Respiration

Once the data was converted to Excel™ format, several corrections to the data had to be made; adjustments to the RR interval were corrected to compensate for removed beats; QT interval, ST segment, and T interval were corrected for heart rate. Ponemah™ defines the RR interval as the interval from one marked R-wave to the next R-wave; as such if one beat (and thus the R-wave) was deleted due to a breath (or other anomaly) the corresponding RR interval would be twice the normal value. To correct the RR interval where beats had been removed the RR value was divided by the number of beats removed to correct it. After the RR interval was corrected a corrected QT interval was then calculated (relabelled as QT_c) using the derived QT interval and a method which normalizes the interval to 100 beats per minute (Mitchell, *et al.* 1998). This is required due to high resting heart rates of mice.

$$QT_c = QT / \sqrt{RR/100} \quad (1)$$

To analyze abnormalities in ST segments and T intervals (from T peak to T end) novel methods had to be created due to the lack of a true ST segment in mice, unlike that of humans. It has been noted that in mice there is an early peaking in the T-wave (which

is also merged with the end of the S-wave), (Horenziak 2010). To overcome these issues Van Acker *et al.* developed a method by which depressions and elevations of the ST segment (which is defined as the distance from end of S to the T peak) can be measured (van Acker, et al. 1996, Horenziak 2010). The T interval is defined as the difference between, T peak and T end, accordingly the following formula were used to determine ST_c and T_c .

$$ST_c = \frac{(QT - QRS - T \text{ peak})}{\sqrt{RR/100}} \quad (2)$$

$$T_c = \frac{T \text{ peak}}{\sqrt{RR/100}} \quad (3)$$

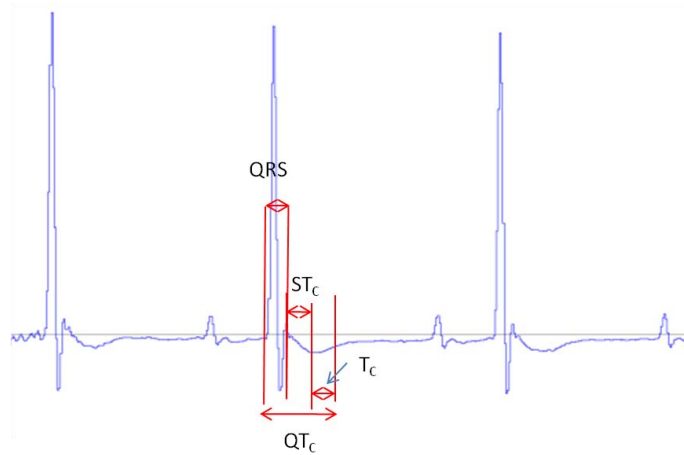


Figure 15. Parameters Used in and Derived from the Equations (1, 2, and 3)

Unlike in the study by Horenziak, the T peak was used in place of the QTAN due to large numbers of inverted T-waves (see Figure 15). Using all the data from the 30 second intervals, averages were obtained for the following parameters (QRS, QT_c, T peak to T end, T_c, ST_c) for each group for statistical analysis.

Echocardiography Methods

ECHOs were done on every mouse studied during the various weeks *in vivo* studies were conducted. The methods used to conduct the measurements and analyze them were exactly the same as those used in the study by Horezniak. However they were unable to be used due system recording and copying failures.

Statistical Methods

All data was analyzed using JMP® 8, and checked for normality, outliers, and equal variances prior to ANOVA and t testing. After the data was checked for normality, outliers, and equal variances the appropriate statistical test was conducted to determine if significance existed. All comparisons were checked for significance using a α of 0.05. All graphs show the standard error unless otherwise noted.

IV. Results

Results of Body and Tissue weights Analysis

Mouse body weights showed marked differences based on whether they were exposed to sarin or not. Sarin mice were approximately two grams lighter than those not exposed throughout the study with a significance at an α of 0.05 (see Figure 16).

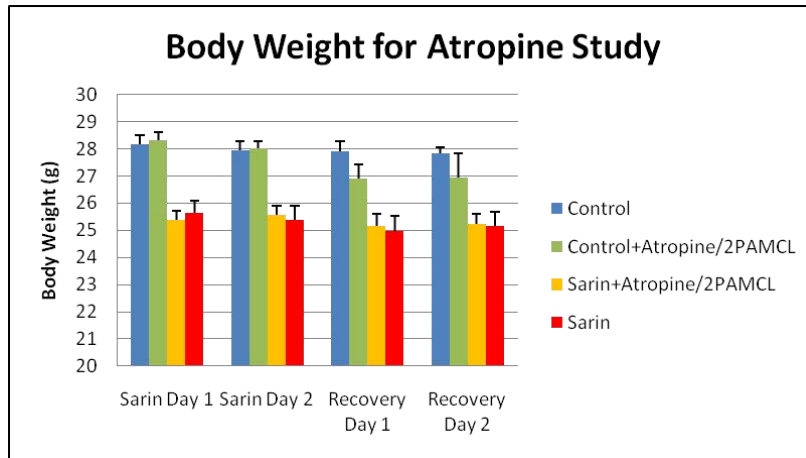


Figure 16. Body Weight Measurements from the Atropine Study

Another noticeable trend was after the atropine/2PAMCL injection of the control mice there was weight loss of around one gram in the group (although not significantly less). There was no loss of weight in the control mice over the same time period. The control+atropine mice did recover the lost weight by week seven when compared to the control mice (see Figure 17). There was also a slight loss of weight in the sarin treated mice after the second injection; however, it was not significant at the 0.05 level. There was also no difference in body weight in the first four days between the sarin and sarin+atropine groups. However, by week seven the sarin+atropine mice showed much

lower body weight than the sarin group, but it was not significant at the 0.05 level ($p = 0.054$), (see Figure 18).

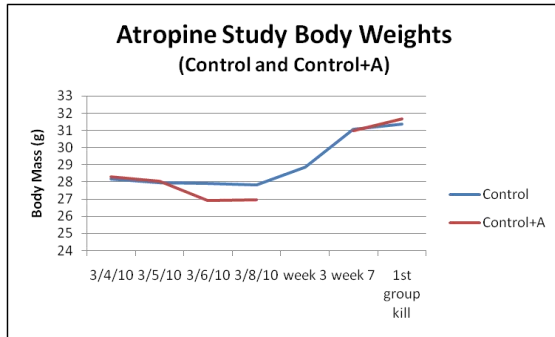


Figure 17. Comparison of Control and Control+Atropine/2PAMCL groups Body Weight

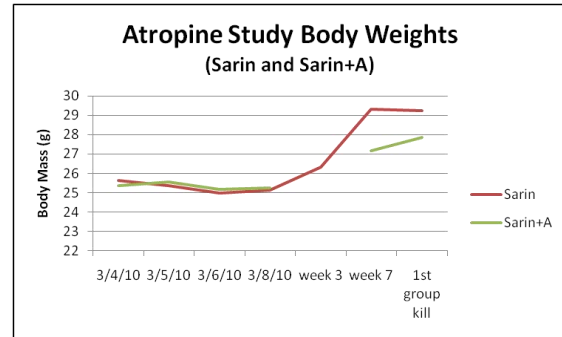


Figure 18. Comparison of Sarin and Sarin+Atropine/2PAMCL groups Body Weights

In the β adrenergic drug therapy study there was a significant difference between the isoproterenol and the propranolol treatment group during week nine at the 0.05 level (see Figure 19). Body weights of the week 10 control mice from the Horenziak were taken and compared to the week nine and 11 body weights of the β adrenergic drug therapy study (the mice from the Horenziak study were treated in the same manner as the mice in this study). The control body weights averaged $38.22g \pm 1.25g$ the continued lower body weight of sarin treated mice to control mice was still observed at both weeks nine and 11. At the time of sacrifice the sarin+propranolol group's body weight was found to be significantly lower than both the other β adrenergic drug therapy groups ($p = 0.0001$ (iso) and 0.002 (saline), see Table 3, Appendix A).

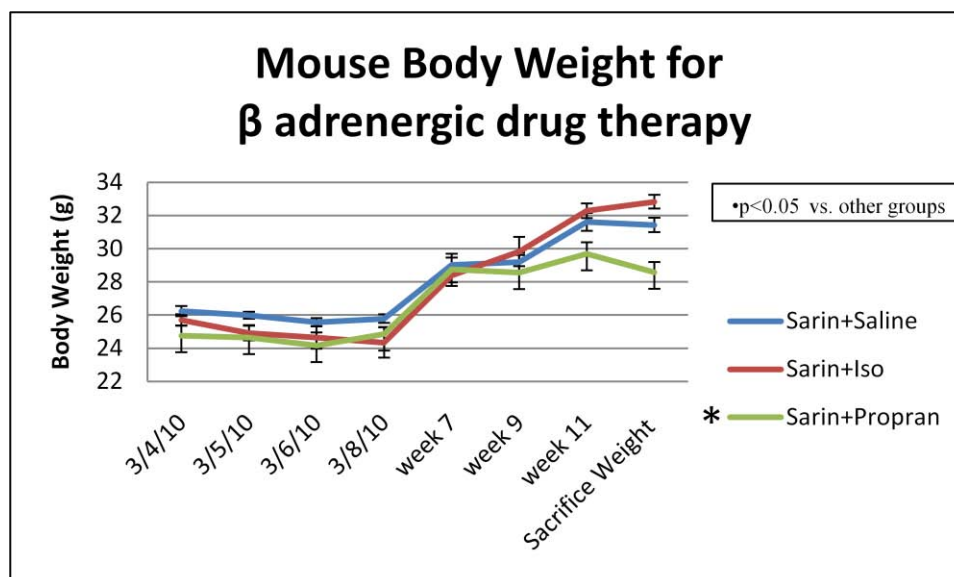


Figure 19. β adrenergic drug therapy study Body Weight Comparisons

Heart weights were normalized to mice body weights (HW/BW) and analyzed to compare overall heart size. The mice in the atropine study showed no significance between any of the treatment groups versus the control group ($\alpha = 0.05$, see Table 2).

Table 2. Atropine Study HW/BW Ratios

	Atropine Study HW/BW Ratio			
	Control	Sarin	Conrol+A	Sarin+A
Average	4.75	4.98	5.08	5.13
SEM	0.11	0.20	0.19	0.16

Additionally, there was no significance between any of the treatment groups (F prob = 0.8546). The overall trend in the atropine study mice, while not significant, shows a slight increase in the ratio of HW/BW from control, sarin, control+atropine/2PAMCL, sarin+atropine/2PAMCL (see Figure 20). The β adrenergic drug therapy study mice groups had no control group from this study, so the control group (sacrificed at week 10)

from the study done by Horenziak was used for comparison. Each treatment group (saline, isoproterenol, propranolol) showed significantly higher ratios versus the control group at the 0.05 level ($p = 0.0334, 0.0014, 0.0078$). There was also a significant difference between the isoproterenol group ($5.58 \pm .32$) and the saline and propranolol groups ($4.52 \pm 0.04, 4.70 \pm 0.07$), ($F \text{ prob} = 0.0018$). Additionally, there was no noticeable affect by the propranolol treated mice compared to the saline treated mice ($p = 0.75$).

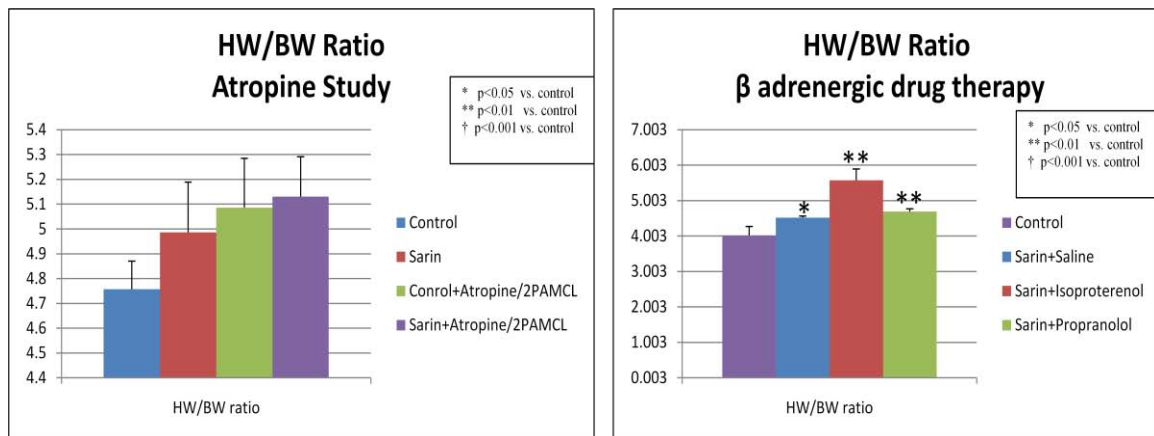


Figure 20. HW/BW Ratios for the Atropine and β Adrenergic Drug Treatment Studies

Results of Cell Size Analysis

The atropine study group showed significant increases in cell size of all treatments versus the control at the 0.05 level (see Figure 21). Additionally an one-way ANOVA between the treatment groups showed a significant difference between sarin only and those treated with atropine/2PAMCL ($F \text{ prob} = 0.0014$).

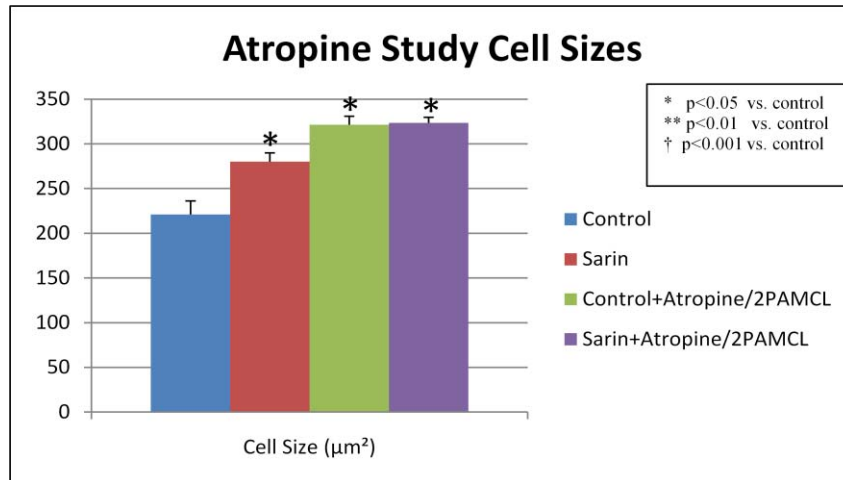


Figure 21. Atropine Study Cardio Myocyte Size Results

When compared to the Atropine study the β adrenergic drug therapy study shows a trend to larger cell size. Due to slight differences between analysis techniques in this study and Horenziak's, his week 10 H&E control data could not be compared with data from the β adrenergic drug therapy study. The β adrenergic drug therapy study showed a decrease in the cell size of the isoproterenol ($339\mu\text{m}^2 \pm 12 \mu\text{m}^2$) treated mice versus the saline ($383\mu\text{m}^2 \pm 5 \mu\text{m}^2$) treated mice ($p = 0.0296$). Additionally, there was a trend showing reduced cell size in the propranolol group versus the saline treated group (see Figure 22). Examples of the cell sizes can be seen in the pictures in Appendix C.

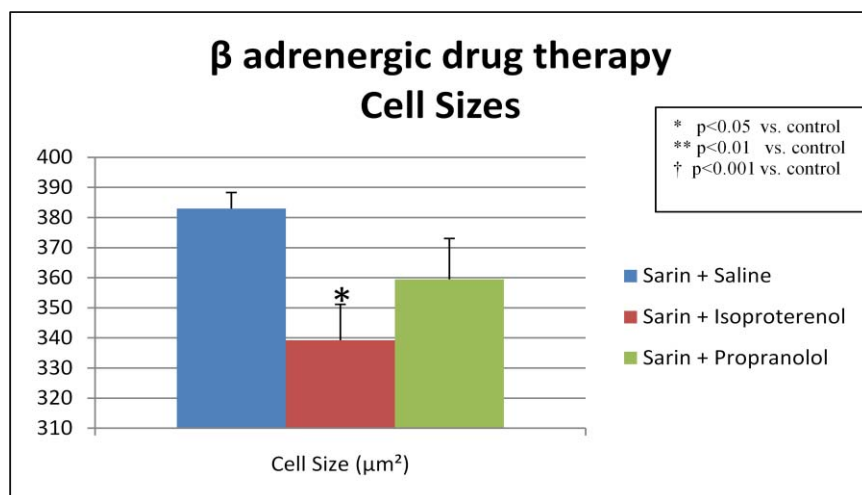


Figure 22. β Adrenergic Drug Therapy Cardio Myocyte Size

Results of ECG Analysis

The results of the ECG analysis are twofold; first, the actual changes in QTc, STc, and Tc intervals pre and post dobutamine stress testing and second, the changes in T-wave morphology pre and post dobutamine stress testing. Of the two, the T-wave morphology provides the most meaningful indicators of the possible remodeling that was occurring and the extent. The week three ECG recordings showed a trend for larger percent differences in the sarin treated mice versus control mice for QTc and Tc intervals, however only the QTc difference was significantly different (p = 0.049). There was also a noticeable decrease in the STc segment in both groups (see Figure 23).

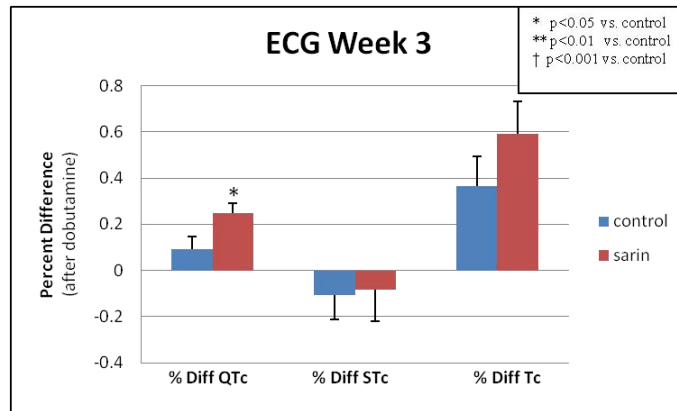


Figure 23. ECG Results from Week 3

At week seven the sarin and control+atropine/2PAMCL groups showed a decrease in the percent differences of the QTc intervals versus the control (ChiSq = 0.005 and 0.045). The STc segments for the treatment groups were lower than the control groups, however; they were not statistically significant at the 0.05 level. Additionally, the percent difference of the Tc segment from the control group was larger than all three treatment groups, significantly so, compared to the sarin and sarin+atropine/2PAMCL groups (ChiSq = 0.049 and 0.022), (see Figure 24).

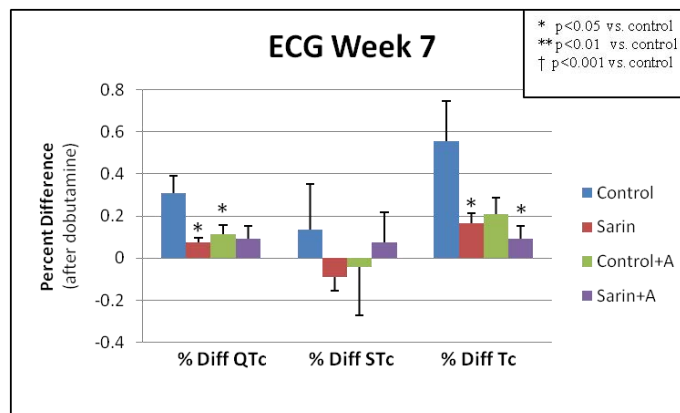


Figure 24. ECG Results from Week 7

At week nine all mice were essentially sarin treated mice as defined by study protocol. After dobutamine stress testing large numbers of mice began showing signs of T-wave inversion. All mice that showed T-wave inversion had larger percent differences for QTc, STc, and Tc, compared to the STc depressed mice ($p = 0.07, 0.10, 0.07$ respectively), (see Figure 25).

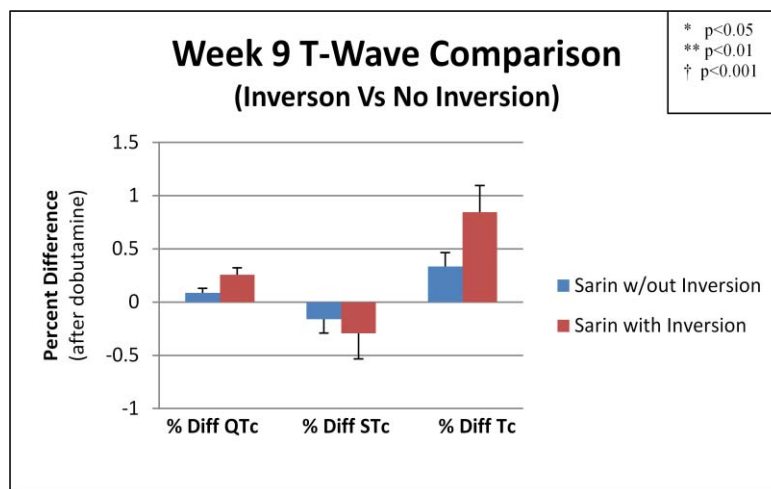


Figure 25. Comparison Between Mice with and without T-Wave Inversion at Week 9

The ECG results for week 11 (after two weeks of drug treatment) showed significance in the QTc segment difference at the 0.05 level for all groups (saline, isoproterenol, propranolol) versus the control (ChiSq = 0.025, 0.004, and 0.029). There was no difference found between the STc segment percent differences, however, the isoproterenol and propranolol groups did show significantly lower values versus the control for the Tc segment (ChiSq = 0.004 and 0.01), (see Figure 26).

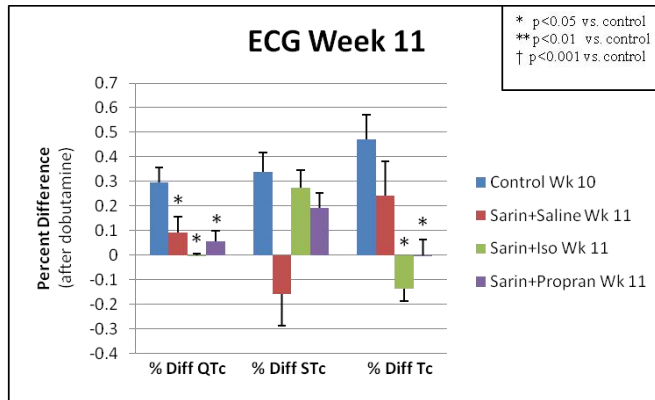
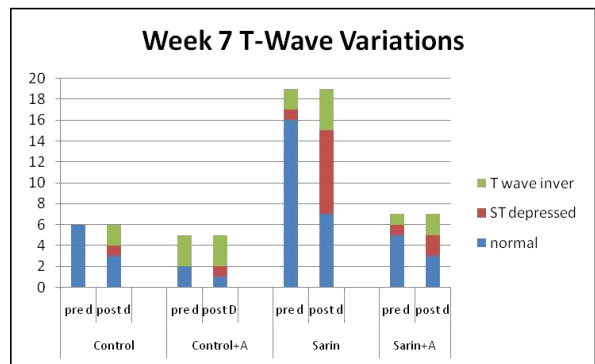
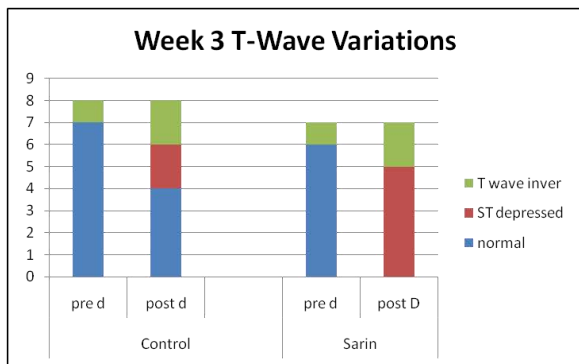


Figure 26. ECG Results from Week 11

Additional analysis of the T-waves on the ECG readouts showed increased levels of T-wave depression and inversion in the sarin exposed mice versus the controls in weeks three and seven. Comparison of week nine and week 11 T-waves showed the effects of the drug treatments on the mice. The isoproterenol treated mice showed increased heart stress, pre-dobutamine, while the propranolol treated mice showed no T-wave inversions after treatment compared to four out of seven mice at week seven (Appendix B has examples of T-wave morphology in the ECG readings).



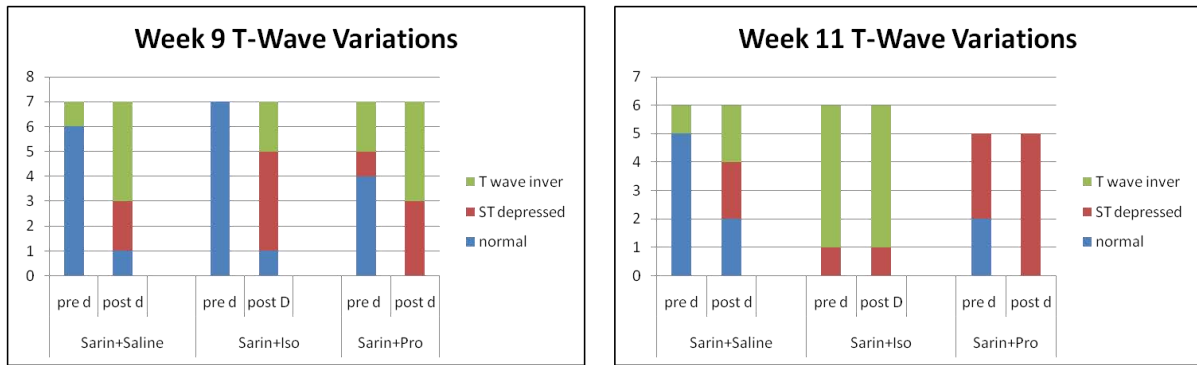


Figure 27. Comparison of T-Wave Variations at Week 3, 7, 9, and 11

Comparison of the week seven and nine sarin groups shows a continued increase in the morphology of the T-wave post dobutamine injection with little to no change in pre dobutamine indicators for sarin treated mice (see Figure 28).

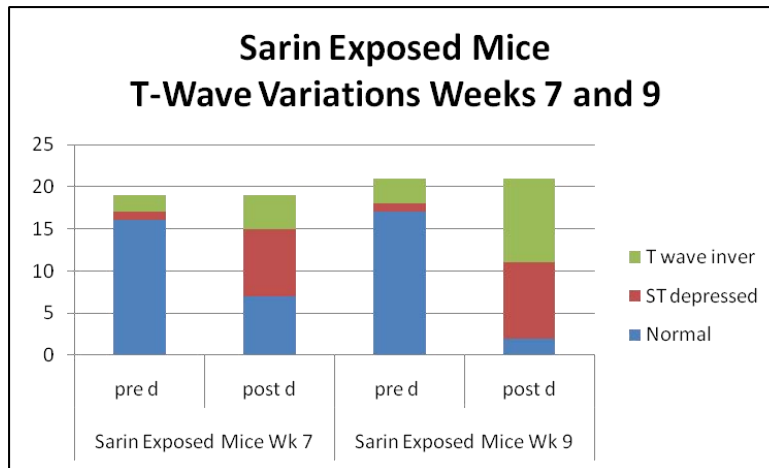


Figure 28. Weeks 7 and 9 T-wave Comparisons

Results of BNP Analysis

BNP staining was done at the end of this study. Initial qualitative examination of the slides showed increases in BNP levels for sarin and atropine/2PAMCL treated mice which was later confirmed by analysis of the atropine study group (see Figure 29).

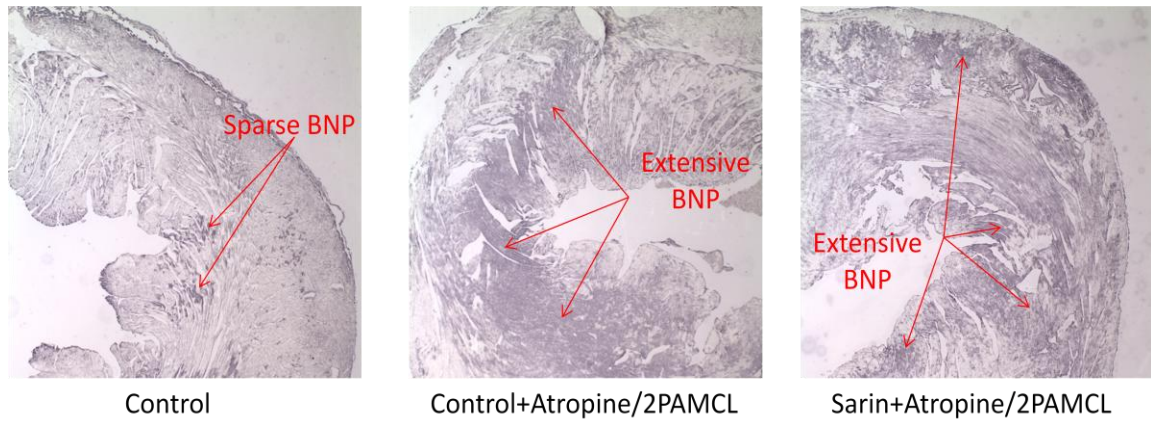


Figure 29. Examples of BNP perfusion in Control, Control+Atropine/2PAMCL, and Sarin+Atropine/2PAMCL mice ventricles

There were also possible increases in the week 11 mice BNP levels in the sarin+saline mice and decreases in the isoproterenol and propranolol treated mice versus the control. The ratio of BNP to total cell area showed significant differences between the atropine/2PAMCL treated mice (both control and sarin) versus the control ($p = 0.028$ and 0.0001 respectively), (see Figure 30).

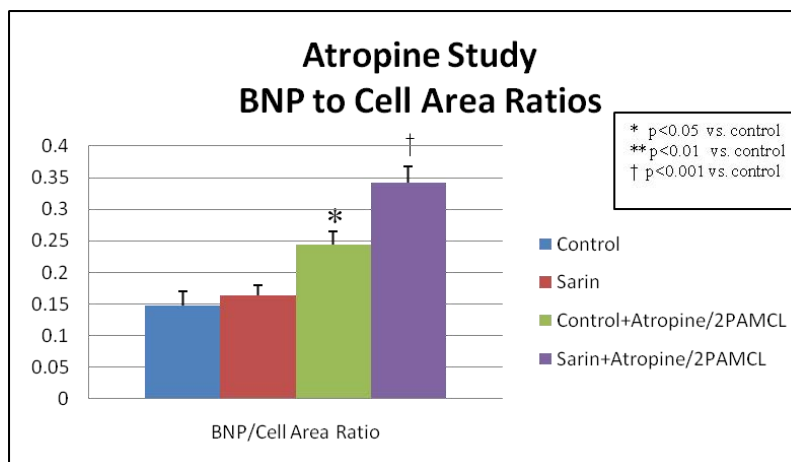


Figure 30. Results of BNP Analysis from the Atropine Study

There was even a significant difference between the sarin+atropine/2PAMCL and sarin treated mice ($p = .0001$). Comparison of BNP levels in the β adrenergic drug treatment groups were not significant at the 0.05 level (see figure 31).

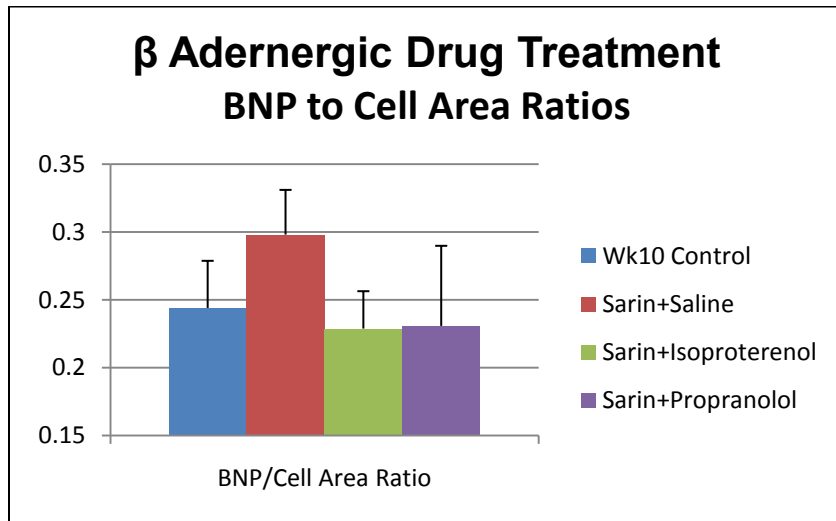


Figure 31. Results of BNP Analysis form the B adrenergic drug therapy study

IV. Discussion

Introduction

Results of previous studies have shown that a single exposure to asymptomatic levels of sarin nerve agent can lead cardiac remodeling. This study looked to determine whether the standard treatment for nerve agent exposure, (atropine/2PAMCL) could protect the heart from long term remodeling. Additionally sarin exposed mice were treated with β receptor agonists and antagonists to look at their effects on cardiac performance and morphology.

Atropine/2PAMCL Study Discussion

Change in body weight can be an indicator of any number of harmful conditions. There was a significant difference in body weights between the control and sarin exposed mice ($28.17g \pm .33$ vs. $25.78g \pm .34$). The atropine/2PAMCL treated groups showed decreases of approximately 1g in body weight post treatment (Table 4, Appendix A). By week three the two different sarin groups had both regained their weight. This effect of weight loss after sarin exposure has been well documented (Bide & Risk, 2004; Grauer, et al., 2008; Shih, et al., 2006). All groups showed a continued increase in body weight into week seven. Due to the steady (there was no sudden increase in body weight noticed) weight gain over time after the initial exposures and treatments, it is not believed to be a factor in the noticed cardiac performance differences between groups.

Heart weight is an important indicator of overall performance and heart health. There was a noticeable upward trend in HW/BW ratios from control, sarin, control+atropine/2PAMCL, sarin+atropine/2PAMCL (Table 3, Appendix A), which cannot be explained by cholinergic insult. While noticeable, the upward lacked

significance at the 0.05 level due to large variances which were caused by the small sample size. All physiological data collected from the sarin group up to week seven were consistent with previous findings which show induced long term hypertrophy in mice after sarin exposure. The physiological results showed that the atropine/2PAMCL treatment had more profound markers for hypertrophy than the sarin data, which was not expected.

Along with slightly larger HW/BW ratios the cell size analysis of the atropine/2PAMCL groups also showed that the cardio myocytes of the two groups are significantly larger than both the control and sarin groups at week seven (Table 3, Appendix A). This increase in cell size is also an indicator of hypertrophy (Takeda, et al. 2010, Harada, et al. 1997). Analysis of BNP levels in heart tissue is used as an indicator of cardiac insult and possible damage. The results of the BNP analysis also point to greater cardiac insult due to atropine/2PAMCL treatment. The higher levels of BNP in both groups treated with atropine/2PAMCL ($34.2\% \pm 5.6$ and $24.4\% \pm 2.1$) versus the control ($14.7\% \pm 2.4$) support this. Of interest is the difference even between control and sarin atropine/2PAMCL groups which suggests a possible additive affect of the two agents.

ECG's are used to identify changes in the electrical impulse propagation in the heart. Anomalies in the recordings allow for *in vivo* observation of cardiac performance. The ECG data in the atropine study shows markers which are indicative of hypertrophy in both sarin and atropine/2PAMCL treated mice. The atropine/2PAMCL groups showed an increase in QTc intervals which is a sign of possible hypertrophy (Abraham, et al. 2001). The Tc segment changes were also noted which is due to the presence of ST

depression and inverted T-waves after dobutamine stressing. Two-thirds of the atropine/2PAMCL nearly two-thirds of the sarin treated mice showed ST point depression or T-wave inversion, which are both signs of ischemia in mice (Lambiase, et al. 2003, Chu, et al. 2001, Stoller, et al. 2007, Tomai, et al. 1999). Ischemia has also been shown to be an indicator of hypertrophy due to inability of the coronary circulatory system to adequately supply blood to hypertrophic cardiac muscle tissue (Smits and Smits 2004). These results of mice treated with atropine/2PAMCL might be the result of long term damage to the muscarinic receptors caused by the atropine treatment, which caused an increase in heart rate at the time of treatment (possibly stressing the heart).

β Adrenergic Drug Therapy Study Discussion

Treatment of various heart conditions (including hypertrophy) involve the use of drugs which either stimulate or block the normal function of β adrenergic receptors. By looking at the effects of these drugs on sarin exposed mice insights into therapeutic treatments can be gained. The mice in the β adrenergic drug therapy study were treated with a 0.4LD₅₀ dose of sarin and experienced similar changes in weight loss and gain as the sarin mice in the atropine study. After the administration of the drug treatments at week nine all groups showed increases in body weight by week 11, however overall body weights were still significantly lower than the week 10 control mice, these results are consistent with the previous studies results (Horezniak 2010).

After normalization of the heart weights from week 11, using the body weight, and comparing them to the week 10 control mice, all treatment groups showed significantly larger HW/BW ratios than the control mice (see Table 3, Appendix A). The isoproterenol group also showed significantly larger HW/BW ratios than either treatment

group (see Table 3, Appendix A), this was expected as long term treatment with isoproterenol is known to induce hypertrophy (Ramos, et al. 2003, Hoffman 2001). The propranolol group showed significant change versus the control, but not against the sarin group. This lack of hypertrophic reversal is most likely due to the low level of propranolol used (20mg/kg), a study in rats showed hypertrophic reversal at doses of 40 and 80 mg/kg body weight, but not at 10mg/kg (Marano, et al. 2002). Cell size analysis showed lower cell size in the isoproterenol and propranolol groups versus the saline treated mice. Only the isoproterenol group showed statistical significance (see Table 5, Appendix A). The smaller size of the isoproterenol group is most likely due to increased cell division of the cardio myocytes (Nadal-Ginard, et al. 2003).; This assumption was unable to be confirmed. The decrease in the propranolol is likely the beginning of possible hypertrophic reversal but again was unable to be verified.

The BNP analysis showed similar results as the cell size analysis. The BNP levels were lower in both the isoproterenol and propranolol versus the saline group (but for different reasons). The isoproterenol results are most likely due to the increase in cardio myocytes which would result in a decrease in cardiac stress and thus a decrease in BNP levels. The decrease in propranolol BNP levels is likely due to the anti-hypertrophic effects of the drug which leads to lower cardiac stress and lower BNP levels.

At week nine there were noticeable differences between mice which underwent T-wave inversion and those which showed ST depression post dobutamine stressing. While none of the differences (QTc, STc, Tc) were significant at the 0.05 level ($p = 0.07, 0.11, 0.07$), the mix between the two affected the statistical comparisons of the segment

intervals. The week 11 data for the saline treated group showed similar results as the weeks seven and nine sarin mice, which are all indicative of hypertrophy (see Table 6, Appendix 4). Looking at the mice T-waves pre and post dobutamine stressing shows the lack of anomalies pre dobutamine stress testing, and their presence after dobutamine stressing. The isoproterenol treated mice showed almost exclusive T-wave inversion pre and post dobutamine stressing which indicates that the heart was already stressed (which is expected after treatment with isoproterenol). This initial stressed state of the hearts resulted in no change of the QTc interval and reversed trends in STc and Tc differences after the injection of dobutamine. The results show that after treatment with isoproterenol the mice lacked any cardiac reserve and failed to respond to the dobutamine stimulus. The propranolol treated mice showed no T-wave inversion (it was present at week nine), but instead showed complete ST depression after dobutamine stress testing. ST depression is a sign of remodeling in heart tissue and may indicate the beginnings of hypertrophic reversal from the propranolol treatment. There was no difference in the Tc interval in propranolol treated mice and a small increase in the QTc interval, but they showed an increase in the STc interval.

Limitations of the Study

Due to the inability to conduct ECHO recordings on all mice during the data collection weeks, not all mice underwent dobutamine stress testing in weeks three and seven. This resulted in a lack of complete ECG and body weight data for these weeks, preventing meaningful timeline analysis using these parameters. This incomplete testing of all groups at weeks three and seven also lowered the overall n for each group, thus lowering the ANOVA statistical power. Another limitation of this study is the lack of

baseline data for the ECG recordings for each mouse before sarin exposure. Obtaining an ECG baseline, would have allowed for more in-depth analysis on the timeline of cardiac changes.

Additional limitations of the studies were that there was only one treatment (dose) level of atropine/2PAMCL and propranolol in the studies. Without being able to test multiple dose levels it was not possible to determine if their effects could be beneficial at different dose levels to either prevent or treat hypertrophy in cardio myocytes after exposure to sarin at asymptomatic doses.

This study looked at BNP levels in the cardiac tissue post sacrifice. There are assay kits which measure BNP levels in blood plasma in mice. Utilizing these kits pre and post sarin exposure would have allowed for a more complete analysis of the onset of cardiac remodeling.

Future Work

To more accurately determine if the atropine/2PAMCL treatment could be beneficial in treating possible low dose exposure to sarin, a range of treatment doses should be tested. The same study should be done with propranolol to determine if larger doses per kg of body mass would result in reversal of the hypertrophic effects of sarin. Further studies should also continue to look at the mechanism of action of sarin on the cardiac system. Additional studies of possible treatment protocols should also be explored to see if there is one that can effectively prevent or treat the cardiac remodeling caused by low dose sarin exposure. Additionally, the use of BNP levels in the blood should be analyzed to look at levels over time to see if they change over time and correspond to other markers of cardiac remodeling.

Conclusion

All data obtained during the two studies reinforced the findings by Horezniak, that acute low dose sarin exposure causes hypertrophy in the hearts of mice. The standard antidote treatment dose of atropine/2PAMCL (10 and 25 mg/kg) in rodents is not effective in preventing hypertrophy in cardiac tissue of mice, in fact, the data collected on the mice treated in this manner showed stronger markers of hypertrophy than the sarin treated mice. The increased stress on the heart from isoproterenol and increased cardiac load of sarin mice after dobutamine injection indicate that the low dose sarin exposure does not damage the β_1 adrenoreceptor cascade. This suggests that it would be possible to treat the hypertrophic effects caused by sarin with propranolol possibly reversing the hypertrophy if proper dose levels can be determined.

The ECG recordings of these studies continued to show the insidious nature of low dose sarin exposure by appearing normal prior to dobutamine stress testing. These results are of extreme interest to the military community for several reasons. First, most early warning equipment employed by the military are designed and employed to detect levels of CWA that pose a direct threat to life or limb. These levels are higher than symptomatic doses, thus, they would not be able to detect nerve agents at the asymptomatic dose levels used in this experiment. Second, based on the normal cardiac output that is present at a non-stressed state, personnel exposed to asymptomatic levels would show no issues during normal activities, such garrison or non-field operations. However, this would quickly change during periods of high cardiac output, i.e., physical fitness training, deployment operations, and field operations, which, could affect heart performance or result in heart failure of the Soldier. Third, current post deployment

physicals occur far before these symptoms would present themselves and annual physicals do not include a heart stress test unless there are extenuating circumstances. It is therefore extremely important that any possible (no matter how minor) exposure to nerve agents be reported and be included in Soldiers' medical records to ensure proper medical care in the future. These physical concerns of heart performance, holes in the current medical screening process, and detection limitations of CWA's demonstrate the need for further examination of low dose toxicological effects of sarin.

Appendix A: Data Tables

Table 3. HW/BW Ratio Data

Atropine Study HW/BW Ratio				
	Control	Sarin	Conrol+A	Sarin+A
Average	4.75	4.98	5.08	5.13
SEM	0.11	0.20	0.19	0.16

β adrenergic drug therapy HW/BW Ratio				
	Control*	Sarin	Iso	Prop
Average	4.02	4.52	5.58	4.70
SEM	0.25	0.04	0.32	0.07

* Indicates data used from a separate study

Table 4. Body Weight Data

Atropine Study Groups Body Weights								
		3/4/10	3/5/10	3/6/10	3/8/10	week 3	week 7	Sacrifice
control	Average	28.17	27.96	27.92	27.82	28.88	31.05	31.36
	SEM	0.33	0.32	0.35	0.22	0.54	0.96	0.83
sarin	Average	25.78	25.75	25.31	25.42	26.34	30.68	29.25
	SEM	0.34	0.36	0.31	0.33	0.46	0.75	0.45
control+A	Average	28.32	28.03	26.92	26.96	No Data	31.00	31.68
	SEM	0.29	0.26	0.49	0.87	No Data	0.65	0.54
sarin+A	Average	25.37	25.56	25.16	25.25	No Data	27.17	27.86
	SEM	0.34	0.35	0.45	0.34	No Data	0.72	0.53

Drug Treatment Study Groups Body Weights									
		3/4/10	3/5/10	3/6/10	3/8/10	week 7	week 9	week 11	Sacrifice
Sarin + saline	Average	26.24	25.99	25.57	25.79	29.02	29.20	31.61	31.43
	SEM	0.30	0.21	0.25	0.27	0.44	0.56	0.53	0.43
Sarin + isoprtrenol	Average	25.70	24.91	24.65	24.34	28.38	29.83	32.28	32.83
	SEM	0.35	0.44	0.67	0.91	0.41	0.89	0.45	0.41
Sarin + propranolol	Average	24.76	24.64	24.17	24.85	28.75	28.56	29.70	28.59
	SEM	0.62	0.75	0.78	0.44	0.95	1.07	0.69	0.61

Appendix A: Data Tables

Table 5. Cardio Myocyte Cell Size Data

Atropine Study Groups Cell Sizes (μm^2)				
	Control	Sarin	Control+Atropine/2PAMCL	Sarin+Atropine/2PAMCL
Average	221	280	321	324
SEM	15	10	9	6

β adrenergic drug therapy Cell Sizes (μm^2)			
	Sarin + Saline	Sarin + Isoproterenol	Sarin + Propranolol
Average	383	339	360
SEM	5	12	14

Table 6. T-Wave Comparison Data

Week 3 Atropine Study T-Wave Types				
	Control		Sarin	
	pre d	post d	pre d	post D
normal	7	4	6	0
ST depressed	0	2	0	5
T wave inversion	1	2	1	2

Week 7 Atropine Study T-Wave Types								
	Control		Control+Atropine		Sarin		Sarin+Atropine	
	pre d	post d	pre d	post D	pre d	post d	pre d	post d
normal	6	3	2	1	16	7	5	3
ST depressed	0	1	0	1	1	8	1	2
T wave inversion	0	2	3	3	2	4	1	2

Week 9 Drug Treatment Study T-Wave Types								
	Sarin+Saline		Sarin+Isoproterenol		Sarin+Propranolol		Combined	
	pre d	post d	pre d	post D	pre d	post d	pre d	post d
normal	6	1	7	1	4	0	17	2
ST depressed	0	2	0	4	1	3	1	9
T wave inversion	1	4	0	2	2	4	3	10

Week 11 Drug Treatment Study T-Wave Types						
Wave Type	Sarin+Saline		Sarin+Isoproterenol		Sarin+Propranolol	
	pre d	post d	pre d	post D	pre d	post d
normal	5	2	0	0	2	0
ST depressed	0	2	1	1	3	5
T wave inversion	1	2	5	5	0	0

Appendix A: Data Tables

Table 7. Atropine Study ECG Data

		QRS pre	QRS post	Tpe pre	Tpe post	HR pre	HR post	QTc pre	QTc post	STc2 pre	STc2 post	Tc2 pre	Tc2 post	% diff QTc	% diff STc2	% diff Tc2	% diff HR
Control	Average	14.88	14.40	24.99	29.46	454.26	548.90	51.14	55.41	17.57	14.81	21.27	27.90	0.09	-0.11	0.36	0.22
Week 3	STD	3.33	2.80	6.12	7.17	49.28	53.36	4.44	6.04	5.24	3.75	4.90	6.04	0.16	0.30	0.37	0.14
Sarin	Average	15.46	15.14	24.73	32.39	395.79	537.25	47.17	58.55	14.76	13.59	19.84	30.61	0.25	-0.08	0.59	0.38
Week 3	STD	1.69	1.95	5.76	3.61	54.36	40.71	4.91	5.34	3.27	5.66	3.73	3.46	0.11	0.37	0.37	0.20
Control	Average	15.36	15.59	21.43	28.13	446.68	577.46	44.36	57.30	12.81	14.59	18.29	27.41	0.31	0.14	0.56	0.31
Week 7	STD	1.04	1.53	5.91	7.19	52.25	47.02	4.71	4.28	1.20	6.38	4.24	6.28	0.21	0.52	0.47	0.17
Sarin	Average	15.93	15.93	27.84	29.72	492.39	550.80	51.35	54.38	11.62	10.65	25.33	28.49	0.06	0.00	0.18	0.13
Week 7	STD	1.92	1.98	8.43	3.82	63.07	46.27	5.41	7.14	6.07	6.61	8.48	4.19	0.10	0.52	0.21	0.09
Iso+Prop	Average	14.89	14.97	25.74	26.92	460.61	590.66	49.35	54.08	13.75	12.61	22.58	26.63	0.11	-0.10	0.28	0.29
+Sal	STD	1.30	1.08	6.72	4.99	42.38	54.10	5.67	2.87	2.86	4.99	6.10	4.81	0.17	0.29	0.54	0.14
Combine	Average	15.22	15.27	26.41	28.06	470.64	578.07	49.98	54.17	13.08	11.99	23.45	27.21	0.10	-0.07	0.25	0.24
d Sarin	STD	1.55	1.44	7.13	4.73	50.32	53.93	5.52	4.43	4.09	5.44	6.82	4.59	0.15	0.37	0.46	0.14
Sarin	Average	15.27	15.28	25.80	26.54	464.84	580.18	50.05	53.67	13.96	12.88	22.68	25.78	0.07	-0.09	0.17	0.26
Minus T	STD	1.21	1.08	5.51	3.54	50.32	59.98	3.22	4.40	3.98	5.40	5.14	3.33	0.09	0.26	0.18	0.14
Control+	Average	14.67	14.76	30.21	32.01	414.93	540.88	52.00	57.67	14.74	13.33	25.09	30.38	0.11	-0.04	0.21	0.30
Atro	STD	0.85	0.64	1.98	4.72	46.73	69.28	3.34	2.44	2.28	7.05	2.19	5.43	0.10	0.51	0.17	0.06
Sarin+Atr	Average	15.83	15.80	26.23	25.13	490.02	588.78	49.40	53.18	11.87	13.04	23.30	24.53	0.09	0.07	0.09	0.21
o Week 7	STD	1.38	1.39	11.07	7.15	69.49	70.79	11.30	9.43	5.28	6.70	8.70	5.62	0.16	0.38	0.15	0.14

Appendix A: Data Tables

Table 8. β adrenergic drug therapy study ECG Data

		QRS pre	QRS post	Tpe pre	Tpe post	HR pre	HR post	QTc pre	QTc post	STc2 pre	STc2 post	Tc2 pre	Tc2 post	% diff QTc	% diff STc2	% diff Tc2	% diff HR
Sarin+Saline Week	Average	15.83	16.01	29.76	31.25	404.68	589.32	51.63	59.67	14.24	12.87	24.41	30.94	0.16	0.02	0.28	0.46
	STD	1.38	2.07	4.56	9.03	30.36	28.99	4.90	6.16	3.29	6.64	3.79	8.84	0.15	0.73	0.39	0.11
Sarin+Iso Week 9	Average	16.25	15.69	20.39	31.34	458.99	636.80	46.87	57.50	14.96	9.19	17.71	32.21	0.24	-0.37	0.96	0.40
	STD	1.48	2.08	6.09	9.51	52.70	41.80	5.40	5.00	3.28	7.50	5.08	9.59	0.17	0.55	0.83	0.18
Sarin+Prop Week 9	Average	16.06	15.87	27.74	31.71	468.32	613.58	53.76	58.04	15.19	10.00	24.34	32.01	0.10	-0.33	0.48	0.32
	STD	2.46	2.55	10.29	7.81	45.64	30.14	7.36	9.02	4.27	7.32	8.44	7.69	0.25	0.49	0.60	0.12
Combined Sarin	Average	16.05	15.86	25.97	31.43	444.00	613.23	50.75	58.40	14.80	10.69	22.15	31.72	0.17	-0.22	0.58	0.39
	STD	1.75	2.13	8.13	8.36	50.61	37.99	6.40	6.64	3.48	6.99	6.62	8.32	0.19	0.60	0.67	0.15
Sarin Minus T	Average	15.56	15.26	26.87	28.07	463.46	626.07	51.64	55.80	14.62	11.65	21.38	28.59	0.09	-0.16	0.33	0.36
	STD	1.61	1.77	9.21	6.20	43.70	35.90	5.50	7.77	2.95	5.49	9.75	5.90	0.14	0.43	0.43	0.14
Sarin w/ T Inver	Average	16.58	16.51	24.97	35.13	422.59	599.11	49.77	61.27	14.99	9.63	18.97	35.17	0.26	-0.29	0.84	0.43
	STD	1.83	2.39	7.11	9.14	50.97	36.78	7.44	3.69	4.15	8.52	8.33	9.48	0.20	0.76	0.79	0.15
Sarin Week 11	Average	17.48	18.76	28.15	29.76	430.60	541.34	53.89	57.40	15.32	11.26	23.78	28.36	0.09	-0.16	0.24	0.27
	STD	0.83	1.73	2.09	7.05	35.95	33.55	4.41	7.44	4.78	3.43	1.04	7.10	0.16	0.31	0.35	0.08
Sarin+Iso Week 11	Average	16.77	16.87	33.96	29.80	555.60	557.08	62.14	62.05	13.68	17.48	32.29	28.29	0.00	0.28	-0.14	0.00
	STD	1.65	1.74	8.04	9.48	64.66	62.12	1.72	2.03	3.78	5.54	6.05	7.93	0.02	0.17	0.13	0.02
Sarin+Prop Week	Average	16.75	16.32	48.08	40.81	384.05	503.28	63.26	66.09	11.89	14.12	38.06	37.16	0.06	0.19	0.00	0.32
	STD	3.57	3.84	12.12	5.65	54.16	46.47	9.71	4.98	3.51	4.45	8.15	3.60	0.10	0.15	0.15	0.16
Week 10	Average	16.49	17.33	21.19	27.17	395.22	553.55	4.42	5.66	13.78	14.19	17.11	25.84	0.30	0.34	0.47	0.40
Control *	STD	2.42	2.62	4.10	4.47	31.74	242.91	0.53	0.46	4.83	3.34	3.07	3.97	0.17	0.22	0.28	0.25

Appendix B: ECG Pre and Post Dobutamine Recordings

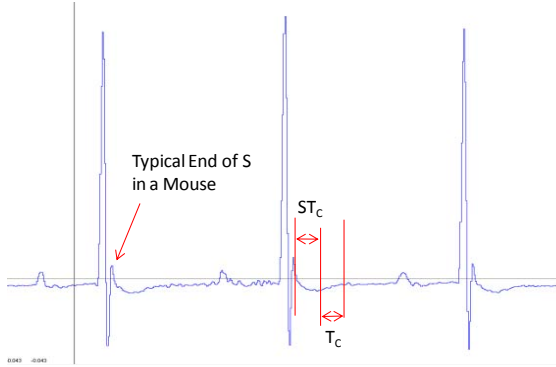


Figure 32. Control Mouse ECG Trace Before Dobutamine Injection

The end of S rises above the iso-electric point and the T-wave tail falls below the iso-electric point before returning (marking the iso-electric relaxation time). Note: as depicted T_c is not corrected by the $(RR/100)^{1/2}$.

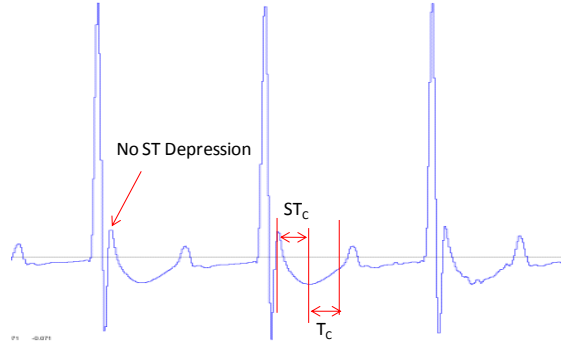


Figure 33. Control Mouse ECG Trace After Dobutamine Injection

The end of S rises above the iso-electric point. The ST_c segment and T_c segments do not change markedly.

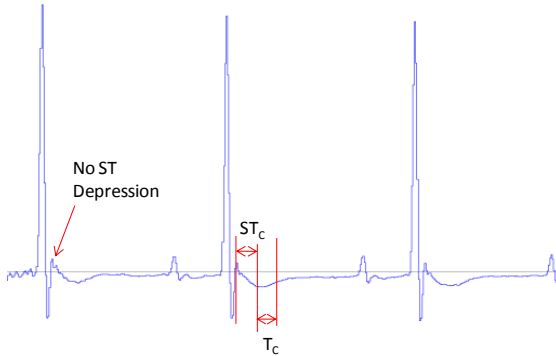


Figure 34. Sarin Mouse ECG Trace Before Dobutamine Injection

The end of S rises above the iso-electric point slightly less than the controls (but not markedly).

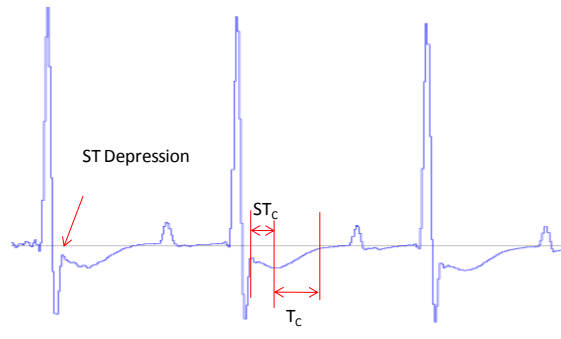


Figure 35. Sarin Mouse ECG Trace After Dobutamine Injection Showing ST Depression

The end of S does not rise to the iso-electric point. The ST_c segment is slightly shortened when compared to the controls and T_c segment is significantly lengthened.

Appendix B: ECG Pre and Post Dobutamine Recordings

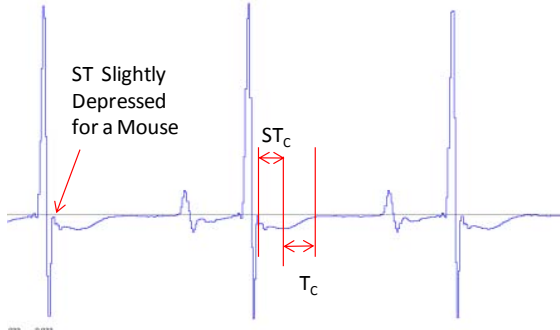


Figure 36. Sarin Mouse ECG Trace Before Dobutamine Injection
The ST is slightly depressed. The T-wave tail falls below the iso-electric point before returning.

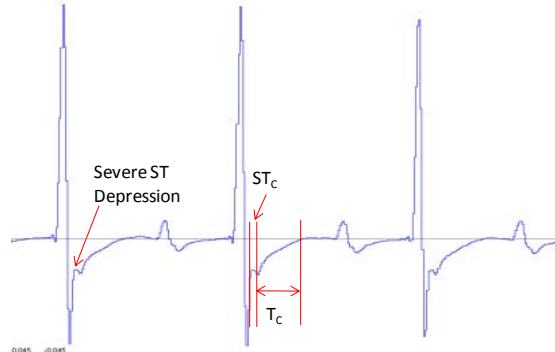


Figure 37. Sarin Mouse ECG Trace After Dobutamine Injection Showing Severe ST Depression
The end of S does not rise to the iso-electric point. The ST_c segment is significantly shortened when compared to the controls and T_c segment is significantly lengthened.

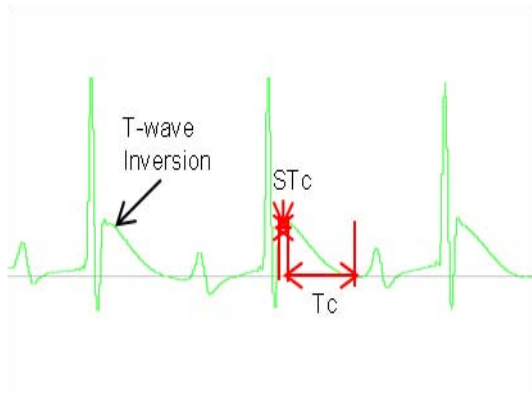


Figure 38. Isoproterenol Treated Mouse ECG Trace Before Dobutamine Injection
The T-wave is already inverted and ends at the start of the P-wave

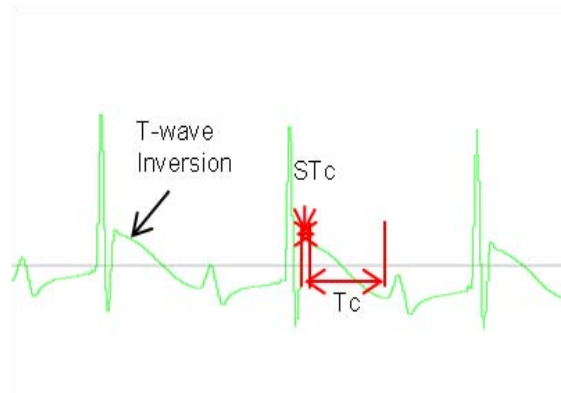
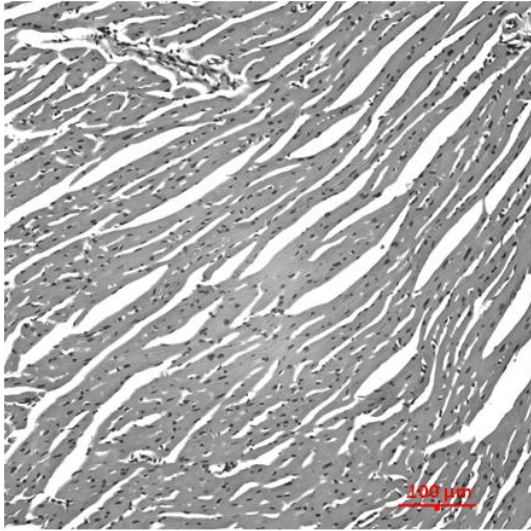
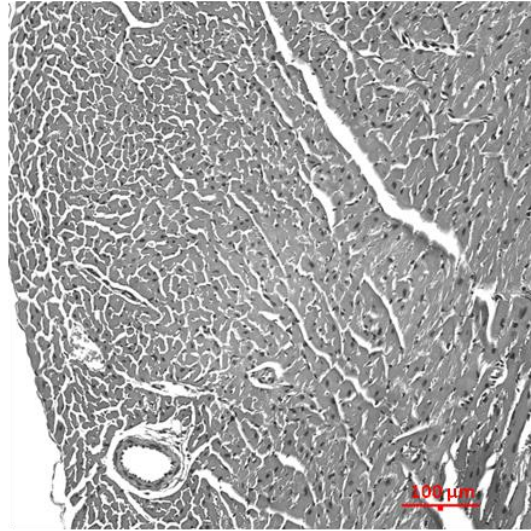


Figure 39. Isoproterenol Treated Mouse ECG Trace After Dobutamine Injection
There was no change in the T-wave even after dobutamine injection

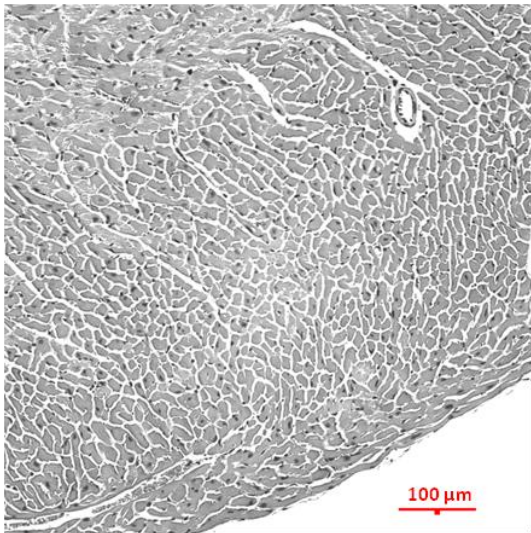
**Appendix C:
H&E Stained Slide Picture Examples Used for Cell Size Analysis**



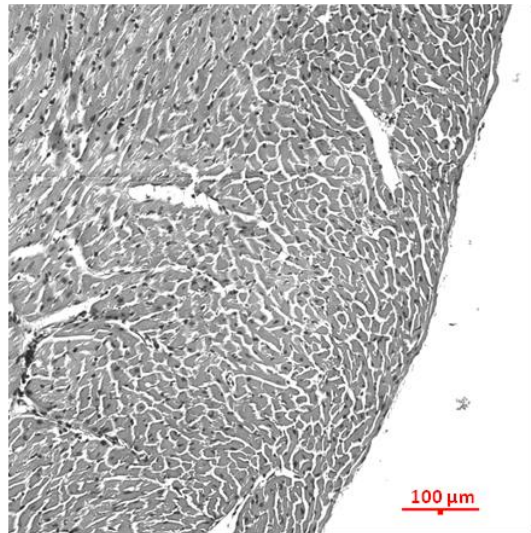
Control Mouse



Sarin Mouse



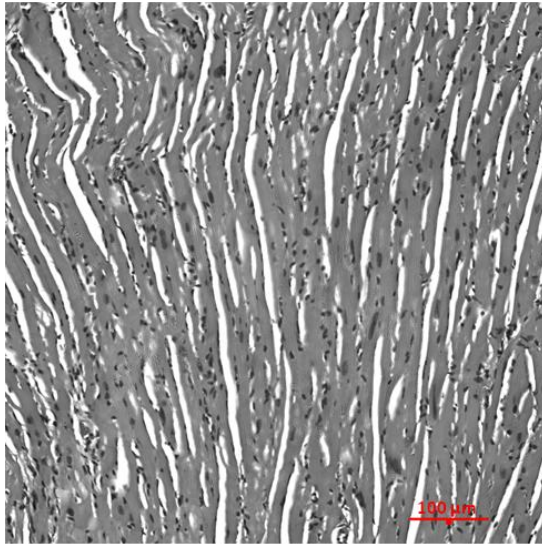
Control+Atropine/2PAMCL Mouse



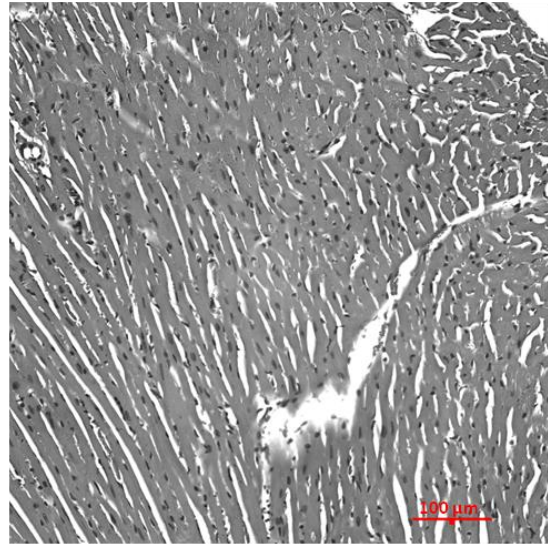
Sarin+Atropine/2PAMCL Mouse

Figure 40. Photos of Each Treatment Group from the Atropine Study

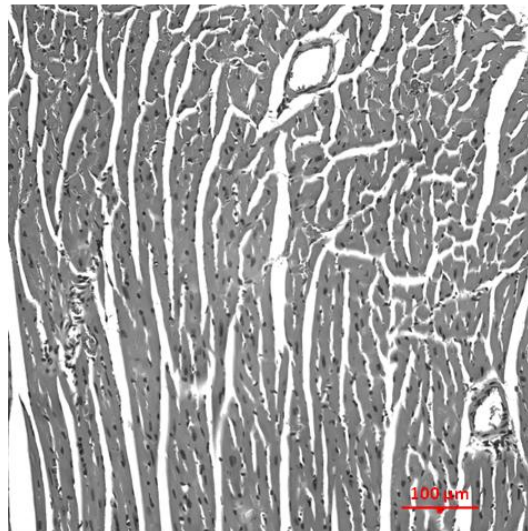
**Appendix C:
H&E Stained Slide Picture Examples Used for Cell Size Analysis**



Isoproterenol Treated Mouse



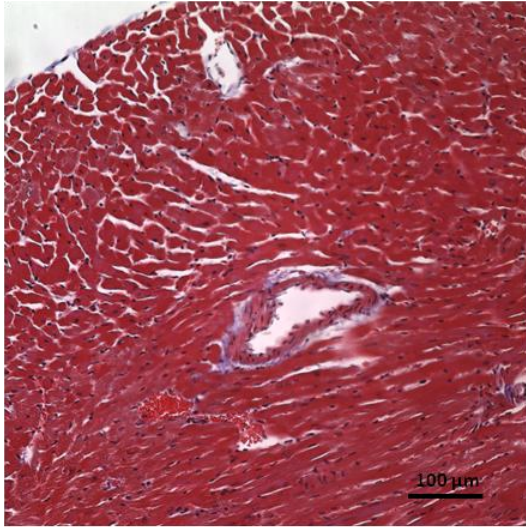
Propranolol Treated Mouse



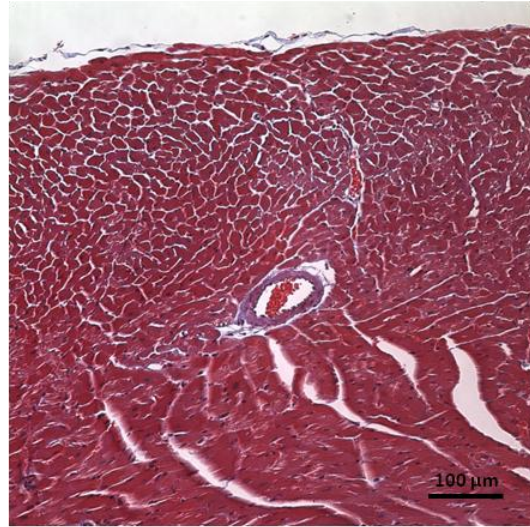
Saline Treated Mouse

Figure 41. Photos From the β Adrenergic Drug Treatment Study

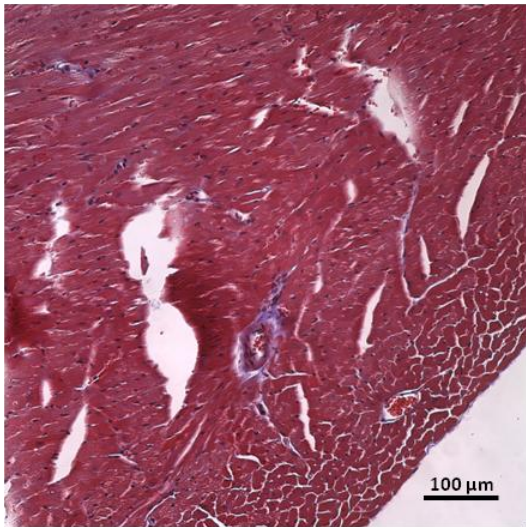
Appendix D: Masson's Trichrome Photos



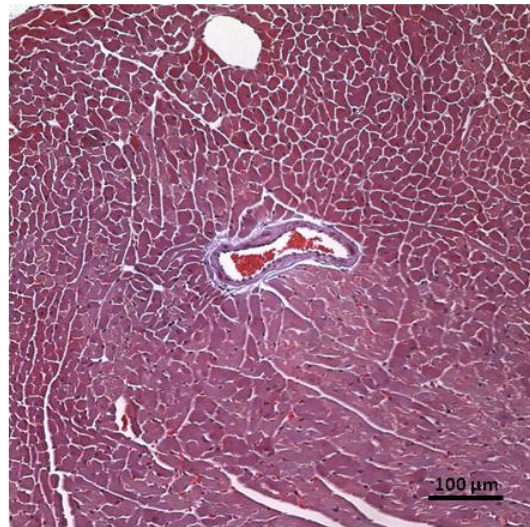
Control



Sarin



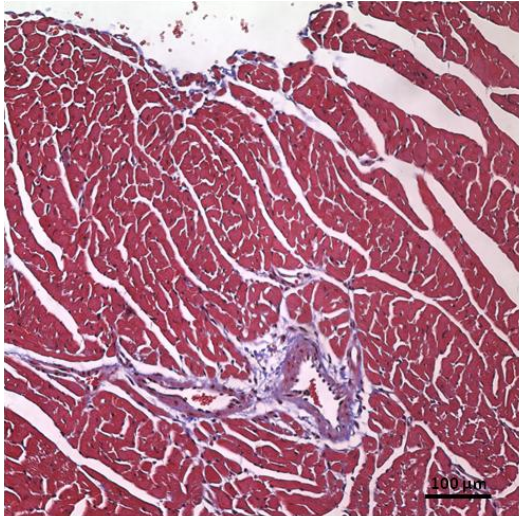
Control+Atropine/2PAMCL



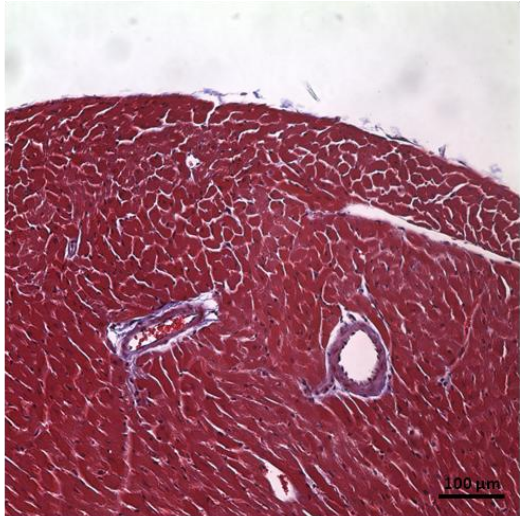
Sarin+Atropine/2PAMCL

Figure 42. Masson's Trichrome Photos from Atropine Study

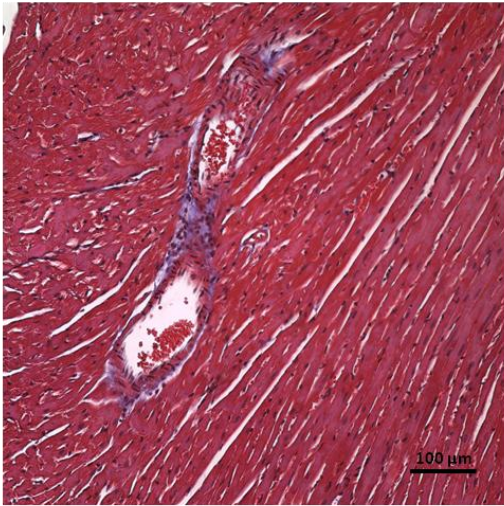
Appendix D: Masson's Trichrome Photos



Isoproterenol



Propranolol



Saline

Figure 43. Masson's Trichrome Photos from β Adrenergic Drug Treatment Study

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REPORT DOCUMENTATION PAGE

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6. AUTHOR(S) Miller, Joshua T. MAJ, USA				5d. PROJECT NUMBER	
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14. ABSTRACT Recent studies have shown that a single asymptomatic dose exposure to the nerve agent sarin can lead to long term cardiac dysfunction. This study looked at immediate and delayed treatment therapies post exposure on cardiac function. The mice were studied using electrocardiography (QTc, STc, and Tc intervals) and histological techniques (hematoxylin and eosin staining and brain natriuretic peptide (BNP) staining). Male C57BL/6J mice were injected with sarin (0.4LD ₅₀) and studied up to 11 weeks. Mice which were given a single dose of the nerve agent treatment atropine/2PAMCL, showed significantly higher cell size and BNP levels than the control (p = 0.001) and increased QTc and Tc intervals, all of which are indicators of cardiac insult. Mice which received drug treatments after the onset of cardiac remodeling showed increased (thus worsening) normalized heart size for isoproterenol (β agonist) treated mice (p = 0.0018) versus saline treated mice along with inverted T-waves pre cardiac stressing. Propranolol (β blocker) treated mice showed only T-wave depression and no difference in normalized heart size versus the saline group. These results suggest that standard treatment methods for nerve agent exposure are not effective in preventing long term cardiac dysfunction and in fact cause them.					
15. SUBJECT TERMS GB, sarin, low dose, beta blockers, atropine, oximes					
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