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14. ABSTRACT The research goal was to undertake a study to define radiofrequency/microwave (RF/MW) exposure parameters that produce non-thermal effects both on catecholamine release, using cultured adrenal chromaffin cells as an <i>in vitro</i> model system, and on skeletal muscle contraction, using intact skeletal muscle strips. Major accomplishments included 1) implementation of experiments following completion of the design, characterization and construction both of a waveguide-based exposure system (0.75-1 GHz frequency range) and a free space exposure system (1-6 GHz frequency range) for on-line monitoring of catecholamine release during exposure of chromaffin cells to RF/MW fields; 2) implementation of experiments following completion of the design, characterization and construction of a waveguide-based exposure system for monitoring skeletal muscle contraction during exposure to 0.75-1 GHz RF fields, and 3) preliminary data showing apparent non-thermal effects on both biological endpoints. The research, which had been transitioned into FA9550-04-1-0194 and FA9550-05-1-0308, has been presented at several international meetings and has culminated in three peer-reviewed published papers and one Master's thesis. Personnel involved in the project included a neurobiologist and an electrical engineer as principal investigators, an associate engineer, research assistants and graduate students.					
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FINAL PERFORMANCE REPORT

Technical Proposal entitled: “Exploring non-thermal radiofrequency bioeffects for novel military applications”

Award Number: F49620-03-1-0262

Start Date: 01 June 2003

Termination Date: 30 November 2006

Principal Investigator: Gale L. Craviso, Ph.D.
Associate Professor of Pharmacology
Dept. of Pharmacology
Howard Building, Room 219
University of Nevada School of Medicine
Reno, NV 89557
Phone: 775-784-4118
Fax: 775-784-1620
Email: gcraviso@medicine.nevada.edu

ABSTRACT

The goals of the research were to undertake a study to define radiofrequency/microwave (RF/MW) exposure parameters that produce non-thermal effects both on catecholamine release, using cultured adrenal chromaffin cells as the *in vitro* model system, and on skeletal muscle contraction, using intact skeletal muscle strips. Major accomplishments included 1) implementation of experiments following completion of the design, characterization and construction both of a waveguide-based exposure system (0.75 – 1 GHz frequency range) and a free space exposure system (1 - 6 GHz frequency range) for on-line monitoring of catecholamine release during exposure of chromaffin cells to RF/MW fields; 2) implementation of experiments following completion of the design, characterization and construction of a waveguide-based exposure system for monitoring skeletal muscle contraction during exposure to 0.75 – 1 GHz RF fields and 3) preliminary data showing apparent non-thermal effects on each biological endpoint. The research, which had been transitioned into FA9550-04-1-0194 and FA9550-05-1-0308, has been presented at several international meetings and has culminated in three peer-reviewed published papers and one Master's thesis. Personnel involved in the project included a neurobiologist and an electrical engineer as principal investigators, an associate engineer, research assistants and graduate students.

SUMMARY

Objectives:

There were two main objectives. The first objective was to define radiofrequency/microwave (RF/MW) exposure parameters that produce non-thermal effects on catecholamine release, using adrenal chromaffin cells as the *in vitro* model system. The frequency ranges to be explored were 0.75 – 1 GHz (experiments conducted in a waveguide) and 1 - 6 GHz (experiments conducted in free space inside an anechoic chamber). The second objective was to begin to define the RF exposure parameters that can produce non-thermal effects on skeletal muscle contraction, using intact skeletal muscle strips (toe muscle of mouse). The frequency range to be explored was from 0.75 to 1 GHz using a waveguide-based exposure system.

Accomplishments:

Objective one

1. Using Finite-Difference Time-Domain (FDTD) numerical modeling, we have completed the design, numerical modeling, and characterization of a free space exposure system for monitoring catecholamine release on-line during exposure of chromaffin cells to RF/MW fields in the frequency range 1 - 6 GHz and have also completed its construction. Experiments are underway.
2. We have identified apparent non-thermal effects on catecholamine release using our waveguide-based exposure system.

Objective two

3. Using FDTD numerical modeling, we have completed the design, numerical modeling, and characterization of a waveguide-based exposure system for assessing the effect of RF fields in the 0.75 – 1 GHz frequency range on skeletal muscle contraction and have also completed its construction. Experiments are underway.
4. We have characterized the effects of supraphysiological temperatures on skeletal muscle contraction, examining effects on both single twitches and tetanic force.

New Findings:

1. Using a waveguide-based exposure system and on-line monitoring of catecholamine release from chromaffin cells, we have observed apparent non-thermal increases in nicotinic receptor stimulated catecholamine release when the cells are exposed to either amplitude modulated or pulse-modulated RF fields in the 0.75 - 0.85 GHz frequency range.
2. We have obtained preliminary data showing apparent non-thermal effects (decrease) on skeletal muscle contractile force by 0.75 GHz EMFs. The effect is observed only for tetanic force, not for single twitches.

Publications:

Hagan, T., Chatterjee, I., McPherson, D. and Craviso, G.L. "A novel waveguide-based radiofrequency/microwave exposure system for studying non-thermal effects on neurotransmitter release - Finite-Difference Time-Domain modeling". IEEE Transactions on Plasma Science 32:1668-1676.

Lambrecht, M.R., Chatterjee, I., McPherson, D., Quinn, J., Hagan, T. and *Craviso, G.L.* Design, characterization and optimization of a waveguide-based RF/MW exposure system for studying nonthermal effects on skeletal muscle contraction. IEEE Transactions on Plasma Science 34:1470-1479, 2006.

Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G.L. Design, characterization and optimization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells exposed to microwave radiation: Finite-Difference Time-Domain Technique. IEEE Transactions on Plasma Science 34:1455-1469, 2006.

Thesis:

Michael Lambrecht completed his M.S. in Electrical Engineering at the University of Nevada, Reno in May 2005; his thesis is titled "Design, Characterization, and Optimization of a Waveguide-Based RF/MW Exposure System for Studying Non-Thermal Effects on Skeletal Muscle Contraction". Michael is continuing to work toward a Ph.D. in Electrical Engineering at the University of New Mexico at Albuquerque.

Interactions/Transitions:

a) Oral Presentations:

Craviso, G. L., Brouse, D., Hagan, T., McPherson, D. and Chatterjee, I. Effect of radiofrequency electromagnetic fields on catecholamine release from cultured bovine adrenal chromaffin cells. Selected presentation at the "Hot Topics" session of the Gordon Research Conference on Bioelectrochemistry, New London, CT, July 2004.

Lambrecht, M., Chatterjee, I., Quinn, J., McPherson, D. and Craviso, G.L. Design and optimization of a radiofrequency/microwave exposure system for assessing effects on skeletal muscle contraction 4th International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases (ElectroMed 2005), Portland, OR, May 2005.

Yoon, J., Chatterjee, I., McPherson, D. and *Craviso, G.L.* Design and characterization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells due to non-thermal levels of 1 – 6 GHz continuous and pulsed microwave radiation - Finite-Difference Time-Domain computations. 4th International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases, Portland, OR, May 2005.

b) Poster Presentations:

Craviso, G. L., Brouse, D., Hagan, T., McPherson, D. and Chatterjee, I. Effect of radiofrequency electromagnetic fields on catecholamine release from cultured bovine adrenal chromaffin cells. Gordon Research Conference on Bioelectrochemistry, New London, CT, July 2004.

Craviso, G.L., Brouse D., Hagan, T., McPherson, D. and Chatterjee, I. "Investigations into non-thermal radiofrequency effects on the release of catecholamines from adrenal chromaffin cells". 4th International Symposium on Nonthermal Medical/Biological Treatments using Electromagnetic Fields and Ionized Gases (ElectroMed 2005), Portland, OR, May 2005.

Lambrecht, M., Chatterjee, I., McPherson, D., Quinn, J. and Craviso, G.L. Finite-difference time-domain computations of SAR distribution within an exposure system for studying the effects of radiofrequency/microwave fields on skeletal muscle contraction. Joint Meeting of the Bioelectromagnetics Society and the European BioElectromagnetics Association, Dublin, Ireland, June 2005.

Craviso, G. L., Brouse, D., Hagan, T., McPherson, D. and Chatterjee, I. Use of cultured adrenal chromaffin cells as an *in vitro* model system to study non-thermal effects of RF radiation on exocytosis. Joint Meeting of the Bioelectromagnetics Society and the European BioElectromagnetics Association, Dublin, Ireland, June 2005.

c) Other forums:

Hagan, T., Chatterjee, I., McPherson, D. and Craviso, G.L. Simulation aids first on-line monitoring of RF effects on neuroendocrine cells. Featured in Xsite, The Newsletter of Remcom, Inc., Fall 2005.

The research was also featured in the December 2006 issue of Popular Mechanics (page 32).

d) Consultative and advisory functions: None

e) Transitions: The research was begun under AFOSR grant F49620-02-1-0306 and transitioned into AFOSR grant FA9550-04-1-0194, and then into AFOSR grant FA9550-05-1-0308. Due to these transitions that resulted in concurrent funding, there is overlap in the reporting of the status of the projects in the Performance Reports for these grants.

New Discoveries, Inventions or patent disclosures: None

Honors/Awards: None

Personnel Involved in the Project:

Gale L. Craviso, Ph.D., Associate Professor of Pharmacology - Principal Investigator
Indira Chatterjee, Ph.D., Professor of Electrical Engineering - Co-Principal Investigator
Dana McPherson, Associate Engineer, Dept. of Electrical Engineering
Jeff Quinn, Research Assistant
David Brouse, Research Assistant
Paulo Vandenberg, M.S. graduate student in Electrical Engineering
Bindya Dumpala, M.S., Biomedical Engineering – graduated December 2006
Todd Hagan, M.S. (2005), currently a Ph.D. graduate student in Electrical Engineering

Personnel Supported by State of Nevada Matching funds

Michael Lambrecht, M.S. Electrical Engineering – May 2005
Jihwan Yoon, M.S. (2004), currently a Ph.D. graduate student in Electrical Engineering

COMPREHENSIVE TECHNICAL SUMMARY

Rationale

Exploring the interaction of RF/MW fields with living systems has been the focus of much research both in the United States and abroad. Of particular interest to us are studies that have provided evidence of non-thermal bioeffects produced by RF/MW exposure at low specific absorption rates (SARs). This is because the feasibility of developing non-lethal, RF/MW-based “stunning/immobilizing” weaponry is critically dependent on the ability of applied RF/MW radiation to selectively target relevant physiological processes in a highly controlled, non-deleterious manner, rather than cause tissue heating, which would affect many cellular constituents and molecules non-selectively and potentially result in tissue damage as well.

The research is based on the hypothesis that integral plasma membrane proteins such as neurotransmitter receptor binding sites, ion channels and other proteins that have moieties that extend extracellularly beyond the lipid bilayer will be a likely site of cellular interaction with applied RF/MW electromagnetic (EMF) fields. Two types of electrically excitable cells are used for exploring non-thermal effects of RF/MW exposure on plasma membrane proteins, and effects are monitored either on-line or in real-time.

1) Monitoring basal and nicotinic receptor stimulated catecholamine release from chromaffin cells during RF exposure of the cells to 0.75 – 1 GHz fields.

The research was first supported by AFOSR grant F49620-02-1-0306 and the goal was to explore RF/MW bioeffects on catecholamine release, using an on-line monitoring approach and a waveguide-based RF exposure system for the 0.75 – 1 GHz frequency range (described in Hagan et al., IEEE Transactions on Plasma Science, 2004). The research transitioned fully into this DoD EPSCoR funded grant as of June 1, 2005. As summarized in the final performance report for grant F49620-02-1-0306 that covered the period 01 June 2002 – 31 May 2005, we had

obtained experimental results that show apparent non-thermal increases in nicotinic receptor stimulated catecholamine release from chromaffin cells exposed to pulse and amplitude modulated RF fields in the 0.75 – 0.85 GHz frequency range (Appendix). These results were obtained using a matched waveguide in which chromaffin cells were immobilized on a glass fiber filter that was placed within a cell perfusion chamber to allow for continuous superfusion of the cells with a preheated physiological salt solution during the exposures. The amount of catecholamines released into the perfusate was monitored on-line by electrochemical detection. In preparing these results for publication, we determined not only that additional experiments were needed to complete the data but that before more experiments were conducted, our efforts would best be served if the exposure system was modified to address certain concerns of the existing setup. These concerns include a thermal gradient across the glass fiber filter on which the chromaffin cells are immobilized (determined by thermal modeling and actual temperature measurements), non-optimal perfusion of the cells with the nicotinic agonist, electric (E) field magnitudes that are not high enough to produce robust and consistent effects, and other factors that could contribute to erroneous interpretation of data (e.g., pressure variations during the perfusion). Work is currently underway to improve the exposure system, employing both Finite-Difference Time-Domain (FDTD) numerical modeling and experimentation.

2) Monitoring basal and nicotinic receptor stimulated catecholamine release from chromaffin cells during RF/MW exposure of the cells to 1 - 6 GHz fields.

For these experiments we originally proposed to use two waveguides consisting of a 12” section of WR430 (cross-section 10.922 cm X 5.461 cm) for the frequency range 1.70 – 2.6 and adapt existing MW generating equipment for use at 2.45 GHz. However, because the inside of these waveguides would not be large enough to accommodate the cell perfusion apparatus used for the 0.75 – 1 GHz frequency range exposures, this meant that experiments would be carried out in which chromaffin cells are placed in the inner chamber of organ culture dishes under static conditions. This in turn meant that on-line monitoring of catecholamine release during 2.45 GHz exposure of the cells would not be possible and we would instead have to rely on off-line post-exposure measurements. With funds obtained from other sources (AFOSR grant FA9550-04-1-0194 and a Defense University Research Instrumentation Program (DURIP) grant), our research plan was modified so that we can expose chromaffin cells to RF/MW fields from 1 GHz up to 6 GHz in free space while continuing to use on-line monitoring of catecholamine release during exposure of the cells. Details are given in the Progress Report for FA9550-04-1-0194.

Using FDTD numerical modeling, we have completed the design, numerical modeling, and characterization of the free space exposure system for the frequency range 1 - 6 GHz and have also completed its construction. A complete description of this setup has been published in IEEE Transactions on Plasma Science (Yoon et al., 2006). Major instrumentation for this work, which includes a RF/MW signal generator, broadband power amplifier, broad-band horn, high-power cables and a computer to control the instrumentation and experimental protocols, is set up in the screen room adjacent to the microwave anechoic chamber (located in the Harry Reid Engineering Laboratory building) where the broad-band horn antenna is placed and hence where cell exposures are carried out. We have also completed measurements of both the near- and far-field antenna radiation pattern of the broad-band horn antenna over the frequency range 1 - 6

GHz. These measurements are important for quantifying the incident field exposure on the cells.

A great deal of trial and error effort went into fine-tuning the system to both monitor and control for any conditions (fleeting changes in flow rate, temperature or pressure, etc.) that could influence the output of the electrochemical detector and thus produce misleading results. We have just recently completed many of these modifications and although experiments have just gotten underway, more modifications are needed. Because this exposure system utilizes on-line monitoring of catecholamine release using the same cell perfusion apparatus as the waveguide-based system, some of the needed modifications are the same as those identified for that setup (e.g., a thermal gradient across the glass fiber filter; non-optimal perfusion of the cells with the nicotinic receptor agonist). Others pertain to the specific frequency range being investigated (e.g., non-uniform E-fields over the 1 to 6 GHz frequency range). FDTD numerical modeling that was used throughout the design of the exposure system will continue to be utilized in conjunction with experimentation for further optimization and refinement of the exposure setup.

3) Defining RF exposure parameters in the 0.75 – 1 GHz frequency range that alter skeletal muscle contraction.

Using FDTD numerical modeling, we have completed the design, characterization and construction of a waveguide-based exposure system for assessing the effect of RF fields in the 0.75 – 1 GHz frequency range on skeletal muscle contraction. The setup consists of a tissue-organ bath system placed inside a waveguide, the same waveguide as that used for on-line monitoring of catecholamine release from chromaffin cells during RF exposure of the cells to 0.75 – 1 GHz fields. The muscle that serves as the source of skeletal tissue for experiments, *flexor digitorum brevis (FDB)* from mouse hindfoot (Figure 1), is suspended inside the inner chamber of the organ bath and muscle contraction elicited by electrical stimulation (Appendix).

Using FDTD numerical modeling, we have determined the optimal conditions for stimulating the muscle electrically during RF exposure so that (1) the RF fields that the skeletal muscle will be exposed to are homogeneous to within 30%, and, (2) the stimulating electrodes (platinum) that are used to elicit contractions do not significantly perturb the RF fields. We have also fully automated the exposure system and addressed logistical issues that arose due to our conducting muscle contraction experiments inside the waveguide. We have also constructed a feedback temperature monitor system that will allow us to deliver the greatest amount of power into the waveguide during RF exposures while maintaining the temperature of the Tyrode solution perfusing the tissue at a constant temperature. The description of the system appears in IEEE Transactions on Plasma Science (Yoon, et al., 2006) and actual photographs of the system are given in Figures 2-4.

As a prelude to our experiments, we have carried out a series of experiments in which we have characterized how skeletal muscle contraction, both single twitches and tetanic force (fused twitches), are affected by increases in temperature. This information will help us distinguish non-thermal from thermal effects. We also have obtained preliminary data showing that tetanic force, but not single twitches, is decreased when the muscle is exposed to 750 MHz EMFs (Appendix). No apparent change in temperature was found to occur in the Tyrode solution, suggesting that

the response is non-thermal in nature. However, we are exploring the nature of this effect to insure that it is not an artifact.

4) Ongoing work/Future directions.

Because this work has transitioned into other currently funded AFOSR projects, we are actively engaged in modifying our existing exposure systems for assessing RF/MW effects on catecholamine release from chromaffin cells, exploring approaches that are “outside the box” yet employ experimental conditions in which the cells are maintained as close as possible to the physiological state. Studies are also well underway examining the effects of RF exposure on skeletal muscle contraction and we expect significant progress to be made on these studies.

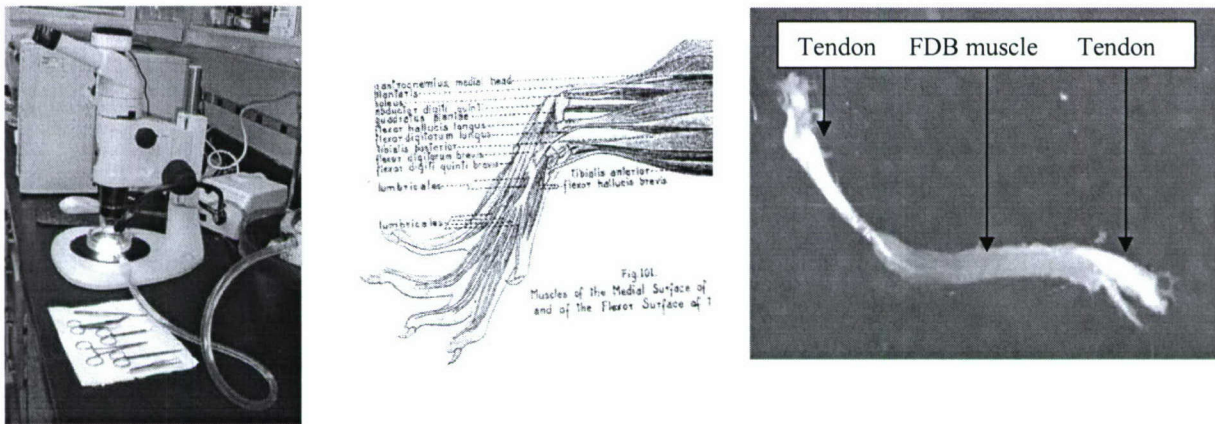


Figure 1. (Left) Photograph of the stereoscopic microscope setup for obtaining an intact FDB muscle by dissection; (middle) schematic drawing of the location of the FDB muscle in the mouse hindfoot; (right) photograph of a dissected FDB with tendons attached. A suture attached to one tendon tethers the muscle to the bottom of the organ bath and a suture attached to the other tendon tethers the muscle to the force transducer.

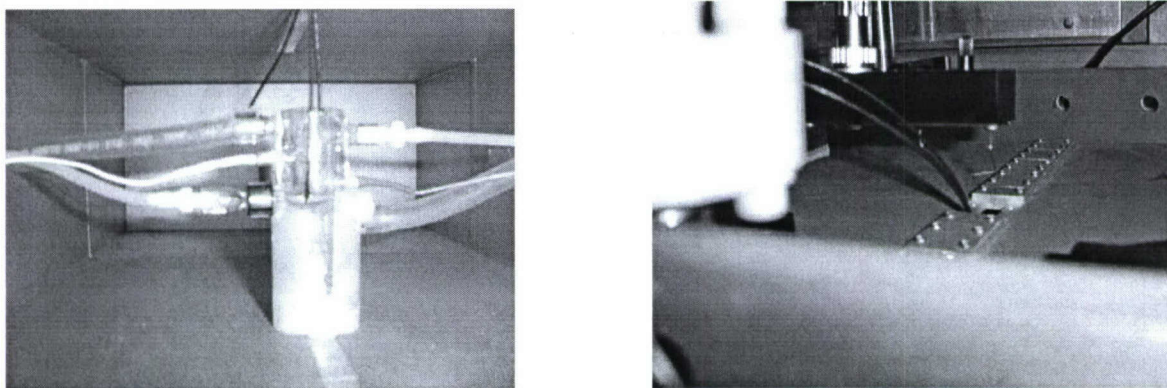


Figure 2. Photographs of: (left) the organ bath inside the waveguide showing the fluoroptic temperature probes, perfusion tubing, electrode wires, etc. exiting the waveguide via non-radiating slots; (right) a top view of the waveguide showing the fluoroptic temperature probes exiting the waveguide.

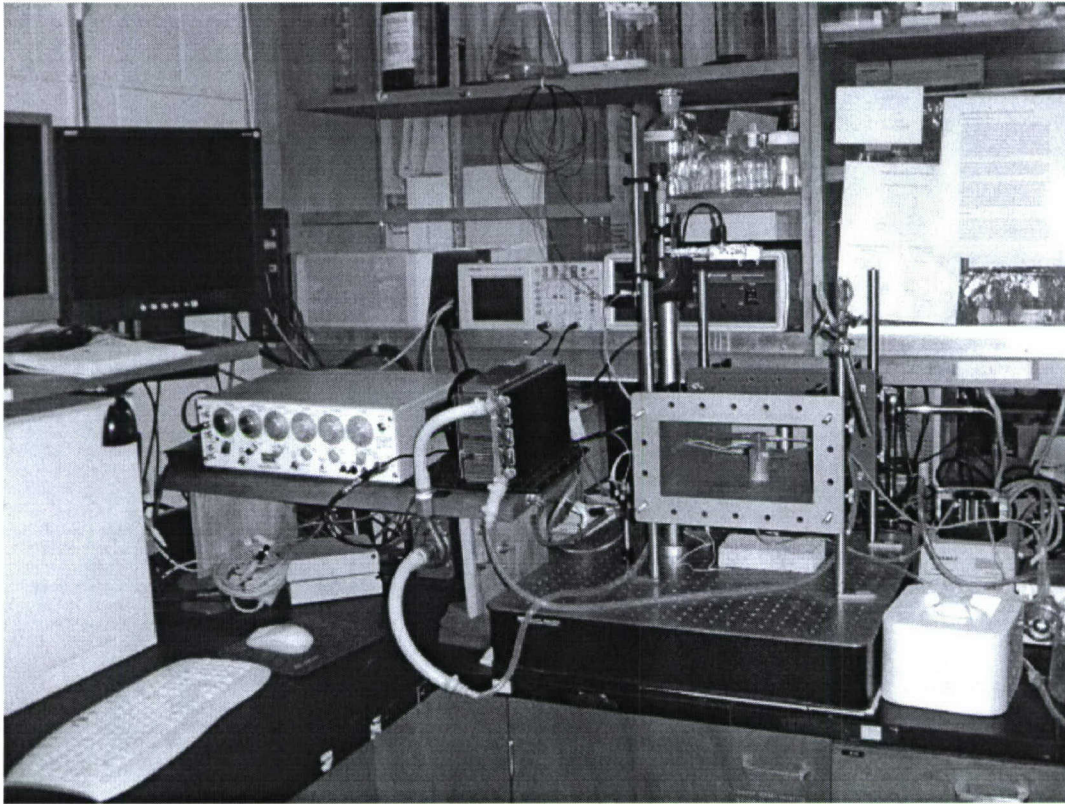


Figure 3. Photograph of entire waveguide-based exposure setup.

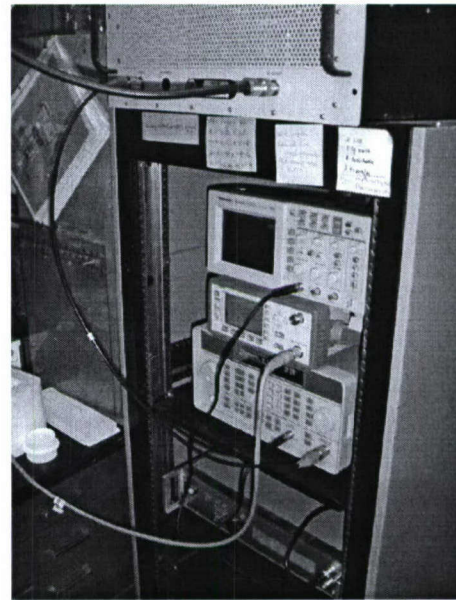
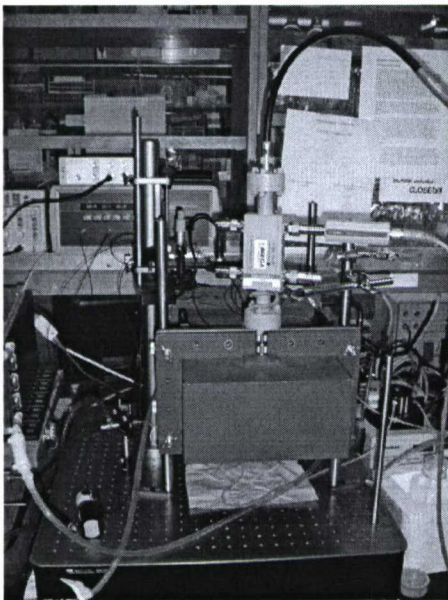
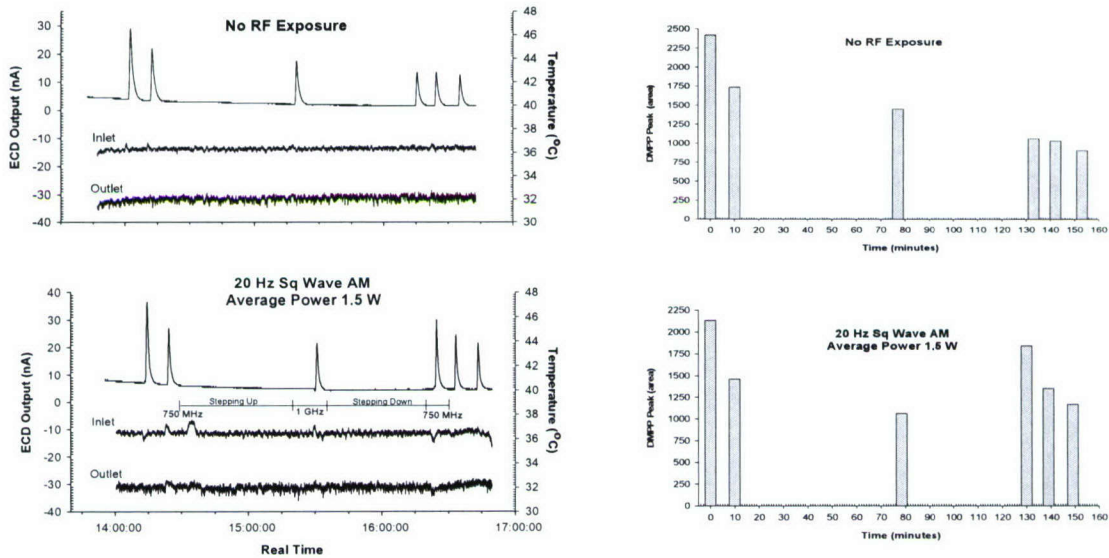


Figure 4. Photographs of: (left) the exposure system when the RF field is being delivered; (right) the RF generating equipment.

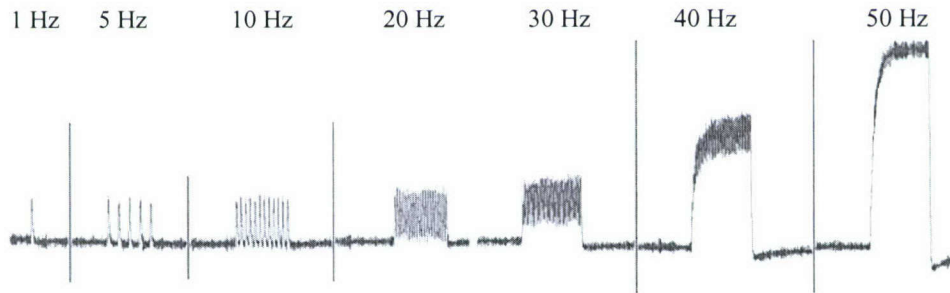
Appendix

Data from experiments studying the effects of RF exposure on catecholamine release from chromaffin cells.



Data from a representative experiment showing that RF exposure at 750 MHz (bottom panels) leads to an increase in catecholamine release stimulated by the application of a nicotinic receptor stimulus (represented by the spikes in the electrochemical (ECD) output in the figure at the left, and the areas of the spikes, plotted to the right).

Data from experiments studying the effects of RF exposure on contractile activity of mouse DFB muscle.



Data from a representative experiment showing contractile force of an FDB muscle at low to high frequencies of electrical stimulation. As frequency increases, contractile force increases when single twitches fuse to form tetanic force.



Data from a representative experiment showing the contractile response of an FDB muscle to electrical stimulations at 40, 50, 75 and 100 Hz both in the absence (top panel) and presence (bottom panel) of 750 MHz continuous wave RF fields. These data show a decrease in tetanic force in the presence of the RF field.