Air Pollution Role in Hypertension and Heart Attacks

- **Co-Investigators:**
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- **Goals: Test two main hypotheses**
  - Exposure to ultrafine (< 0.18 micrometer) ambient particles causes physiologic and pathologic damage to hearts by direct routes (not involving the lung) and by indirect mechanisms due to effects of particles on the respiratory system, and
  - Differences in susceptibility will be conferred by differences in genetic expression between sensitive populations.

Approach

- We will examine isolated working hearts to test for <u>direct effects</u> of administered ultrafine ambient particles in:
  - ☐ Normal (young adult versus senescent hearts),
  - ☐ Hypertrophied (hearts from spontaneously hypertensive rats) or
  - ☐ Hearts that had been subjected to myocardial infarction due to proximal coronary artery occlusion
- We will examine the effects of chronic inhalation exposure to ambient UFP to determine:
  - ☐ Whether progression of disease is accelerated,
  - ☐ Whether the differential susceptibility of aged and hypertensive individuals can be explained by differences in gene expression.
- By identifying genes that appear to confer susceptibility, we hope to identify possible treatments to mitigate pollutant effects.

Relevant Background

- Heart disease is the leading cause of death in the U.S.
- Exposure to air pollutants may represent an important preventable cause of both morbidity and mortality for those living in polluted environments.
- There are strong and relatively consistent associations between exposure to ambient particulate matter (PM) and the development or exacerbation of cardiovascular morbidity and mortality.
- Particulate matter (PM) is a mixture of solid particles and liquid droplets that vary in size and origin.
  - Coarse PM (between 2.5 and 10 microns) is typically derived from attrition, erosion, or dispersion of soil, road dust and construction debris or agglomeration of smaller combustion particles.
  - Fine PM (less than 2.5 micrometers) and ultrafine PM (smaller than 0.15 micrometers in diameter) are emitted, or formed, from combustion products of fossil fuels (e.g., from vehicle engines, power plants, and refineries).

Background (Cont.)

- Ultrafine particles are capable of inducing the greatest amount of pulmonary inflammation per unit of PM mass due to:
  - □ High particle number,
  - □ High pulmonary deposition efficiency,
  - □ High surface area that can carry adsorbed or condensed toxic air pollutants (oxidant gases, organic compounds and transition metals) having pro-inflammatory effects.

- Ultrafine PM can avoid phagocytosis by alveolar macrophages and enter pulmonary interstitial sites, including the endothelium.

- Ultrafine PM may induce pulmonary inflammation at both epithelial and interstitial sites, as well as enter the circulation to reach other target sites, including cardiovascular tissue.

EXPERIMENTAL DESIGN FOR IN-VIVO STUDIES

- Normal and compromised rats will be exposed, whole body, to CAPs or filtered air for 6 hours/day, 5 days per week, for up to 3 months.

- CAPs @ ~10x ambient aerosol concentrations

  - Low pressure impactor Inlet will be used to remove PM larger than 0.15 μm

  - Exposures conducted in Riverside California

    - California Air Quality Monitoring Site

    - Peak hourly PM concentrations during high pollution season over 600 μg/m3 were measured in 2003.

  - Initial Dose-Response to set Conditions for Overall Study

- Filtered air exposure controls

- Lungs and Hearts will be examined for:

  - Markers of inflammation and oxidative stress

  - Histological changes (cardic cell hypertrophy, pulmonary lesions)

  - Changes in Gene Expression

Figure 8
Suspended Particulate Matter (PM10) - 1996
Annual Arithmetic Mean, μg/m³

SCAQMD
AIR MONITORING NETWORK

.... SOUTH COAST
AIR BASIN (SCAB)

—·—·— COUNTY LINES

● AIR MONITORING
STATION

0 ——————— 25
MILES

JUNE 1996 VERSION

– – – Federal Standard
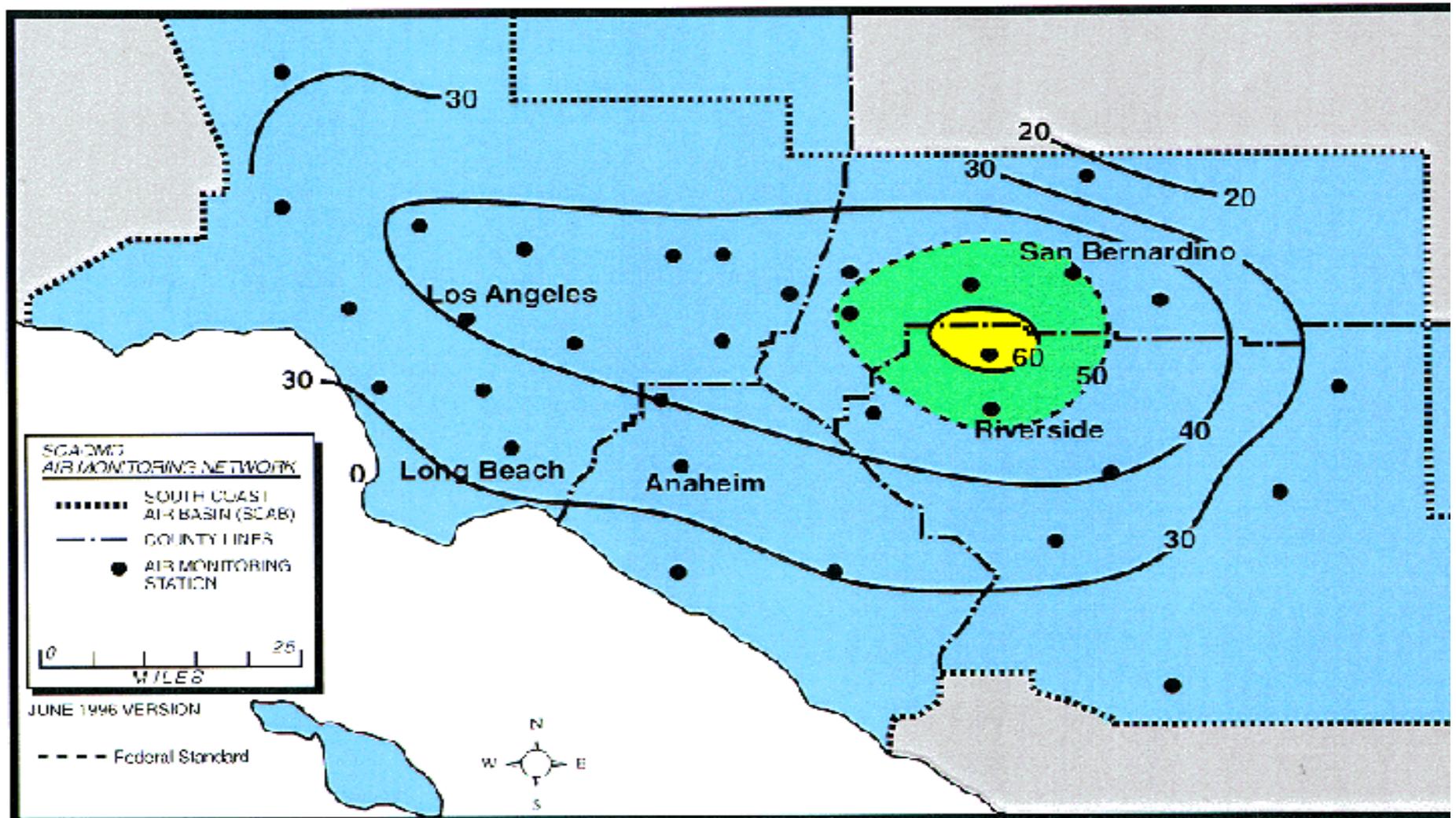
NOT-EXCEEDED   1 - 5   5 - 19   20 OR MORE

EXPERIMENTAL DESIGN FOR EX-VIVO (LANGENDORFF) STUDIES

- Hearts from healthy and compromised animals will be obtained and perfused with buffer or serum-based buffer.
- $PM_{0.15}$ CAPs will be collected at Riverside CA using the system developed by Sioutas for in-vitro sample collection.
- Perfused hearts will be exposed to CAPs
- Tissue and perfusate will be examined for:
  - ☐ Inflammatory Biomarkers
  - ☐ Changes in Gene Expression
  - ☐ Histological Changes

Ambient Particle Concentrating and Exposure Systems

Ambient Air → **Low-Pressure Impactor Inlet (0.15 μm cut)** → **VACES (Sioutas, et al) Humidifier, Cooler, and Virtual Impactors** → **Animal Exposure Chambers (ECG telemetry monitoring)**
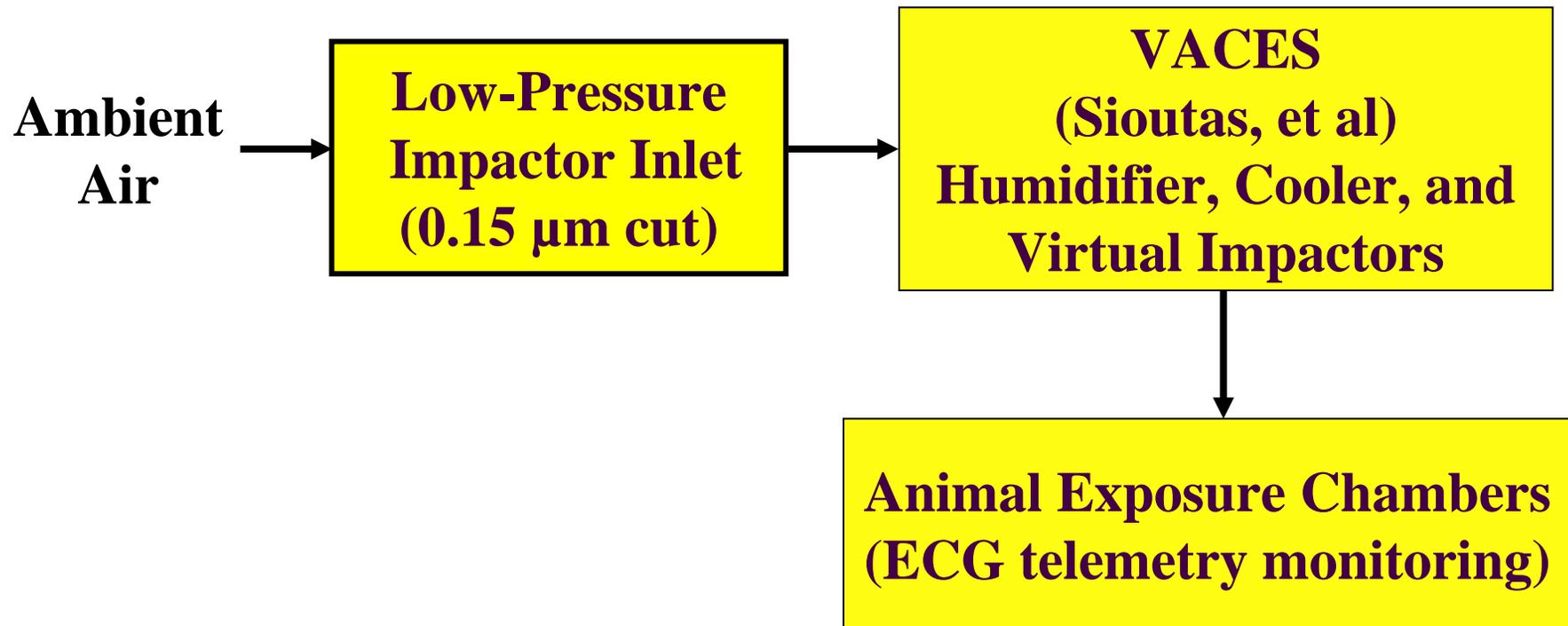
Identical exposure chambers with an air purifier (Charcoal, Purafil,HEPA Filter) at the inlet is used for sham exposure.

Exposure atmosphere sampling and methods of analyses

Exposure Atmospheres	Ambient Air
PM$_{0.15}$ Concentrations	Temperature
Ambient (post impactor inlet)	Relative Humidity
Exposure Chambers	PM$_{2.5}$ -TEOM
Gravimetric	EC -Aethalometer
DataRAM (MIE)	EC/OC -R&P 5400
PM Compositions	VOC -PUFF-GC/MS
XRF for elements	Ozone
IC (Dionex) for ions	NOx
Exposure chamber	SO$_2$
Temperature, Particle Size and Mass	
Concentration	
DataRAM (MIE)	
Particle Number Concentration -	
CNC (TSI)	

Experimental Animals

- Sprague-Dawley: "Infarcted Rats"

- Sprague-Dawley: "Normal Rats"

  - Age-matched to Infarcted Rats

  - Aged to "50%" Mortality (Geriatric)

- Spontaneously Hypertensive Rats (SHR)

  - Progressive hypertension and Cardiomyopathy

- WKY Rats

  - Age-matched 'normotensive' controls for SHR

  - May develop cardiac pathology after PM exposure

Subchronic and Chronic Effects of CAPs on Heart Rate and Core Body Temperature

- The differences between CAPs (or specific components) and control will be used to estimate the daily effects of CAPs (or components) using a time-varying model.

- The daily effects will be used to estimate the chronic trend of CAPs (or components) exposure using a Bayesian inference model, which has been described by Chen et al. (2004).

$$y_{ijt} = \gamma_{0t} + \gamma_{1t}I(i \in Disease) + \gamma_{2t}I(j \in PM) + \gamma_{3t}I(i \in Disease \text{ and } j \in PM)$$
$$+ \sum_{l=1}^{t-h} \varphi_{tl}(y_{ij,t-l} - \hat{y}_{ij,t-l}) + e_{ijt}$$

$$\theta_t = \delta + \alpha \times [1 - e^{-\lambda \times \max(t-\omega,0)}] + \eta \times I(C_t > 0) + \beta \times I(C_t > \psi) + \phi \times I(t \in [a,b]) + \varepsilon_t$$
$$= \mu_t(\delta, \alpha, \lambda, \omega, \eta, \beta, \psi, \phi) + \varepsilon_t$$

PRELIMINARY RESULTS

1. Geriatric (22 month) Rats were exposed to $PM_{2.5}$ CAPs or Purified Air.
   - □ BP, HR, Inflammatory Biomarkers, Free Radical Production were measured.

2. Geriatric (22 month) Rats were exposed to EC Particles or Purified Air.
   - □ BP, HR, HRV were measured.

3. Normal and Infarcted Rats were exposed to $PM_{0.15}$ (~1-10 μg) directly via jugular vein.
   - □ BP, HR, ECG changes were measured.
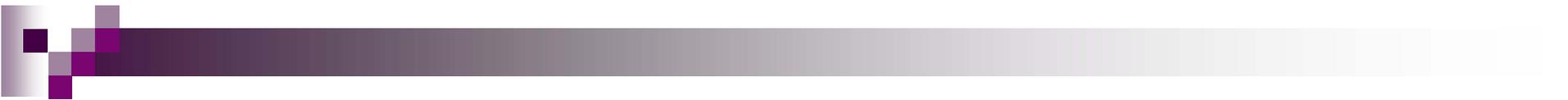
1. CAPs-Exposed Geriatric Rats

- Animals exposed for 3 consecutive days.
  - □ Implanted animals for BP and HR.
  - □ Non-implanted for necropsy.
- Animals were killed 24 hours after the last exposure. (Implanted rats were not killed at that time).
- Lungs were lavaged. BAL was analyzed for cytokines.
- Macrophages were isolated and free radical (superoxide) production was measured.

Findings

- Exposures were low - 140 μg/m^3

- Average heart rate and blood pressure data were measured before and after control and CAPs exposures.

- Cytokines were assayed using a Cytokine Array Processor.

- Free radical production was measured using chemiluminescence.

# CAPs Exposures Cause Changes in BP, HR and Cytokine Production
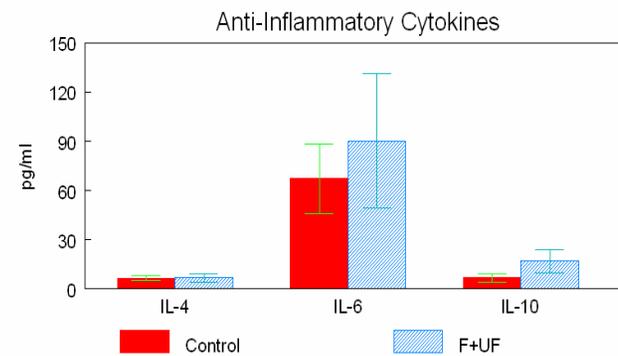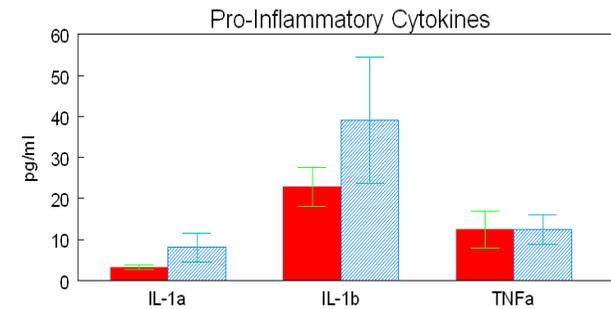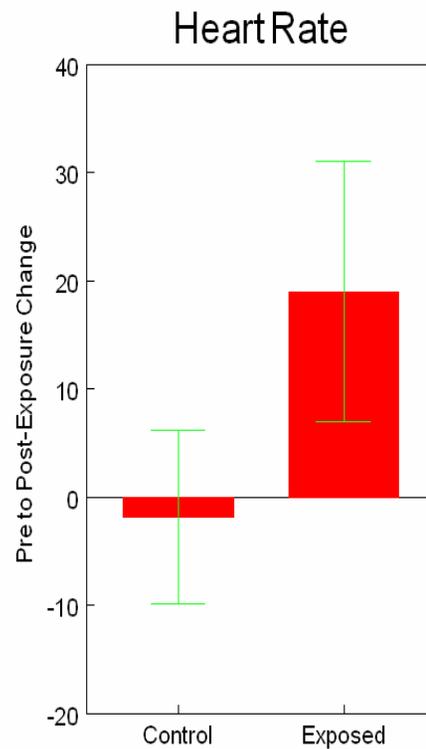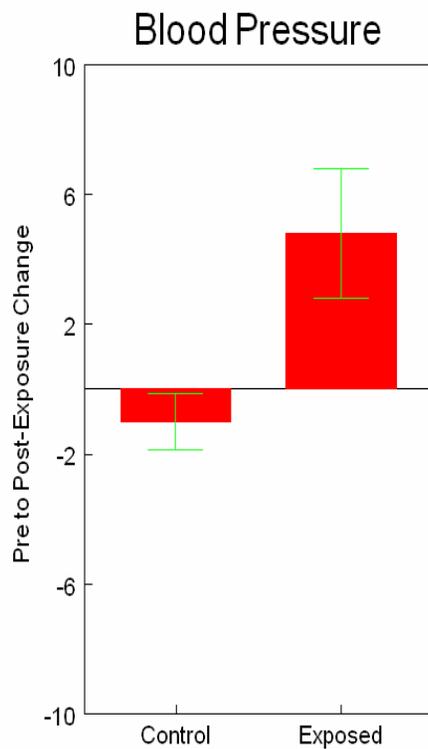
Superoxide production was increased in macrophages after CAPs exposure in Aged Rats
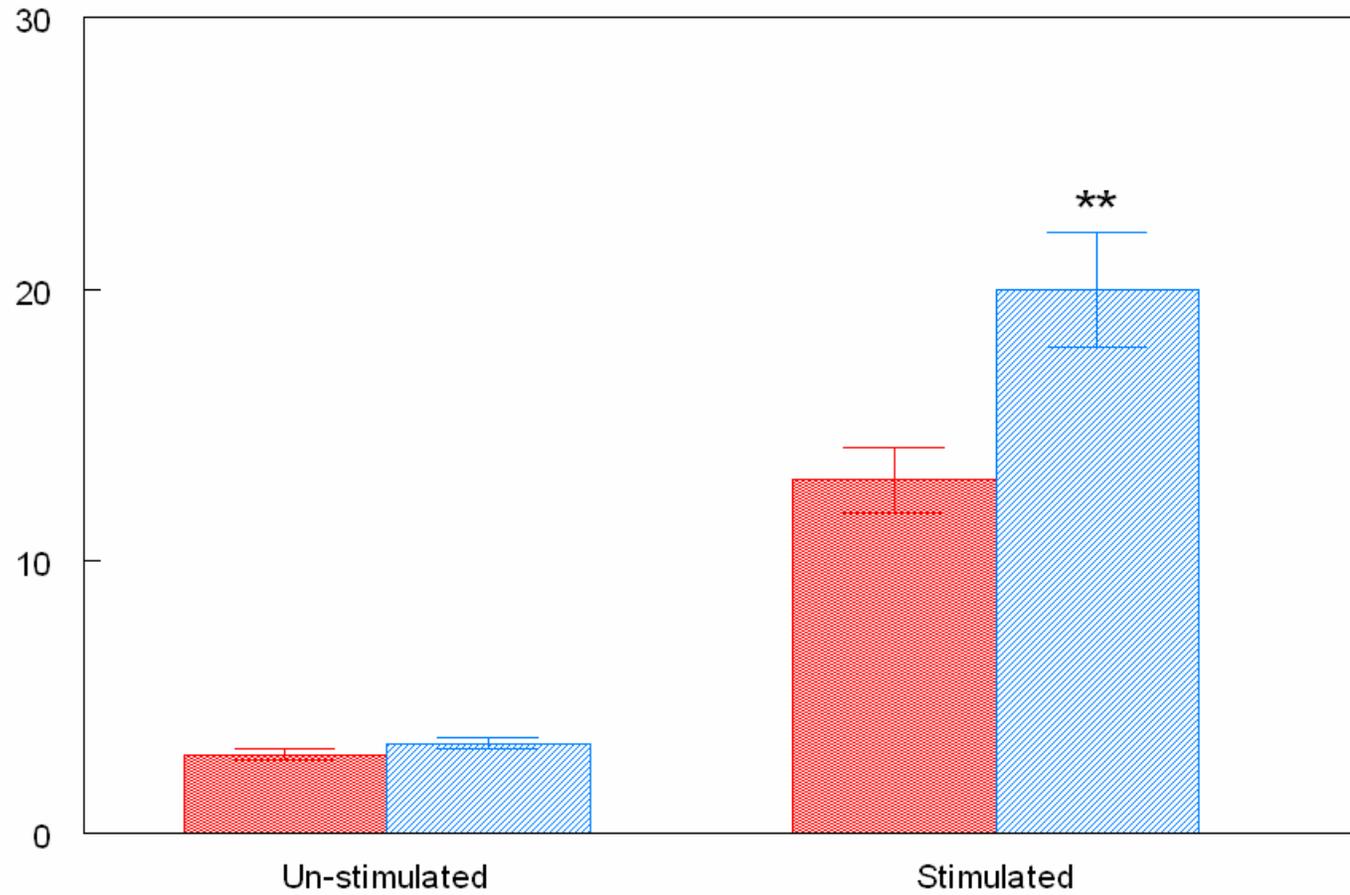
2. EC-Exposed Geriatric Rats

- Animals exposed 3 consecutive days.
  - Implanted animals for BP and HR.
- BP and ECG Data acquired before and after exposure.
- HRV determined as Standard Deviation of Normal Beat to Beat Intervals.

Heart Rate Variability Significantly Decreased by EC Exposure
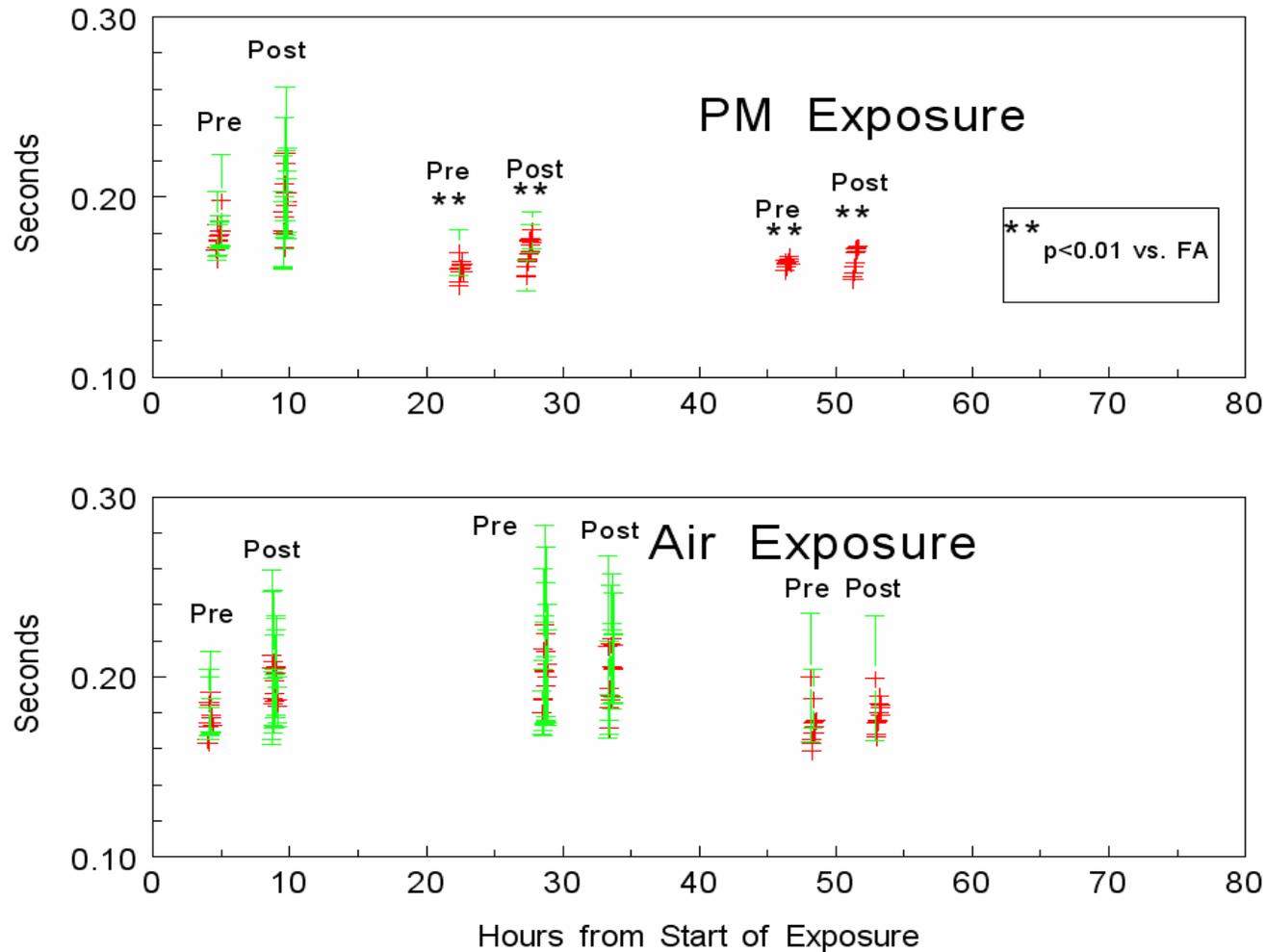
3. Normal and Infarcted Rats exposed to $PM_{0.15}$
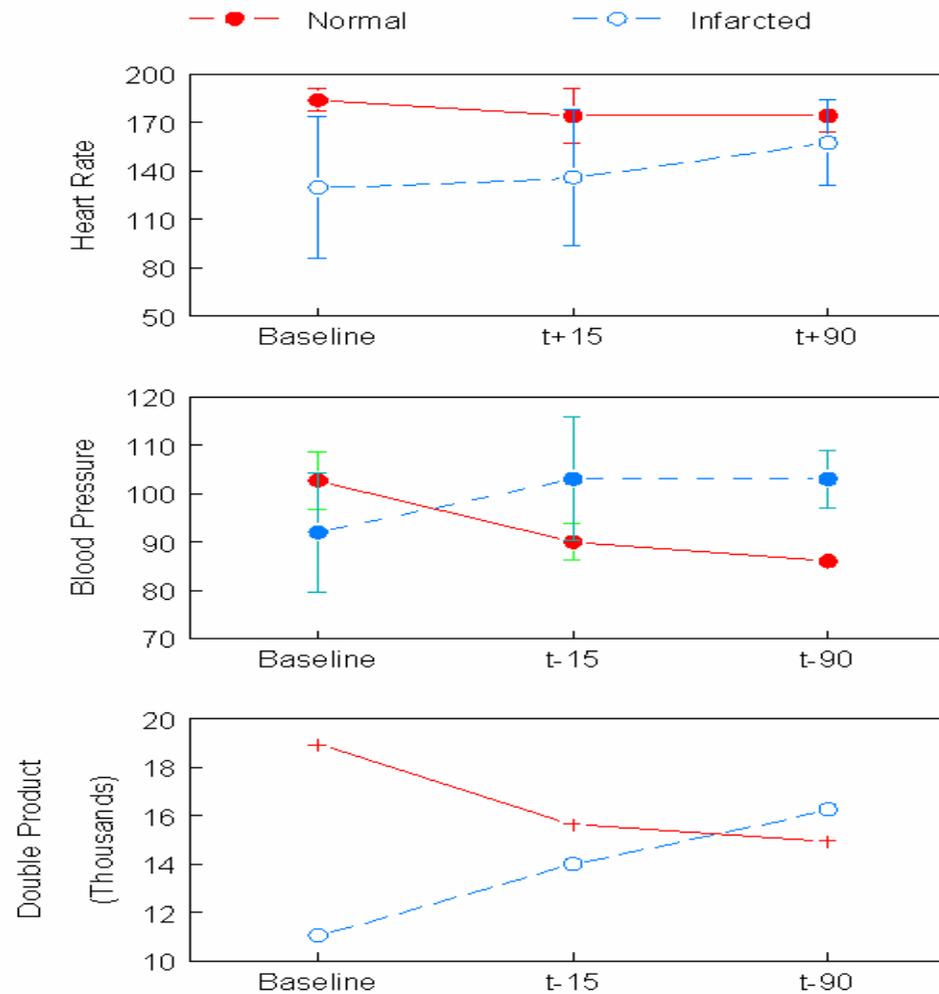
Conclusions

- This study will try to better elucidate:
  - Mechanisms by which air pollutants affect the cardiovascular system, resulting in increased morbidity and mortality,
  - Will seek to define factors which lead to differential susceptibility for certain groups and individuals.
- We will use innovative models and model systems and state-of-the-art technologies
  - To examine the role of ultrafine particles in the development or aggravation of heart disease in susceptible populations, and
  - To evaluate the utility of interventions to prevent or reduce the health impacts