Subchronic CAPs Exposures in Mice:
Biological Endpoints and Exposure Assessment

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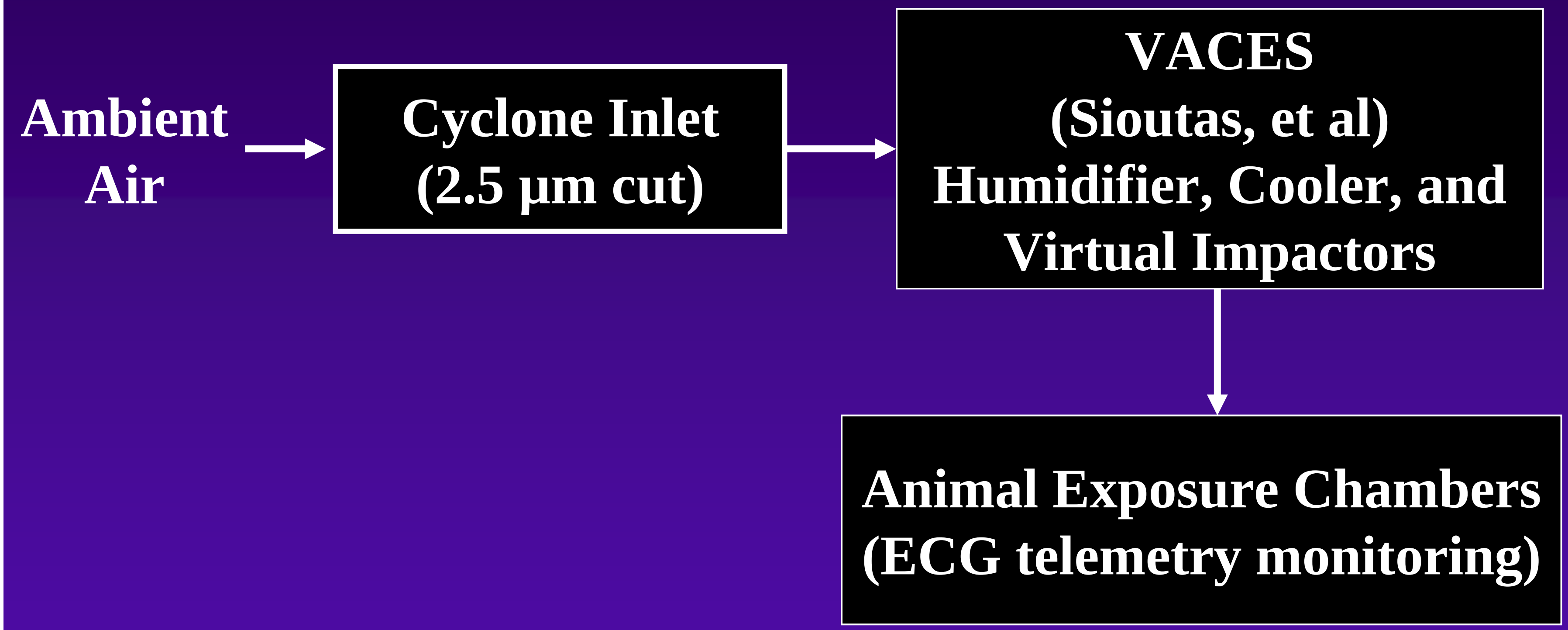
Issues Addressed:

- Does long term exposure to $PM_{2.5}$ cause cumulative damage to cardiovascular, pulmonary and other tissues?
- What is the relationship between the temporal variations in CAPs concentrations/composition and acute changes in cardiac function?

EXPERIMENTAL DESIGN

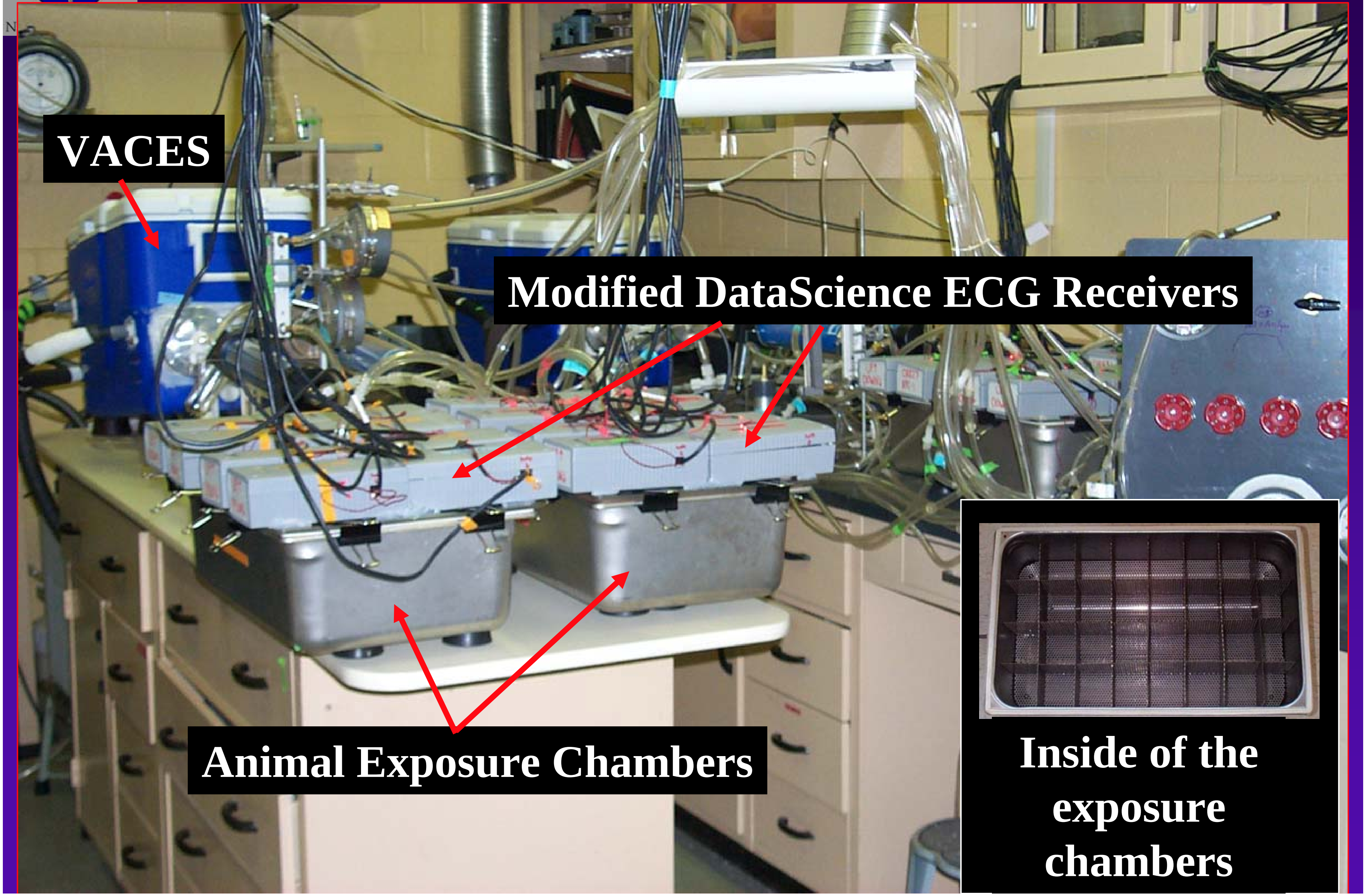
- Normal and compromised mice are exposed, whole body, to CAPs or filtered air for 6 hours/day, 5 days per week, for up to 6 months.
- CAPs @ ~10x ambient Northeastern U.S. background aerosol concentrations
  - Cyclone Inlet was used to remove PM larger than 2.5 μm
- Filtered air exposure controls
  - PM is removed prior to the virtual impactor concentrator system.

Ambient Particle Concentrating and Exposure Systems



An identical system with a HEPA filter at the inlet (to remove ambient particles) is used for sham exposure.

Aerosol Processing, Inhalation Exposure, and ECG Monitoring Systems



Exposure atmosphere sampling and methods of analyses

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Exposure Atmospheres

$PM_{2.5}$ Concentrations
- Ambient (post cyclone)
- Exposure Chambers
  - Gravimetric
  - DataRAM (MIE)

PM Compositions
- XRF (Jordan Valley) for elements
- IC (Dionex) for ions
- Single Particle Mass Spec (RSMS III)

Humidifier Air
- Temperature and dew point

Exposure chamber
- Temperature, Relative Humidity
- Particle Size and Mass Concentration
  - DataRAM (MIE), SMPS (TSI)
- Particle Number Concentration
  - CNC (TSI)

Ambient Air

Temperature

Relative Humidity

$PM_{2.5}$ -TEOM

EC -Aethalometer

EC/OC -R&P 5400

VOC -PUFF-GC/MS

Ozone

NOx

SO_2

Experimental Animals

- C57BL/6: "Normal mice"
- C57BL/6J ApoE$^{-/-}$
  - Hyperlipidemia, progressive atherosclerosis on a high fat diet
- B6;129 ApoE$^{-/-}$ LDLr$^{-/-}$
  - Hyperlipidemia, progressive atherosclerosis and coronary artery disease on a high fat diet.
  - Myocardial ischemia within 6 months.
  - Males have more severe disease than females.
  - A model of humans with severe atherosclerosis and coronary artery disease.

Exposure Parameters

- Average Concentration During Exposure
  - 110 ± 79 ug/m^3
    - equivalent to 20 ug/m^3 annually
- Median Concentration
  - 91 ug/m^3 (1.2 - 426 ug/m^3)
- Particle Size
  - 0.43 ± .13 µm (SMPS, Volume distribution)
- Concentrating factor
  - 9.6 ± 3.8

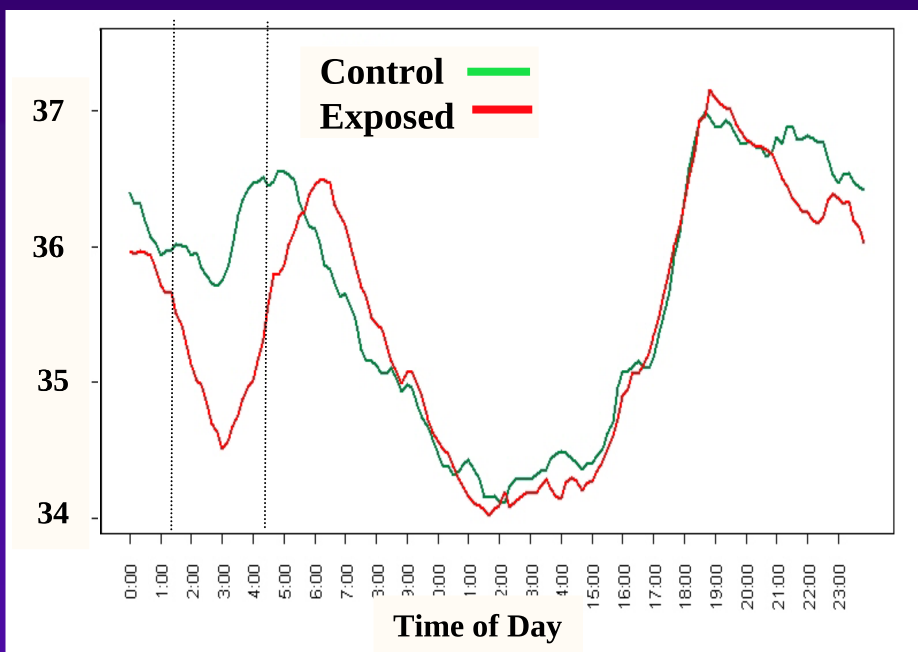
Subchronic Effects of CAPs on the Lungs

- No change in lavage cell counts, differentials, LDH, protein
- No histological change
- No change in immunological endpoints

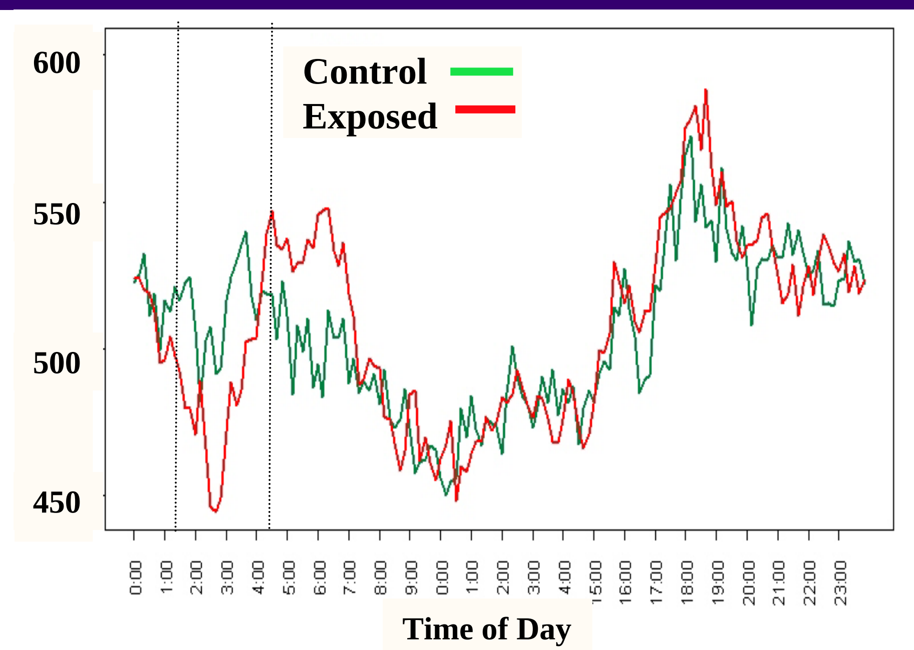
No change in serum troponin and IL-6 levels.

Subchronic Effects of CAPs on Heart Rate and Core Body Temperature

Body Core Temperature (°C)



Heart Rate (beats/min)



- The time of day that significant effects of CAPs at the end of exposure occurred was found using the “Fishing License” method, based on the averages of the 5 post exposure days.

Subchronic Effects of CAPs on Heart Rate and Core Body Temperature

- The time of day that significant effects of CAPs at the end of exposure occurred was found using the "Fishing License" method, based on the averages of the 5 post exposure days.
- The differences between CAPs and control at that time were used to estimate the daily crude effects of CAPs using a time-varying model.

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

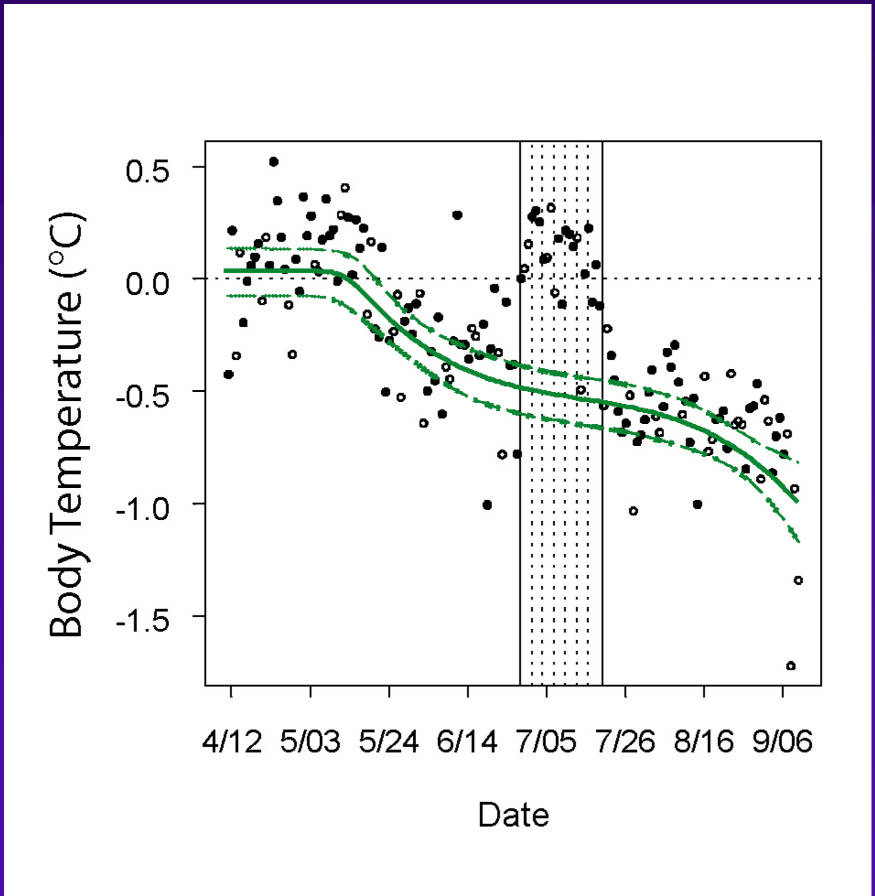
- The daily crude effects were used to estimate the chronic trend of CAPs exposure using a Bayesian inference model.

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

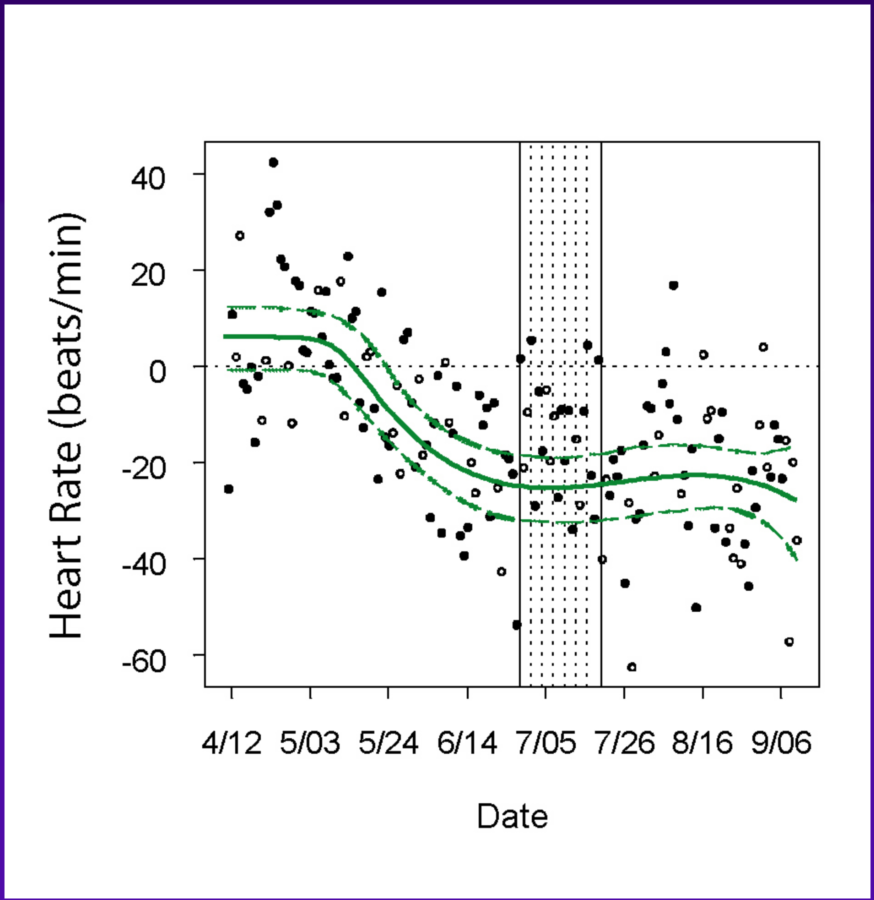
Subchronic Effects - ApoE$^{-/-}$

1:30-4:30 AM

Body Core Temperature (°C)



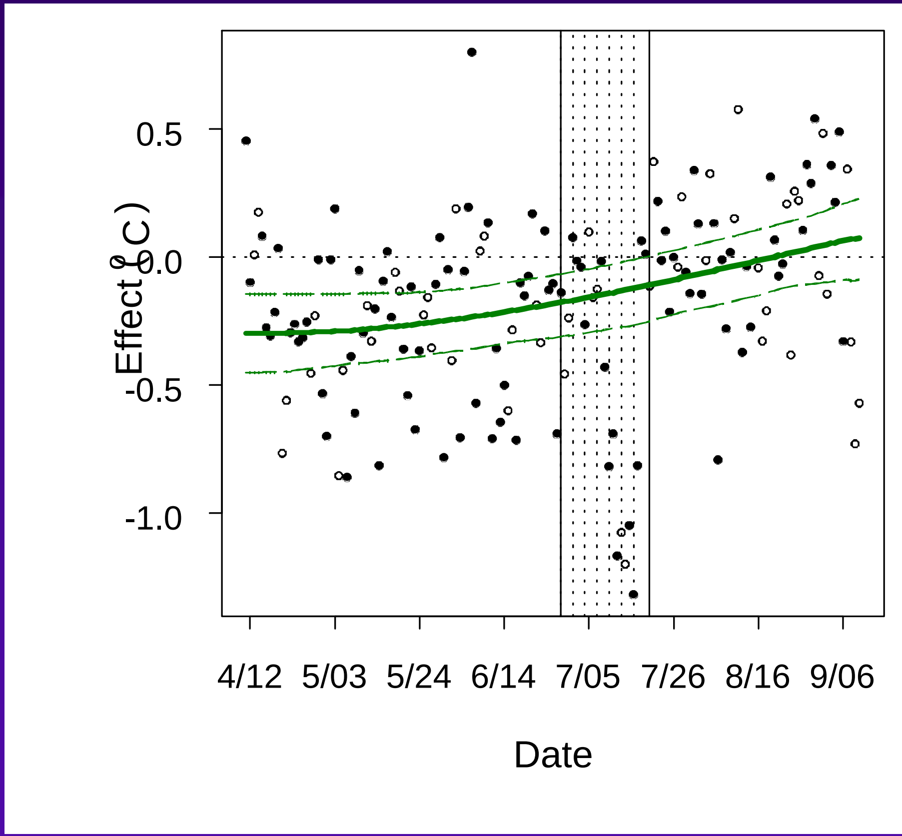
Heart Rate (beats/min)



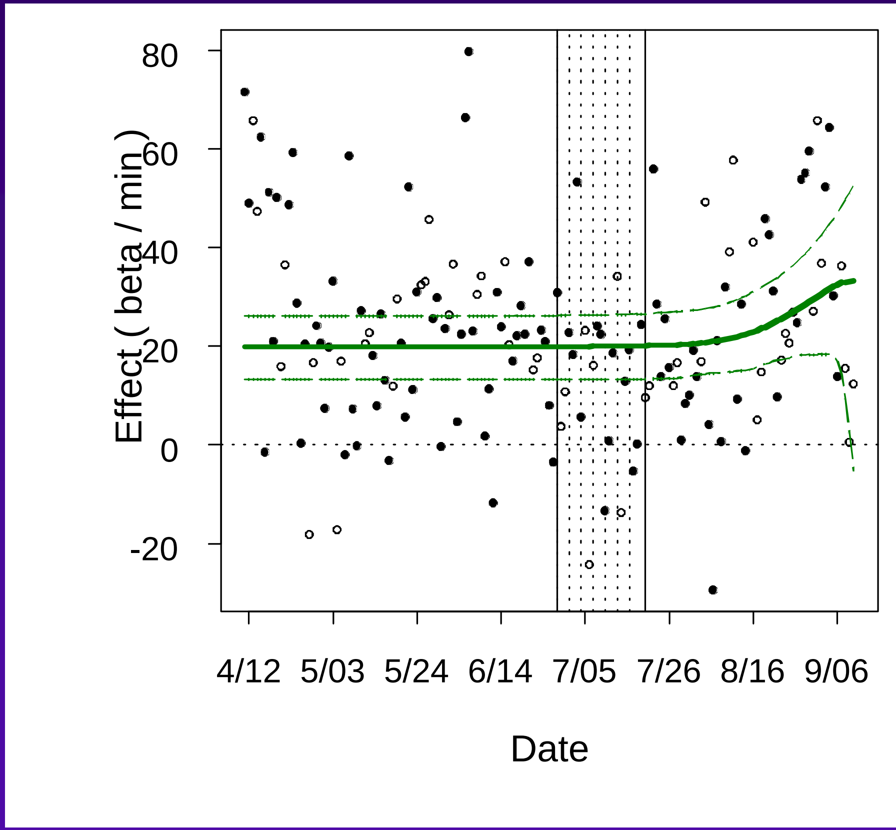
Subchronic Effects - C57

1:30-4:30 AM

Body Core Temperature (°C)



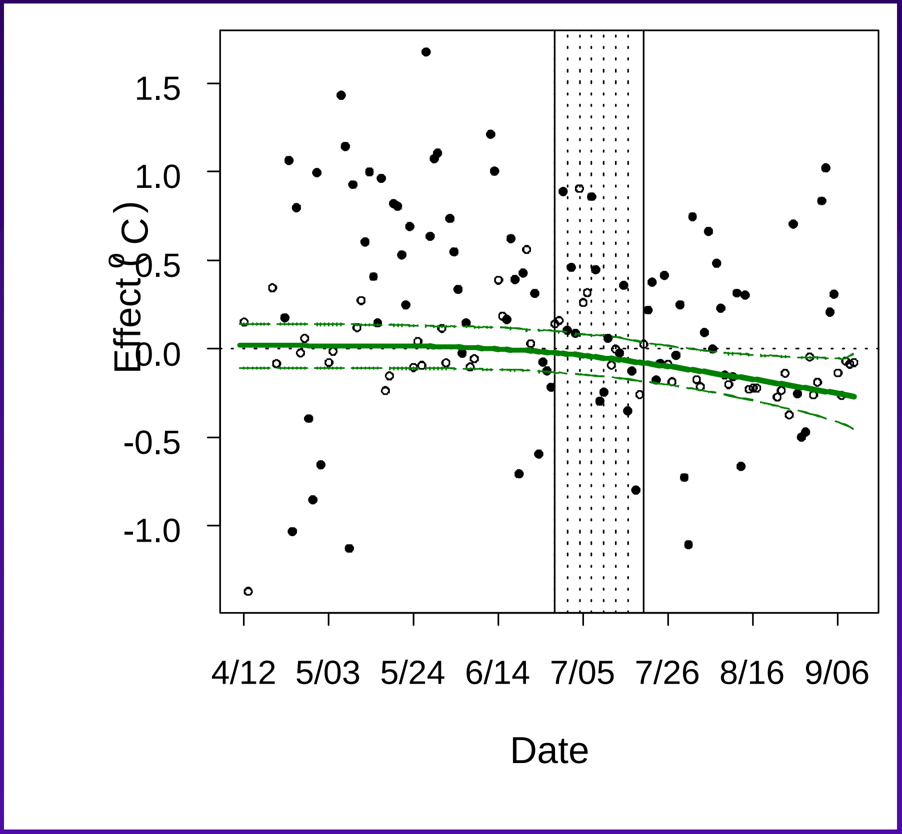
Heart Rate (beats/min)



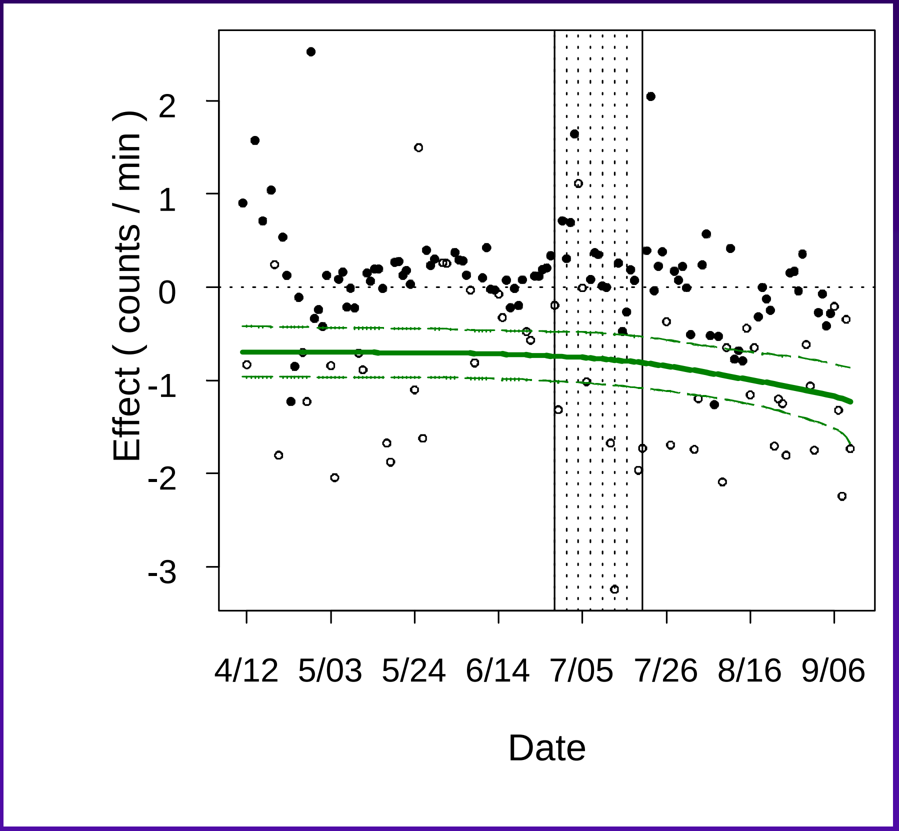
Trend of Daily Acute Effects - ApoE$^{-/-}$

11:00 AM - 1:30 PM

Body Core Temperature (°C)



Heart Rate (beats/min)

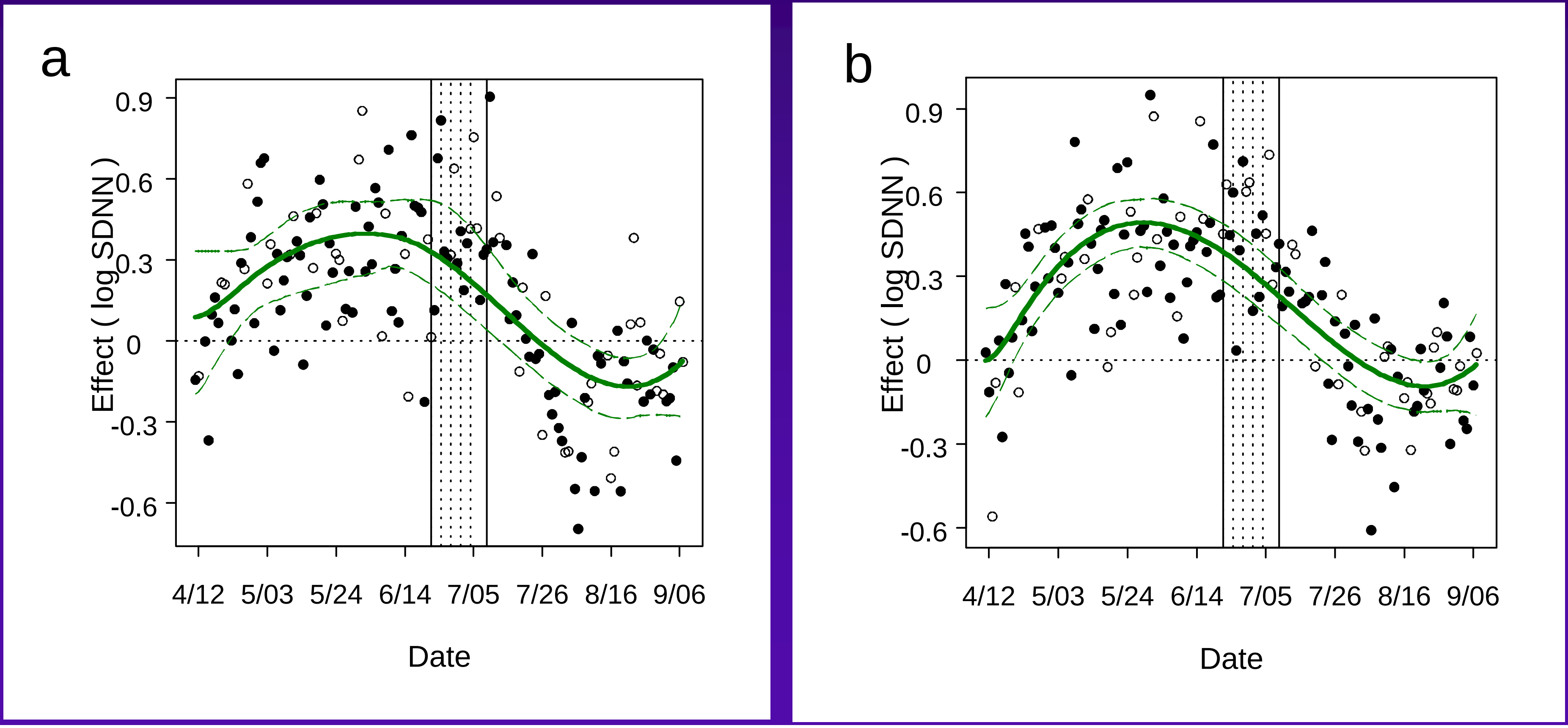


Summary Effects of CAPs on Core Temperature and Heart Rate

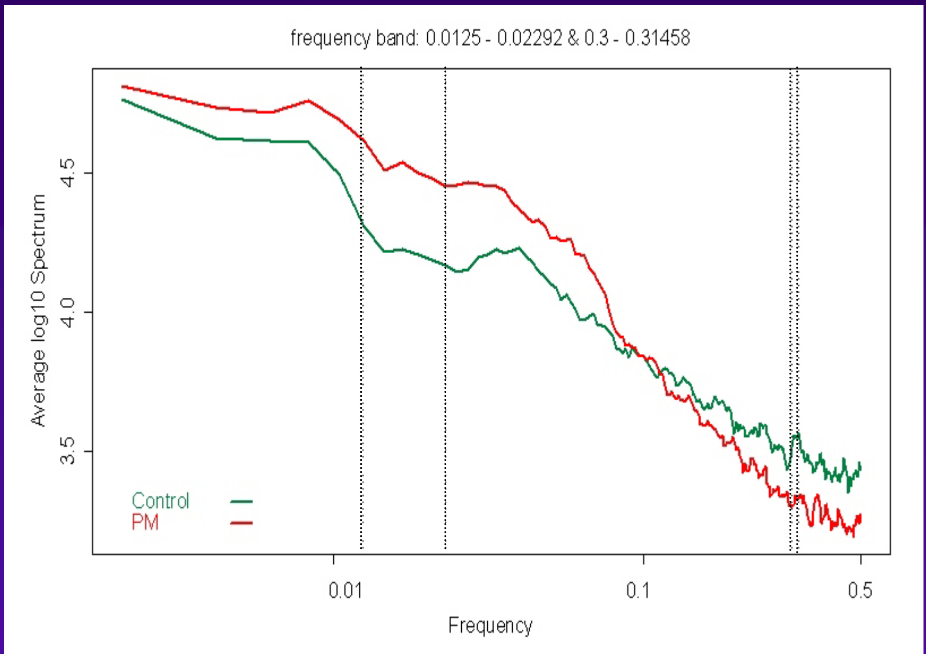
	Effects of CAPs (1:30 am - 4:30 am)				Effects of CAPs (11:00 am - 1:00 pm)	
	Core temp (°C)		Heart rate (beats/min)		Core temp (°C)	Heart rate (beats/min)
	ApoE	**C57**	**ApoE**	**C57**	**ApoE only**	
Onset of the chronic effects calendar days into exposure)	26.3 (9.1, 37.1)	76.7 (31.8, 106)	32.4 (18.7, 49.1)	98.2 (2.9, 148.3)	83.2 (54.6, 110.8)	66.4 (9.7, 113.2)
Magnitude of change at the end of exposure	-0.86 (-0.73, -1.02)	0.3 (0.15,0.45)	-29.7 (-22, -38)	4.4 (-41, 26)	-0.25 (-0.4,-0.1)	-7.1 (-14.6,0.12)
Average acute effect of CAP	0.1 (-0.15, 0.28)	-0.01 (-0.8, 0.17)	3.8 (-2.4,9.9)	0.5 (-13.5, 9.3)	-0.04 (-6.84, 1.17)	-12.5 (-18.1, -5.8)

Effects of CAPs on Heart Rate Variability

Changes of log SDNN for ApoE$^{-/-}$ mice during (a) 16:00 – 18:00 and (b) 1:30 – 4:30 obtained from the Bayesian model in the 2nd stage.



Effects of CAPs on Heart Rate Variance ApoE$^{-/-}$

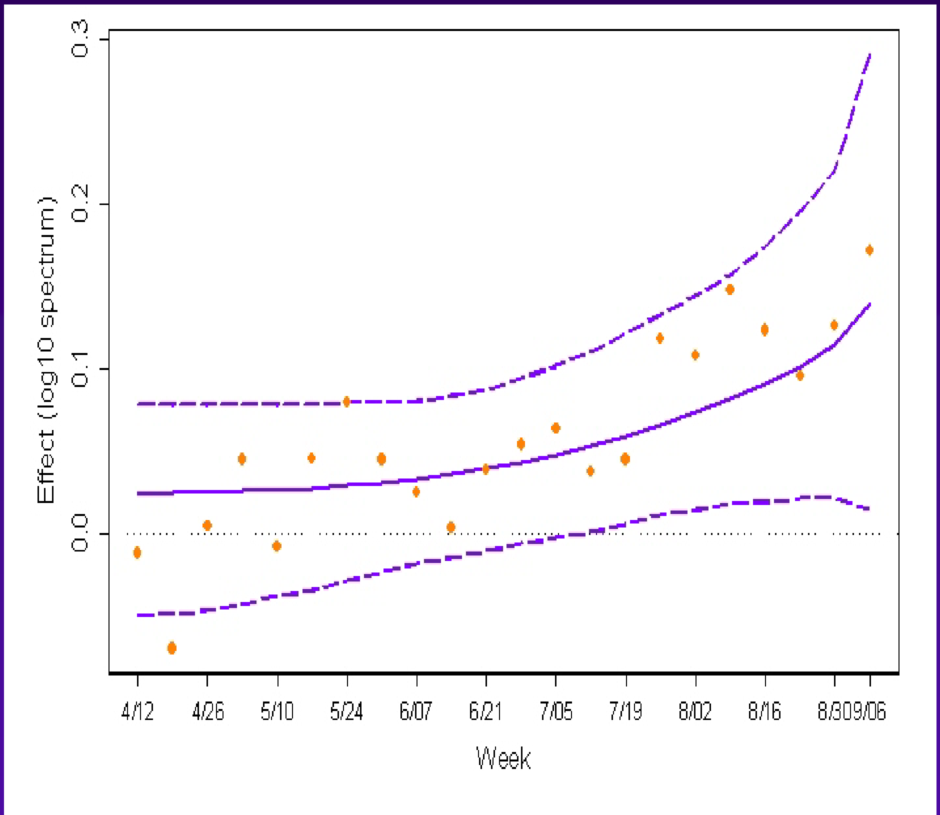


- Fast Fourier transform with Daniell smoother was used to estimate the power spectral densities for the 5-min average HR collected on the last non-exposed days (weekends).
- Fishing license method was used to estimate the frequency band during which the mean $\log_{10}$ transformed powers differed most significantly between CAPs and air.
- Two Stage method was used to estimate the effects of CAPs on heart rate variance over the 22 weekends.

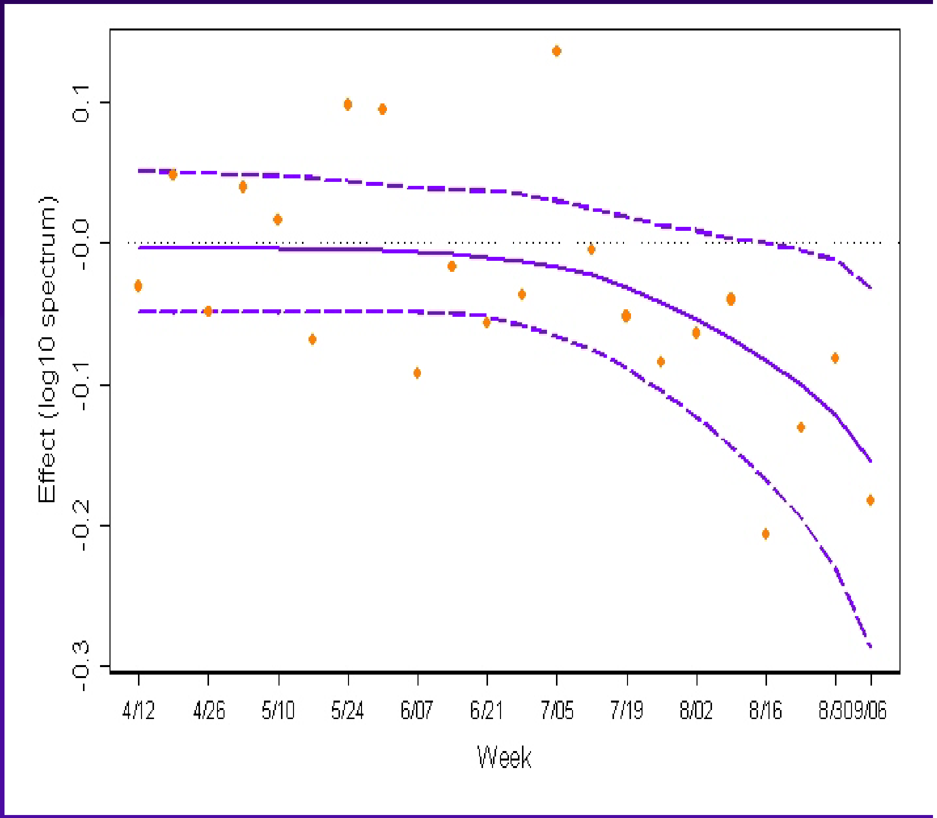
No change in Heart Rate Variance in C57

Subchronic effects of CAPs on Heart Rate Variance-ApoE$^{-/-}$

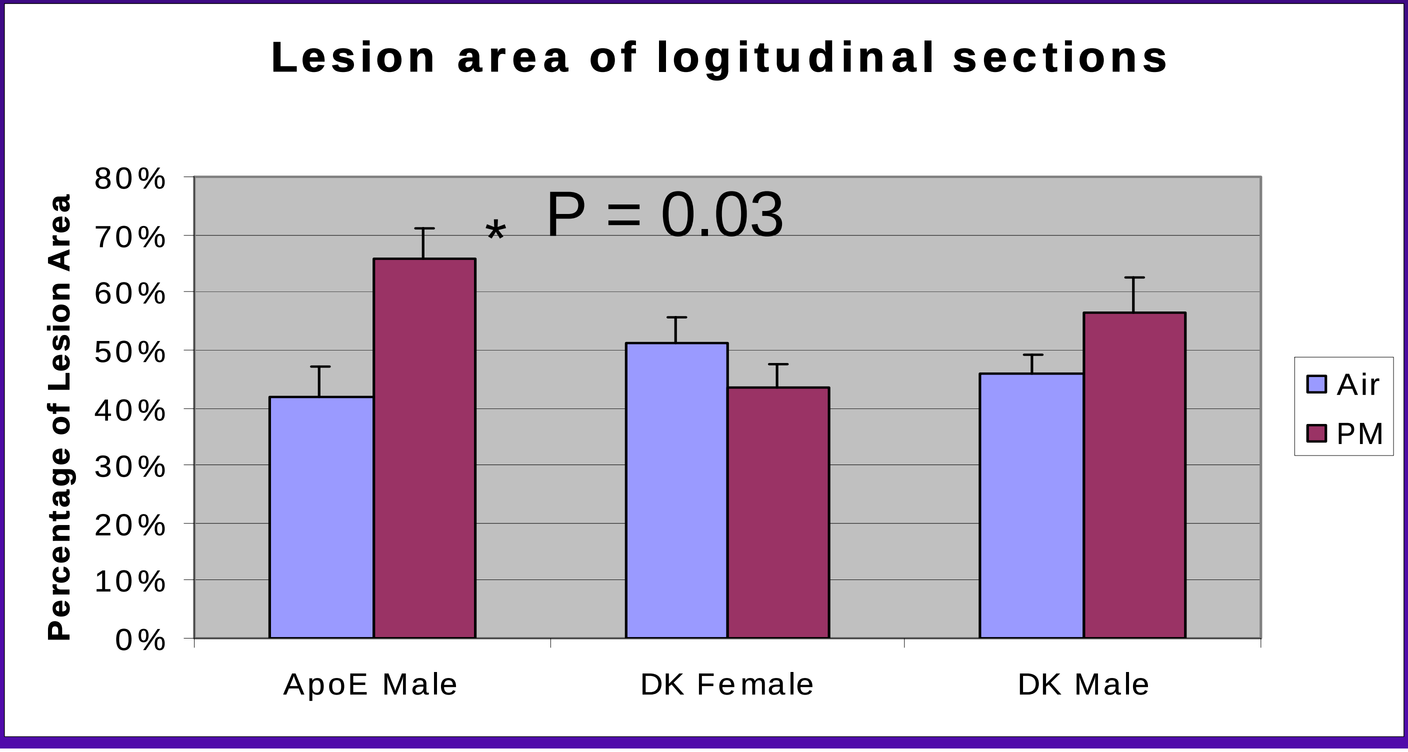
Low Frequency (3.6-6.7 hr)



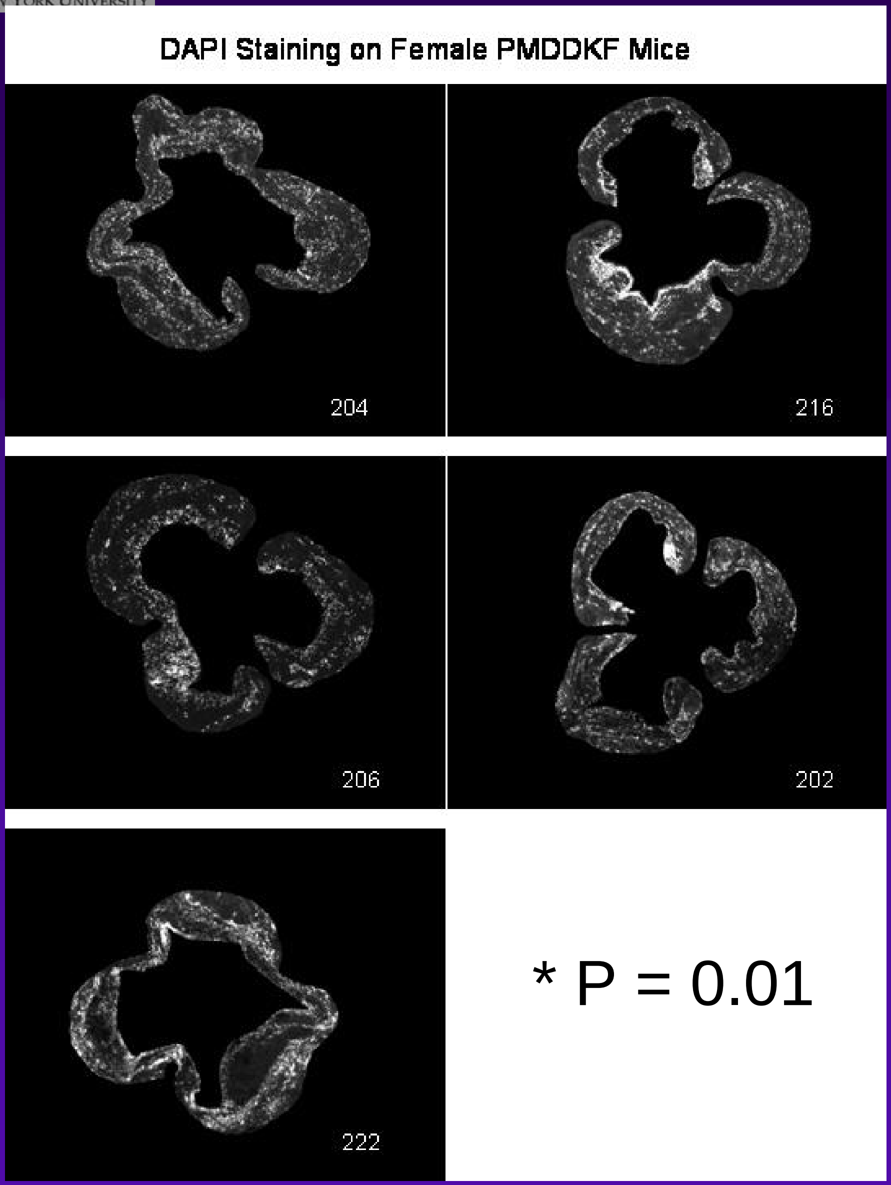
High Frequency (16 minutes)



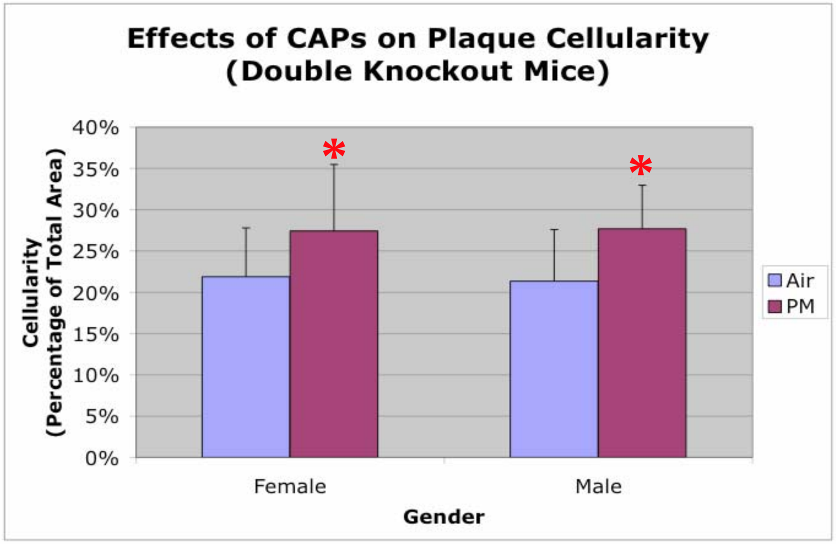
Effects of CAPs on aorta plaque size



Effects of CAPs on aorta plaque cellularity



* P = 0.01



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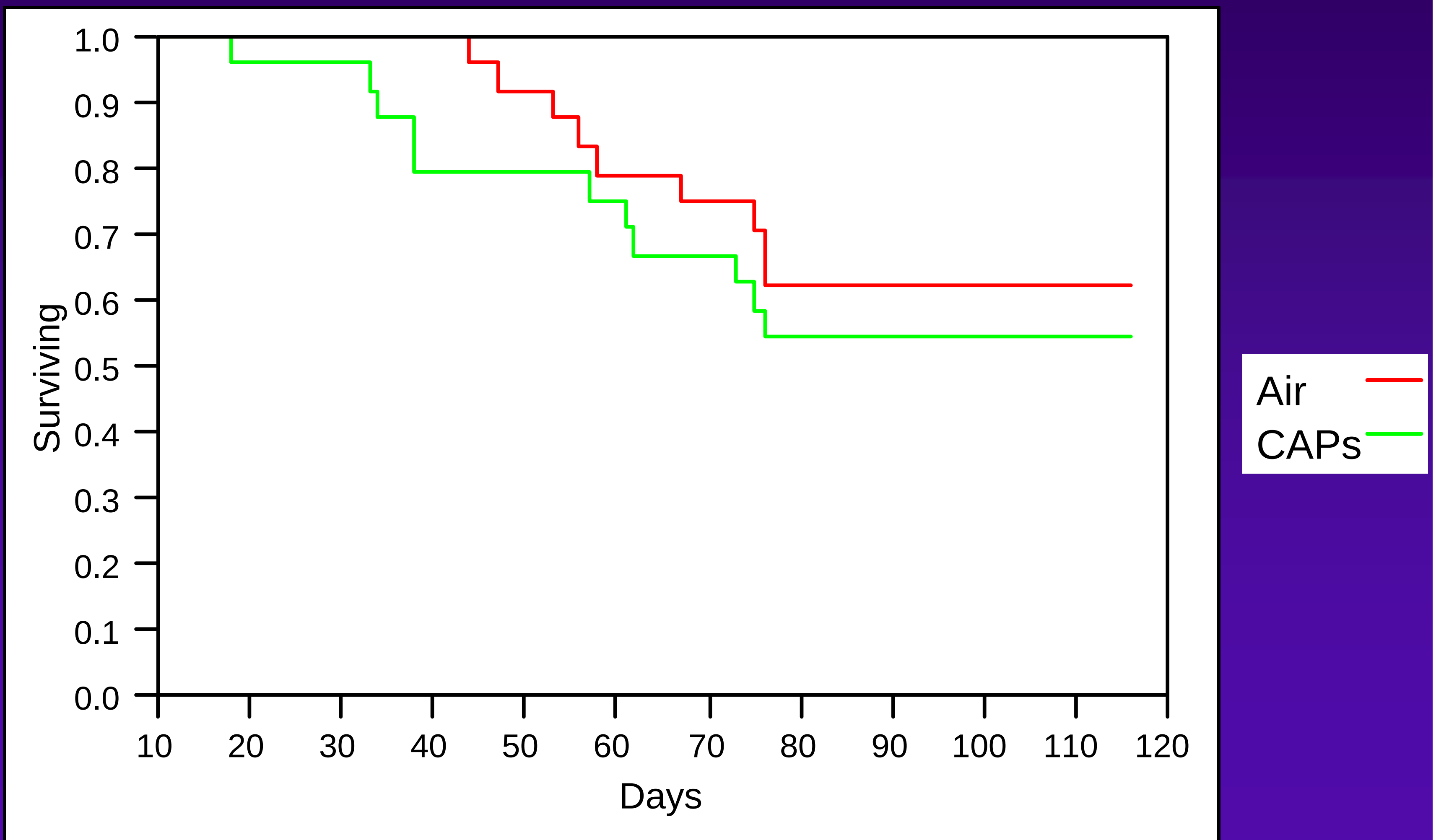
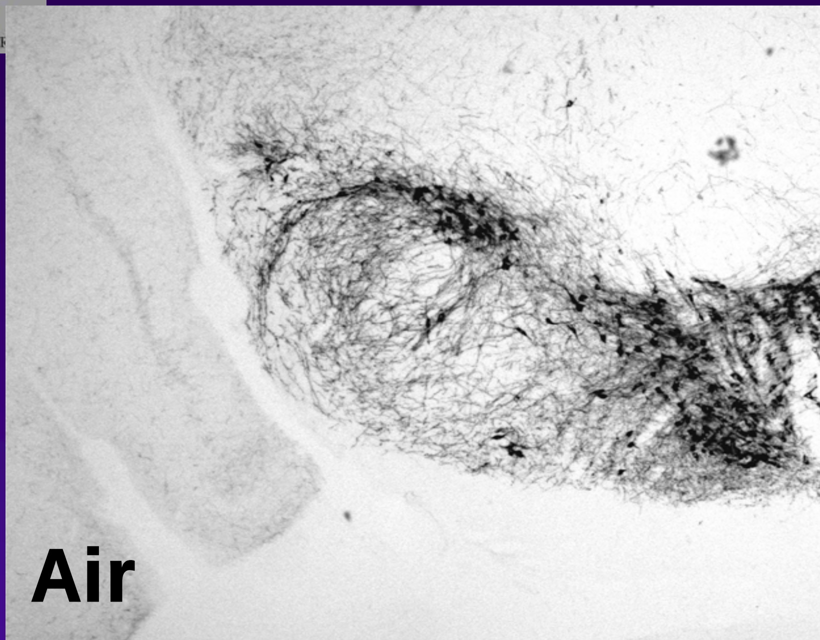
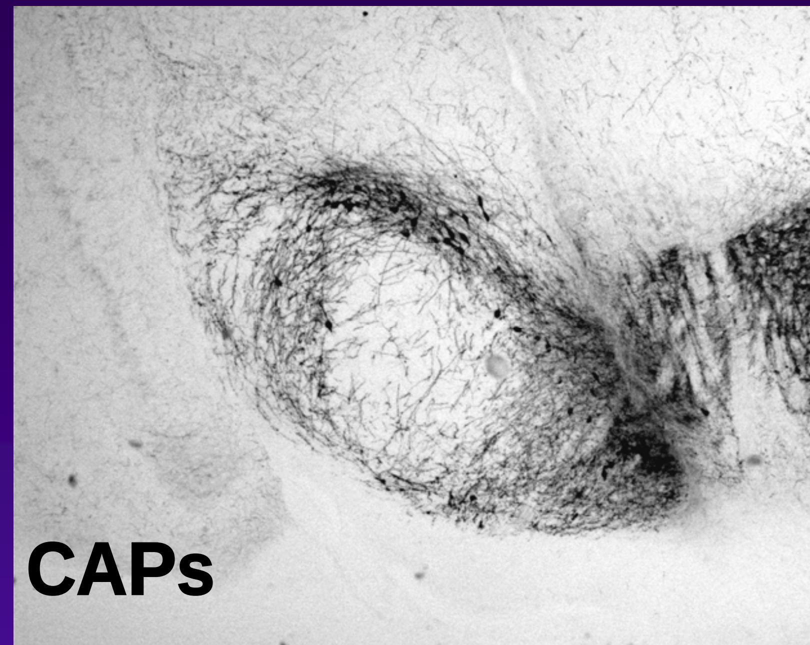
Longevity of the $ApoE^{-/-}$ $LDLr^{-/-}$ double knockout mice reduced by CAPs

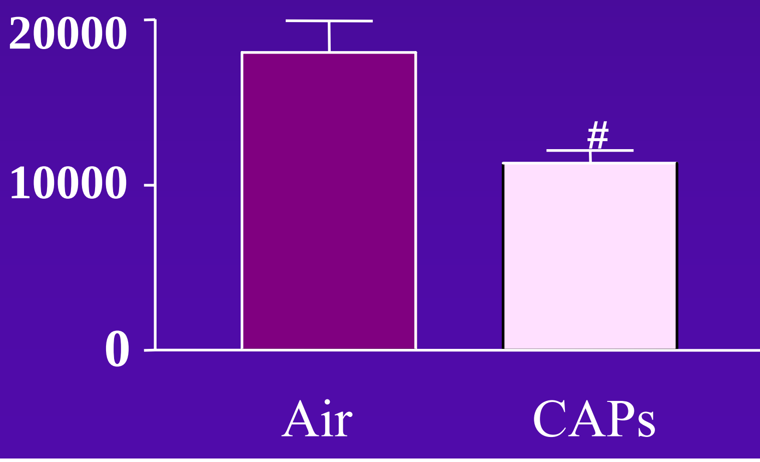


Effects of CAPs on substantia nigra of ApoE$^{-/-}$ Mice

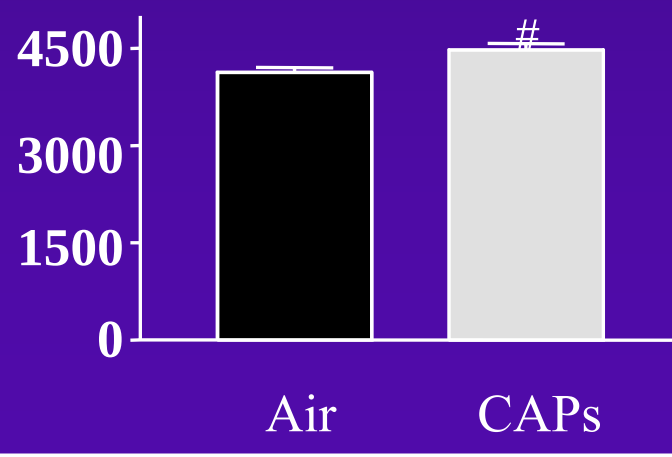




Tyrosine Hyrolase Stained Cells
(number of pixels)



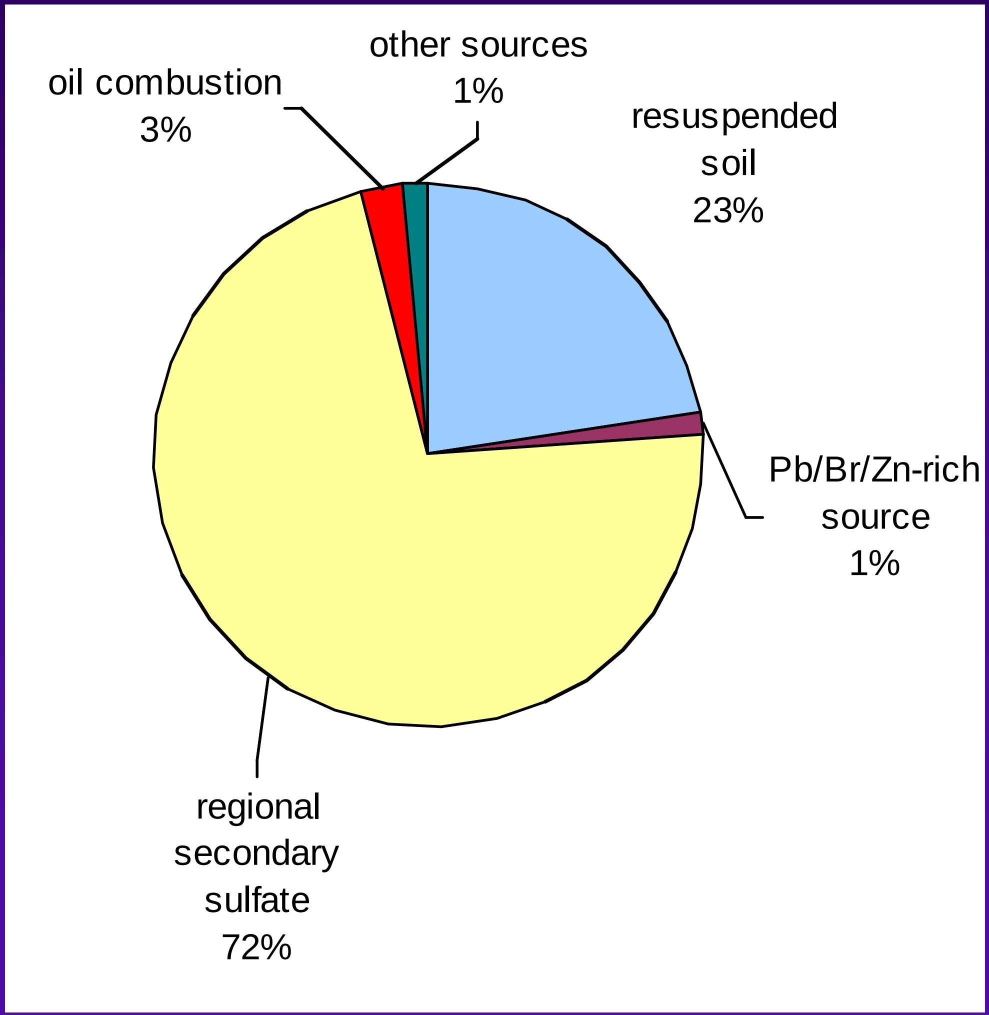
GFAP (glial fibrillary acidic protein)
Stained Cells (number of pixels)



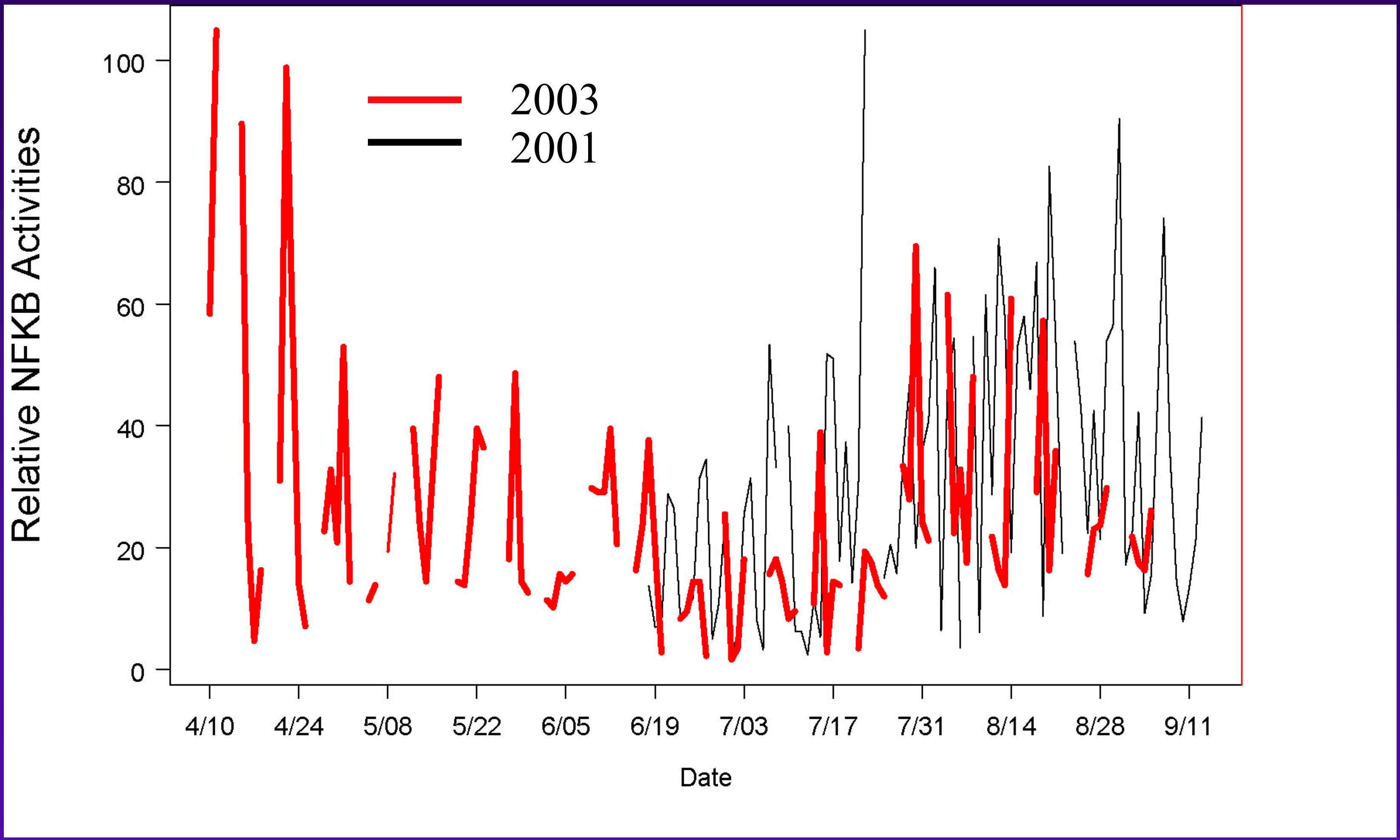
Effects of CAPs on gene expression in lung and heart

- Affymetrix GeneChips
- 3 animals/strain/exposure group/tissue
- "Heart" genes altered by subchronic CAPs exposure
  - Pyruvate dehydrogenase kinase
    - Energy regulation
  - 6 ion channels for K^+, Na^+, and Ca^{2+}
    - Ion balance
  - Myosin heavy chain
    - muscle
  - Albumin D-element binding protein
    - Circadian rhythm
  - Heat shock proteins
    - Protection from injury

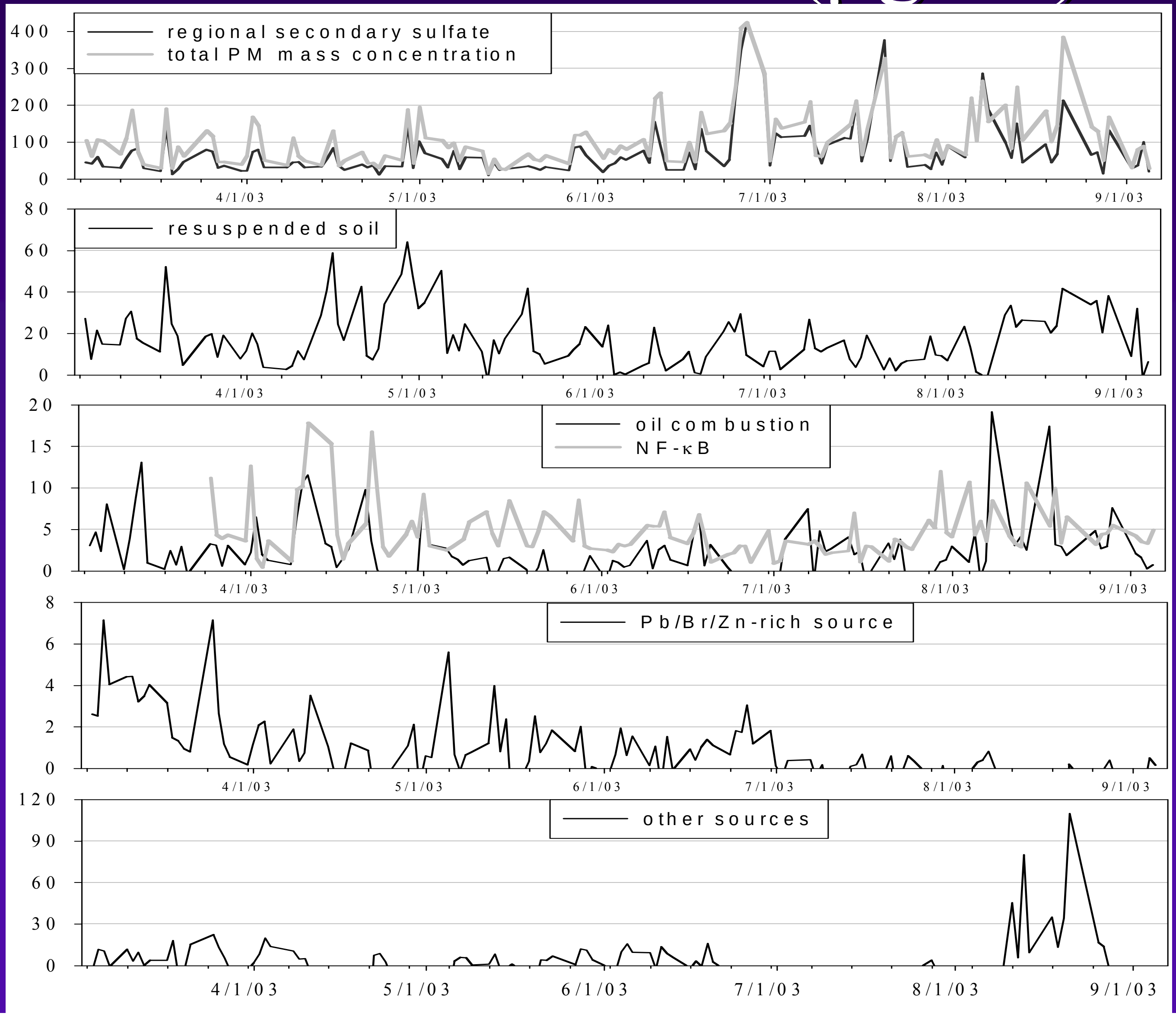
Average contribution of individual sources to CAPs mass concentration



Daily CAPs NF-kB Activities



Time series plot of each source mass contribution (μg/m^3)



Correlation coefficients (r) of the cellular response NF-kB with the sources identified by factor analysis

	NF-kB	Secondary sulfate	Resuspended soil	Oil combustion	Pb/Br/Zn-rich source	Other sources
NF-kB	1	-0.179	0.003	**0.326**	-0.044	0.039
Coal combustion		1	-0.026	-0.003	0.096	-0.059
Resuspended soil			1	-0.005	-0.056	-0.011
Oil combustion				1	-0.137	0.015
Pb/Br/Zn-rich source					1	-0.135

Correlation between NF-kB and CAPs Components

(Stepwise Regression)

	Coefficient	t	Pr(>|t|)
(Intercept)	11.83	5.29	0.000
V	0.24	2.25	0.027
Ni	0.44	2.86	0.005
Cu	0.50	2.89	0.006
As	0.29	2.07	0.041

N=99, R^2=0.3343

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There were significant associations between HRV parameters with NFκB

Strain	Time Interval	HRV Measure	Mean (95% C.I.)
$ApoE^{-/-}$	1600-1800	Log SDNN	-0.0190 (-0.0353, -0.0014)
		Log RMSSD	-0.0271 (-0.0449, -0.0089)
	0130-0430	Log SDNN	-0.0051 (-0.0183, 0.0083)
		Log RMSSD	-0.0031 (-0.0203, 0.0129)
C57	1600-1800	Log SDNN	-0.0149 (-0.0342, 0.0048)
		Log RMSSD	-0.0253 (-0.0471, -0.0025)
	0130-0430	Log SDNN	-0.0105 (-0.0258, 0.0046)
		Log RMSSD	-0.0145 (-0.0320, 0.0037)

Summary of the Subchronic CAPs Exposure Studies

- Cumulative changes in body core temperature, HR, and HRV.
- Animals prone to develop atherosclerosis appeared to be more sensitive to CAPs exposure than normal mice.
- Marked acceleration of plaque development and affected plaque characteristics.
- Alterations in the brain that are consistent with Parkinson's disease.
- Alterations in gene expression in heart and lung tissue.
- PM derived from oil combustion sources most closely associated with NF-kB response *in vitro* and depressed HRV in animals.

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