

**Long-Term Exposure to Concentrated Ambient PM_{2.5}
Increases Mouse Blood Pressure through Abnormal
Activation of the Sympathetic Nervous System:
A Role for Hypothalamic Inflammation**

**Zhekang Ying, Xiaohua Xu, Yuntao Bai, Jixin Zhong,
Minjie Chen, Yijia Liang, Jinzhuo Zhao, Dongyao Liu,
Masako Morishita, Qinghua Sun, Catherine Spino,
Robert D. Brook, Jack R. Harkema, and Sanjay Rajagopalan**

<http://dx.doi.org/10.1289/ehp.1307151>

Received: 29 May 2013

Accepted: 13 November 2013

Advance Publication: 15 November 2013

Long-Term Exposure to Concentrated Ambient PM_{2.5} Increases Mouse Blood Pressure through Abnormal Activation of the Sympathetic Nervous System: A Role for Hypothalamic Inflammation

Zhekang Ying,^{1,2} Xiaohua Xu,^{1,3} Yuntao Bai,³ Jixin Zhong,¹ Minjie Chen,^{1,2} Yijia Liang,¹ Jinzhuo Zhao,³ Dongyao Liu,³ Masako Morishita,⁴ Qinghua Sun,^{1,3} Catherine Spino,⁴ Robert D. Brook,⁴ Jack R. Harkema,⁵ and Sanjay Rajagopalan¹

¹Davis Heart & Lung Research Institute, the Ohio State University, Columbus, Ohio, USA;

²Department of Cardiology, East Hospital, Tongji University School of Medicine, Shanghai, PR China; ³Division of Environmental Health Sciences, Colleges of Medicine and Public Health, Ohio State University, Columbus, Ohio, USA

⁴University of Michigan, Ann Arbor, Michigan, USA

⁵Michigan State University, East Lansing, Michigan, USA

Address correspondence to:

Zhekang Ying, PhD

Department of Cardiology, East Hospital, Tongji University School of Medicine, Shanghai, PR China

Tel: 614-247-2532

Fax: 614-293-5614

E-mail: yingzhekang@hotmail.com

Short title: CAPs exposure increases blood pressure

Acknowledgements: This study is supported by NIH grants (R01ES013406 and R01ES015146 and R21 DK088522) and EPA grant (R834797) to Dr. Rajagopalan. Zhekang Ying is also supported by AHA (11POST7640030 and 13SDG17070131) and the National Natural Science Foundation of China (Grant No. 81270342).

Conflict(s) of interest/disclosure(s) statement: None of the authors has any actual or potential competing financial interests.

Disclaimer: This publication's contents are solely the responsibility of the grantee and do not necessarily represent the official views of the US EPA. Further, US EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

Abstract

Background: Particulate matter less than 2.5 μ m in diameter (PM_{2.5}) exposure increases blood pressure (BP) in humans and animal models. Abnormal sympathetic nervous system (SNS) activation has been implicated in the acute BP response to PM_{2.5} exposure. The mechanisms responsible for SNS activation and its role in chronic sustenance of hypertension (HTN) in response to PM_{2.5} exposure are currently unknown.

Objectives: We postulated that central nervous system (CNS) inflammation may be implicated in chronic PM_{2.5} exposure-induced increases in BP and SNS activation.

Methods: C57Bl/6j mice were exposed to concentrated ambient PM_{2.5} (CAPs) for 6 months, and BP was analyzed by radioactive telemetric transmitters. Sympathetic tone was assessed by measurements of low frequency blood pressure variability (LF-BPV) and urinary norepinephrine (NE) excretion. Additionally the effects of acute pharmacologic inhibitors of the SNS and parasympathetic nervous system were tested.

Results: Our results show that long term CAPs exposure significantly increased basal blood pressure, paralleled by increases in LF-BPV and urinary NE excretion. The increased basal blood pressure was attenuated by the centrally acting α 2A agonist guanfacine, suggesting a role of increased sympathetic tone in CAPs exposure-induced hypertension. The increase in sympathetic tone was accompanied by an inflammatory response in the arcuate nucleus of the hypothalamus, evidenced by increased expression of pro-inflammatory genes and inhibitor kappaB kinase (IKK)/nuclear factor-kappaB (NF-kB) pathway activation.

Conclusion: Long-term CAPs exposure increases BP through activation of SNS, which may involve hypothalamic inflammation.

Background

Hypertension (HTN) is the leading risk factor contributing to global disease burden (Lim et al. 2012). Over the last few decades, a focused scientific effort on modifiable lifestyle risk factors such as sodium intake, diet, obesity, and inactivity has resulted in fundamental insights into how these may increase propensity for hypertension. The link between exposure to pervasive environmental factors such as air-pollution and propensity to hypertension has only gained recent attention (Dong et al. 2013).

Controlled exposure studies in humans and animal models have suggested both acute effects and chronic sustained increases in blood pressure (BP) in response to inhaled particulate matter less than 2.5 micrometers in diameter (PM_{2.5}) (Dvonch et al. 2009; Brook et al. 2009; Brook and Rajagopalan 2009; Sun et al. 2008; Ying et al. 2009a). It has further been postulated that the mechanisms for the acute (within hours) BP increase in response to PM_{2.5} exposure likely differ from mechanisms responsible for sustained increases in BP in response to chronic PM_{2.5} exposure (Brook and Rajagopalan 2009; Brook et al. 2010). Imbalanced activation of autonomic nervous system (ANS) in particular has been postulated to play a role in the pathogenesis of acute increases in BP in response to PM_{2.5}, primarily on account of the rapidity of the effects and the association with components of heart rate variability reflective of sympathetic activation (Brook et al. 2009; Cosselman et al. 2012). BP effects in response to chronic PM_{2.5} exposure on the other hand in controlled exposure studies in animals has been attributed to a variety of mechanisms including dysfunctional NOS, production of vasoactive mediators such as ET-1 and Rho/ROCK activation, inflammatory cellular infiltration and vascular remodelling (Brook and Rajagopalan 2009; Sun et al. 2008; Ying et al. 2009a; Thomson et al. 2005; Kampfrath et al. 2011).

In light of accumulating evidence that sympathetic activation may modulate not only acute variations in BP but could play a dominant role in chronic sustenance of HTN via mechanisms such as immune activation, renal sodium handling and vascular remodelling (DiBona 2013), we investigated the importance of sympathetic activation in the genesis of HTN with chronic concentrated ambient PM_{2.5} (CAPs) exposure.

Material and Methods

Whole-body ambient inhalational CAPs exposures protocol

The protocol of animal experiments was approved by IACUC of the Ohio State University, and all the animals were treated humanely and with regard for alleviation of suffering. Animal exposure and the monitoring of exposure atmosphere and ambient aerosol were performed as previously described using a versatile aerosol concentration enrichment system that was modified for long-term exposures (Kampfrath et al. 2011). Briefly, C57Bl6j mice (8-week-old, male, n=12/group and 24 in total) were bought from Jackson Laboratory, and were housed in standard cages in a mobile trailer with a 12 light/12 dark cycle, temperatures of 65-75°F, humidity of 40-60%. After one week of acclimation, mice were exposed to CAPs or filtered air (FA) in “Ohio air pollution exposure system for the interrogation of systemic effects” chamber at The Ohio State University in Columbus, OH.. All mice including both FA and CAPs groups were exposed at exactly the same time. FA-exposed mice received an identical protocol with the exception of a high-efficiency particulate air filter (Pall Life Sciences, East Hills, NY) positioned in the inlet valve to remove CAPs in the filtered air stream, as detailed described previously (Kampfrath et al. 2011). The exposure protocol comprised of exposures for 6 h/day, 5 days/wk

(no exposure during the weekend). The C57Bl/6j mice were exposed for a total duration of 6 months from December 2011 to May 2012.

Sampling and analyses of Ambient PM_{2.5} and CAPs in the exposure chamber

To calculate exposure mass concentrations of ambient PM_{2.5} and CAPs in the exposure chambers, samples were weekly collected on Teflon filters (PALL Life Sciences Teflo, 37 mm, 2 µm pore, Ann Arbor, MI) and weighed before and after sampling in a temperature- and humidity-controlled weighing room using Mettler's microbalance (Mettler Toledo - 11106057). Ambient PM_{2.5} and CAPs samples collected on Teflon filters were wetted with ethanol and extracted in 1% nitric acid solution. The extraction solution was sonicated for 48 h in an ultrasonic bath, and then allowed to passively acid-digest for a minimum of two weeks. Sample extracts were then analyzed for a suite of trace elements using inductively coupled plasma-mass spectrometry (ICP-MS) (ELEMENT2, Thermo Finnigan, San Jose, CA) (Morishita et al. 2004).

BP, heart rate (HR), and locomotor activity measurement

FA/CAPs-exposed C57Bl/6j mice (n=6/group) were implanted with radiotelemetric transmitter as previously described (model TA11PA-C10; Data Sciences International) (Tallam et al. 2005). To prevent the effects of surgery, we allowed the mice to recover for ten days (No exposure to CAPs in the first four days). By this time the mice had regained their circadian BP and HR and the surgery- and anesthesia-dependent initial changes in BP and HR were followed by stable values (Figure 1B). We then began monitoring mean arterial pressure (MAP) and HR as outlined in Figure 1A. Basal MAP, HR and locomotor activity were recorded for 3 days (18 hours/day). The MAP, HR, and locomotor activity data in a 16-hour period (5PM-9AM) were analyzed and presented.

To evaluate autonomic control of BP, the following drugs were applied as previously described (Gross et al. 2005): muscarinic blocker atropine (2 mg/kg), β -adrenergic receptor blocker propranolol (4 mg/kg), and alpha2- adrenergic agonist guanfacine (1 mg/kg). To prevent the potential interference of the preceding administration of drug, mice were allowed to rest for at least 22 hours. All drugs were administered intraperitoneally between 4:00 and 6:00 pm. Continuous beat-by-beat values of BP were recorded for 30 minutes before administration of drugs and 2 h after administration of drugs. As previously described (Gross et al. 2005), the values from the 45th to the 60th minute after drug injection were used to characterize the responses to drugs and avoid the measurement of stress-induced BP and HR changes.

To determine whether chronic CAPs exposure alters the MAP and HR response to an acute pressor stress, we utilized a previously described model of acute stress that combines restraint with pulsatile, unavoidable bursts of air to the forehead for 1 min (do Carmo et al. 2011). Briefly, all mice were acclimatized to the restrainer tube for one hour prior to actual experimentation to minimize trauma associated with entry into the restrainer tubes. BP was monitored by telemetry for 15 min. Air jet stress consisted of pulses (2 s in duration delivered every 10 s for 1 min) of compressed air (15 lb/in²) aimed at the forehead from an opening at the front of the tube. After the 1-minute air-jet stress, MAP was measured for an additional 30-minute recovery period. BP response during the air-jet stress and recovery period following the air-jet stress were calculated as the changes compared to baseline period. We also calculated the areas under the MAP curve (AUC) during the air-jet stress and recovery periods.

Spectral analysis

The power spectra of systolic BP (SBP), pulse interval time series, and the cross spectra were calculated using fast fourier transform (FFT). Beat-to-beat values of detected R-R intervals and BP values were interpolated, low-pass filtered (cutoff 6 Hz), and re-sampled at 12 Hz. Data segments of 43 s were used for spectral analysis. Linear trends were removed, and power spectral density was estimated with the FFT-based Welch algorithm using segments of 512 data points with 50% overlapping and Hanning window. The power in the frequency range of low frequencies (LF, 0.25 to 0.6 Hz) was calculated. Five representative intervals were chosen for spectral analysis by a researcher who did not know the treatment of the animals and averaged according to the following criteria: (1) steady state conditions, (2) no large sudden BP changes, and (3) no artifacts.

Myograph

The mesenteric bed was removed from FA/CAPs-exposed mice (6/group, exposed to CAPs as described above, but not subject to telemetric transmitter implantation surgery and all subsequent analysis, and euthanized one week later than others), and mesenteric arteries (2 mm segments of second order branch of the superior mesenteric artery) were dissected free of fat and connective tissue and mounted in wire myograph chambers (DMT 620M). Vessels were maintained at 37°C in physiological Krebs' buffer that was bubbled with 95% O₂/5% CO₂ to maintain the buffer at pH 7.4. After a 30 min equilibration period, vessel tension was increased to 1 mN. A further 30 min after resting tension was established, mesenteric arteries were maximally contracted with a K⁺-depolarizing solution (KPSS: 123 mmol/L KCl, 1.17 mmol/L MgSO₄, 2.37 mmol/L KH₂PO₄, 2.5 mmol/L CaCl₂, 11.1 mmol/L D-glucose and 0.026 mmol/L EDTA). The vessels were subjected to graded doses of vasoconstrictors phenylephrine (PE; 10⁻⁹ to 10⁻⁵ mol/l) and U46619

(10^{-8} to $10^{-6.5}$ mol/l). The results were expressed as % of contraction by 120 mM. To test the acetylcholine-induced vasodilatation, mesenteric arterial rings were pre-contracted by phenylephrine (1 μ M). After a stable contraction plateau was reached with PE, the vessels were exposed to endothelium-dependent vasodilator ACh (10^{-8} to 10^{-5} mol/l). Results were expressed as % of pre-contraction by PE.

Urine collection and noradrenaline analysis

Urine was collected in the week before implantation of telemetry transmitters. After the daily exposure, mice (6/group) were placed into urine collection cages. The urine was then collected the next morning. Every mouse was subject to two separate collection and the urine from the same animal pooled and stored at -80°C until analysis. The concentration of noradrenaline in urine was analyzed with Norepinephrine ELISA Kit (Abnova, CA) per the instruction of manufacturer.

Immunofluorescence analysis

A subgroup of FA/CAPs-exposed mice (the same animals as described in Myograph, euthanized in the week following completion of blood pressure analysis) used for immunofluorescence analysis were anesthetized with isoflurane and blood was collected through the large retro-orbital plexus. After ligation and separation of the thoracic aorta with hemostat, perfusion was performed with 30 ml PBS injected intra-cardiac. The vasculature was then pre-fixed through perfusion of 15 ml paraformaldehyde (4%). The brains were rapidly removed and fixed at 4°C for one day in paraformaldehyde (4%), and cryoprotected in 30% sucrose-PBS for 3 days, frozen and sectioned in 6 μm thick coronal slices. IKK phosphorylation and c-fos expression were performed according to the protocols previously described (Purkayastha et al. 2011). Both rabbit

phospho-IKK (Ser176/180) and c-fos antibodies were procured from Cell Signaling (EMD Millipore, MA).

Quantitative RT-PCR

Total RNA of hypothalamus (from the same animals as described in BP Measurement) was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA). Four microgram of total RNA was reverse transcribed by random hexamers and ThermoScript RT-PCR System (Invitrogen). Quantitative real-time PCR was performed with the Stratagene Mx3005 using SYBER Green PCR Master Mix (Applied Biosystems, Carlsbad, CA). The sequences of primers used in the present study were as follows: GAPDH: sense, 5'-TGA ACG GGA AGC TCA CTG G-3', antisense, 5'-TCC ACC ACC CTG TTG CTG TA-3'; TNF α : sense, 5'-GGC ACC ACT AGT TGG TTG TCT TTG-3', antisense, 5'-AGA AAT GCA GTC AGC ACC ATC AAG-3'; IL-6: sense, 5'-TGA TGC TGG TGA CAA CCA CGG C-3', antisense, 5'-TAA GCC TCC GAC TTG TGA AGT GGT A-3'); E-selectin: sense, 5'-GGC CAG CGC AGG TTG AAT GC-3', antisense, 5'-ATG TTG CCC TGC TGT GGC GC-3'; ICAM-1: sense, 5'-CCG GTC CTG ACC CTG AGC CA-3', antisense, 5'-ATT GGA CCT GCG GGG TGG GT-3'; IL-10: sense, 5'-CAC AAA GCA GCC TTG CAG AA-3', antisense, 5'-CTG GCC CCT GCT GAT CCT-3'; and VCAM-1: sense, 5'-GGA GAC CTG TCA CTG TCA ACT G-3', antisense, 5'-TCC ATT TCA CCA CTG TGT AAC C-3'. The relative expression level was obtained as previously described (Ying et al. 2009b).

Statistical Analysis

All data are expressed as means \pm standard deviation (SD) unless otherwise mentioned. Statistical tests were performed using one-way or two-way ANOVA followed by Bonfferoni

correction or unpaired t test using GraphPad Prism (version 4.1.2). The significant level was set at $P < 0.05$.

Results

CAPs exposure data

The average ambient daily $PM_{2.5}$ concentration during the period was $9 \pm 1 \mu\text{g}/\text{m}^3$. The average CAPs concentration in CAPs chambers was $107 \pm 6 \mu\text{g}/\text{m}^3$ versus $3 \pm 1 \mu\text{g}/\text{m}^3$ in FA chambers. As the exposures were performed for 6 h/day, 5 days/wk, the normalized daily CAPs concentration was $26.5 \mu\text{g}/\text{m}^3$, which was significantly higher than the annual national ambient air quality standard at $12 \mu\text{g}/\text{m}^3$ set by the U.S. Environmental Protection Agency (NAAQS, 2012). The elemental composition during the exposure period is presented in Table 1. Black carbon in ambient $PM_{2.5}$ and CAPs during the exposure period was 492.5 ± 283.4 and $5269.5 \pm 2616.2 \text{ ng}/\text{m}^3$, respectively. Among all chemical components in Table 1, S and Se had the two highest correlations with $PM_{2.5}$ mass (0.94 and 0.84, respectively). While identifying $PM_{2.5}$ emission sources is beyond the scope of this paper, the chemical characterization data showed that the study site was most strongly impacted by secondary aerosol, which is likely to include emissions from coal-fired utility boilers located regionally (Reff et al. 2009).

CAPs exposure increases mouse BP

All animals were weighed before sacrificing. No significant difference in body weight was observed between FA- and CAPS-exposed groups following exposure (34.7 ± 2.3 and 32.5 ± 2.9 g, FA and CAPs respectively). As shown in Figure 1B and C, CAPs exposure significantly

increased basal mean arterial BP (MAP). In contrast, the exposure did not significantly induce changes in HR (Figure 1D) or locomotor activity (Figure 1E).

CAPs exposure induces vascular dysfunction

Since resistance but not conduit arteries primarily determine total peripheral resistance and BP, we isolated mesenteric arteries and assessed their functional response in mice that underwent the same exposure but were not subject to intra-arterial catheter placement. Figure 2 reveals that CAPs exposure significantly increased the contractile response of mesenteric arteries to phenylephrine (a selective α 1-adrenergic receptor agonist) and U-46619 (a thromboxane A2 receptor agonist), and reduced relaxation responses to acetylcholine (an endothelium-dependent vasodilator). logEC50 and peak responses are enumerated in Table 2. However, the aortic mRNA expression of α 1 adrenergic receptors was not changed by CAPs exposure (Supplemental Material, Figure S1).

CAPs exposure activates sympathetic nervous system

Abnormal activation of the SNS is implicated in the pathogenesis of human HTN (Paton et al. 2013). We observed increased BP variability in CAPs-exposed animals (the lower panel of Figure 1B). Since low frequency variation of BP (LF-BPV) is an index of sympathetic tone, we assessed these changes in response to exposure. Figure 3A reveals that CAPs exposure significantly increased low frequency variation of BP. We also analyzed catecholamine production by measuring excretion of the sympathetic neurotransmitter norepinephrine (NE). As shown in Figure 3B, CAPs exposure significantly increased urine excretion of NE. In contrast, there was no significant difference in collected urine volume (FA vs CAPs: 0.5 ± 0.2 vs 0.4 ± 0.2 ml, n=6, p=0.6, student's t test)

Consistent with the increased sympathetic tone in response to long term CAPs exposure, a non-selective beta-blocker propranolol significantly decreased HR in CAPs-exposed but not FA-exposed animals (Figure 3C and Supplemental Material, Table S1). In contrast, atropine did not induce a significant HR changes in both FA-exposed and CAPs-exposed animals (Figure 3D and Supplemental Material, Table S1). Both propranolol and atropine did not have significant effects on BP. This is consistent with previous reports that neither propranolol nor atropine induces significant BP responses in various situations (Rodrigues et al. 2011; Fazan et al. 2005). To assess if the increased sympathetic tone in CAPs-exposed animals contributes to hypertension, we acutely inhibited SNS with a selective α_2A receptor agonist guanfacine. Figure 3E demonstrates that administration of guanfacine resulted in a significantly increased BP drop in CAPs-exposed animals.

CAPs exposure increases the hypertensive response to air jet stress

Air jet stress typifies psychoemotional stress and invokes a characteristic “defense reaction” typified by an increase in MAP mediated through ANS. Figure 4 demonstrates that compared to control mice, CAPs-exposed mice had significantly increased peak BP response to air jet, and the area under curve was also significantly increased.

CAPs exposure induces hypothalamic inflammation

An inflammatory response in hypothalamus has been shown to play a critical role in the activation of SNS in response to diverse pathological stimuli and mediate the obesity-induced increase in BP (Kang et al. 2009; Yu et al. 2012; Purkayastha et al. 2011). We therefore isolated mouse hypothalamus and assessed the expression of pro-inflammatory genes by real-time RT-PCR. Figure 5A demonstrates that CAPs exposure significantly increased hypothalamic mRNA

expression of endothelial-leukocyte adhesion molecule 1 (E-selectin), tumor necrosis factor alpha (TNF α) and intercellular Adhesion Molecule 1 (ICAM-1). Inhibitor kappaB kinase (IKK)/NF-kappaB signalling pathway is central in inflammatory responses (Gamble et al. 2012). Figure 5B reveals that similar to the response to high fat diet (Purkayastha et al. 2011), the phosphorylation of IKK in mouse hypothalamic arcuate nucleus (ARC) was markedly increased in response to chronic CAPs exposure. In contrast, long-term CAPs exposure induced only a non-significant trend towards an increase in IKK phosphorylation in hypothalamic paraventricular nucleus (PVN). Expression of c-fos an indirect marker of neuronal activity was markedly increased in response to CAPs exposure in hypothalamic ARC (Dragunow and Faull 1989). Figure 5C shows that consistent with the level of IKK phosphorylation. Long-term CAPs exposure also significantly increased the number of c-fos positive cells in hypothalamic PVN.

Discussion

In the present study, we demonstrate the following key findings: 1) long term CAPs exposure significantly increased BP in C57Bl/6j mice, paralleled by marked alterations in resistance vessel tonal responses to agonist stimulation; 2) long term CAPs exposure induced HTN and abnormal activation of the SNS, as evidenced by increased LF-BPV, increased urinary NE excretion and attenuation of BP with guanfacine; 3) SNS activation is paralleled by an inflammation in the hypothalamus, suggesting that the latter may play a permissive role in chronic CAPs exposure-induced HTN.

Epidemiologic and empirical evidence strongly support short-term effects of air pollution on the cardiovascular system with a magnification of these effects with chronic exposure.(Brook et al. 2010; Miller et al. 2007) This is consistent with the concept that chronic and cumulative

exposure to air-pollution that occurs over a life time of an individual should result in more pronounced effects, perhaps owing to sustained effects of particle exposure (Miller et al. 2007). From a BP perspective, models that provide a unified understanding of short-term effects, that also help reconcile chronic effects of PM_{2.5} exposure on HTN and their mechanisms are needed. Acute exposure to both PM_{2.5} and diesel exhaust particles triggers rapid changes in BP associated with abnormalities in indices of HRV traditionally associated with sympathetic activation (Brook et al. 2009; Cosselman et al. 2012). BP effects in response to chronic CAPs exposure in animals on the other hand has been attributed to a variety of mechanisms including dysfunctional NOS, production of vasoactive mediators such as ET-1 and Rho/ROCK activation, inflammatory cellular infiltration in the perivascular adipose and vascular remodelling (Brook and Rajagopalan 2009). The notion that SNS may modulate chronic hypertension through inflammation in key regulatory areas in the CNS represents a paradigm shift in our understanding of HTN (Andersson and Tracey 2012; Olofsson et al. 2012; Harrison et al. 2011; Leibowitz and Schiffrin 2011; Elenkov et al. 2000). This shift has resulted in a change in our view of the ANS as an arbitrator of acute changes in heart rate and BP to a facilitator of chronic functional and structural changes in HTN (Harrison et al. 2011; Abboud et al. 2012). Studies from several groups suggest that diverse stimuli including stress may lead to modest elevations in blood pressure (pre-hypertensive state) via SNS mechanisms (Marvar et al. 2011). This early phase may then lead to a more protracted chronic phase driven by inflammatory mechanisms leading to perpetuation of HTN. Thus SNS appears to mediate both short term changes in BP but also pathways that may lead to perpetuation of HTN (Abboud et al. 2012). Our studies suggest that chronic exposure to PM_{2.5} may represent yet another factor that leads to chronic sympathetic activation and hypertension. The increase in sympathetic tone in response to chronic PM_{2.5} exposure is

supported by increased LF-BPV, heightened NE excretion and abnormal hypertensive response to air jet stress, a model of hypertension associated with sympathetic activation. Furthermore, the central sympathetic inhibitor guanfacine ablated the BP response to chronic CAPs exposure. SNS activation in the CNS is accomplished via alteration of pre-synaptic α 2-adrenergic receptors, which are critical in determining central catecholamine levels and activation. The adrenergic α_{2a} receptors are almost exclusively expressed in the CNS, and have sympathoinhibitory effects, while α_{2b} has sympathoexcitatory function (Makaritsis et al. 1999; Makaritsis et al. 2000; MacMillan et al. 1996). The effects of guanfacine but not propranol or atropine in lowering BP, suggests that central SNS activation likely plays an important role in $PM_{2.5}$ effects on BP.

There is emerging data that hypothalamic inflammation plays an important role in regulation of sympathetic tone in response to diverse pathophysiological stimuli, such as high fat diet, angiotensin II, and heart failure (Kang et al. 2009; Yu et al. 2012; Purkayastha et al. 2011). Interestingly, a recent study showed that chronic CAPs exposure increased NF-kappaB activation and pro-inflammatory gene expression paralleled by an increase of neurotransmitter NE in hypothalamic PVN (MohanKumar et al. 2008). Our data while somewhat consistent with these findings, also found increased expression of pro-inflammatory genes and IKK activation in the ARC and less so in the PVN. Importantly, the local increase in IKK activation was correlated to neural activity of these locations, as evidenced by the increased number of c-fos positive cells. While, these are suggestive of a role for inflammation in increased sympathetic tone and consequent hypertension in response to chronic $PM_{2.5}$, definitive proof may require additional experiments such as disruption of IKK/NF-kappaB signaling pathway in the hypothalamus and assessment of how this affects chronic $PM_{2.5}$ exposure-induced increase in BP. An additional

interpretative difficulty is the relative importance of specific circumventricular organs that may differentially contribute to HTN. For instance, the POMC neurons have been shown to be involved with high fat diet mediated BP effects while AgRP neurons have not (Purkayastha et al. 2011). Further, inflammation in sites such as the nucleus tractus solitarius (NTS) and other vagal centers (dorsal ventral lateral medulla and the nucleus ambiguus) may also play a role (Teslovich et al. 2010; Waki et al. 2011). In several models of hypertension, enhanced activation of circuits involving GABA and increased glutamate receptor activation in the PVN and RVLM, contribute to the elevated SNS activity and BP. In this regard, it is interesting to postulate that air pollution may activate both rapid and slow CNS pathways regulating sympathoexcitation.

Although our results provide evidence that chronic CAPs exposure increases sympathetic tone and BP, CAPs exposure did not increase HR. This is consistent with previous studies showing that acute CAPs while elevating BP, did not affect cardiac output in both humans (Brook et al. 2009) and dogs (Bartoli et al. 2009). While the reasons for the selective modulation of presser responses without alterations in HR are unclear at this time, one potential explanation may relate to the fact that sympathetic control may be differentially modulated in various organs in response to diverse stimuli (do Carmo et al. 2011; Esler et al. 2006). Thus the effects of CAPs exposure on sympathetic tone may also be organ-specific. Therefore, it is important in the future to assess the sympathetic tone of different organs, in particular those central in BP regulation such as kidney.

Our CAPs exposure level after normalization was still higher than the annual national ambient air quality standard set by the U.S. Environmental Protection Agency. Although these mean exposure levels are unusual in North America these days, it is not rare in cities with heavy air pollution. For instance in 2005, 89% of the world's population lived in areas where the World Health Organization Air Quality Guideline of $10 \mu\text{g}/\text{m}^3$ $\text{PM}_{2.5}$ (annual average) was exceeded.

Globally, 32% of the population lived in areas exceeding the WHO Level 1 Interim Target of 35 $\mu\text{g}/\text{m}^3$, driven by high proportions in East (76%) and South (26%) Asia (Brauer et al. 2012).

Our study has a number of important limitations that must be acknowledged. These include the fact that we have not provided cause and effect relationship between inflammation in the hypothalamus, SNS activation and BP responses. This will require additional experiments targeting inflammation in the hypothalamus and concomitantly measuring SNS activation and BP. We have not provided any data on the time course of SNS activation, inflammation and BP response. Finally our interventional experiments with sympathetic and parasympathetic blockade were performed acutely and it remains to be seen if chronic blockade with guanfacine attenuates the development of BP in response to CAPs. Another limitation is the fact that we have not done comparative assessment of other areas in the brain, such as NTS and centers such as the dorsal ventral lateral medulla and the nucleus ambiguus, which may also have played a role (Teslovich et al. 2010; Waki et al. 2011).

Conclusion

In summary, our data provide evidence that increased sympathetic tone mediates the hypertensive action of chronic CAPs exposure, which may involve an inflammatory response in hypothalamus.

Reference

- Abboud FM, Harwani SC, Chapleau MW. 2012. Autonomic neural regulation of the immune system: implications for hypertension and cardiovascular disease. *Hypertension* 59(4):755-762.
- Andersson U, Tracey KJ. 2012. Reflex principles of immunological homeostasis. *Annu Rev Immunol* 30:313-335.
- Bartoli CR, Wellenius GA, Diaz EA, Lawrence J, Coull BA, Akiyama I, et al. 2009. Mechanisms of inhaled fine particulate air pollution-induced arterial blood pressure changes. *Environ Health Perspect* 117(3):361-366.
- Brauer M, Amann M, Burnett RT, Cohen A, Dentener F, Ezzati M, et al. 2012. Exposure assessment for estimation of the global burden of disease attributable to outdoor air pollution. *Environ Sci Technol* 46(2):652-660.
- Brook RD, Rajagopalan S. 2009. Particulate matter, air pollution, and blood pressure. *J Am Soc Hypertens* 3(5):332-350.
- Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* 121(21):2331-2378.
- Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, et al. 2009. Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. *Hypertension* 54(3):659-667.
- Cosselman KE, Krishnan RM, Oron AP, Jansen K, Peretz A, Sullivan JH, et al. 2012. Blood pressure response to controlled diesel exhaust exposure in human subjects. *Hypertension* 59(5):943-948.
- DiBona GF. 2013. Sympathetic nervous system and hypertension. *Hypertension* 61(3):556-560.
- do Carmo JM, da Silva AA, Cai Z, Lin S, Dubinion JH, Hall JE. 2011. Control of blood pressure, appetite, and glucose by leptin in mice lacking leptin receptors in proopiomelanocortin neurons. *Hypertension* 57(5):918-926.
- Dong GH, Qian ZM, Xaverius PK, Trevathan E, Maalouf S, Parker J, et al. 2013. Association between long-term air pollution and increased blood pressure and hypertension in China. *Hypertension* 61(3):578-584.

- Dragunow M, Faull R. 1989. The use of c-fos as a metabolic marker in neuronal pathway tracing. *J Neurosci Methods* 29(3):261-265.
- Dvonch JT, Kannan S, Schulz AJ, Keeler GJ, Mentz G, House J, et al. 2009. Acute Effects of Ambient Particulate Matter on Blood Pressure. Differential Effects Across Urban Communities. *Hypertension* 53(5):853-859.
- Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. 2000. The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev* 52(4):595-638.
- Esler M, Straznicky N, Eikelis N, Masuo K, Lambert G, Lambert E. 2006. Mechanisms of sympathetic activation in obesity-related hypertension. *Hypertension* 48(5):787-796.
- Fazan R, Jr., de Oliveira M, da Silva VJ, Joaquim LF, Montano N, Porta A, et al. 2005. Frequency-dependent baroreflex modulation of blood pressure and heart rate variability in conscious mice. *Am J Physiol Heart Circ Physiol*. 289(5):H1968-1975.
- Gamble C, McIntosh K, Scott R, Ho KH, Plevin R, Paul A. 2012. Inhibitory kappa B Kinases as targets for pharmacological regulation. *Br J Pharmacol* 165(4):802-819.
- Gross V, Tank J, Obst M, Plehm R, Blumer KJ, Diedrich A, et al. 2005. Autonomic nervous system and blood pressure regulation in RGS2-deficient mice. *Am J Physiol Regul Integr Comp Physiol* 288(5):R1134-1142.
- Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, et al. 2011. Inflammation, immunity, and hypertension. *Hypertension* 57(2):132-140.
- Kampfrath T, Maiseyeu A, Ying Z, Shah Z, DeJulius JA, Xu X, et al. 2011. Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways. *Circ Res* 108(6):716-726.
- Kang YM, Ma Y, Zheng JP, Elks C, Sriramula S, Yang ZM, et al. 2009. Brain nuclear factor-kappa B activation contributes to neurohumoral excitation in angiotensin II-induced hypertension. *Cardiovasc Res* 82(3):503-512.
- Leibowitz A, Schiffrin EL. 2011. Immune mechanisms in hypertension. *Curr Hypertens Rep* 13(6):465-472.

- Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380(9859):2224-2260.
- MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE. 1996. Central hypotensive effects of the alpha_{2a}-adrenergic receptor subtype. *Science* 273(5276):801-803.
- Makaritsis KP, Handy DE, Johns C, Kobilka B, Gavras I, Gavras H. 1999. Role of the alpha_{2B}-adrenergic receptor in the development of salt-induced hypertension. *Hypertension* 33(1):14-17.
- Makaritsis KP, Johns C, Gavras I, Gavras H. 2000. Role of alpha(2)-adrenergic receptor subtypes in the acute hypertensive response to hypertonic saline infusion in anephric mice. *Hypertension* 35(2):609-613.
- Marvar PJ, Lob H, Vinh A, Zarreen F, Harrison DG. 2011. The central nervous system and inflammation in hypertension. *Curr Opin Pharmacol* 11(2):156-161.
- Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, et al. 2007. Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med* 356(5):447-458.
- MohanKumar SM, Campbell A, Block M, Veronesi B. 2008. Particulate matter, oxidative stress and neurotoxicity. *Neurotoxicology* 29(3):479-488.
- NAAQS (National Ambient Air Quality Standards), updated on December 14, 2012. Available: <http://www.epa.gov/air/criteria.html> [accessed 5 November 2013].
- Olofsson PS, Rosas-Ballina M, Levine YA, Tracey KJ. 2012. Rethinking inflammation: neural circuits in the regulation of immunity. *Immunol Rev* 248(1):188-204.
- Paton JF, Sobotka PA, Fudim M, Engleman ZJ, Hart EC, McBryde FD, et al. 2013. The carotid body as a therapeutic target for the treatment of sympathetically mediated diseases. *Hypertension* 61(1):5-13.
- Purkayastha S, Zhang G, Cai D. 2011. Uncoupling the mechanisms of obesity and hypertension by targeting hypothalamic IKK-beta and NF-kappaB. *Nat Med* 17(7):883-887.
- Purkayastha S, Zhang H, Zhang G, Ahmed Z, Wang Y, Cai D. 2011. Neural dysregulation of peripheral insulin action and blood pressure by brain endoplasmic reticulum stress. *Proc Natl Acad Sci U S A* 108(7):2939-2944.

- Reff A, Bhave PV, Simon H, Pace TG, Pouliot GA, Mobley JD, et al. 2009. Emissions inventory of PM_{2.5} trace elements across the United States. *Environ Sci Technol* 43(15):5790-5796.
- Rodrigues FL, de Oliveira M, Salgado HC, Fazan R, Jr. 2011. Effect of baroreceptor denervation on the autonomic control of arterial pressure in conscious mice. *Exp Physiol* 96(9):853-862.
- Sun Q, Yue P, Ying Z, Cardounel AJ, Brook RD, Devlin R, et al. 2008. Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of Rho/ROCK. *Arterioscler Thromb Vasc Biol* 28(10):1760-1766.
- Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. 2010. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 466(7307):707-713.
- Thomson E, Kumarathasan P, Goegan P, Aubin RA, Vincent R. 2005. Differential regulation of the lung endothelin system by urban particulate matter and ozone. *Toxicol Sci* 88(1):103-113.
- Waki H, Gouraud SS, Maeda M, Raizada MK, Paton JF. 2011. Contributions of vascular inflammation in the brainstem for neurogenic hypertension. *Respir Physiol Neurobiol* 178(3):422-428.
- Ying Z, Kampfrath T, Thurston G, Farrar B, Lippmann M, Wang A, et al. 2009b. Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species. *Toxicol Sci* 111(1):80-88.
- Ying Z, Yue P, Xu X, Zhong M, Sun Q, Mikolaj M, et al. 2009a. Air pollution and cardiac remodeling: a role for RhoA/Rho-kinase. *Am J Physiol Heart Circ Physiol* 296(5):H1540-1550.
- Yu Y, Zhang ZH, Wei SG, Weiss RM, Felder RB. 2012. Peroxisome proliferator-activated receptor-gamma regulates inflammation and renin-angiotensin system activity in the hypothalamic paraventricular nucleus and ameliorates peripheral manifestations of heart failure. *Hypertension* 59(2):477-484.

Table 1. Elemental data for ambient PM_{2.5} and CAPs (mean ± SD in ng/m³)

Element	Ambient	FA	CAPs
Rb	0.07 ± 0.02	0.03 ± 0.02	0.66 ± 0.23
Sr	0.37 ± 0.09	0.12 ± 0.04	3.81 ± 1.28
Mo	0.33 ± 0.12	0.13 ± 0.11	2.93 ± 1.35
Cd	0.24 ± 0.09	0.5 ± 0.37	1.8 ± 1.02
Sb	0.68 ± 0.25	0.06 ± 0.04	6.39 ± 2.84
Ba	2.41 ± 0.59	0.65 ± 0.21	25.92 ± 9.24
La	0.03 ± 0.01	0 ± 0	0.33 ± 0.18
Ce	0.04 ± 0.01	0.02 ± 0.01	0.44 ± 0.21
Pb	2.8 ± 0.62	2.06 ± 0.78	23.3 ± 6.14
Na	54.37 ± 25.62	73.46 ± 25.17	453.74 ± 303.19
Mg	18.72 ± 4.69	7.76 ± 2.85	202.36 ± 61.84
Al	22.89 ± 8.09	19.77 ± 13.38	201.34 ± 68.75
P	10.2 ± 2.15	17.55 ± 3.1	64.72 ± 10.42
S	790.17 ± 455.19	33.69 ± 16.58	7556.13 ± 4759.81
Ca	70.95 ± 19.94	74 ± 39.23	698.49 ± 220.44
Ti	0.82 ± 0.17	0.1 ± 0.04	9 ± 2.33
V	0.24 ± 0.15	0.02 ± 0.01	2.25 ± 1.47
Cr	3.22 ± 0.48	6.86 ± 0.99	13.59 ± 1.54
Mn	1.92 ± 0.82	0.18 ± 0.15	19.11 ± 9.57
Fe	43.26 ± 12.69	11.59 ± 10.21	462.3 ± 145.59
Co	0.09 ± 0.1	0.11 ± 0.1	0.81 ± 1.02
Ni	0.38 ± 0.43	0.42 ± 0.44	2.61 ± 1.43
Cu	2.33 ± 0.62	1.58 ± 1.42	23.98 ± 7.57
Zn	10.3 ± 3.17	6.81 ± 5.66	90.07 ± 29.94
K	34.96 ± 11.13	22.49 ± 8.11	313.02 ± 103.66
As	0.56 ± 0.19	0.03 ± 0.01	5.26 ± 2.31
Se	0.74 ± 0.28	0.05 ± 0.02	7.27 ± 3.59

PM_{2.5} was collected weekly and the average during the 6 months of exposure is presented.

Table 2. The logEC50s and peak responses of mesenteric arteries from FA/CAPs-exposed mice (Mean \pm SD).

	logEC50		Peak Response	
	FA	CAPs	FA	CAPs
Phenylephrine	-7.1 \pm 0.2	-6.9 \pm 0.2	117.3 \pm 11.3	183.8 \pm 18.7*
Acetylcholine	-7.1 \pm 0.2	-7 \pm 0.2	-88.3 \pm 4.6	-61.1 \pm 3.7*
U-46619	-7.5 \pm 0.3	-9.4 \pm 0.9*	197.3 \pm 27	181.1 \pm 24

*p<0.05 vs FA, student's t test. -

Figure legends

Figure 1. CAPs exposure increases basal BP. (A) The experimental time scheme. P, propranolol; A, atropine; G, guanfacine; S, air jet stress. (B and C) After exposure to FA/CAPs for 6 months, C57Bl/6j mice (n = 6/group) were implanted with DSI radiotelemetry transmitter, and the basal BP was analyzed after the recovery from surgery. (B) The representative recordings, the basal BP was recorded after daily FA/CAPs exposure for about 18 hours/day and 3 continuous days (upper, FA; lower, CAPs); (C) the quantization of 16-hour mean arterial blood pressure (MAP, 5pm-9am); (D) the quantization of heart rate (HR); (E) the quantization of mouse activity. *p < 0.05 versus FA, two-way ANOVA.

Figure 2. CAPs exposure induces resistance arterial dysfunction. After sacrifice, mesenteric arteries were isolated and mounted onto wire myograph, and responses to phenylephrine (PE, A), acetylcholine (B), and U-46619 (C) were analyzed. *p < 0.05 versus FA, two-way ANOVA.

Figure 3. CAPs exposure increases sympathetic tone. (A) The LF-BPV was calculated from the 3 days of basal blood pressure recording. (B) Urine of FA/CAPs-exposed mice were collected and norepinephrine (NE) excretion was assessed. (C-E) After analysis of the basal BP in FA/CAPs-exposed mice with DSI radiotelemetry transmitter, these mice were treated with propranolol (C), atropine (D), and guanfacine (E), and changes in either HR (propranolol and atropine) or BP (guanfacine) is presented. *p < 0.05 versus FA, student's t test.

Figure 4. CAPs exposure increases stress-induced hypertension. FA/CAPs-exposed mice were stimulated with air jet and BP response was analyzed with DSI radiotelemetry transmitter. (A) the BP response curve. (B) the peak increase in BP. (C) the under curve area of BP response curve. *p < 0.05 versus FA, student's t test.

Figure 5. CAPs exposure induces hypothalamic inflammation. FA/CAPs-exposed mice were sacrificed, and hypothalamic expression of pro-inflammatory genes was analyzed by real-time RT-PCR (A). (B and C) Immunostaining of mouse hypothalamus with anti-phospho-IKK2 (B) and anti-c-fos (C). * $p < 0.05$ versus FA, student's t test. Scale bar = 25 μm .

Figure 1

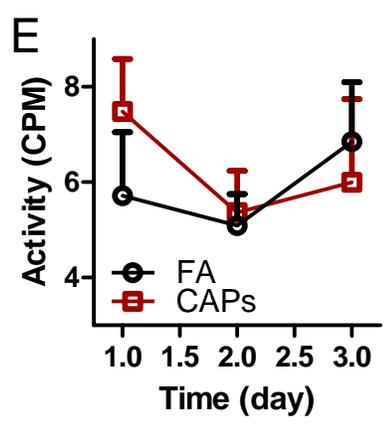
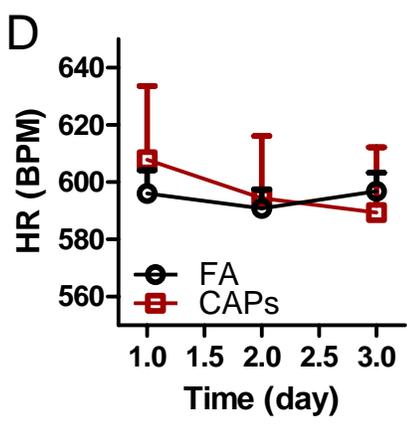
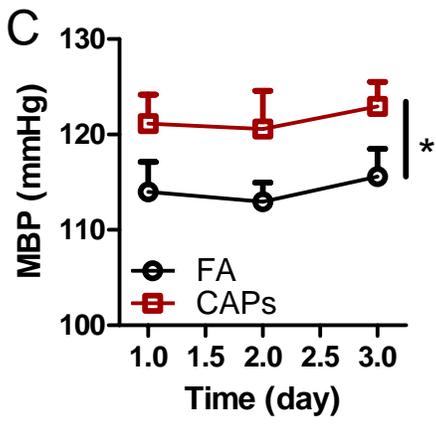
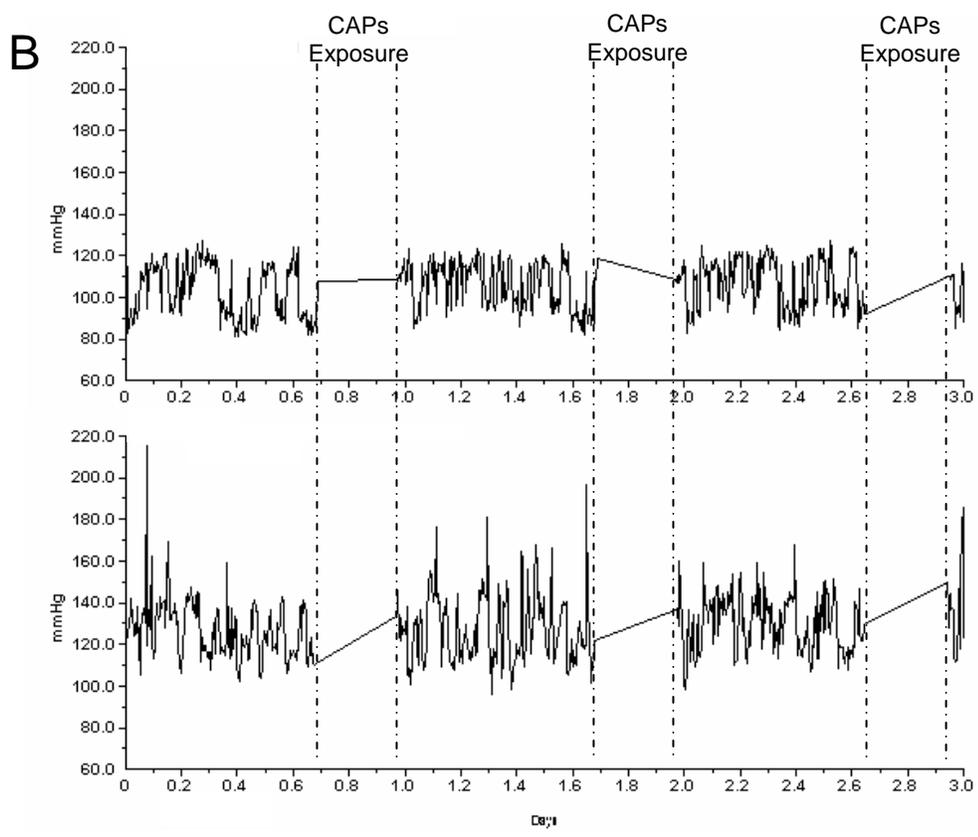
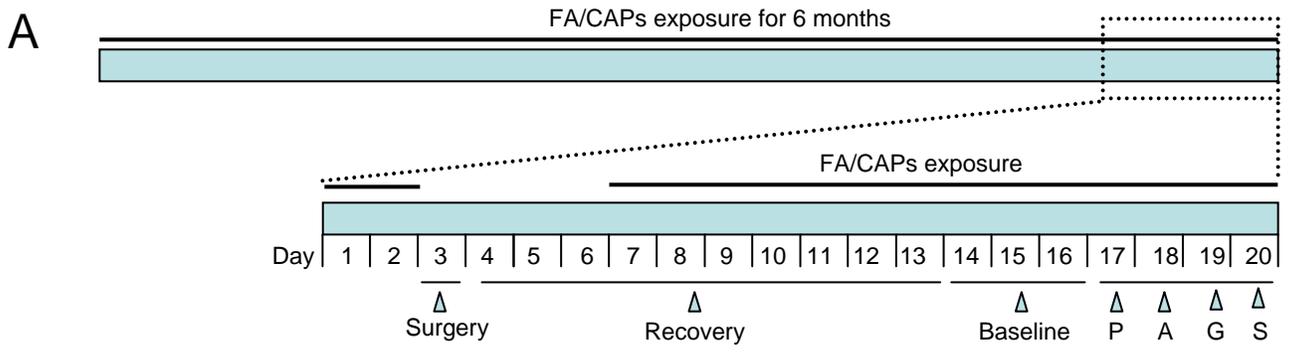


Figure 2

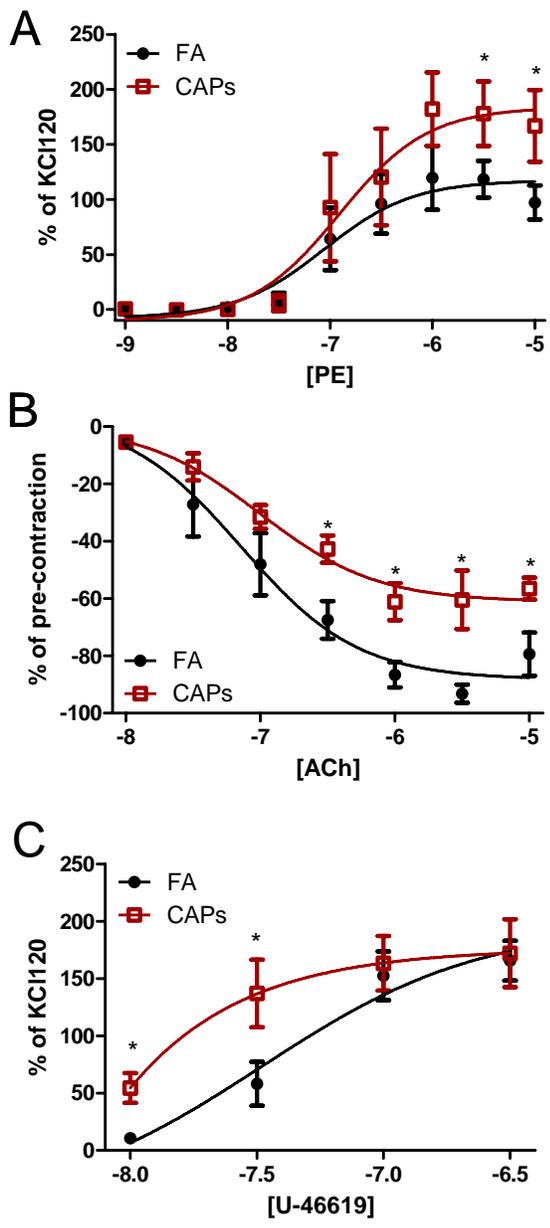


Figure 3

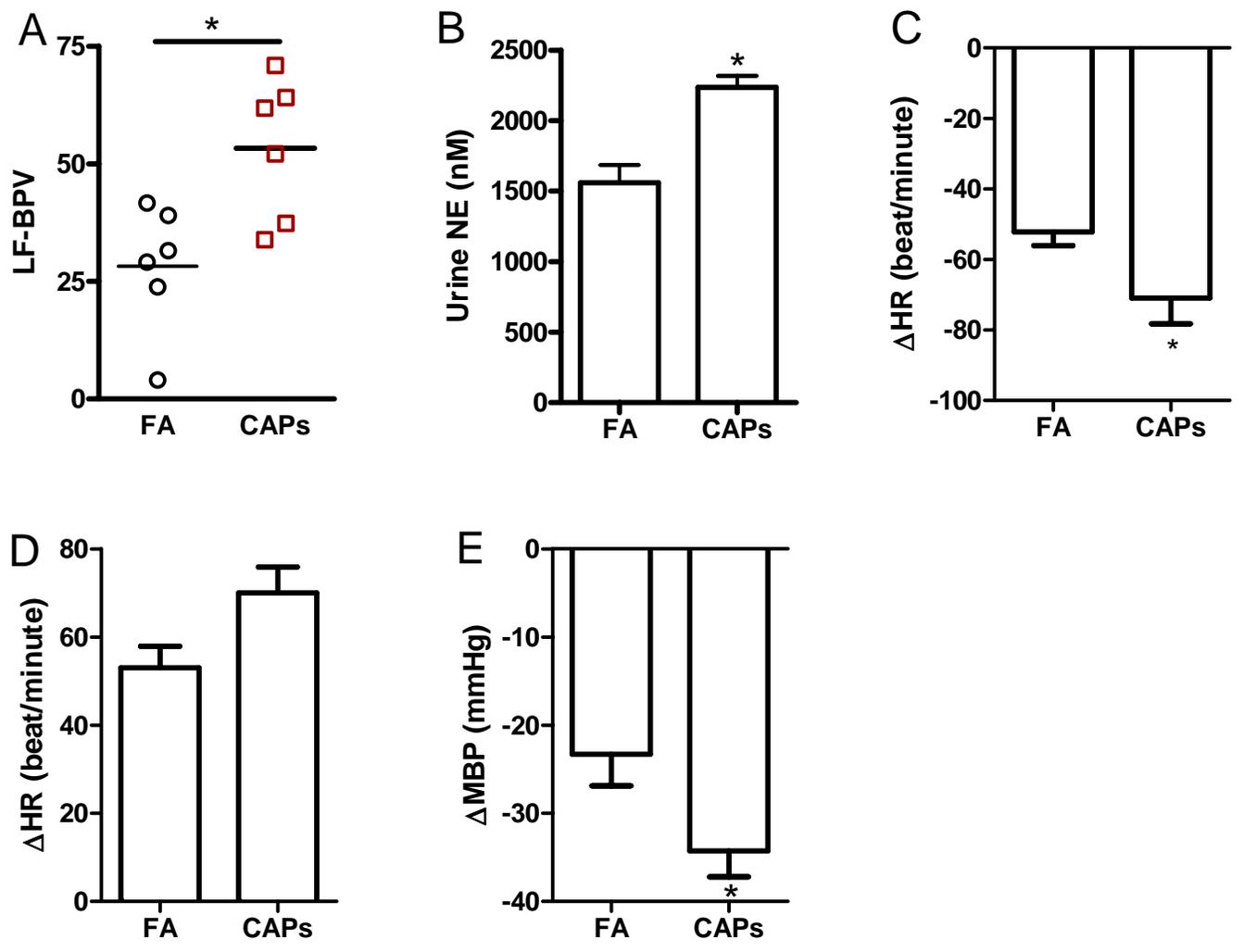


Figure 4

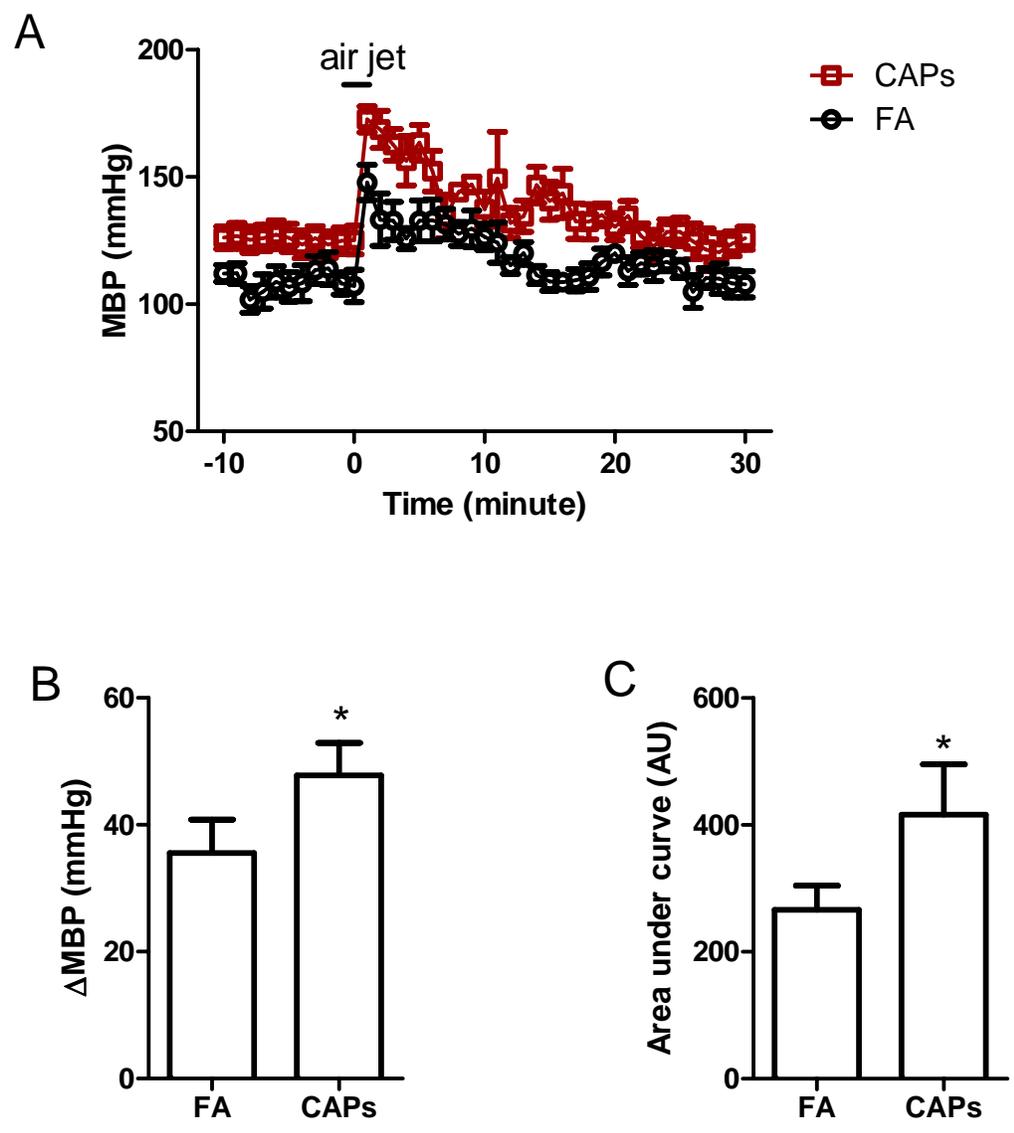
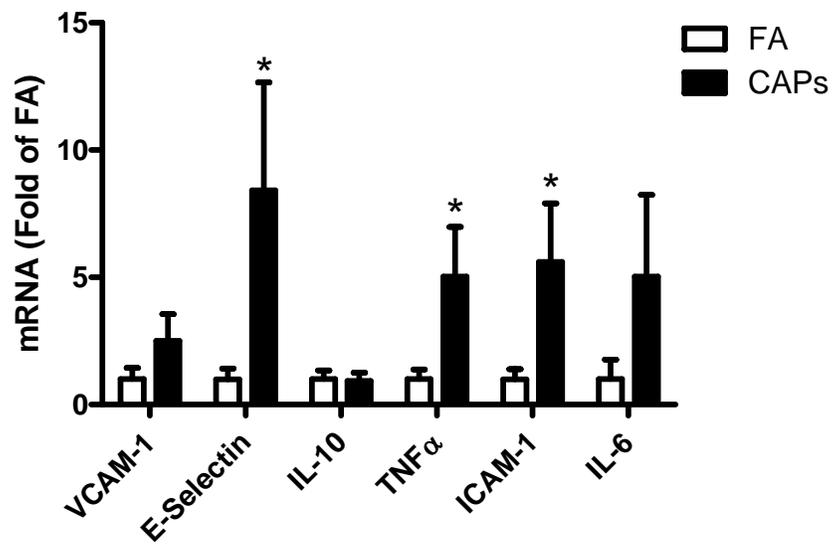
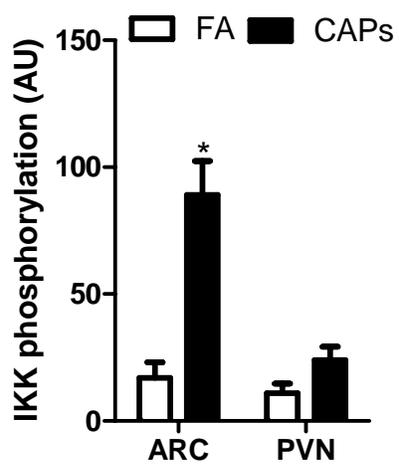
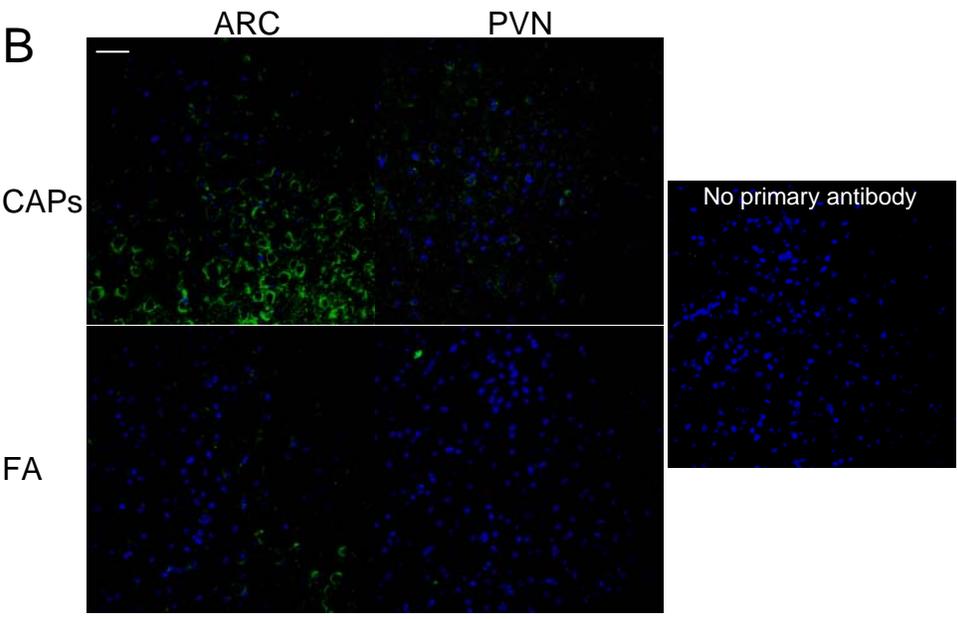


Figure 5

A



B



C

