Online Supplement

THE CAFETERIA DIET INCREASES FAT MASS AND CHRONICALLY ELEVATES LUMBAR SYMPATHETIC NERVE ACTIVITY IN RATS

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Short Title: Cafeteria Diet and Sympathoexcitation

METHODS

Implantation and Use of Telemetry Devices

After one week of habituation, over-night fasted rats were anesthetized (isoflurane, 1.4% in oxygen), were given analgesic (ketoprofen, 5 mg/kg) and antibiotic (Baytril, 10 mg/kg), and prepared for placement of a recording electrode on the lumbar nerve and an arterial catheter using sterile techniques. In brief, a midline abdominal incision was made, the intestines were retracted, and the abdominal aorta and vena cava were pulled aside to expose the left lumbar nerve (Figure S1). A 2 mm section of the nerve was carefully dissected from underlying connective tissue using glass probes. Electrode wires from a telemetry device that measures blood pressure and SNA (model TR46SP, Telemetry Research, Auckland, New Zealand) were anchored to the aorta using approximately seven 6-O sutures placed through the aortic adventitia. Then, the 2 ends from the wires were placed underneath the sympathetic trunk (between L3 and L4) and the ends and nerve were covered with a silicone elastomer (Kwik-Sil, WPI, Sarasota, FL). The ground electrode from the device was sutured to the abdominal muscles just exterior to the wound. The quality of the signal was monitored during surgery by visualizing raw nerve activity and from assessing sound quality using an audiomonitor (Grass model AM8, Warwick, RI). After this, a section of the abdominal aorta caudal to the nerve electrode was carefully separated from the vena cava. The tip of the catheter from the radiotelemetry device was inserted through a hole made by a 23-gauge needle, and the catheter was then fixed to the artery with cyanoacrylate cement (Vetbond, 3M, St. Paul, MN) so as to not occlude the flow of blood.

Lumbar SNA signals were sampled at 500 Hz and continuously displayed on the PowerLab system. The original lumbar SNA signal was amplified, filtered between 50-2,000 Hz, full-wave rectified, and integrated using a low-pass filter with a 20-ms time constant. Chronic lumbar SNA was analyzed using a method adapted from Yoshimoto and colleagues [1]. In brief, the area under the integrated SNA curves was calculated every 1 sec using the PowerLab system, and the background noise level was set to be the lowest value from each 1 sec segment of the integrated SNA curves. The validity of this assumption was tested by comparing these lowest values during normal recording with values obtained after euthanasia in the same rat. The lack of difference obtained during the two conditions indicated that the lowest level during each 1 sec SNA segment was a good estimate of the background noise. Using this method, the background noise was therefore subtracted from the total activity every second. A post-study analysis of background noise demonstrated that these levels were not different between the 2 groups and did not change during the study (Figure S2). Means of the area under the integrated SNA curves were calculated for every hour of the 17-day recording period. To quantify lumbar SNA responses to the control and cafeteria diets, percentage changes in SNA were calculated by taking the mean of these values during the 2-day baseline period as 100%.

For the control group, there were 7 successful recordings and 11 recordings that failed and were removed from the study. For the cafeteria group, there were 7 successful recordings

and 8 recordings that failed and were removed from the study. To determine nerve viability, we preformed daily written records for each subject that included raw noise voltages and spike voltages, integrated noise voltages and spike voltages, as well as assessment of nerve quality through sound. Criteria for inclusion in the study were 21 days of nerve recordings in each rat showing the following on a daily basis: 1) signal to noise ratio > 2:1 from the raw nerve signal where the signal is the microvolt value (peak to peak) of the lumbar SNA bursts and the noise is the microvolt value (peak to peak) of the background noise, 2) clearly visible SNA spikes on the raw nerve signal having a typical lumbar SNA appearance, which includes conspicuous SNA bursts occurring in a semi-cyclic fashion with the signal falling to the level of the background noise in-between SNA bursts, 3) clearly visible SNA bursts on the integrated signal showing the same characteristics, 4) audible nerve bursts that correspond exactly to the visible bursts on both the raw and integrated channels. Recordings that contained electrocardiogram signals or electrical noise or movement artifact (continuous voltage increases of 1 sec. or longer) were excluded from the protocol.

At study completion, SNA signal quality was assessed in isoflurane anesthetized rats by examining lumbar nerve responses to intravenous sodium nitroprusside, hexamethonium bromide, and to euthanasia. To do this, a catheter was placed in the left femoral vein and baseline blood pressure and SNA were recorded for 15 min. Then, sodium nitroprusside (200 µg/ml, iv) was given as 2.5 to 80 µg/kg/min over 1-2 min, followed by recovery of blood pressure, and then administration of hexamethonium (30 mg/kg, iv). Euthanasia was achieved by an overdose of sodium pentobarbitone (100 mg/kg, iv). Accuracy of blood pressure calibration in the TR46SP devices was tested in each unit before and after implantation against a mercury manometer.

REFERENCES

1. Yoshimoto M, Miki K, Fink GD, King A, Osborn JW. Chronic angiotensin II infusion causes differential responses in regional sympathetic nerve activity in rats. *Hypertension*. 2010; 55: 644-657.

Values are per 1g of food item	Total Kcal	Fat Kcal	Total Fat g	Total Carb g	Protein g	Dietary Fiber g	Sugars g	Saturated Fat g	Cholesterol mg	Sodium mg
Lab Diet 500 l (Purina)	4.07	0.45	0.05	0.49	0.24	0.05	0.00	0.02	0.00	4.00
Kit-Kat bar (Hersheys)	5.00	2.10	0.26	0.64	0.07	0.02	0.50	0.16	0.11	7.10
Ritz cracker (Nabisco)	5.00	2.50	0.28	0.62	0.06	0.00	0.06	0.06	0.00	6.50
Vanilla wafers (Nabisco)	4.60	1.66	0.20	0.70	0.02	0.00	0.36	0.05	0.16	3.80
Mini-wheats cereal (Kelloggs)	3.45	0.18	0.01	0.83	0.09	0.10	0.20	0.00	0.00	0.00
Peanuts (Planters)	6.07	4.28	0.50	0.17	0.25	0.07	0.07	0.07	0.00	5.70
Cheetos (UTZ)	5.35	2.85	0.32	0.57	0.07	0.03	0.03	0.09	0.00	9.20
Buttered popcorn (UTZ)	6.07	3.90	0.42	0.46	0.07	0.07	0.03	0.10	0.00	8.90
Salami (El Cerdito Supremo)	2.30	1.70	0.19	0.04	0.13	0.00	0.00	0.89	0.63	10.50
Sweetened condensed milk (La Fe)	3.33	0.76	0.07	0.56	0.07	0.00	0.56	0.05	0.25	1.10
Smoked fish (Shop Rite)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fruit punch (Shop Rite)	0.58	0.00	0.00	0.14	0.00	0.00	0.14	0.00	0.00	0.06

NA, not available

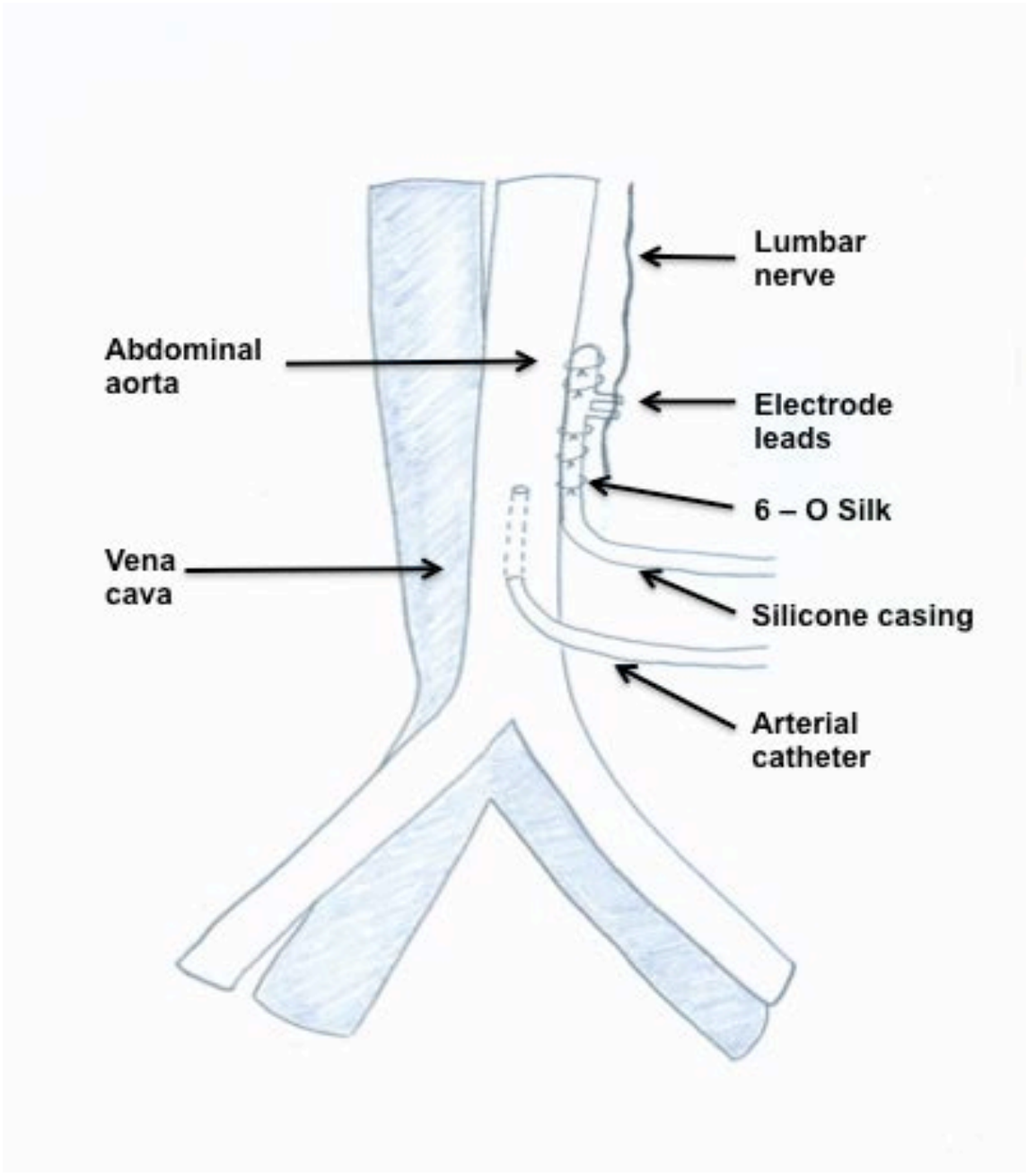


FIGURE S1.

Schematic representation of the lumbar sympathetic electrode and arterial catheter. The 6 – O silk was anchored through the adventitia of the abdominal aorta.

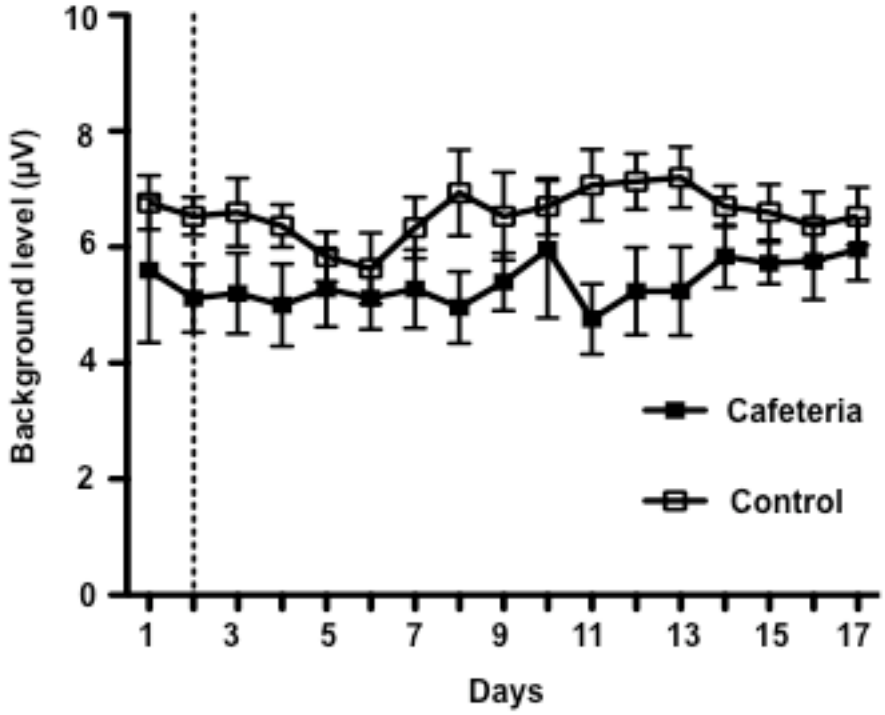


FIGURE S2.

Group data showing background noise level in the 2 groups. Background noise was set to be the lowest value from each 1 sec segment of the integrated SNA curves. Means of these values were calculated for every hour of the 17-day recording period.

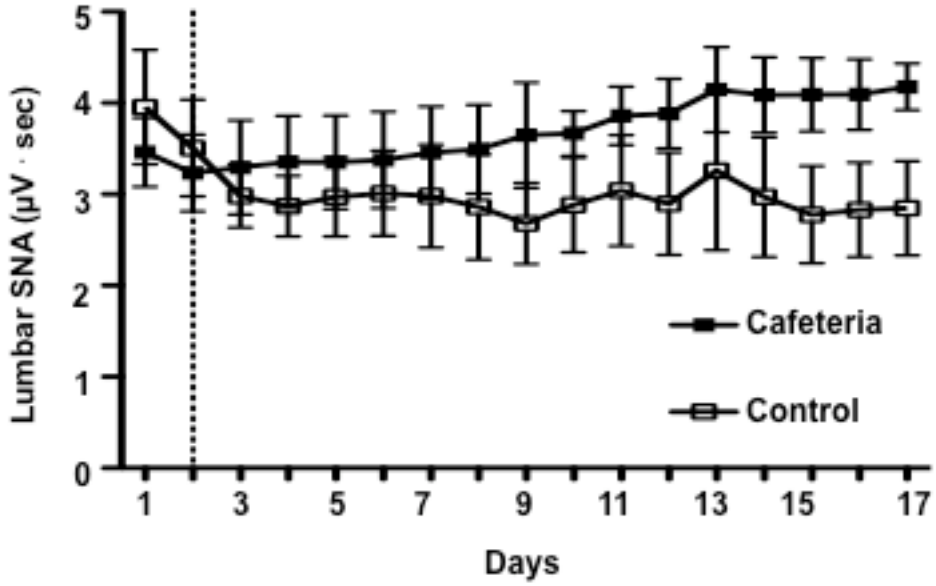


FIGURE S3.

Group data showing lumbar sympathetic nerve activity (LSNA) responses as area under the integrated SNA curves, both before (days 1-2) and during (days 3-17) control diet or cafeteria diets in rats. Values are means ± SEM.

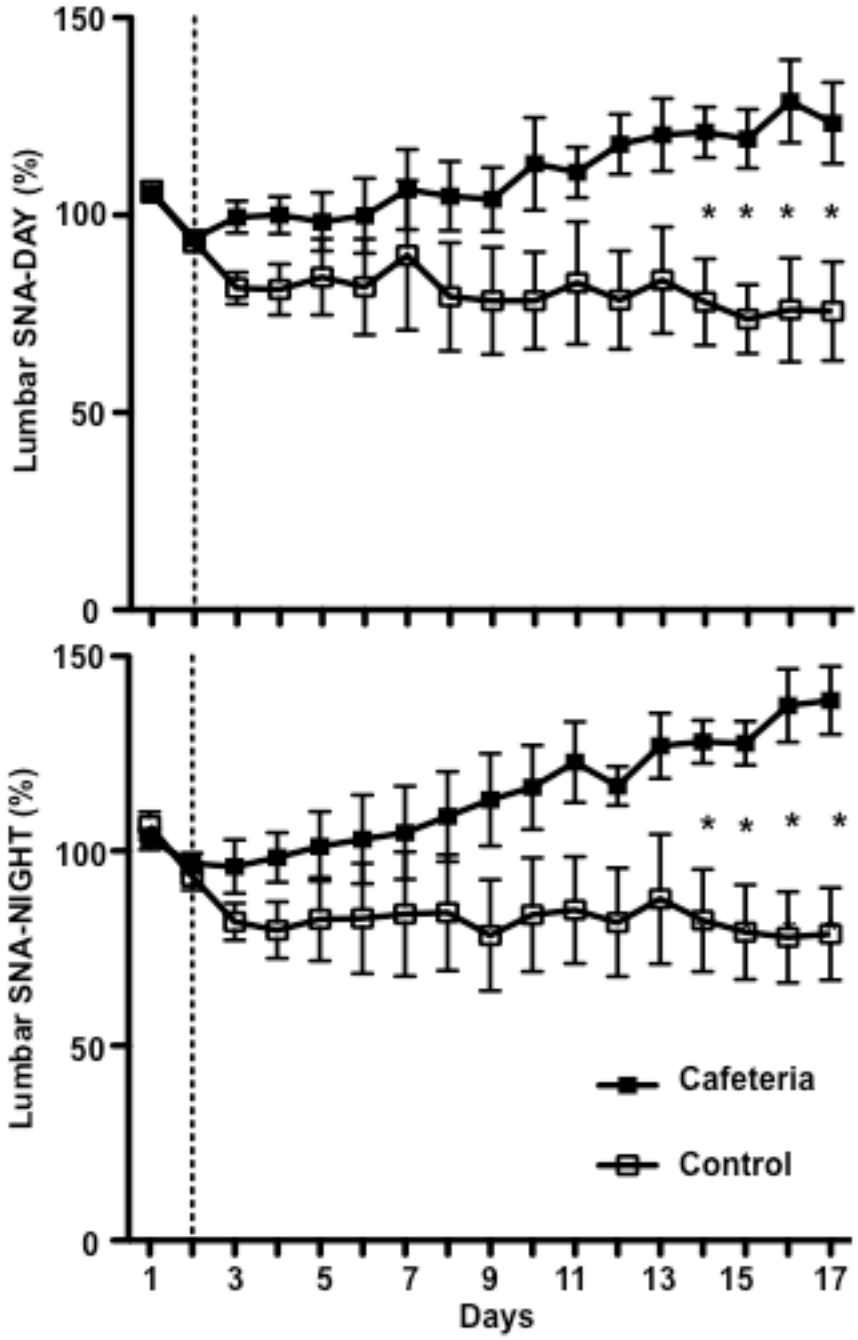


FIGURE S4.

Lumbar sympathetic nerve activity (LSNA) responses before (days 1-2) and during (days 3-17) control diet or cafeteria diets during the 12-hour day periods (top) and the 12-hour dark periods (bottom). Values are means ± SEM. * $P < 0.05$, cafeteria rats vs. control rats.

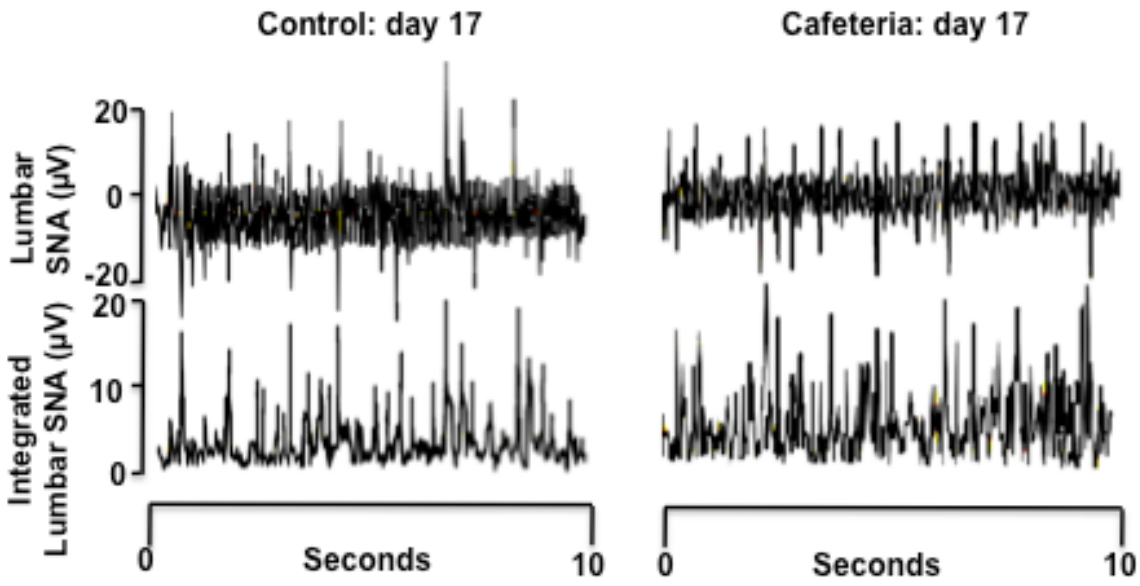
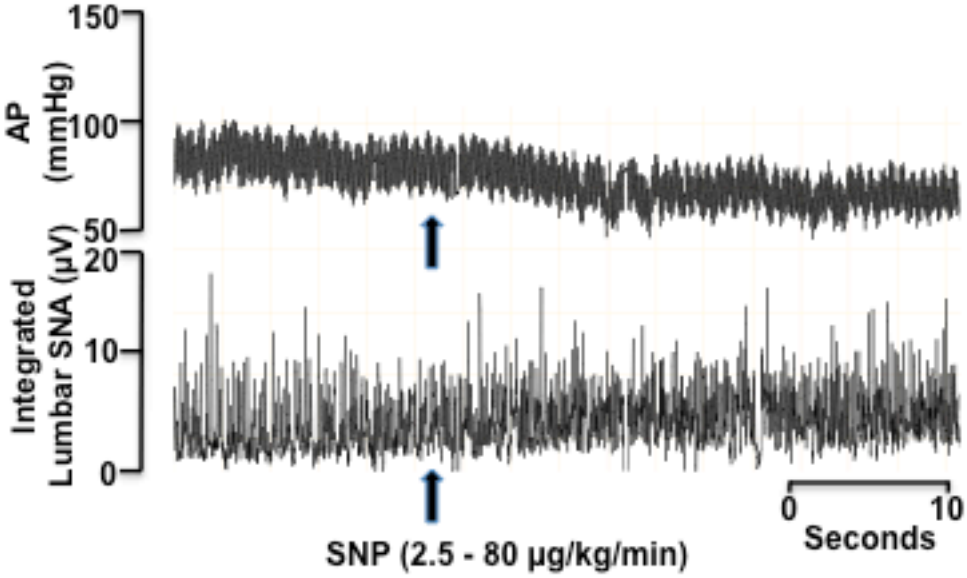


FIGURE S5

Representative raw (top) and integrated (bottom) lumbar SNA signals from a control and cafeteria subject on the final day of the study (day 17).



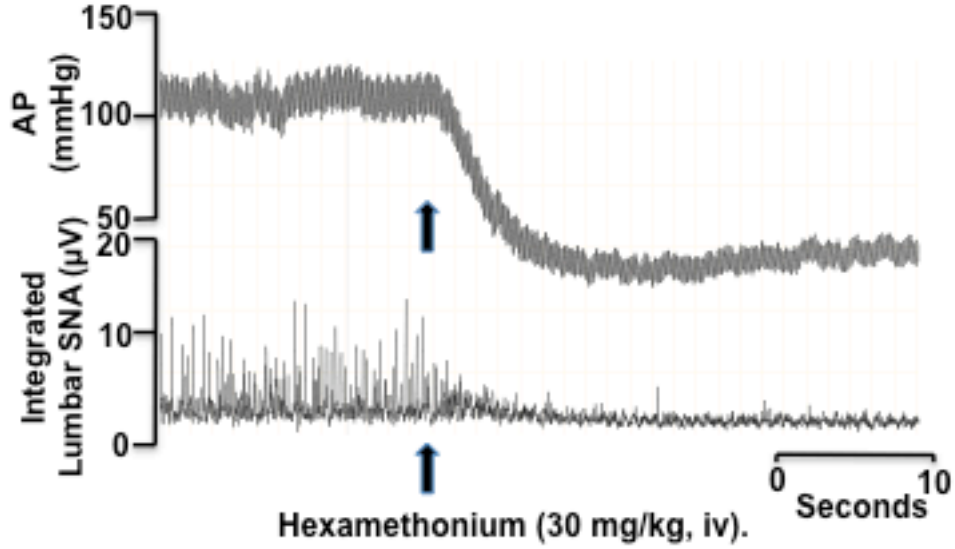


FIGURE S6

Representative arterial pressure (AP) and integrated lumbar SNA responses to sodium nitroprusside (SNP)(top) and to hexamethonium bromide (bottom) from a control subject on the final day of the study (day 17). The increase in SNA to SNP can be seen as an increase in spike density (frequency) rather than an increase in amplitude.