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The dmNTS is not the source of increased blood pressure variability in baroreflex denervated rats

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INTRODUCTION

The arterial baroreceptor reflex is a powerful and rapid buffer mechanism that stabilizes the moment-to-moment blood pressure (BP). The arterial BP sensors, or baroreceptors, are located at the aortic arch and carotid sinus; their axons travel in the aortic depressor nerve (ADN) and the carotid sinus nerve (CSN), to the second order cells in the dorsal medial nucleus of the solitary tract (dmNTS). From the dmNTS, the common pathway diverges into sympathetic and parasympathetic branches. The sympathetic branch projects to the caudal ventrolateral medulla (CVLM), and then to the rostral ventrolateral medulla (RVLM). The output of RVLM neurons produces the sympathetic tone and largely determines the resting level of arterial pressure (Granata, Ruggiero et al. 1985; Granata and Reis 1987). For the parasympathetic branch, the baroreflex dmNTS neurons project to the dorsal motor nucleus of vagus (dmnX), and the nucleus of ambiguous (NA). Axons from the dmnX and NA innervate the intrinsic plexus of the heart and influence both rate and contractile strength.

Across species, e.g., dogs, cats, rats and rabbits, in conscious or lightly anesthetized states, eliminating baroreflex inputs, either by sinoaortic denervation (SAD) or by bilateral lesions of the NTS, substantially increases blood pressure variability (BPV) (Alper, Jacob et al. 1987; Jacob, Alper et al. 1991; Schreihof and Sved 1994; Sved, Mancini et al. 1994; Jacob, Ramanathan et al. 1995; Oosting, StruikerBoudier et al. 1997; Dworkin, Dworkin et al. 2000; Dworkin, Tang et al. 2000; Fazan, de Oliveira et al. 2005); however the source of the increased BPV has not been identified. Trapani (Trapani 1984) found that “the variability of arterial pressure produced by SAD was unchanged by (pre-collicular) decerebration and was equivalent to that seen after SAD alone or in sham decerebrate baroreceptor-denervated rats (Table 10 in (Trapani 1984))”, which effectively excluded the brain regions rostral to the colliculus and pointed to the caudal brain stem as the noise source

In the 80s and the early 90s, a series of studies (Alper, Jacob et al. 1987; Alper, Jacob et al. 1987; Jacob, Alper et al. 1989; Jacob, Alper et al. 1991) demonstrated that, after SAD, complete restoration of BPV to the level seen in intact rats required simultaneous administration of a chlorisondamine ganglionic block and an angiotensin-converting enzyme inhibitor or a vasopressin antagonist, which appeared to indicate that at least a part of the post-SAD BPV

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was generated peripherally as mechanical or hydraulic noise in the vasculature; however, because 75% of the variability is eliminated by the chlorisondamine (Alper, Jacob et al. 1987) alone, the central system undoubtedly is an important source of post-denervation BPV. In this paper we will examine the likelihood that a substantial part of the central variability is due to noise generated in the dmNTS.

Previously, using a chronically neuromuscular blocked (NMB) rat preparation, by systematically analyzing the major baroreflex afferent aortic depressor nerve (ADN) to BP open-loop frequency transfer function, we found (Dworkin, Dworkin et al. 2000; Dworkin, Tang et al. 2000) that the maximum baroreflex effectiveness (gain) was in the very low frequency (VLF) range of 0.01–0.2 Hz (Fig 4 & Fig 5 in (Dworkin, Tang et al. 2000)); correlatively, destroying baroreflex by complete SAD resulted in a large increase of the power of the systolic blood pressure noise spectrum in the VLF range (Fig 2 in (Dworkin, Tang et al. 2000)). In the present study, using the same preparation, we found that, although SAD significantly increased the VLF power in the expiratory systolic blood pressure (EsBP) spectrum, it decreased the VLF power of the expiratory heart inter-beat-interval (EIBI) spectrum (partial data were previously published in the *American Journal of Physiology* (Dworkin, Tang et al. 2000)). Because dmNTS is the only major common node of the vascular sympathetic and cardiac parasympathetic pathways, if the noise source were in the dmNTS, after SAD, the noise would be expected to appear in both sympathetic and parasympathetic pathways, and thus increase the VLF powers in both the EsBP and EIBI spectra. The observed directionally opposite changes in the post-SAD VLF power of the EsBP and EIBI spectra precludes that; thus, the noise is more likely generated distal to the dmNTS, for example, in the CVLM, and/or the RVLM nodes of the baroreflex. Here we show that, compared with the dmNTS evoked responses to single pulse ADN stimuli, the corresponding evoked systolic blood pressure responses (Δ sBP) have a larger error variance, which is what would be expected, if noise that is generated distal to the dmNTS were being summed with baroreceptor input.

METHODS

Surgical Preparations

Data from 5 female Long-Evans (LE) (225–275g), and 17 female Sprague-Dawley (SPD) (230–270g) rats were used in this study. The LE rats had complete SAD, and the 17 SPD rats did not; and the same rats were used in (Dworkin, Tang et al. 2000) and (Tang and Dworkin 2007), respectively. Data from the LE rats were used for EsBP and EIBI power spectral analyses, and those from the SPD rats were used for ADN-dmNTS and ADN-BP evoked response analyses. Detail information about the LE and SPD preparations is described in (Dworkin, Dworkin et al. 2000), and (Tang and Dworkin 2007), respectively. The following is a brief summary: Each ventilated, NMB rat was individually maintained in a special “ICU” facility and monitored around the clock, using life support, and analgesic protocols as stringent as those that are accepted as adequate for critical care of human adults and infants. All actual surgery, or physical manipulation was done with full sterile technique and under precisely controlled and monitored, deep (>1.5%) isoflurane anesthesia. The experimental protocols were supervised and certified to be in compliance with NIH and APS guidelines by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee (IACUC).

Both preparations required 2 days to complete the surgical procedures, which were the same for the first day, but different for the second day. Surgeries started in the morning, and finished before 1:00 PM for the first day, and before 3:00 PM for the second day. During all procedures, the isoflurane anesthetic level was >1.5% ensuring that: (1) the electroencephalogram (EEG) was synchronized and dominated by high-voltage slow-wave activity, (2) mean BP was <100 mmHg, and heart rate (HR) was <420 beats/min, and (3) there were no evident EEG, BP, or

HR responses to surgical manipulations. Isoflurane was delivered into the inspiratory gas stream by precision mass flow controller. During the first day, surgery included following implantations: EEG electrodes for brain activity measurements; a transurethral bladder cannula to allow drainage and measurement of urine production rate; a pair of subcutaneous precordial electrocardiogram (ECG) silver wire electrodes, a femoral artery cannula for arterial blood pressure (ABP) measurement, and a femoral vein cannula for administering parenteral solutions and recording of venous pressure (VBP). Core temperature was servo-regulated at 37°C. Following induction of paralysis with a 75 µg i.v. bolus of α -cobratoxin, the rats were ventilated with positive pressure at 72 breaths/min (inspiratory : expiratory = 1:2), at a volume of 180–220 cc/min, of 48.5% O₂, 47% N₂ and 3% CO₂, and 1.5% isoflurane. Neuromuscular block was maintained by continuous infusion of α -cobratoxin (250 µg/day).

During the second surgical day, for both the SPD and LE rats, the left ADN was dissected caudal to the carotid bifurcation, and placed on an anodized Ta-Ta₂O₅ capacitance electrode (Dworkin, Dworkin et al. 2000; Tang and Dworkin 2007), which was driven by a computer controlled optically isolated constant current unit (CCIU-8, FHC Inc., Bowdoinham, ME). For the LE rats, the right ADN was cut, the left sinus was denervated by thoroughly stripping the neural and connective tissues around the carotid bifurcation areas, and a stainless steel and silicone balloon was inserted into the right carotid sinus. The effective SAD was verified by no detectable bradycardia to 10 µg phenylephrine (i.v.). For the SPD rats, a 1–2 MΩ glass insulated tungsten microelectrode (Alpha-Omega, Alpharetta, GA) was advanced into the dmNTS, using a motorized microdrive (FHC Inc., Bowdoinham, ME). The baroreflex cardiovascular area in the dmNTS was located by atlas coordinates, and confirmed by reference to the somatotopic features of the gracile nucleus, which is 50–150 µm dorsal to the baroreflex cells of the dmNTS. The pre-amplifier (XCELL-3 X 4, 40-#40-8B, FHC, Bowdoinham, ME) gain was 20k, and signals were bandpass filtered at 0.3–1.0 kHz, and digitized at 10 kHz using Spike2® and a Power 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK).

A low (analgesic ≤ 0.5) level of isoflurane was maintained between surgical days, and 3- to 4-days post-surgery. On day 5, isoflurane was gradually reduced to zero for the LE rats, and to 0.5% level for the SPD rats; and then maintained at those levels for the remainder of the experiment. Multiple protocols were scheduled for the both preparations (Dworkin, Dworkin et al. 2000; Tang and Dworkin 2007), and the average useful life of the preparations, including the surgical and experimental periods, was 20 days for the LE rats, and 12 days for the SPD rats, probably because, for the SPDs, the brainstem was exposed for multi-unit central recordings.

Data Acquisition

For LE and SPD rats, data were continuously acquired 24 hours/day, 7 days/wk. Data for the LE rats were acquired in 1998. Because of computer storage limitations at that time, data were only acquired at a resolution of 2.5 s (see detail in (Dworkin, Dworkin et al. 2000)). In brief, the data acquisition cycle was triggered 100 ms before each inspiratory peak, and 4 samples were collected in each data cycle. The first sample was during the first systolic BP maximum, the second sample during the following diastolic minimum, the third during the first systolic maximum after the beginning of expiration, and fourth during the following diastolic minimum. Each of these samples included BP, hind-paw blood flow, 4-band EEG spectral power density, core temperature, substrate heater temperature, urine output rate, expiratory CO₂, and intratracheal pressure. Inter-beat-interval (IBI) samples were at the beginning of inspiration and the end of expiration. A new data cycle was initiated 2.5 s (3 inspirations $\times$ 0.833 s) after the start of previous cycle, i.e. one third of the respiratory cycles were sampled.

For the SPD rats, the same variables were recorded using Spike2® and a Power 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK), but at higher sampling rates, e.g., BP at 250 Hz, ERs at 10kHz, and EKG with the “wavemark” mode in Spike2® (see detail in (Tang and Dworkin 2007)).

Experimental Protocols

Effects of SAD on expiratory systolic BP(EsBP) and expiratory inter-beat-interval (EIBI) variabilities (*the LE data were used for the protocol*)—We choose to use measurements from expiratory phase, because during expiratory phase, intrathoracic pressure is near atmospheric and intrinsically more constant. We do not expect data from expiratory phase differ significantly from those from inspiratory phase. Data from 7 pre-, and 7 post-SAD hours were used for analysis. The pre-SAD data were from the last 7 hours immediately prior to the beginning of the second day surgery, which was ~12 hours after the end of the first day surgery. Anesthesia was at a <0.5% level. The 7 hour post-SAD data were acquired on day 4 or 5, after anesthesia was reduced to ~0.

Transduction curves—Data were collected from 12 SPD rats, on day 4 or 5, after isoflurane was reduced to a level of 0.5%. Transduction curves were used to characterize the relationships of the ADN stimulus amplitude to the magnitude of dmNTS ERs, and the ADN stimulus amplitude to the magnitude of BP decreases. Two patterns were used: “ascending” which consisted of 10 incrementally increasing stimuli (300 μ s, inter-pulse interval = 15s), and the “symmetric” which consisted of 20 stimuli: 10 incrementally increasing, and 10 symmetrically decreasing, (300 μ s, inter-pulse interval = 15s). The 10 different stimulus amplitudes were equally distributed between 110%, the pattern maximum, and 10%, the pattern minimum, of the dmNTS ER saturation level (see detail in (Tang and Dworkin 2007)).

The coefficient of variations (CVs)—Data were collected from 10 SPD rats (5 SPD rats were common for the CV and TC analyses) on day 4 or 5, after isoflurane was reduced to a level of 0.5%. CV allows comparison of the variation of populations that have significantly different mean values; and is calculated as the ratio of the standard deviation (SD) to the mean of the variable measurements. In this study, CVs were used to compare the reliability of the dmNTS ERs with the BP responses (decreases) to single pulse (300 μ s, inter-pulse interval = 6 $\pm$ 1s) ADN stimuli. The single pulse stimuli were applied for 5 hours, and current amplitude was chosen at the level, which elicited distinct ER complexes in a 10 min averaged ER trace, and which produced >0.4 mmHg BP decreases.

Data Analysis

The EsBP and EIBI amplitude spectra—For each pre- and post- SAD hour, EsBP and EIBI power spectra were obtained by a FFT (8.33 mHz resolution) on Hanning-windowed EsBP, and EIBI signals, respectively (the square root of power spectrum is the amplitude spectrum). The VLF (0.01–0.2 Hz) power is the integral of the EsBP, or EIBI, amplitude spectrum value between 0.01 to 0.2 Hz.

The ER magnitude—The ER magnitude was determined from the 1-hr stimulus triggered ensemble averaged ER trace (see detail in (Tang and Dworkin 2007)). After removing DC, the ER complex area and the matching baseline area symmetric to the trigger, were calculated by integration of the absolute (rectified) value of the signal. The ER magnitude was defined as the difference between the ER and the baseline areas.

The magnitude of BP response—The magnitude of systolic BP change (Δ sBP) was calculated, from a stimulus triggered ensemble averaged sBP trace, as the difference between

the minimum systolic value, occurring during the 1–3 s post-stimulus period, and the mean systolic value of the 1 s pre-stimulus period.

Stimulus amplitude vs. dmNTS ER magnitude and stimulus amplitude vs. BP response transduction curves—Magnitudes of ensemble averaged dmNTS ER and BP responses were calculated for each stimulus level. To combine rats, for each rat, the stimulus amplitudes were converted to the percent of maximum amplitude used in the pattern, and the average response magnitudes were converted to the percent of maximum response elicited by the pattern. Data were pooled across all rats, and means and SE of the responses were calculated for each percentage-of-maximum stimulus level (see detail in (Tang and Dworkin 2007)).

The coefficient of variations (CVs)—For each rat, the averaged magnitudes of the ER and the Δ sBP response were calculated every 10 min (100 sweeps) for 5 hours; and CVs, for both the ER and the Δ sBP, were calculated as the ratio of the SD to the mean of the corresponding 5 h of 10 min averaged magnitudes, and multiplied by 100%.

Statistical Analysis—Student's *t*-test, with two-tailed distribution, two-sample unequal variance, was used for statistical analysis (SigmaStat, version 2.03, SPSS Inc); and $p < 0.05$ is considered as a significant level.

Results

Effects of SAD on EsBP and EIBI variabilities

Table 1 provides the grand averages of the means and the variabilities, represented as standard deviations (SDs), of the EsBP, EIBI measurements of the 5 LE rats, during the 7 pre- and 7 post-SAD hours. Compared with the pre-SAD period, the post-SAD period had both significantly increased mean and variability for the EsBP (Δ EsBP mean = 30.7 mmHg, $df = 8$, $t = 2.924$, $p < 0.05$; Δ EsBP SD = 11.9 mmHg, $df = 8$, $t = 2.406$, $p < 0.05$); but, not for the EIBI. (Δ EIBI mean = -4 ms, $df = 8$, $t = -1.139$, $p > 0.05$; Δ EIBI SD = -0.624 ms, $df = 8$, $t = 0.705$, $p > 0.05$). For each rat, the Δ change for each value, e.g. the mean EsBP, was calculated as the difference of the measurements between the 7 h post- and 7 h pre-SAD periods, i.e., Δ EsBP = mean (EsBP)_{post-SAD} - mean (EsBP)_{Pre-SAD}.

Effects of SAD on the VLF power of the EsBP and EIBI amplitude spectra

In Fig. 1 and Fig 2, the upper panels show, respectively, the averaged EsBP and EIBI amplitude spectra (mean $\pm$ SE) of the 7 pre-(gray) and 7 post-SAD (black) hours, for each individual LE rat; and the bottom panels show their corresponding VLF powers. SAD significantly increased the VLF powers in the EsBP spectra for all 5 LE rats (Fig. 1); but decreased the VLF powers in the EIBI spectra for 4 of the 5 LE rats. The remaining one rat showed an increase in EIBI VLF power (Fig. 2), but, the increase was not statistically significant. Overall, SAD has produced an opposite effect in the VLF powers of the EsBP and EIBI spectra: a large increase in the EsBP VLF power, but a decrease in the EIBI VLF power.

The dmNTS and sBP evoked responses

Data in Fig. 3 and Fig 4 were acquired from the 12 SPD rats, in which ADN stimulation elicited ERs were recorded at the dmNTS. Fig. 3 summarizes important features of the dmNTS ERs (data from the same subjects were previously published in (Tang and Dworkin 2007)): (a) is a typical ensemble averaged ER trace ($N = 589$ sweeps), from a single dmNTS recording site, which is composed of complexes that arrive at the dmNTS in the time ranges of 4–20 ms (median ~8 ms), and 30–50 ms (median 38 ms). The stereotaxically measured Pythagorean distance between the dmNTS recording and ADN stimulation sites was 11.5 – 16.5 mm; given the known conduction velocities of ~5 – 10 m/s for myelinated A-fiber, and ~0.3 – 0.5 m/s for

unmyelinated C-fibers (Fan and Andresen 1998), it is unlikely that C-fiber elicited ERs could arrive in <20 ms. Thus, based on latency and threshold, it is almost certain that the early complex contains only A-fiber conducted ERs, and the complex is defined as A-complex; the high threshold later complex is defined as HTL complex (see detail in (Dworkin, Tang et al. 2000)). (b) and (c) are the average stimulus amplitude – ER magnitude, and stimulus amplitude – Δ sBP transduction curves, respectively. Both the A- and HTL complex curves are sigmoidal (b); however, the Δ sBP curve was approximately linear ($r^2=0.98$; $n=11$) over the full amplitude range (c). The Δ sBP response, to 300 μ s, single pulse ADN stimuli, at 100% amplitude was only ~1.0 mmHg, and for individual rat, the relationship between amplitude and Δ sBP was not consistently reliable. For the 12 SPD rats, within the threshold-to-saturation stimulus amplitude ranges, the mean and the SE of the r^2 values were 0.96, and 0.0086, respectively, for both the A and the HTL complexes. In contrast, for Δ sBP over the same amplitude ranges, the mean r^2 values were much smaller and more variable (see Table 1 in (Tang and Dworkin 2007)): over the A linear range: mean $r^2 = 0.30$, SE of the $r^2 = 0.098$; over the HTL complex linear range, mean $r^2 = 0.52$, SE of the $r^2 = 0.098$. Thus, the evoked BP change was much less reliable and robust than the evoked dmNTS response.

The coefficient of variations (CVs)

were used to compare the reliability of the dmNTS ER with the sBP responses to the single pulse ADN stimuli. The CV was calculated as the ratio of the standard deviation to the mean of the measurement variable. Fig. 4 shows the CVs (mean $\pm$ SE) of the Δ sBP, and the dmNTS ER magnitudes to the 5 hour, 10 min single pulse (300 μ s, inter-pulse interval = 6 ± 1 s) ADN stimuli, in 10 SPD rats. The CV of the Δ sBP was significantly larger ($t=4.652$, $df = 18$; $p<0.001$) than that of the dmNTS ERs, which indicates that the dmNTS ERs were much more reliable than the Δ sBP in response to the ADN stimulation.

Discussion

Independent of behavioral state, complete removal of baroafferent inputs producing exaggerated BPV has been observed consistently across animal species (Guazzi and Zanchetti 1965; Cowley, Liard et al. 1973; Korner, Head et al. 1984; Alper, Jacob et al. 1987; Alper, Jacob et al. 1987; Jacob, Alper et al. 1991; Schreihofner and Sved 1994; Sved, Mancini et al. 1994; Dworkin, Dworkin et al. 2000; Dworkin, Tang et al. 2000), including our NMB preparation, in which respiration, core temperature are strictly controlled, and skeletal activity is absent. Mechanisms of the enhanced post-SAD BPV are not completely understood. We (Dworkin, Dworkin et al. 2000) showed that chlorisondamine, a ganglionic blocker, significantly reduced the SAD increased BPV. Our observation is consistent with the findings by Alper et al. (Alper, Jacob et al. 1987), who found that a combination of chlorisondamine, and either an angiotensin-converting enzyme inhibitor or a vasopressin antagonist, to return variability to the level seen in intact animals, which indicates that both the central and the peripheral mechanisms contribute to the post-SAD increased BPV (Alper, Jacob et al. 1987; Jacob, Alper et al. 1991).

Julien et al., (Julien, Zhang et al. 1993) demonstrated that, in chlorisondamine ganglionically blocked SAD rats, BPV can be largely restored by infusion of a vasoconstrictor agent (phenylephrine or angiotensin II), and moreover, there is little correlation between BPV and renal sympathetic nerve activity (RSNA) (Barres, Lewis et al. 1992; Julien, Chapuis et al. 2003). These results suggest that SNA is not directly responsible for BPV in SAD rats, but, rather plays a permissive role in maintain vascular tone at a nearly normal level.

However, Jacob et al. (Jacob, Alper et al. 1989) showed that graded sustained increases in pressure (+10 to +82 mmHg) produced by constant infusion of angiotensin II, phenylephrine, or vasopressin did not affect lability in SAD rats. The discrepancy between the two studies is

unresolved. It is also not clear the degree to which RSNA represents the overall sympathetic nervous output in SAD rats. There is, indeed, evidence that the RSNA does not accurately track the general sympathetic outflow: (1) Given the evidence for unaltered vascular sensitivity in chronic SAD rats (Barres, Cheng et al. 2004; Schreihöfer, Ito et al. 2005), air-jet stress induced similar increases in RSNA in both control and SAD rats, but a much larger increase in MAP in chronic SAD rats (Barres, Cheng et al. 2004), thus in this case at least, RSNA does not correlate with the general SNA; (2) both Jacob et al., (Jacob, Alper et al. 1991) and Trapani et al., (Trapani, Barron et al. 1986) showed that hemodynamic changes in individual vascular beds, including hindquarter, renal and mesenteric vascular beds, did not correlate with pressure lability, however, the sum of the changes in resistance was significantly correlated with the pressure lability. Possibly sympathetic activity is similar, and in chronic SAD rats, the sum of the changes of the sympathetic activity is correlated with MAP, but not that of any particular sympathetic outflow; (3) SAD may lead to changes in efferent sympathetic output/pathway. Zhang ZQ et al. (Zhang, Julien et al. 1996) found that, in response to air-jet stress, intact rats showed vasoconstriction in mesentery and vasodilatation in hindquarters, and that SAD markedly increased mesenteric vasoconstrictor responses, but blunted hindquarters vasodilatation. It is not known whether RSNA is a good index of SNA in chronic SAD rats. It is, however, shown in dogs that RSNA does not well represent SNA during hemorrhage (Morita and Vatner 1985).

Mannard and Polosa (Mannard and Polosa 1973) recorded T1 cervical spinal sympathetic units in the cat, and demonstrated that variability range of the unit background firing was significantly reduced after the spinal cord was transected at the cervical level, which implies a large supraspinal influence on the variability of the sympathetic preganglionic neurons. Furthermore, it is shown that, in ganglion-blocked SAD rats, restoration of the initial MAP, by infusion of a vasoconstrictor agent (phenylephrine or angiotensin II), did not return BPV to the control level (Julien, Zhang et al. 1993), which suggests a substantial involvement of the sympathetically mediated central nervous system activity in the post-SAD BPV.

Trapani (Trapani 1984) examined effects of pre-collicular decerebration on BPV produced by SAD, and reported that “the variability of arterial pressure produced by SAD was unchanged by (pre-collicular) decerebration and was equivalent to that seen after SAD alone or in sham decerebrate baroreceptor-denervated rats (Table 10 in (Trapani 1984))”. These results excluded the brain regions rostral to the colliculi as the potential noise sources, and suggest that brain stem is likely where the noise originates. The dmNTS is the only major common anatomic node for the sympathetic and cardiac parasympathetic nervous systems in the brainstem; we suppose that if the noise source were at the dmNTS, after SAD, the noise at the dmNTS would have been similarly conducted in both the sympathetic and parasympathetic pathways, leading to both increased the BPV, and increased heart rate variability. Indeed, studies in several species have found a significant post-SAD increase in BPV; however, these same studies, found the HRV to be unchanged, or even decreased (Alper, Jacob et al. 1987; Mancina, Parati et al. 1999; Fazan, de Oliveira et al. 2005). Similarly, in NMB rats, SAD substantially increased BPV, but did not affect the overall HRV (Table 1). More specifically, Fig. 1 & Fig 2 show that SAD produces a significant increase in the VLF power of the EsBP spectra for all 5 experimental LE rats; but a decrease in the VLF power of the EIBI spectra in 4 of the 5 LE rats (the SAD did not affect the EIBI VLF power in the 5th LE rat). Thus, the noise is more likely at loci further along the baroreflex pathway, for example, the CVLM and/or RVLM.

It is possible that, the peak gain area of the parasympathetic cardiac baroreflex is at higher frequency than the VLF region, and because of the low sampling rate (0.4 Hz) of our old data acquisition system we did not detect the increased power with SAD; however, this is not likely because given the results of other studies on the effects of SAD on BPV and HRV (Buchholz and Nathan 1984).

Another possibility is that there may be distinct visceral afferent subtypes at the NTS (Andresen, Doyle et al. 2004), and the cardiac and BP baroreflexes may involve different neuron populations; thus SAD could increase noise in the BP population, but not in the cardiac population; but if so, bilateral NTS electrolytic lesions, which eradicate the area, should remove the noise sources at the NTS, and decrease the BPV; however, Buchholz et al. (Buchholz and Nathan 1984) showed a 380% increase in BPV after bilateral NTS electrolytic lesions. Conversely, bilateral electrolytic lesions at the RVLM do not produce obvious changes in the BP and HR variabilities (Cochrane and Nathan 1989): the SDs of the MAP were 5.7 mmHg (n=8) for the RVLM lesion group and 12.7 mmHg (n = 18) for the control group; and the SDs of the HR were 46 bpm (n=8) for the lesion group, and 72 bpm (n=18) for the control group. Correlatively, Granata et al. (Granata, Ruggiero et al. 1985) found that bilateral electrolytic lesions of the RVLM reduced both BPV and HRV (Table 1 in (Granata, Ruggiero et al. 1985)): For 8 experiments, the SD for MAP was ± 17.53 mmHg before the lesion, and ± 2.82 mmHg after the lesion; the SD for HR was ± 94.75 bpm before the lesion, and ± 46.95 bpm after the lesion. The different effects of bilateral NTS and RVLM lesions on the BPV support our hypothesis that the key central noise source for the large post-SAD BPV is not at the NTS, and point to the RVLM as a likely site.

Furthermore, the transduction curves of the stimulus amplitude vs dmNTS ER, and amplitude vs systolic BP change (Δ SBP) (Fig. 3(b) & (c)) showed that the evoked dmNTS neuronal responses were much more reliable and robust than the evoked Δ SBP responses (see detail in Table 1 in (Tang and Dworkin 2007)) to the single pulse stimuli. One of the likely reasons for the dmNTS ERs to be more reliable and robust than the Δ SBP responses is that, although some fibers of carotid and contralateral aortic inputs reach the ER recording site, the variance contribution that these trigger-random inputs make to the ER is proportionally less than the net depressor response, where the four inputs are equally represented. Another likely reason is that, the activity of the RVLM, which is the basis of the sympathetic tone (Poree and Schramm 1992), has intrinsic variability which is superimposed on the basal BP and sums with an evoked BP response; in contrast, when the ADN stimulus locked response is measured at the dmNTS, the RVLM variability is not superimposed, the variance is less, and response appears to be more stable. Accordingly, the coefficient of variation (CV) for the Δ SBP responses to single pulse ADN stimuli is much larger than that for the dmNTS ERs (Fig. 4). Because the maximum to single pulse BP response is ~ 1 mmHg, when a mean is close to zero, the CV becomes sensitive to the SD; thus, it could be argued that the large CV for the Δ SBP response is an artifact the CV method. However, in (Fig. 1, top, (Dworkin, Dworkin et al. 2000)), we showed that the mean and the SD of Δ SBP responses, calculated as the difference between the mean sBP during 30s of baseline and 30s of stimulation, to repeated ADN stimulation with a Ta-Ta₂O₅ electrode at 100 μ A, 300 μ s, and 20 impulses/s was -10 mmHg, and ± 8.7 mmHg, respectively. The CV would thus be $(8.7 \div 10) \times 100\% = 87\%$, which is quite comparable to the CV = 78% for the Δ SBP responses to single pulse ADN stimuli, and significantly larger than the CV= 19% for the dmNTS ERs.

Finally, we acknowledge that two different strains of rats, i.e., LE and SPD, were used, but have no evidence that the strain differences affect the outcome. LE rats used in the earlier studies, because we believed that ADN dissection was easier in LE rats than in SPD rats, but with more experience, we have found this not true. Because SPD rats are in more common use, we switched to SPD rats for our recent experiments.

Perspective

The most consistent observation following complete SAD or the bilateral NTS lesions is an increased variability of arterial pressure; the source, and the purpose, if any, of the endogenous noise, however, are not known. In (Tang and Dworkin 2007), we demonstrated that following

simultaneously high frequency activation of the ADN A and C-fibers, the size of the A-fiber evoked responses at the dmNTS is enhanced. Physiologically, because A and C-fiber receptors are collocated at the same area, and exposed to the same pressure, we suspect that the fluctuations (mostly the increases) of the BP, generated by the variability in the BP, could potentially simultaneously activate the A- and the C-fiber receptors, and thus, similar to what was shown in (Tang and Dworkin 2007), enhance baroafferent A-fiber gain at the dmNTS. We suspect that blood pressure variability may have an important role in the baroreflex gain adjustment, and BP regulation.

Better understanding BPV is also clinically important: For over decades, extensive clinical effort has been invested in describing cardiovascular variability changes in a range of physiological and pathological conditions, such as coronary artery disease (Casolo, Stroder et al. 1995), and hypertension; but unfortunately, up to now, no test using cardiovascular variability has been found to provide better prognostic and diagnostic information than conventional tests (Malpas 2002). This may be due to lack of appreciation of the sources and fundamental causes of cardiovascular variability.

Reference

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Fig. 1.

The SAD effects on the VLF power of the pre- and post-SAD EsBP amplitude spectra. The upper panel shows, for each individual LE rat, the averaged EsBP amplitude spectra of the 7 pre- (gray) and the 7 post- (black) SAD hours; and the bottom panel shows the corresponding VLF (0.001–0.2 Hz) power (mean $\pm$ SE) of each spectrum. Following the SAD, the VLF power significantly increased for all 5 rats (Rat LE 1: Δ PSD= 43 mmHg/√Hz, df=12, $t=3.026$, $p<0.05$; Rat LE 2, Δ PSD=74.12 mmHg/√Hz, df=12, $t=8.71$, $p<0.001$; Rat LE 3, Δ PSD=45.12 mmHg/√Hz, df=12, $t=3.35$, $p<0.01$; Rat LE 4, Δ PSD=146.39 mmHg/√Hz, df=12, $t=8.171$, $p<0.001$; and Rat LE 5, Δ PSD=76.78 mmHg/√Hz, df=12, $t=12.05$, $p<0.001$). The Δ value was calculated as the differences of the mean VLF powers between the 7 post- and the 7 pre-SAD hours.

Fig. 2.

The SAD effects on the VLF power of the pre- and post-SAD EIBI amplitude spectra. The upper panel shows, for each individual LE rat, the averaged EIBI amplitude spectra of the 7 pre- (gray) and the 7 post- (black) SAD hours; and the bottom panel shows the corresponding VLF (0.001–0.2 Hz) powers (mean $\pm$ SE) of each spectrum in the upper panel. For 4 of the 5 rats, SAD significantly decreased the VLF powers in the EIBI amplitude spectra (Rat LE 1, Δ PSD = -6.43 ms/√Hz, $df=12$, $t=-2.933$, $p<0.05$; Rat LE 2, Δ PSD = -15.06 ms/√Hz, $df=12$, $t=-7.26$, $p<0.001$; Rat LE 3, Δ PSD = -25.94 ms/√Hz, $df=12$, $t=-8.48$, $p<0.001$; and Rat LE 4, Δ PSD = -15.1 ms/√Hz, $df=12$, $t=-2.382$, $p<0.05$). SAD increased VLF powers in the 5th LE rat, but, the increase was not statistically significant (Δ PSD = 8.5 mmHg/√Hz, $df=12$, $t=1.88$, NS).

Fig. 3.

(a) A typical ensemble averaged ER trace from the NTS baroreflex region to single pulse ($300 \mu s$, $400 \mu A$, inter-pulse-interval = $6 \pm 1 s$; $n=589$) ADN stimuli. Because A- and C-fibers have different conduction velocities, activity arriving in the NTS within 4–20 ms is almost certainly from A-fibers, and is defined as A-complex. The later complex in the averaged ER trace is defined as high threshold later (HTL) complex. (b) The average stimulus amplitude – ER magnitude transduction curve, and (c) the average stimulus amplitude – systolic BP change (ΔsBP) transduction curve. To combine rats, for each rat, the stimulus amplitudes were converted to the percent of maximum amplitude used in the pattern, and the average response magnitudes were converted to the percent of maximum response elicited by the pattern. Data were pooled across all 12 SPD rats, and each point is the mean $\pm$ SE.

Fig. 4.

The average coefficient of variations (mean $\pm$ SE) of the NTS ERs and the ΔsBP responses to 5 hour single pulse ADN stimuli (300 μ s, inter-pulse interval = 6 ± 1 s) in 10 SPD rats. CV was calculated as the ratio of the standard deviation to the mean of the 5 h 10 min dmNTS ER and the ΔsBP responses. The ΔsBP (black bar) had a significantly larger CV compared with that of the ERs (gray bar) ($t=4.652$, $df = 18$; $p<0.001$).

Table 1

Effects of SAD on EsBP and EIBI variabilities.

	Pre-SAD	Post-SAD
Mean EsBP (mmHg)	90.3 ± 4.2	121.11 ± 8.43 [*]
Variability (mmHg)	11.3 ± 2.2	23.24 ± 3.84 [*]
Mean EIBI (msec)	134 ± 2.37	130 ± 2.98
Variability (msec)	3.87 ± 0.58	3.25 ± 0.54

The grand averages of the means and the variabilities, represented as standard deviations (SDs), of the EsBP, EIBI measurements of the 5 LE rats, during the 7 pre- and the 7 post-SAD hours. Data are given as mean ± SE.

^{*} reliably different from the pre-SAD measurement ($p < 0.05$).