Published in final edited form as:

Auton Neurosci. 2011 February 24; 160(1-2): 42–52. doi:10.1016/j.autneu.2010.11.010.

Daily voluntary exercise alters the cardiovascular response to hemorrhage in conscious male rats

Joslyn K. Ahlgren and Linda F. Hayward

Department of Physiological Sciences, University of FL, Gainesville, FL 32610

Abstract

The present study tested the hypothesis that voluntary wheel-exercised rats would better tolerate severe hemorrhage (HEM) compared to age matched sedentary (SED) controls. Conscious rats housed with (EX, n=8) or without (SED, n=8) a running wheel for 6 weeks underwent a 30% total blood volume HEM over 15 min. and were euthanized 90 min later and brain tissue processed for Fos-like immunoreactivity (FLI). Both EX and SED groups displayed typical responses to HEM (initial tachycardia followed by decreased HR and MAP) but at the end of HEM, mean arterial pressure (93 ± 6 vs 58 ± 3 mmHg) and heart rate (316 ± 17 vs. 247 ± 22 bpm,) were higher in the EX vs. SED animals and 60 min following the end of HEM HR remained significantly elevated in the EX vs SED animals. The altered HR response to HEM in the EX animals was linked to a significant difference in sympathovagal drive identified by heart rate variability analysis and an augmented baroreflex response to hypotension tested in a separate group of animals (n=4–5/group). In many of the brain regions analyzed EX rats displayed lower levels of FLI compared to SED rats. Significantly lower levels of FLI in the EX vs SED rats were identified in the middle and caudal external lateral subnucleus of the lateral parabrachial nucleus and the dorsal cap of the hypothalamic paraventricular nucleus. These results suggest that enhanced tolerance to HEM following daily exercise may result from EX-induced reduced excitation or exaggerated inhibition in central circuits involved in autonomic control.

1. INTRODUCTION

The hemodynamic response to severe blood loss or hemorrhage (HEM) follows a tri-phasic pattern. At the onset of HEM, the initial loss of blood volume, and associated deviation in arterial pressure (AP), is sensed by the arterial baroreceptors which stimulate a reflex increase in heart rate (HR) and sympathetic drive to the vasculature to maintain a normotensive state. This first “compensatory” phase of HEM is sustained until blood loss reaches ~15–20% of total blood volume (TBV) (Schadt and Ludbrook 1991). When blood loss exceeds this critical value, the body’s initial compensatory response quickly transitions into a “decompensatory” or sympathoinhibitory phase which triggers a sudden decline in both HR and AP (Schadt and Ludbrook 1991; Hasser and Schadt 1992; Evans, Ventura et al. 2001). These changes in autonomic drive trigger a HEM-induced hypotension that is paralleled by increasing plasma levels of renin, vasopressin, and epinephrine (Schadt and Ludbrook 1991). These neurohumoral factors aid in the third phase of HEM, or the “recovery” phase, which follows the offset of blood loss. During the recovery phase,

Corresponding Author: Linda Hayward, Department of Physiological Sciences, Box 100144, HSC, University of Florida, Gainesville, FL 32610, Telephone: (352)-294-4048, Fax: (352)-392-5145, haywardl@ufl.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

sympathetic tone is restored to the vasculature (Scrogin 2003). The longer the body is exposed to sub-standard perfusion pressures during the sympathoinhibitory phase, the chance of recovery is reduced (Kauvar, Lefering et al. 2006). Thus, interventions that can modulate central or peripheral mechanisms activated during HEM to delay the onset and limit the magnitude of the sympathoinhibitory phase and/or facilitate the recovery phase would be beneficial to survival outcomes.

Exercise has been shown to ameliorate a host of cardiovascular pathologies including hypertension (Grassi, Seravalle et al. 1992; Sutoo and Akiyama 2003), heart failure (Coats, Adamopoulos et al. 1992; Bensimhon, Adams et al. 2007), and a number of other diseases in which there is marked autonomic dysregulation (Warburton, Nicol et al. 2006; Souza, Flues et al. 2007; Felber Dietrich, Ackermann-Liebrich et al. 2008). While the peripheral effects of exercise are well documented (Blomqvist 1983; Lund, Yu et al. 2002; Bensimhon, Adams et al. 2007) and constitutes a large portion of exercise research, recent work has begun identifying centrally-mediated adaptations that also contribute to enhanced health outcomes associated with chronic exercise (Zhu, Gao et al. 2004; Nelson, Juraska et al. 2005; Bakos, Hlavacova et al. 2007; Mueller 2007; Kleiber, Zheng et al. 2008). Moreover, current evidence suggests that chronic exercise can affect many areas of the brain involved in autonomic control of AP and HR (Zhu, Gao et al. 2004; Nelson, Juraska et al. 2005; Bakos, Hlavacova et al. 2007; Mueller 2007; Kleiber, Zheng et al. 2008). Some of these same central sites have been identified to be involved in mediating autonomic changes during the different phases of severe HEM (Ward and Darlington 1987; Krukoff, MacTavish et al. 1995; Krukoff, MacTavish et al. 1997; Chan and Sawchenko 1998; Jhamandas, Harris et al. 1998; Buller, Smith et al. 1999; Kakiya, Arima et al. 2000; Pelaez, Schreihof et al. 2002). This raises the possibility that exercise training may impact the body's ability to withstand severe hemorrhage via a change in neuronal responsiveness of specific brain nuclei known to play a role in HEM.

The present study was undertaken to test the hypothesis that wheel-exercised rats would display altered autonomic responses to severe HEM. Based on previous studies showing that exercise training can either slightly enhance [(Brum, Da Silva et al. 2000; Sakuragi 2006; Harthmann 2007)], attenuate (Chen 1996), or not change (Liu 2002) baroreflex sensitivity to hypotension, we hypothesized that chronic exercise would only modestly alter the compensatory response of hemorrhage. Alternatively, it was hypothesized that daily exercise would significantly alter the decompensatory phase of severe HEM based on recent work demonstrating that cardiac afferent activity (Di Carlo 1990), hormonal responses to hypotension (Mueller 2008) and stress (Sasse 2008) are all altered by daily exercise, in addition to central changes involving sympathoexcitatory (Zheng 2005; Mueller 2007) and sympathoinhibitory (De Souza 2001; Mueller and Hasser 2006) circuits in the brain. Accordingly, we also hypothesized that voluntary exercise training would alter the pattern of cellular activation (as marked by c-Fos immunoreactivity) in rostral brainstem regions identified to be involved in autonomic control during HEM including the locus coeruleus (LC; compensation), ventrolateral periaqueductal gray (vlPAG; decompensation) and the lateral parabrachial nucleus (LPBN, recovery).

2. METHODS

2.1 General Preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida. Male Sprague-Dawley rats (n=36; 175–200 g, Harlan Industries, Minneapolis, IN) were randomly placed into one of two groups: exercise (EX) or sedentary (SED). Animals were pair-housed for six weeks in cages that did (EX) or did not (SED) contain a running wheel equipped with an electronic monitor coupled to a

computer for 24 hr activity measurement (Lafayette Instruments, IN). Animals were lightly handled and weighed weekly and were maintained in a 12 hr lights on: 12 hr lights off, temperature controlled environment with food and water ad libitum. Once a week, distance run per cage was recorded and counters were reset. Average daily distance run per rat was then estimated by dividing the weekly total by 14 (7 days $\times$ 2 rats).

Following 6 weeks of daily exercise each animal was surgically instrumented with bilateral femoral arterial catheters (PE-10 connected to PE-50 tubing, Braintree Scientific, Braintree, MA), and in some instances unilateral femoral venous catheters (n=9), under isoflurane anesthesia (4% $\rightarrow$ 2–2.5%). Catheters were subcutaneously routed and exteriorized between the scapulae, filled with heparinized saline (100 IU/ml), and plugged with stainless steel obturators (23-gauge, Braintree Scientific, Braintree, MA). Analgesics (Rimadyl, 0.5 ml/kg; Buprenorphine, 0.01 ml/kg) were administered subcutaneously following catheterization and animals were allowed 48 hours to recover. During recovery, animals were housed singly. The day following catheter placement, animals were brought to the lab to ensure catheter patency and for acclimation to the testing chamber. Animals were weighed, lightly handled, and allowed to sit quietly for 2–3 hrs in the testing chamber (9 $\times$ 9 inch bucket) to be used on the day of the experiment. Animals were then returned to their home cages for another 24 hrs of recovery. EX animals were not allowed access to their running wheels on the day before the experiment to eliminate the confounding short-term effects of exercise. Animals that lost more than 10% of their body weight during the post-surgical recovery-period following surgery were excluded from the study (n=2; 1 EX and 1 SED).

2.2 Experimental Protocol

On the day of the experiment, animals were brought to the lab, weighed, and catheters were connected to additional heparinized saline-filled tubing (10–50 IU/ml; PE-50). The animal was then placed in the testing chamber and the catheters were routed through a hole in the lid of the testing chamber in such a way that the animal could move freely within the testing chamber but not excessively twist the catheters. One of the arterial catheters was connected to a calibrated pressure transducer in-series with an amplifier (Stoelting, Wooddale, IL). Both pulsatile and mean arterial pressures (MAP) were recorded on-line at 100 Hz using a Cambridge Electronics Design computer interface and Spike2 data software. HR was derived on-line from the interval between peak systolic pressure waves in the arterial pressure (AP) trace. The peak detection was performed via automated detection software (Spike2).

AP, MAP, and HR were collected for 30–60 min during which the animal was undisturbed in order to ensure a stable baseline measurement. Animals were then subjected to either a 30% TBV HEM over 15 min (n=8 per group), were allowed to sit quietly over the same time course (non-hemorrhaged controls, n=4–5/group) or underwent baroreflex testing only (n=4–5/group). In the HEM group blood loss was induced by steady withdrawal of blood at a rate of 2% TBV/min from the second arterial catheter into a heparinized syringe (Troy, Heslop et al. 2003; Ahlgren, Porter et al. 2007; Shafford 2007; Porter, Ahlgren et al. 2009). TBV was estimated using a previously reported equation for estimation of rat blood volume: $(0.06 \text{ ml/g}) \times (\text{body weight in g}) + (0.77)$ (Lee and Blaufox 1985). In a separate group of animals, baroreflex responses were elicited by intravenous injections of sodium nitroprusside (0.2 ml; 20 $\mu\text{g/kg}$; i.v.) followed injection of phenylephrine (0.2 ml; 80 $\mu\text{g/kg}$; i.v.) when MAP and HR had returned to baseline. Drug delivery altered MAP at a rate of 1–3 mmHg/s (Liu, Yeo et al. 2000).

Ninety min following the offset of the offset of blood withdrawal (or the equivalent time period for non-hemorrhaged controls) all animals were administered an overdose of sodium pentobarbital (100–150 mg/kg). All animals, except those in the baroreflex group were

transcardially perfused with gravity-driven heparinized saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 24 hours followed by 24-48 hr of immersion in cryoprotectant solution (30% sucrose) prior to cryostat sectioning.

2.3 Fos Immunocytochemistry

Extracted brains were cut into 30-micron coronal sections and processed for Fos-like immunoreactivity (FLI) as previously described (Hayward and Von Reitzenstein 2002). Briefly, free floating sections were washed in sodium phosphate buffered saline (PBS, pH 7.4) followed by a second wash in a 3% goat serum-PBS-triton X100 solution (3% GS-PBS-TX) to prevent nonspecific binding. Sections were then incubated for 24 hr in rabbit anti-c-Fos primary antibody (1:2000 dilution, sc-52r, Santa Cruz Biotechnology) at 4°C. Following another wash in 1% GS-PBS-TX, sections were incubated in goat anti-rabbit biotin (Jackson ImmunoResearch Laboratories, Inc., 111-065-144) for 2 hr and re-washed (1% GS-PBS-TX) prior to being placed in avidin-biotin peroxidase complex (ABC Vectastain Kit, Vector, Burlingame, CA). Sections were put through a final 1% GS-PBS-TX wash followed by visualization of the FLI with a chromagen solution (0.05% diaminobenzidine hydrochloride [DAB], 2.5% ammonium sulfate, 0.033% hydrogen peroxide in 0.05 M Tris-HCl, Vector). Sections were then mounted onto glass slides, air-dried, dehydrated in a graded alcohol and CitriSolv (Fisher Scientific) series, and coverslipped.

2.3 Neuroanatomical Identification and Quantification of FLI

For each animal, two representative sections from each brain area of interest, matched by neuroanatomical landmarks, were identified and imaged by a technician blinded to the experimental treatment group (Axioskop, Carl Zeiss; 5-40X). A stereotaxic rat brain atlas was referenced for identification of all areas imaged and quantified (Paxinos and Watson 2005). Rostral, middle, and caudal aspects of the LPBN were imaged at approximately 9mm, 9.16mm, and 9.3mm caudal to bregma, respectively (Figure 5). Criteria used for selecting specific sections of the LPBN included the shape of the superior cerebellar peduncle, the width of the LPBN from the superior peduncle to the ventral spinocerebellar column, and the width of the ventral spinocerebellar tract. Caudal VLPAG sections were imaged at approximately 8mm caudal to bregma (Figure 6A). Other areas imaged and quantified for FLI included the Kolliker-Fuse nucleus (KF, imaged in the same section and at the level of the rostral LPBN; Figure 5A), LC (rostral: 9.3–9.68mm caudal to bregma; caudal: 9.8mm caudal to bregma; Figure 6B), and the main body of the paraventricular nucleus of the hypothalamus (PVN; 1.8mm caudal to bregma; Figure 6C). Selected brain images were then imported into Adobe Photoshop and standardized masks or outlines of each nucleus/subnucleus were placed over the image (see Fig 4) and the number of FLI neurons present were counted. Following quantification the slide was decoded and assigned a treatment group (Ahlgren, Porter et al. 2007).

2.4 Cardiovascular Measurements

In the HEM treated animals, MAP and HR values averaged over 1 min intervals, beginning 1 min prior to, and ending 30 min following the onset of HEM, as well as at 60 min following the onset of HEM were used for determining group averages.

In the baroreflex tested animals, baroreflex function was determined from the linear relationship between the change in HR for a given change in MAP from baseline (consecutive 3 s averages) during sodium nitroprusside and phenylephrine, separately (Liu, Yeo et al. 2000). The slope of the relationship ($\Delta\text{HR}/\Delta\text{MAP}$) for each drug was then determined using a linear regression model.

Changes in the autonomic control of HR between EX and SED animals were also evaluated using HR variability (HRV) analysis. HRV was analyzed during three different 3 min time windows including: pre-HEM, (within 10 min *prior* to the onset of HEM); peak (the 3 min interval just preceding the drop in AP and HR associated with hemorrhagic sympathoinhibition); and nadir (3 min period when HR was lowest near the end of HEM). Within each time window, the inter-peak-interval for successive systolic pressure waves was determined. The resultant tachogram was then manually checked to insure accurate peak detection (Borges 2008; Sugimua 2008). The tachogram was then analyzed in the frequency domain using HRV software (Biosignal Analysis Group; University of Kuopio, Finland) (Niskanen, Tarvainen et al. 2004). The tachogram was interpolated at 10 Hz, detrended via the smoothness priors formulation ($\alpha=1000$) (Tarvainen, Ranta-Aho et al. 2002; Niskanen, Tarvainen et al. 2004). The autoregressive model was set to the 40th order and the Welch's Periodogram window width was designated to 512 points with an overlap of 256 points in the Hanning window. In the rat, the frequency components of HRV are designated by the following frequency ranges: 0.16-0.6 Hz (Low Frequency, LF), and 0.6-3.0 Hz (High Frequency, HF) (Japundzic, Grichois et al. 1990). Frequency domain characteristics analyzed included the power within the LF and HF components (area under the curve) and the ratio of LF/HF power.

2.5 Statistical Analysis

Within treatment groups, data were averaged and reported as the mean $\pm$ SEM. A one-way ANOVA was used to determine if there were any significant differences in baseline MAP or HR between treatment groups. A two-way ANOVA with repeated measures was used to identify the effects of experimental treatment (HEM) and group (EX vs. SED) on MAP, HR and HRV across time. When indicated, a one-way ANOVA with a Scheffe Post Hoc or multiple paired t-tests with a Bonferonni adjustment for the number of comparisons (time points within groups) was utilized to determine significance. FLI data from all brain regions evaluated were analyzed using a one-way ANOVA to compare differences between HEM experimental groups (EX vs. SED). Prior to analysis all CON values within each subnucleus were combined following determination that there was no statistical difference between SED-CON and EX-CON groups in any brain region, $P>0.5$. Baroreflex data was analyzed using a one-way ANOVA to identify differences between groups (EX vs SED) and reflex responses to changes in MAP. Differences were considered significant when $P<0.05$ for all statistical analyses performed.

3. RESULTS

Figure 1 illustrates the typical weekly average distance run per day from EX animals ($n=13$) and difference in body weight between the SED ($n=12$) and EX animals during the 6 week "training period." As illustrated, the average km/day increased steadily during the first 3 wks of training and then leveled off. During the same time period the average body weight gained per wk increased more in the SED group compared to the EX group (interaction, $P<0.0001$) and by the 3rd wk the average body weight was significantly different between groups ($P<0.01$). Despite significant body weight differences, there was no evidence of a training-induced bradycardia or changes in MAP at rest in the EX animals compared to the SED group (see Table 1).

3.1 Effect of EX on the hemodynamic response to HEM

Figure 2 (left panel, A&B) illustrates the typical cardiovascular response of a SED vs an EX animal to 30% HEM. During the initial period of HEM both animals elicited a robust increase in HR (peak) and MAP was maintained. However after 15–20% TBV withdrawal HR and MAP suddenly began to decline. The peak drop in MAP and HR occurred around

the end of HEM (nadir). Following the offset of HEM both MAP and HR slowly returned to baseline. Overall, SED animals displayed a greater drop in MAP and HR in response to HEM compared to EX animals.

The averaged MAP and HR over time for the EX (n=8) and SED (n=8) animals that underwent 30% HEM is shown on the right of Figure 2(C.). Statistical analysis of the HR response to HEM identified a significant effect of training ($P=0.016$), time ($P<0.001$), and a significant interaction between training and time ($P=0.015$). Subsequent analysis of HR identified a trend for HR in the SED animals to be significantly lower than HR in the EX animals between min 15 and 30, but at no point was the difference between the groups significant when the P value was adjusted for multiple comparisons ($0.002<P<0.05$). In a separate comparison, at 60 min. following the onset of HEM, HR in the EX animals was identified to be significantly greater than HR in the SED animals (368 ± 9 vs 327 ± 5 bpm, respectively, $P=0.02$).

Statistical analysis of MAP during HEM in EX and SED animals also identified a significant effect of training ($P=0.005$), time ($P<0.001$), and a significant interaction between training and time ($P<0.001$). From the beginning of blood withdrawal, both EX and SED groups maintained MAP through min 10 and there was no significant difference between groups. Around min 11 both groups displayed a drop in MAP that continued until the end of HEM. Just prior to the end of blood withdrawal, at min 14 there was a trend for MAP in SED animals to be significantly lower than the EX animal ($P=0.02$) and between min 15 and 20, MAP was significantly lower in the SED vs EX animals ($P<0.002$), after which (min 21 and 25) there was only a trend for MAP to be significantly lower in the SED animals compared to the EX animals be ($0.01<P<0.05$). At 60 min. MAP in the SED group (105 ± 2 mmHg) was not significantly different from MAP in the EX group (110 ± 4 mmHg; $P=0.35$).

3.2 Effect of EX on HRV during HEM

An example of a typical HRV frequency profile at rest (pre-HEM) is shown In Figure 3A. In both EX and SED rats, the area under the curve or power of the HF component was greater than the power of the LF curve at baseline and the LF/HF ratio was less than 1.0 for all animals (Figure 3D, pre). Before HEM there was no significant difference between the EX and SED animals in HF power (Figure 3B; $P=0.53$) or LF/HF ratio (Figure 3C; $P=0.26$).

Figure 2A illustrates the typical position of the 3 min time windows used for HRV analysis during HEM (peak and nadir time points) and Fig. 3B illustrates a typical HRV frequency profile from a SED vs an EX animal at the nadir time point. As illustrated in this example, at the nadir time-point, the HRV frequency profile for the EX and SED groups was noticeably different. Analysis of the effect of EX training on the HF component during HEM (see Fig. 3C) however only identified a significant effect of time ($P<0.008$). Subsequent analysis identified that independent of training (both SED and EX groups combined), HF power at the nadir time point was significantly elevated compared to both the pre and peak time points ($P<0.016$). In contrast, analysis of the LF/HF ratio identified a significant effect of both time ($P<0.0001$) and EX training ($P=0.05$), but the interaction between treatment and time only showed a trend toward significance ($P=0.09$). Subsequent analysis identified that independent of treatment group (SED and EX groups combined) there was a significant increase in the LF/HF ratio at both the peak and nadir time points relative to the pre time point ($P<0.003$).

3.3 Effect of EX on neural response to HEM

To evaluate the impact of exercise on central autonomic control during HEM, three regions of the rostral brainstem and the PVN were evaluated for changes in FLI following HEM in

EX versus SED animals (n=8/group). Two groups of CON animals were added for this analysis, including SED-CON (n=4) and EX-CON (n=5; see Table 1 for resting MAP and HR). No significant difference in FLI between SED-CON and EX-CON ($P>0.5$) as identified following analysis of all brain regions, consequently data from both groups were combined into a single CON group for subsequent analysis.

Figure 4 illustrates the typical FLI pattern observed throughout the LPBN from a SED and an EX animal following HEM. The average FLI within each LPBN subnucleus is shown in Figure 5. In general, HEM induced a greater amount of FLI in the SED-HEM versus both the EX-HEM and CON animals throughout the rostral-caudal extent of the LPBN. For example, FLI in the SED-HEM group was significantly elevated above CON in the external lateral subnucleus and in both the caudal and middle external subnucleus FLI was significantly greater in the SED-HEM compared to the EX-HEM group. FLI was also significantly elevated above CON in both the middle dorsolateral and central lateral subnuclei of the SED-HEM group, but no significant difference from CON was identified for the EX-HEM group in these regions. In fact, in only one region of the LPBN was FLI identified to be significantly elevated above CON in the EX-HEM group. This region was the central lateral subnucleus of the rostral LPBN and a similar increase in FLI above CON was identified in the SED-HEM group.

The average FLI identified in the caudal VLPAG, LC, and the PVN is shown in Figure 6. In all three regions both SED-HEM and EX-HEM animals showed significant increases in FLI compared to CON. In only one region, however, was a significant difference between SED-HEM and EX-HEM animals identified. In the dorsal cap of the PVN, FLI was significantly lower in the EX-HEM animals compared to SED-HEM animals ($P=0.014$).

3.4 Effect of EX on baroreflex function

In a separate group of animals not utilized for FLI analysis or HEM (n=4–5/group; baseline MAP and HR shown in Table 1), baroreflex-induced changes in HR following pharmacologically induced changes in MAP were evaluated. As illustrated in Fig. 7A the increase in HR in response to a comparable decrease in MAP was significantly greater in the EX group compared to the SED group ($P<0.002$). Conversely, the decrease in HR in response to an increase in MAP was not significantly different between groups. Analysis of the slope of the baroreflex response (Fig. 7B) identified that the slope of the reflex change in HR to a decrease in MAP was significantly greater in EX versus SED animals ($p<0.02$) but there was no significant difference in the slopes in response to an increase in AP ($p=0.99$).

4. DISCUSSION

The results of the present study demonstrate for the first time that 6 wks of daily exercise attenuates the cardiovascular decline associated with severe blood loss. The attenuated HEM response was paralleled by signs of putative alterations in autonomic regulation identified by both HRV analysis and changes in central neural activity indicated by differences in FLI in the external lateral LPBN and dorsal cap of the PVN. These results support previous reports that daily exercise can modify neuronal excitability in select central nuclei involved in autonomic regulation (Dufloth 1997; De Souza 2001; Becker 2005; Jackson 2005; Mueller and Hasser 2006; Mueller 2007; Kar 2010) and modulate the cardiovascular response to other types of hypotensive stress (Hung 2008).

4.1 Impact of daily exercise on the compensatory phase of HEM

The initial response to blood loss or compensatory phase of HEM, is primarily mediated by baroreflex adjustments in sympathetic and parasympathetic drive. Previous work in both

animals and humans has provided inconclusive results regarding the impact of daily exercise on baroreflex function. For example, in some instances, exercise training has been reported to modestly enhance baroreflex function (Sakuragi 2006; Harthmann 2007) and baroreceptor afferent sensitivity (Brum, Da Silva et al. 2000), leave reflex (Chen 1996) and receptor function unchanged (Chen 1995), or attenuate reflex function (Negrao 1993; Chen 1995). Some of the differences between studies may be related to training protocols, baroreflex testing methods, gender or anesthesia. However, in general the effects appear to be modest. Accordingly, in the present study 6 wks of daily EX was identified to significantly augment baroreflex-mediated increases in HR in response to nitroprusside-induced hypotension, yet during the initial period of blood withdrawal, the tachycardic response was only slightly elevated in the EX compared to SED animals and the difference was not statistically significant. Furthermore, although HRV analysis identified an elevation of the LF/HF ratio at this peak time point in the EX group, potentially indicative of heightened sympatho-vagal balance (Staass 2003), the change in LF/HF ratio between the EX versus SED groups at the peak time point did not reach significance following adjustment for multiple comparisons. Thus, the impact of EX-induced changes in the compensatory phase during HEM appears to be relatively subtle.

4.2. Impact of daily exercise on the hypotensive phase of HEM

Previous reports from SED rats have identified that the onset of a “decompensatory” response to HEM occurs following approximately 15–20% TBV loss (Porter, Ahlgren et al. 2009). Similarly, in the present study, the onset of the hypotensive response to HEM appeared at the same time point in both EX and SED animals. However, MAP was identified to be significantly higher in the EX versus SED group at the end of HEM. At the same time point HR was noticeably different between groups, however no significant differences in HR between EX and SED groups were identified any specific time point just prior to the end of HEM ($P>0.001$). HRV analysis at the nadir time point identified that both the power in the HF range and the LF/HF ratio were elevated in both groups relative to baseline, indicating that the drop in HR at the nadir was likely to be mediated by changes in both parasympathetic and sympathetic drive respectively. Indeed, previous results from our lab in SED rats has demonstrated that pharmacological blockade of the parasympathetic system prior to the onset of 30% HEM eliminated all HEM-induced decreases in HR and increases in power in the HF range. Furthermore, parasympathetic blockade revealed an underlying tachycardia which, in the absence of competing vagal drive, was associated with either a delayed or attenuated the HEM-induced drop in MAP (Porter, Ahlgren et al. 2009). Interestingly, in the present study, the only difference in HRV between the EX and SED group that was identified to be significant was the LF/HF ratio, suggesting that daily EX may selectively modulate central circuits involved in regulating sympathetic drive during hypotensive HEM. Alternatively, there is some evidence that EX training may also reduce cardiac pacemaker responsiveness to vagal drive (Negrao 1992). Thus, there may also have been parallel changes in vagal efferent drive that were counter-balanced by peripheral changes (Ludbrook and Graham 1984). It should be noted however, that HRV variables, particularly the HF component are also modulated by changes in respiration (Cottin 2004), thus it remains possible that another effect of daily EX is to modulate the respiratory response to HEM (Li 2008; Kung 2010).

4.3. Impact of daily exercise on the recovery response to HEM

The recovery from HEM has been linked to the modulation multiple systems, including elevated levels of circulating vasopressin (Hock 1984), activation of the arterial chemoreflex (Kung 2010), and increases in sympathetic drive (Osei-Owusu and Scrogin 2006). In the present study at the end of HEM, both EX and SED animals showed immediate signs of recovery with spontaneous increases in HR and MAP. Between min 15 and 20 MAP was

identified to be significantly different between EX and SED groups. HR also showed a strong trend to be higher in the EX group during the same time points but did not reach significance. At 60 min however HR was significantly higher in the EX vs SED animals. These observations suggest that greatest effect of daily EX on the response to HEM may occur during the recovery phase of HEM and support recent evidence that EX training for as little as 1 week significantly attenuates the hypotensive effect of endotoxic shock in diabetic rats and improves survival rates (Hung 2008).

Central activation of the parabrachial nucleus (Ward 1989; Blair, Jaworski et al. 2001; Blair and Mickelsen 2006), more specifically, the external subnucleus in the ventrolateral region of the LPBN, has been implicated in both central modulation of baroreflex function (Saleh and Connell 1997; Len and Chan 1999; Len and Chan 2001; Wei 2008) and the recovery from HEM. In the present study, HEM was shown to induce a significant increase in FLI in the external subnucleus of the LPBN in SED animals, supporting previous findings that correlate neural activation within the external LPBN with spontaneous recovery following HEM (Blair, Jaworski et al. 2001; Blair and Mickelsen 2006). Somewhat unexpected however, FLI in the LPBN of the EX group following HEM was not identified to be significantly different from the CON group in most subnuclei of the LPBN examined, with the exception of the central lateral subnucleus of the rostral LPBN. Moreover, in the external lateral subnucleus of the middle and caudal external subnucleus of the LPBN, FLI was significantly lower in EX versus SED rats. To our knowledge this is the first observation that links daily EX to changes in neuronal excitability to the dorsolateral pons.

Previous reports have demonstrated the daily EX modulates both excitatory and inhibitory neurotransmission in other brainstem regions (Mueller and Hasser 2006; Mueller 2007) and can attenuate c-fos expression (Ichiyama 2002). Thus, the overall reduction in FLI we observed in the LPBN of EX animals may reflect a change in excitability or an elevation in tonic inhibition in this region. Indeed, chemical inactivation of the LPBN has been associated with the augmented baroreflex function (Saleh and Connell 1997; Hayward and Felder 1998). It is acknowledged however that additional studies are needed to truly identify the impact of daily EX on neuronal function within the LPBN. Previous studies have also strongly implicated the VLPAG in the hypotensive phase of HEM (Cavun and Millington 2001; Cavun, Goktalay et al. 2004; Dean 2004; Schadt, Shafford et al. 2006). Yet, in the present study FLI in the VLPAG was not significantly different treatment effect within the VLPAG, suggesting that the attenuated HEM response observed in EX was not be due to differences in neuronal activation within this nucleus, but may have been associated with alterations within critical efferent projection sites (Vagg, Bandler et al. 2008). Similarly, both parvocellular and magnocellular subnuclei of the PVN displayed increases FLI following HEM compared to CONs that was similar in both the SED and EX animals. Since both these regions contain vasopressinergic neurons that project to the posterior pituitary (magnocellular region) or within the CNS (parvocellular region) (Swanson and Sawchenko 1983; Badoer 2001) these data support previous work demonstrating that 6–8 weeks of daily EX may not impact vasopressin release in response to hypotension (Mueller 2008). Alternatively, there is evidence that EX training can enhance the excitability of centrally projecting PVN neurons (Jackson 2005). Unfortunately, FLI is not a sensitive marker of changes in neuronal firing characteristics, thus our results simply suggest that neuronal recruitment within the parvocellular region of the PVN in response to HEM was not significantly altered by daily EX.

Interestingly, the number of FLI positive neurons in the dorsal cap of the PVN was identified to be significantly reduced following HEM in the EX vs SED animals. Decreased blood volume, but not euvoletic hypotension, activates these spinally and RVLM-projecting PVN neurons (Badoer, McKinley et al. 1993; Badoer and Merolli 1998). Thus a

decreased activation of the dorsal PVN could translate into a decreased sympatho-excitatory response to HEM in the EX animals, a finding that is paradoxical since EX animals displayed higher mean values for HR and MAP compared to SED animals during recovery from HEM. Similar to our findings in the LPBN and other reports (Mueller and Hasser 2006; Mueller 2007), this reduction in FLI may reflect changes in excitatory or inhibitory tone. Further studies however are needed to fully evaluate the exact mechanisms underlying EX induced differences within the PVN. Nonetheless, our results provide a potential subnuclear region for the focus of these future studies.

4.4 Methodological Considerations

There are two main methodological constraints to consider in the context of the present study. First, in the present study we utilized c-Fos to quantify the effect of daily EX on central neural circuitry involved in the autonomic response to HEM. It is generally accepted that FLI is an accurate indication of neuronal activation in response to sustained stimuli (Chan and Sawchenko 1994), however the exact timing of this change in neural “excitation” cannot be assigned to one specific phase of the HEM. Another limitation of FLI mentioned, is that expression of FLI does not identify whether the excitability of a neuron to a sustained input is altered (Jackson 2005) or whether neuronal inhibition has been altered.

Second, although not evaluated in the current study, the peripheral effects of daily EX cannot be excluded from the possible explanations of the enhanced tolerance to HEM seen in EX rats. First, if the EX animals had developed an increased blood plasma volume, the use of the same equation for TBV estimation based on body weight would be inappropriate. However, other studies using a similar wheel-based model of EX have reported no difference in plasma protein concentration and, presumably, therefore, no functional change in plasma volume between EX and SED rats (Stranahan, Khalil et al. 2006). Additionally, had blood volume been greater in the EX animals in this study, a delay in the onset of the decompensatory stage of HEM might have been expected. Yet, as Figure 2 shows, the decline in MAP and HR in response to HEM occurred at similar time points in both EX and SED animals. Alternatively, there may have been differences in circulating hormone levels, like angiotensin II (Mueller 2008) or differences in cardiac pacemaker sensitivity to vagal input (Negrao 1992; Sant'Ana 2010) or vascular responsiveness which contributed to the attenuated response that contributed to the differences observed.

Conclusions—The results of this study demonstrate that six weeks of voluntary daily EX leads to an enhanced ability to tolerate severe blood loss in conscious male rats. While this study does not pinpoint an exact mechanism or mechanisms of action directly resulting in this protection against hypovolemic shock, but our results support recent results demonstrating that EX training improves cardiovascular function in response to hypotensive challenges (Brum, Da Silva et al. 2000; Hung 2008). The results of this study identify for the first time that EX-induced plasticity may also include the LPBN, particularly the external lateral subnucleus, in addition to previously identified autonomic control regions, including the PVN (Jackson 2005; de Abreu 2009), NTS (Mueller and Hasser 2006; Nelson 2010) and RVLM (Mueller 2007). However, additional studies are needed to further identify any peripheral adaptations that may also be critical for the altered response to HEM seen in trained subjects.

Acknowledgments

Authors thank Ms. Mabelin Castellanos and Ms. Jessica Rivera for their technical help on the project and acknowledge grant support from the American Heart Association to (AHA-Florida Puerto Rico Affiliate, pre-doctoral fellowship) and NIH (HL-76518).

References

- Ahlgren J, Porter K, et al. Hemodynamic responses and c-Fos changes associated with hypotensive hemorrhage: standardizing a protocol for severe hemorrhage in conscious rats. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292(5):R1862–71. [PubMed: 17218446]
- Badoer E. Hypothalamic paraventricular nucleus and cardiovascular regulation. *Clin Exp Pharmacol Physiol*. 2001; 28(1–2):95–9. [PubMed: 11153547]
- Badoer E, McKinley MJ, et al. A comparison of hypotensive and non-hypotensive hemorrhage on Fos expression in spinally projecting neurons of the paraventricular nucleus and rostral ventrolateral medulla. *Brain Res*. 1993; 610(2):216–23. [PubMed: 8319084]
- Badoer E, Merolli J. Neurons in the hypothalamic paraventricular nucleus that project to the rostral ventrolateral medulla are activated by haemorrhage. *Brain Res*. 1998; 791(1–2):317–20. [PubMed: 9593966]
- Bakos J, Hlavacova N, et al. Oxytocin levels in the posterior pituitary and in the heart are modified by voluntary wheel running. *Regul Pept*. 2007; 139(1–3):96–101. [PubMed: 17140677]
- Becker L, Santos RA, Campagnole-Santos MJ. Cardiovascular effects of angiotensin II and angiotensin-(1–7) at the RVLM of trained normotensive rats. *Brain Res*. 2005; 1040:121–128. [PubMed: 15804433]
- Bensimhon DR, Adams GL, et al. Effect of exercise training on ventricular function, dyssynchrony, resting myocardial perfusion, and clinical outcomes in patients with heart failure: a nuclear ancillary study of Heart Failure and A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION); design and rationale. *Am Heart J*. 2007; 154(1):46–53. [PubMed: 17584550]
- Blair ML, Jaworski RL, et al. Parabrachial nucleus modulates cardiovascular responses to blood loss. *American Journal of Physiology (Integrative Comp Physiol)*. 2001; 280:R1141–R1148.
- Blair ML, Mickelsen D. Activation of lateral parabrachial nucleus neurons restores blood pressure and sympathetic vasomotor drive after hypotensive hemorrhage. *Am J Physiol Regul Integr Comp Physiol*. 2006
- Blomqvist CG. Role of exercise training in secondary prevention of ischemic heart disease. *Prev Med*. 1983; 12(1):228–32. [PubMed: 6844311]
- Borges G, Salgado HC, Silva CA, Rossi MA, Prado CM, Fazan R. Changes in hemodynamic and neurohumoral control cause cardiac damage in one-kidney, one-clip hypertensive rats. *Am J Physiol*. 2008; 295:R1904–R1913.
- Brum PC, Da Silva GJ, et al. Exercise training increases baroreceptor gain sensitivity in normal and hypertensive rats. *Hypertension*. 2000; 36(6):1018–22. [PubMed: 11116118]
- Buller KM, Smith DW, et al. NTS catecholamine cell recruitment by hemorrhage and hypoxia. *Neuroendocrinology (NeuroReport)*. 1999; 10:3853–3856.
- Cavun S, Goktalay G, et al. The hypotension evoked by visceral nociception is mediated by delta opioid receptors in the periaqueductal gray. *Brain Research*. 2004; 1019:237–245. [PubMed: 15306258]
- Cavun S, Millington WR. Evidence that hemorrhagic hypotension is mediated by the ventrolateral periaqueductal gray region. *American Journal of Physiology (Integrative Comp Physiol)*. 2001; 281:R747–R752.
- Chan RK, Sawchenko PE. Spatially and temporally differentiated patterns of c-fos expression in brainstem catecholaminergic cell groups induced by cardiovascular challenges in the rat. *J Comp Neurol*. 1994; 348(3):433–60. [PubMed: 7844257]
- Chan RKW, Sawchenko PE. Differential time- and dose-related effects of haemorrhage on tyrosine hydroxylase and neuropeptide Y mRNA expression in medullary catecholamine neurons. *European Journal of Neuroscience*. 1998; 10:3747–3758. [PubMed: 9875353]
- Chen CY, DiCarlo SE. Daily exercise and gender influence arterial baroreflex regulation of heart rate and nerve activity. *Am J Physiol*. 1996; 40:H1840–H1848. [PubMed: 8945899]
- Chen CY, DiCarlo SE, Scislo TJ. Daily spontaneous running attenuated the central gain of the arterial baroreflex. *Am J Physiol*. 1995; 268:H662–H669. [PubMed: 7864192]

- Coats AJ, Adamopoulos S, et al. Controlled trial of physical training in chronic heart failure. Exercise performance, hemodynamics, ventilation, and autonomic function. *Circulation*. 1992; 85(6):2119–31. [PubMed: 1591831]
- Cottin F, Medigue C, Lepretre PM, Papelier Y, Koralsztejn JG, Bilat V. Heart rate variability during exercise performed below and above ventilatory threshold. *Med Sci Sports Exerc*. 2004; 36:594–600. [PubMed: 15064586]
- de Abreu S, Lenhard A, Mehanna A, de Souza HC, Correa FM, Hasser EM, Martins-Pinge MC. Role of paraventricular nucleus in exercise training-induced autonomic modulation. *Auton Neurosci*. 2009; 148:28–35. [PubMed: 19297253]
- De Souza C, Michelini LC, Fior-Chadi DR. Receptor changes in the nucleus tractus solitarius of the rat after exercise training. *Med Sci Sports Exerc*. 2001; 33:1471–1476. [PubMed: 11528334]
- Dean C. Hemorrhagic sympathoinhibition mediated through the periaqueductal gray in the rat. *Neurosci Lett*. 2004; 354(1):79–83. [PubMed: 14698486]
- Di Carlo S, Bishop VS. Exercise training enhances cardiac afferent inhibition of baroreflex function. *Am J Physiol*. 1990; 258:H212–H220. [PubMed: 1967912]
- Dufloth D, Morris M, Michelini LC. Modulation of exercise tachycardia by vasopressin in the nucleus tractus solitarius. *Am J Physiol*. 1997; 273:R1271–R1282. [PubMed: 9362290]
- Evans RG, Ventura S, et al. Neural mechanisms in the cardiovascular responses to acute central hypovolaemia. *Clin Exp Pharmacol Physiol*. 2001; 28(5–6):479–87. [PubMed: 11428384]
- Felber Dietrich D, Ackermann-Liebrich U, et al. Effect of physical activity on heart rate variability in normal weight, overweight and obese subjects: results from the SAPALDIA study. *Eur J Appl Physiol*. 2008; 104(3):557–65. [PubMed: 18597107]
- Grassi G, Seravalle G, et al. Physical exercise in essential hypertension. *Chest*. 1992; 101(5 Suppl): 312S–314S. [PubMed: 1576856]
- Harthmann A, De Angelis K, Costa LP, Senador D, Schann BD, Krieger EM, Irigoyen MC. Exercise training improves arterial baro- and chemoreflex in control and diabetic rats. *Auton Neurosci*. 2007; 133:115–120. [PubMed: 17196889]
- Hasser EM, Schadt JC. Sympathoinhibition and its reversal by naloxone during hemorrhage. *Am J Physiol*. 1992; 262(3 Pt 2):R444–451. [PubMed: 1558215]
- Hayward LF, Felder RB. Lateral parabrachial nucleus modulates baroreflex regulation of sympathetic nerve activity. *American Journal of Physiology (Integrative Comp Physiol)*. 1998; 43:R1274–1282.
- Hayward LF, Von Reitzenstein M. c-Fos expression in the midbrain periaqueductal gray after chemoreceptor and baroreceptor activation. *Am J Physiol Heart Circ Physiol*. 2002; 283(5):H1975–84. [PubMed: 12384476]
- Hock C, Su JY, Lefer AM. Role of AVP in maintenance of circulatory homeostasis during hemorrhagic shock. *Am J Physiol*. 1984; 246:H174–H197. [PubMed: 6696129]
- Hung CH, Chen YW, Shao DZ, Chang CN, Tsai YY, Cheng JT. Exercise pretraining attenuates endotoxin-induced hemodynamic alteration in type 1 diabetic rats. *Appl Physiol Nutr Metab*. 2008; 33:976–983. [PubMed: 18923573]
- Ichiyama R, Gilbert AB, Waldrop TG, Iwamoto GA. Changes in exercise activation of diencephalic and brainstem cardiorespiratory areas after training. *Brain Res*. 2002; 947:225–233. [PubMed: 12176165]
- Jackson K, Silva HM, Zhang W, Michelini LC, Stern JE. Exercise training differentially affects intrinsic excitability of autonomic and neuroendocrine neurons in the hypothalamic paraventricular nucleus. *J Neurophysiol*. 2005; 94:3211–3220. [PubMed: 16049146]
- Japundzic N, Grichois ML, et al. Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J Auton Nerv Syst*. 1990; 30(2):91–100. [PubMed: 1973426]
- Jhamandas JH, Harris KH, et al. Activation of neuropeptide FF neurons in the brainstem nucleus tractus solitarius following cardiovascular challenge and opiate withdrawal. *J Comp Neurol*. 1998; 402(2):210–21. [PubMed: 9845244]
- Kakiya S, Arima H, et al. Effects of acute hypotensive stimuli on arginine vasopressin gene transcription in the rat hypothalamus. *American Journal of Physiology (Endocrinol Metab)*. 2000; 279:E886–E892.

- Kar S, Gao L, Zucker IH. Exercise training normalizes ACE and ACE2 in the brain of rabbits with pacing induced heart failure. *J Appl Physiol.* 2010; 108:923–932. [PubMed: 20093667]
- Kauvar DS, Lefering R, et al. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma.* 2006; 60(6 Suppl):S3–11. [PubMed: 16763478]
- Kleiber AC, Zheng H, et al. Exercise training normalizes enhanced glutamate-mediated sympathetic activation from the PVN in heart failure. *Am J Physiol Regul Integr Comp Physiol.* 2008; 294(6):R1863–72. [PubMed: 18385465]
- Krukoff TL, MacTavish D, et al. Changes in blood volume and pressure induce c-fos expression in brainstem neurons that project to the paraventricular nucleus of the hypothalamus. *Brain Res Mol Brain Res.* 1995; 34(1):99–108. [PubMed: 8750865]
- Krukoff TL, MacTavish D, et al. Activation by hypotension in neurons in the hypothalamic paraventricular nucleus that project to the brainstem. *The Journal of Comparative Neurology.* 1997; 385:285–296. [PubMed: 9268128]
- Kung L, Glasgow J, Ruszaj A, Gray T, Scrogin KE. Serotonin neurons in the caudal raphe nuclei contribute to sympathetic recovery following hypotensive hemorrhage. *Am J Physiol.* 2010; 298:R939–953.
- Lee HB, Blafox MD. Blood volume in the rat. *J Nucl Med.* 1985; 26(1):72–6. [PubMed: 3965655]
- Len WB, Chan JY. Glutamatergic projection to RVLM mediates suppression of reflex bradycardia by parabrachial nucleus. *Am J Physiol.* 1999; 276(5 Pt 2):H1482–92. [PubMed: 10330230]
- Len WB, Chan JY. GABAergic neurotransmission at the nucleus tractus solitarii in the suppression of reflex bradycardia by parabrachial nucleus. *Synapse.* 2001; 42(1):27–39. [PubMed: 11668588]
- Li Y, Ding Y, Agnew C, Schultz HD. Exercise training improves peripheral chemoreflex function in heart failure rabbits. *J Appl Physiol.* 2008; 105:782–790. [PubMed: 18583379]
- Liu JL, Kulakofsky J, Zucker IH. Exercise training enhances baroreflex control of heart rate by a vagal mechanisms in rabbits with heart failure. *J Appl Physiol.* 2002; 92:2403–2408. [PubMed: 12015354]
- Liu J, Yeo HC, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol.* 2000; 89(1):21–8. [PubMed: 10904031]
- Ludbrook J, Graham WF. The role of cardiac receptor and arterial baroreceptor reflexes in control of the circulation during acute change of blood volume in the conscious rabbit. *Circ Res.* 1984; 54(4):424–35. [PubMed: 6713607]
- Lund TD, Yu LC, et al. Repeated massage-like stimulation induces long-term effects on nociception: contribution of oxytocinergic mechanisms. *European Journal of Neuroscience.* 2002; 16:330–338. [PubMed: 12169113]
- Mueller P. Influence of sedentary versus physically active conditions on regulation of plasma renin activity and vasopressin. *Amer J Physiol.* 2008; 295:R727–R732.
- Mueller PJ. Exercise training attenuates increases in lumbar sympathetic nerve activity produced by stimulation of the rostral ventrolateral medulla. *J Appl Physiol.* 2007; 102(2):803–13. [PubMed: 17053106]
- Mueller PJ, Hasser EM. Putative role of the NTS in alterations in neural control of the circulation following exercise training in rats. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290(2):R383–92. [PubMed: 16179489]
- Negrão C, Irigoyen MC, Moreira ED, Brum PC, Freire PM, Krieger EM. Effect of exercise training on RSNA, baroreflex control, and blood pressure responsiveness. *Amer J Physiol.* 1993; 265:R385–R370. [PubMed: 8396354]
- Negrão C, Moreira ED, Santos MC, Farah VM, Krieger EM. Vagal function impairment after exercise training. *J Appl Physiol.* 1992; 72:1749–1753. [PubMed: 1601782]
- Nelson A, Juraska JM, Ragan BG, Iwamoto GA. Effects of exercise training on dendritic morphology in the cardiorespiratory and locomotor centers of the mature rat brain. *J Appl Physiol.* 2010 March 25. e pub.
- Nelson AJ, Juraska JM, et al. Neuroplastic adaptations to exercise: neuronal remodeling in cardiorespiratory and locomotor areas. *J Appl Physiol.* 2005; 99(6):2312–22. [PubMed: 16123206]

- Niskanen JP, Tarvainen MP, et al. Software for advanced HRV analysis. *Comput Methods Programs Biomed.* 2004; 76(1):73–81. [PubMed: 15313543]
- Osei-Owusu P, Scrogin K. Role of the arterial baroreflex in 5-HT_{1A} receptor agonist-mediated sympathoexcitation following hypotensive hemorrhage. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290(5):R1337–44. [PubMed: 16397093]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic; 2005.
- Pelaez NM, Schreihof AM, et al. Decompensated hemorrhage activates serotonergic neurons in the subependymal parapyramidal region of the rat medulla. *Am J Physiol Regul Integr Comp Physiol.* 2002; 283(3):R688–97. [PubMed: 12185004]
- Porter K, Ahlgren J, et al. Modulation of heart rate variability during severe hemorrhage at different rates in conscious rats. *Autonomic Neurosci.* 2009 in press.
- Sakuragi S, Sugiyama Y. Effects of daily walking on subjective symptoms, mood and autonomic nervous function. *J Physiol Anthropol.* 2006; 25:281–189. [PubMed: 16891758]
- Saleh TM, Connell BJ. Modulation of the cardiac baroreflex following reversible blockade of the parabrachial nucleus in the rat. *Brain Res.* 1997; 767(2):201–7. [PubMed: 9367248]
- Sant'Ana J, Pereira MGAG, Dias da Silva VJ, Dambros C, Costa-Neto CM, Souza HCD. Effect of the duration of daily aerobic physical training on cardiac autonomic adaptations. *Auton Neurosci.* 2010 epub.
- Sasse S, Greenwood BN, Masini CV, Nyhuis TJ, Fleshner M, Day HE, Campeau S. Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats. *Stress.* 2008; 11:425–437. [PubMed: 19065456]
- Schadt JC, Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious animals. *Am J Physiol.* 1991; 260(2 Pt 2):H305–H318. [PubMed: 1671735]
- Schadt JC, Shafford HL, et al. Neuronal activity within the ventrolateral periaqueductal gray during simulated hemorrhage in conscious rabbits. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290(3):R715–25. [PubMed: 16195496]
- Scrogin KE. 5-HT_{1A} Receptor Agonist 8-OH-DPAT acts in the Hindbrain to Reverse the Sympatholytic Response to Severe Hemorrhage. *American Journal of Physiology (Regul Integr Comp).* 2003; 284:R782–R791.
- Shafford H, Schadt JC. Visceral pain decreases tolerance to blood loss in conscious female but not male rabbits. *Am J Physiol.* 2007; 293:R721–R728.
- Souza SB, Flues K, et al. Role of exercise training in cardiovascular autonomic dysfunction and mortality in diabetic ovariectomized rats. *Hypertension.* 2007; 50(4):786–91. [PubMed: 17664387]
- Stauss HM. Heart rate variability. *Am J Physiol Regul Integr Comp Physiol.* 2003; 285(5):R927–31. [PubMed: 14557228]
- Stranahan AM, Khalil D, et al. Social isolation delays the positive effects of running on adult neurogenesis. *Nat Neurosci.* 2006; 9(4):526–33. [PubMed: 16531997]
- Sugimura M, Hiose Y, Hanamoto H, Okada K, Boku A, Morimoto Y, Taki K, Niwa H. Influence of acute progressive hypoxia on cardiovascular variability in conscious spontaneously hypertensive rats. *Auton Neurosci.* 2008; 141:94–103. [PubMed: 18599365]
- Sutoo D, Akiyama K. Regulation of brain function by exercise. *Neurobiol Dis.* 2003; 13(1):1–14. [PubMed: 12758062]
- Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci.* 1983; 6:269–324. [PubMed: 6132586]
- Tarvainen MP, Ranta-Aho PO, et al. An advanced detrending method with application to HRV analysis. *IEEE Trans Biomed Eng.* 2002; 49(2):172–5. [PubMed: 12066885]
- Troy BP, Heslop DJ, et al. Haemodynamic response to haemorrhage: distinct contributions of midbrain and forebrain structures. *Autonomic Neuroscience: Basic and Clinical.* 2003; 108:1–11.
- Vagg DJ, Bandler R, et al. Hypovolemic shock: critical involvement of a projection from the ventrolateral periaqueductal gray to the caudal midline medulla. *Neuroscience.* 2008; 152(4):1099–109. [PubMed: 18343586]
- Warburton DE, Nicol CW, et al. Health benefits of physical activity: the evidence. *Cmaj.* 2006; 174(6):801–9. [PubMed: 16534088]

- Ward DG. Neurons in the parabrachial nuclei respond to hemorrhage. *Brain Research*. 1989; 491:80–92. [PubMed: 2765884]
- Ward DG, Darlington DN. Lesions of the caudal periaqueductal gray prevent compensation of arterial pressure during hemorrhage. *Brain Res*. 1987; 407(2):369–75. [PubMed: 3567651]
- Wei S, Lei M, Tong M, Ding J, Han Q, Xiao M. Acute baroreceptor unloading evokes Fos expression in anesthetized rat brain. *Brain Res Bull*. 2008; 76:63–69. [PubMed: 18395612]
- Zheng H, Li Yi-Fan, Cornish KG, Zucker IH, Patel KP. Exercise training improves endogenous nitric oxide mechanisms within the paraventricular nucleus in rats with heart failure. *Am J Physiol*. 2005; 288:H2332–H2341.
- Zhu G, Gao L, et al. ANG II in the paraventricular nucleus potentiates the cardiac sympathetic afferent reflex in rats with heart failure. *J Appl Physiol*. 2004; 97:1746–1754. [PubMed: 15475555]

Figure 1. Evidence of exercise training

(A) Average distance run per day over six weeks of wheel access in pair-housed rats. Data points represent estimated distance run per day for *individual* animals (see methods for further explanation). (B) Average body weights over six weeks for pair-housed SED (n=12) and EX (n=13) rats. * Significant difference between SED and EX rats.

Figure 2. Hemodynamic response to 30% TBV HEM in EX vs. SED conscious rats (A) & (B). Typical arterial pressure (AP) and heart rate (HR) response of one SED and one EX animal in response to hypotensive HEM. Horizontal black bar indicates time of blood withdrawal (2% TBV/min). Vertical dashed boxes indicate approximate 3 min. time windows during which peak and nadir HR values were taken for heart rate variability analysis (HRV) in Fig. 3. (C) Averaged HR and mean AP (MAP; 1 min averages) just prior to (pre), during (min 0–15), and after (min 16–30) HEM. Grey box indicates time of blood withdrawal. * indicates significant training effect ($P < 0.01$). # indicates significant difference between MAP for SED and EX groups at specified time point ($P < 0.002$).

Figure 3. Heart rate variability (HRV) analysis of EX and SED group response to HEM
 (A) Typical power spectrum density (PSD) of heart rate (HR) interval from a conscious EX rat at rest (baseline). Low frequency (LF) and high frequency (HF) peak components are identified. (B). Typical PSD at the offset of HEM (nadir time point) from a SED (black) and EX (shade gray) animal. Note change in scale relative to PSD in A. (C) Average HF power before (pre) and during the peak increase in HR (peak), and at the offset of HEM (nadir) for SED (n=8) and EX (n=8) animals. (D) Average ratio of LF and HF power before HEM (pre) and during the different phases of hemorrhage. * indicates significant training effect (P=0.05). # indicates significant difference between time points indicated (SED and EX groups combined; P<0.0.1);

Figure 4. Representative images of FLI throughout the rostro-caudal extent of the LPBN in both a SED and EX animal following 30% TBV HEM

SCP = superior cerebellar peduncle; CTR = central subnucleus; SUP = superior subnucleus; EXT = external subnucleus.

Figure 5. Average FLI and schematic representations for the rostral (A), middle (B) and caudal (C) LPBN in EX versus SED rats following either 30% TBV HEM (EX-HEM and SED-HEM) or no HEM (CON)

Numbers accompanying the schematics represent distance in millimeters caudal to bregma (adapted from Paxinos and Watson (Paxinos and Watson 2005)). Ctr = central subnucleus; Sup = superior subnucleus; Ext = external subnucleus; KF = Kolliker Fuse nucleus; SCP = superior cerebellar peduncle. * Significant difference from control. # Significant difference between EX and SED-HEM.

Figure 6. Average FLI and schematic representations for the caudal VLPAG (A), LC (B), and hypothalamic PVN (C) in EX versus SED rats following either 30% TBV HEM (EX-HEM and SED-HEM) or no HEM (CON)

Numbers accompanying the schematics represent distance in millimeters caudal to bregma (adapted from Paxinos and Watson (Paxinos and Watson 2005)). Magno = magnocellular; Parvo = parvocellular; Dorsal = dorsal cap. * Significant difference from CON.

Figure 7. Exercise-induced changes in baroreflex control of HR

(A.) Averaged regression lines of tachycardic and bradycardic responses to changes in mean arterial pressure (MAP) induced by i.v. infusion of sodium nitroprusside and phenylephrine (respectively) in SED (n=5) and EX (n=4) rats. (B) Averaged slope of the regression line of the tachycardic and bradycardic responses. * indicates significant difference from SED in response to nitroprusside.

Table 1

Baseline cardiovascular parameters of each animal group

TREATMENT	TRAINING	MAP	HR	n/group
HEM	SED	122±4	327±5	8
	EX	120±3	339±9	8
CON	SED	127±5	352±3	4
	EX	132±5	355±11	5
BAROREFLEX	SED	118±3	353±10	5
	EX	124±3	352±13	4