Physical exercise decreases neuronal activity in the posterior hypothalamic area of spontaneously hypertensive rats

Joseph A. Beatty, Jeffery M. Kramer\textsuperscript{1}, Edward D. Plowey and Tony G. Waldrop\textsuperscript{2}

Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 524 Burrill Hall, 407 South Goodwin Avenue, Urbana, Illinois 61801-3704

Running Head: Exercise and Neuronal Activity

Address for Correspondence:
Joseph A. Beatty
Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 524 Burrill Hall, 407 South Goodwin Avenue, Urbana, Illinois 61801-3704
Voice: 217-244-6618
Fax: 217-333-1133
E-mail: jbeatty@life.uiuc.edu

Present Addresses:
\textsuperscript{1} Iowa Cardiovascular Center and Department of Psychology, University of Iowa, 11 Seashore Hall, E., Iowa City, Iowa 52242-1407

\textsuperscript{2} Department of Cell and Molecular Physiology, University of North Carolina, 5200 Medical Biomolecular Research Building, 103 Mason Farm Road, Chapel Hill, North Carolina 27599-7545
Abstract

Recently, physical exercise has been shown to significantly alter neurochemistry, neuronal function, and increases neurogenesis in discrete brain regions. Although we have documented that physical exercise leads to molecular changes in the posterior hypothalamic area (PHA), the impact on neuronal activity is unknown. The purpose of the present study was to determine if neuronal activity in the PHA is altered by physical exercise. Spontaneously hypertensive rats (SHR) were allowed free access to running wheels for a period of 10 weeks (exercised group) or no wheel access at all (non-exercised group). Single unit extracellular recordings were made in anesthetized in vivo whole animal preparations or in vitro brain slice preparations. The spontaneous firing rates of PHA neurons in exercised SHR in vivo were significantly lower (8.5 ± 1.6 Hz, n=31 neurons) compared to that of non-exercised SHR in vivo (13.7 ± 1.8 Hz, n=38 neurons; p < 0.05). In addition, PHA neurons that possessed a cardiac related rhythm in exercised SHR fired significantly lower (6.0 ± 1.8 Hz, n=11 neurons) compared to non-exercised SHR (12.1 ± 2.4 Hz, n=18 neurons; p < 0.05). Similarly, the spontaneous in vitro firing rates of PHA neurons from exercised SHR were significantly lower (3.5 ± 0.3 Hz, n=67 neurons) compared to those of non-exercised SHR (5.6 ± 0.5 Hz, n=58 neurons; p < 0.001). Both the in vivo and in vitro findings support the hypothesis that physical exercise can lower spontaneous activity of neurons in a cardiovascular regulatory region of the brain. Thus, physical exercise may alter central neural control of cardiovascular function by inducing lasting changes in neuronal activity.

Keywords: Hypertension, Electrophysiology, Plasticity, Cardiovascular, and Autonomic
Introduction

The posterior hypothalamic area (PHA) is a periventricular region located in the caudal-most region of the diencephalon, bordered by the third ventricle, fornix, mammillothalamic tract, and rostrally extends to the level of the dorsomedial hypothalamic area (40). Electrical stimulation or chemical disinhibition, via microinjection of GABA receptor antagonists, within the PHA increases arterial blood pressure, heart rate, and sympathetic nerve activity in rats (4, 14, 33, 50), cats (16), rabbits (19), and dogs (38). Axons of PHA neurons innervate a variety of caudal brain regions involved in cardiorespiratory regulation such as periaqueductal gray, nucleus tractus solitarius, and ventrolateral medulla (3, 23, 31, 41). Afferents terminating within the PHA include locomotor and autonomic inputs from amygdala, basal ganglia, and various cortical areas (1, 53). The PHA is situated to serve as an integrative brain region involved in controlling both autonomic and locomotor functions (3, 4, 14, 23, 31, 33, 41, 50).

Previous studies indicate that the PHA contributes to maintaining the elevated arterial blood pressure routinely observed in spontaneously hypertensive rats (SHR) (12, 18, 24, 28, 37, 42, 45, 51, 52). In particular, we have shown a deficit in glutamic acid decarboxylase mRNA within the PHA that is related to high blood pressure in the SHR animals (24, 44). Neural recordings indicate an increase in spontaneous discharge rates of PHA neurons in SHR compared to normotensive control rats using both in vivo and in vitro preparations. These data support the idea of a greater tonic cardiovascular drive from the PHA (45). Consistent with these findings, metabolic enzyme activities such as hexokinase are elevated, indirectly suggesting an alteration in cellular function (28).

Physical exercise can lower resting arterial blood pressure in hypertensive humans and rats (9, 39, 43, 47). It has been hypothesized that alterations in brain function may partly underlie
the beneficial cardiovascular effects of exercise (27). Physical exercise can upregulate glutamic acid decarboxylase gene transcription in the PHA and thereby partially restore the aforementioned GABAergic deficit in SHR (32). Furthermore, this GABAergic upregulation with physical exercise has been shown to functionally impact blood pressure regulation in SHR (26). To date, however, there is no direct evidence that physical exercise alters the activity of neurons in the PHA. Therefore, the purpose of the present study was to examine if physical exercise impacts the activity of PHA neurons in SHR.
Materials and Methods

Animals

All procedures outlined in this study conform to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Illinois Laboratory Animal Care Advisory Committee.

Male SHR (Harlan; Indianapolis, IN) were received between the ages of 4 to 5 weeks. Upon arrival, animals were paired by body weight and randomly divided into exercise and non-exercise (control) groups. Rats in both groups were initially housed individually for approximately one week in order to obtain baseline resting weights and systolic blood pressures. Following baseline measurements, non-exercised control rats were left in individual home cages for the duration of the training period. SHR assigned to the exercise group were placed individually in home cages similar to controls but with running wheels (diameter 0.98 m) attached. Rats were allowed free access to the wheels (24 hours/day, 7 days/week) for a total of 10 weeks. Magnetic switches (Allied Electronics Corp.) attached to data acquisition equipment (Mini Mitter Company, Inc.) monitored revolutions of the wheels and, therefore, running distances for each animal over each 24-hour period. At the conclusion of the 10-week exercise period, and prior to experimentation, the wheels were locked for 24 hours prior to experimentation to avoid immediate postexercise effects on blood pressure (29). Final resting weights and blood pressures were then taken at which point animals were prepped for either *in vivo* or *in vitro* electrophysiological experiments. In a subset of animals, hearts were harvested, rinsed clean of blood with saline, and weighed. Heart weight-to-body weight ratio (g/kg) was calculated for a peripheral training indicator (11). All rats were kept on a 12-hour light/dark cycle and given free access to standard rat chow and water for the duration of the study.
Resting Systolic Blood Pressure Measurements

Resting systolic blood pressures were measured for exercised and non-exercised groups via tail-cuff plethysmography (IITC) at the beginning and following the 10-week exercise period. Animals were placed in a small transparent tube located in a heated enclosure (27-30 °C), and allowed to acclimate to the chamber for approximately 10 minutes before recording resting systolic blood pressure. Animals were visually monitored during the measurement period to ensure motion artifact was not introduced into the readings. This indirect method has been previously shown to provide reliable and valid systolic blood pressure values (10). A recent study has evaluated the tail cuff method versus intraarterial pressure and found that tail cuff produces a higher systolic pressure versus intraarterial however, the within observer differences for the tail cuff method was very low (25). Three clear and repeatable measurements were used to obtain average resting systolic blood pressure values.

In Vivo Extracellular Recordings

Following the 10-week exercise period, the animals were anesthetized by intraperitoneal injection of a α-chloralose (65 mg/kg) and urethane (800 mg/kg) mixture. An external jugular vein was cannulated (PE-50) for administration of supplemental anesthetic, which was given upon evidence of a withdrawal reflex to noxious paw pinch.

A common carotid artery was cannulated (PE-50) to measure mean arterial pressure with a pressure transducer (Gould) and heart rate was determined from the pulsatile arterial pressure signal with a biotachometer (Gould). A tracheotomy was performed and the animal was allowed to spontaneously breathe room air supplemented with 100% O₂ throughout the experiment. Body temperature was monitored with a rectal temperature probe and maintained between 36.5 and 38.0 °C with a heating pad and radiant heat lamp.
Animals were fixed in a horizontal skull position with a stereotaxic apparatus (Kopf), such that the cranial landmarks, bregma and lambda were within 300 µm of each other in the horizontal plane. A parietal craniotomy was performed bilaterally to allow stereotaxic placement of a microelectrode into the PHA according to the coordinates of Paxinos and Watson (40).

Animals were allowed to stabilize following the surgery for approximately 30 minutes. Sharpened tungsten recording electrodes (3-5 MΩ, Frederick Haer & Co.) were vertically positioned into the PHA and advanced with a motorized micromanipulator (Frederick Haer & Co.) until the action potentials of an individual, spontaneously active neuron were isolated. Single units were isolated in the same manner that were done in previous studies (5, 6, 13, 35, 45). Raw activity was amplified (100,000X; Grass, P511), filtered (300-3000 Hz bandpass) and visualized on an oscilloscope. A window discriminator (Frederick Haer & Co.) was used to isolate single neuronal activity. The waveform of the neuron was monitored on an oscilloscope to ensure a constant waveform indicative of a single unit. A rate monitor (Frederick Haer & Co.) was used to quantify the firing rate of single neurons. Spontaneous neuronal activity and cardiovascular variables were recorded for a minimum of 5 minutes. The neuronal responses to increased mean arterial blood pressure was then tested and recorded. Elevations in mean arterial blood pressure were elicited by intravenous injection of 3-5 µg of the vasoconstrictor phenylephrine (Sigma). Up to six tracts (three on each side), were made bilaterally in the PHA of each rat separated by no less than 500 µm. At the last recording site in the brain, an electrolytic lesion was created by passing direct current (300 µA, 10 sec; D.C. Constant Current Lesion Maker Grass, D.C. LM5A) through the electrode in order to mark the recording site.

At the end of the experiment, animals were administered a lethal intravenous injection of sodium pentobarbital (325 mg/ml). Brains were removed and placed in 10% formalin solution.
for 7 days. Following fixation, the brains were dehydrated in 20% sucrose and the area of the brain containing the hypothalamus was blocked and sliced (50 μm thickness) in coronal sections on a freezing microtome (American Optical Instruments). Alternate sections were stained with cresyl violet. Recording sites were reconstructed from electrolytic lesions and locations were verified by comparison to a rat brain stereotaxic atlas (40).

**In Vitro Extracellular Recordings**

Following the 10-week exercise period, the rats were rapidly decapitated and their brains carefully removed. Brains were bathed with chilled artificial cerebral spinal fluid (ACSF: pH = 7.3 - 7.4, 295 - 305 mOsm) containing Earle's balanced salts solution (Sigma; 116 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂·2H₂O, 0.81 mM MgSO₄, 0.88 mM NaH₂PO₄, 5.55 mM Glucose), glucose (4.45 mM), sodium bicarbonate (26 mM) and HEPES (15 mM). Brains were cut into ~500 μm coronal slices on a tissue chopper. Slices containing PHA were transferred to an inclined plane interface style recording chamber warmed (36-37 °C), humidified, and perfused with oxygenated (95% O₂ / 5% CO₂) ACSF. Also, a humidified gas mixture (95% O₂ / 5% CO₂) was blown continuously over the surface of the slices.

The tissue was allowed to acclimatize for at least one hour after placement in the recording chamber before data collection. Recording pipettes were constructed by pulling capillary glass (World Precision Instruments, Inc., 1.0 mm inner diameter) with a two-stage pipette puller and filled with 4 M NaCl (resistance 3-5 MΩ). The recording electrodes were coupled via a high impedance probe (Grass, HIP5) to a differential amplifier (Grass, P511). A micromanipulator and hydraulic microdrive (Narishige, MO-8) were used to place the recording electrode into the PHA with the aid of a dissecting scope (Nikon) using the wall of the third ventricle, the mammilothalamic tracts and fornix as neuroanatomical landmarks. The electrode
was advanced from the surface of the slice to a maximal depth of 250 \( \mu \)m, until the action potentials of a spontaneously active neuron could be isolated. Raw activity was amplified (100,000X; Grass P511), filtered (300-3000 Hz bandpass) and visualized on an oscilloscope. Neuronal activity was also sent to an audio monitor and storage oscilloscope to help isolate single units. A window discriminator and rate monitor (Frederick Haer & Co.) were used to quantify the firing rate of single neurons. The spontaneous neuronal activity was recorded for a minimum of 5 minutes. Recording sites were documented on pictures adapted from Paxinos and Watson by observing the placement of the electrode tip in the brain slice through a microscope (Nikon) (40).

**Data Analysis**

Data were recorded on a chart recorder (Gould), computer acquisition equipment (ADI Power Lab Chart 3.4.2 with a 200 Hz sampling rate) and videotape (Vetter Instruments) for storage and off-line analyses. Systolic blood pressure and body weight values of exercised and non-exercised groups were calculated and statistically analyzed with a two-way ANOVA (one factor repetition). Mean neuronal firing rates were determined from the average firing rate throughout the 5-minute collection time (both *in vivo* and *in vitro* experiments). Differences between mean neuronal firing rates, heart weight-to-body weight ratio, and mean arterial blood pressure responses to phenylephrine in exercised versus non-exercised groups were tested with Student’s \( t \)-tests. Computer averaging (Run Technologies DataPac III 1.61) coupled with double blind analysis were used in the *in vivo* preparation to determine if neurons displayed cardiac-related rhythms (45). Data presented as mean \( \pm \) SEM. In all cases, statistical significance was reached at \( p < 0.05 \).
Results

A total of 41 SHR were used in this study. Twenty-one SHR were allowed access to running wheels for 10 weeks while 20 served as non-exercised controls. Of the 21 exercised SHR, 8 were used in the in vivo experiments while the remaining 13 exercised SHR were used in the in vitro experiment. Of the 20 non-exercised SHR, 8 were used in the in vivo experiments and the remaining 12 were used for in vitro experiments.

Prior to allowing rats access to running wheels, there was no difference in body weights between the exercise (96 ± 4 g, n=21) and non-exercise groups (94 ± 5 g, n=20; Table 1). Following the 10-week wheel access, body weights still did not differ between groups (321 ± 6 g, n=21 for exercised and 332 ± 5 g, n=20 for non-exercised; Table 1). The exercised rats gradually increased running distances for approximately 5-6 weeks at which time they reached peak levels (~7000-9000 m/day; Fig. 1). Rats did show a peripheral cardiovascular response to wheel running. In a subsection of the groups, the effects of physical exercise on heart weights were determined. Total heart weights from exercised rats (1.42 ± 0.04 g, n=15) were significantly greater than non-exercised rats (1.35 ± 0.03 g, n=15, p < 0.02). Similarly, the exercised group had a significantly greater heart weight to body weight ratios (4.5 ± 0.1 g/kg) when compared to the non-exercised group (3.9 ± 0.1 g/kg, p < 0.005; Table 1).

Prior to providing wheel access to the rats, resting systolic blood pressure did not differ between the exercise group (141 ± 3 mmHg, n=21) and non-exercise group (137 ± 3 mmHg, n=20; Table 1). Following the 10-week exercise period, the exercised group had a significantly lower final resting systolic blood pressure (197 ± 5 mmHg, n=21) compared to the non-exercised group (209 ± 4 mmHg, n=20, p < 0.02; Table 1). Both groups demonstrated significant
elevations of arterial blood pressure from 4-5 weeks of age until final measurements at 14-15
weeks, which is typical for this model of hypertension (36, 37, 52).

In vivo extracellular recordings were obtained from a total of 31 PHA neurons in
exercised SHR (n=8 animals) and 38 neurons in non-exercised SHR (n=8 animals). The mean
spontaneous firing rate of PHA neurons in exercised SHR was 8.5 ± 1.6 Hz, which was
significantly lower than that of neurons in non-exercised SHR (13.7 ± 1.8 Hz, p < 0.05; Fig 2A).
As a control, the spontaneous firing rates of 16 thalamic neurons in exercised SHR and 10
thalamic neurons in non-exercised SHR were recorded along with 8 dorsal medial hypothalamic
neurons in exercised SHR and 9 dorsal medial neurons in non-exercised SHR. The mean
spontaneous firing rate of the thalamic neurons showed no significant difference between the two
groups (12.4 ± 2.6 Hz for the exercise group versus 6.8 ± 2.3 Hz for the non-exercise group). In
addition, the mean spontaneous firing rate of the dorsal medial hypothalamic neurons also
showed no significant difference between the two groups (12.2 ± 2.9 Hz for the exercise group
versus 9.5 ± 2.9 Hz for the non-exercise group).

Neurons were then tested for responsiveness to increases in mean arterial blood pressure.
In non-exercised SHR injection of phenylephrine produced a significant rise in mean arterial
blood pressure (185 ± 5 mmHg versus 223 ± 3 mmHg, p < 0.001). A change in spontaneous
firing rate did not accompany the increase in mean arterial blood pressure (13.9 ± 2.3 Hz versus
15.0 ± 2.5 Hz, p > 0.05). Similar results were obtained in the exercised SHR. The mean arterial
blood pressure was significantly increased by phenylephrine injections (179 ± 6 mmHg versus
223 ± 5 mmHg, p < 0.001) whereas spontaneous firing rates did not change (9.1 ± 2.0 Hz versus
9.3 ± 2.2 Hz, p > 0.05).
Computer averaging demonstrated that 35% (11/31) of PHA neurons recorded in exercised SHR displayed a discharge rhythm temporally related to the cardiac cycle, while 47% (18/38) of PHA neurons recorded in non-exercised SHR displayed a similar characteristic. The mean firing rate of the cardiac cycle related neurons was significantly lower in exercised SHR (6.0 ± 1.8 Hz) in contrast to similar neurons in non-exercised SHR (12.1 ± 2.4 Hz, \( p < 0.05 \); Fig. 2C). Histological reconstruction of recording sites demonstrated a similar distribution between exercised and non-exercised groups (Fig. 3B).

*In vitro* extracellular recordings were obtained from a total of 67 PHA neurons from exercised SHR (n=13) and 58 PHA neurons from non-exercised SHR (n=12). The mean spontaneous firing rate of neurons in exercised SHR was 3.5 ± 0.3 Hz, which was significantly lower than that of PHA neurons in non-exercised SHR (5.6 ± 0.5 Hz, \( p < 0.001 \); Fig. 4). The recording sites, again, showed similar distributions within the PHA between exercised and non-exercised SHR (Fig. 5).
Discussion

The key finding of these experiments is that physical exercise can decrease neuronal activity in a brain region involved in cardiovascular regulation. Using both *in vivo* and *in vitro* extracellular single unit recordings we have demonstrated that physical exercise decreases spontaneous activity of PHA neurons in SHR. The decreases in activity were parallel with a significant decrease in resting systolic blood pressure. The PHA projects to both brainstem presympathetic and spinal preganglionic neurons (3). We believe the reduced PHA activity results in decreased sympathetic tone via these efferent connections and thus decreasing resting systolic blood pressure. Importantly, this decrease in neuronal activity occurs in a model of spontaneous hypertension and is associated with a reduced resting systolic blood pressure. Specifically, our findings demonstrate that physical exercise can reduce the discharge rate of neurons located in the PHA of the SHR, and this change is present in both *in vivo* and *in vitro* preparations.

The reduced PHA neuronal discharge in response to physical exercise was observed *in vivo* demonstrating that alterations are present in a fully intact organism. The decreases in neuronal activity were specific to the PHA, because control neurons located outside the PHA did not show similar decreases in activity in physically exercised animals. Interestingly, PHA neurons that showed a discharge rate temporally related to the cardiac cycle also showed significant decreases in discharge rate. The latter findings do not provide causative evidence of a relationship between the PHA discharge rate and resting systolic blood pressure, but do show that a specific population of neurons within the PHA that are linked to the cardiovascular system are altered with physical exercise.
Similar findings of physical exercise induced alterations in neuronal activity were observed in an in vitro brain slice preparation. The persistence of the exercised induced decrease in PHA activity suggests that the alteration observed in the whole animal may be due to local diencephalic changes. At this point, we cannot conclude that alterations in activity in intrinsic properties of PHA neurons themselves. Rather, our findings suggest that at least some alterations occur in relatively isolated sections of the PHA. Further electrophysiological experiments are required to more fully understand alterations in synaptic function and/or membrane properties associated with the decreased activity.

To our knowledge these findings are the first to demonstrate alterations in neuronal activity in a cardiovascular related brain region with physical exercise. Alterations in neuronal functioning following physical exercise are just now starting to be elucidated. Some laboratories have begun to demonstrate alterations in neurotransmitter and/or neurotransmitter metabolite levels, neurotransmitter binding, and up regulation or down regulation of genes involved in several neurotransmitter systems following physical exercise (2, 12, 15, 17, 20, 21, 30, 34). Other studies have demonstrated angiogenesis in the cerebellum and motor cortex of normotensive rats following running wheel activity and suggest that it occurs in parallel with increased neural activity (8, 46). van Praag and colleagues have found that physical exercise results in a significant increase in the number of newly born neurons in the dentate gyrus of adult normotensive rats (49). In addition, they have also demonstrated similar neurogenesis and an increase in LTP following physical exercise in the dentate gyrus of mice (48). Another study has documented electrophysiological alterations in basic motor neuron biophysical properties following physical exercise (7). Our findings are similar to the above-mentioned studies suggesting that physical exercise is capable of eliciting alterations in brain function.
Similar to previous research in SHR, physical exercise reduced resting systolic blood pressure in the exercised group compared to the non-exercised group (11, 22, 26, 39). In the current study, SHR were given wheel access at 4-6 weeks of age, a time when resting systolic blood pressure is not significantly elevated above normotensive control animals (36, 37, 52). During the period of wheel access (from 5-15 weeks of age) rats still developed a significant rise in resting systolic blood pressure. Since, wheel access was introduced before animals were hypertensive, the current experimental paradigm is investigating an interaction between wheel exposure and the development of hypertension in this animal. Although wheel running had a statistically significant reduction on the development of hypertension, rats still showed a high level of resting systolic blood pressure. It would be interesting to test other models of hypertension to see if exercise such as wheel running, can impact resting systolic blood pressure to a greater extent. It would also be beneficial to provide wheel access to hypertensive rats at a developmental state at which hypertension has already been developed, in order to test the impact of physical exercise apart from critical windows of hypertension development. Lastly, the duration of the positive effects of physical exercise on resting systolic blood pressure remains unexplored. Therefore, detraining studies may provide important information on the mechanisms of adaptation as well as provide information on how to sustain the positive effects.

We have shown that physical exercise can significantly reduce PHA neuronal activity in SHR. The PHA is a heterogeneous brain nucleus (1). However, we believe that the neurons we have recorded from are the glutamatergic projection neurons of the PHA. We came to this conclusion based on the firing patterns of the neurons and the fact that the majority of PHA neurons are glutamatergic with a smaller population of GABA positive neurons (1). We have previously shown a marked increase in glutamic acid decarboxylase gene transcription in the
PHA following physical exercise (32). Our working hypothesis is that physical exercise increases GABAergic input on to PHA projection neurons thus lowering their activity. This change in PHA activity reduces sympathetic input onto brainstem presympathetic neurons and thus spinal preganglionic neurons resulting in reduced sympathetic tone and resting blood pressure. The underlying cellular mechanisms of the reduced firing rates remain unknown, although possibilities range from a direct alteration in intrinsic neuronal properties to changes in afferent synaptic activity. Future studies will be required to more conclusively determine the cellular mechanisms altered by physical exercise. Important issues to address include, 1) determining the cellular mechanisms by which physical exercise can induce alterations in central neural regulation of cardiovascular function, and 2) elucidate systemic mechanisms that induce changes in neurons involved with autonomic regulation. In so doing, we will be able to use physical exercise and stimuli evoked during activity as a model for determining strategies by which to improve cardiovascular health.
Acknowledgements

The authors are grateful to C. L. Cox for critical review of the manuscript.

This work was supported by grants from the National Institutes of Health (NIH HL06296 and NIH T32GM07143) and the American Heart Association (AHA Grant-in-Aid).
References


26. **Kramer JM, Beatty JA, Little HR, Plowey ED and Waldrop TG.** Chronic exercise alters caudal hypothalamic regulation of the cardiovascular system in hypertensive rats.


Figure legends

Figure 1. Average daily running distances for SHR allowed access to running wheels for 10 weeks. Values are presented as means ± SEM.

Figure 2. (A) Mean firing rates for all recorded neurons from exercised (black bar) and non-exercised (white bar) SHR in the in vivo preparation. PHA=posterior hypothalamic area, DMH=dorsomedial hypothalamus, THAL=thalamus. (B) An example of the computer averaging of arterial pressure and unit firing rate of a neuron displaying a cardiac related rhythm. (C) Mean firing rates for PHA neurons that display a cardiac related rhythm. The mean firing rates of PHA neurons from exercised SHR were significantly lower than the mean firing rate of PHA neurons from the non-exercised SHR in both A and C. Number of neurons recorded is shown on each bar. Values are presented as means ± SEM. * p < 0.05.

Figure 3. (A) Photomicrograph of representative brain slice illustrating recording electrode placement. (B) Histological location of recording sites in exercised (■) and non-exercised SHR (●) for the in vivo preparation. The numbers to the right of the diencephalic diagrams are relative to bregma. 3V=third ventricle, mt=mammillothalamic tracts, f=fornix, DM=dorsomedial hypothalamus, PMV=ventral premammillary nuclei, VMH=ventromedial hypothalamic nucleus, Arc=arcuate hypothalamic nucleus, LH=lateral hypothalamus, STh=subthalamic nucleus and PHA=posterior hypothalamic area. Hypothalamic figures were adapted from Paxinos and Watson (40). (C) An example of an action potential from the storage oscilloscope taken from a non-exercised SHR. The location of this cell is marked with a ★ in panel B.

Figure 4. Mean firing rates for PHA neurons from exercised (black bar) and non-exercised (white bar) SHR in the in vitro preparation. The mean firing rates of PHA neurons from exercised SHR were significantly lower than the mean firing rate of PHA neurons from the non-
exercised SHR. Number of neurons recorded is shown on each bar. Values are presented as means ± SEM. * p < 0.001.

**Figure 5.** Histological location of recording sites in exercised (■) and non-exercised SHR (●) for the *in vitro* preparation. 3V=third ventricle, mt=mammilothalamic tracts, f=fornix, DM=dorsomedial hypothalamus, PMV=ventral premammillary nuclei, VMH=ventromedial hypothalamic nucleus, Arc=arcuate hypothalamic nucleus, LH=lateral hypothalamus, and PHA=posterior hypothalamic area. The numbers to the right of the slices are relative to bregma. Hypothalamic figures were adapted from Paxinos and Watson (40).
Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise</th>
<th>After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exercised SHR</td>
<td>Non-exercised SHR</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>96 ± 4</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Heart weight to body weight ratio (g/kg)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>141 ± 3</td>
<td>137 ± 3</td>
</tr>
</tbody>
</table>

Values are presented as means ± SEM. * Indicates significant difference between exercised and non-exercised SHR after exercise; * \( p < 0.02 \) and ** \( p < 0.005 \).
Figure 1
In vivo

A

all neurons

<table>
<thead>
<tr>
<th></th>
<th>Non-exercised</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td>38</td>
<td>31</td>
</tr>
<tr>
<td>DMH</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>THAL</td>
<td>10</td>
<td>16</td>
</tr>
</tbody>
</table>

Mean Firing Rate (Hz)

B

Arterial Pressure

Unit Firing Rate

C

cardiac rhythm-related PHA neurons

<table>
<thead>
<tr>
<th></th>
<th>Non-exercised</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

Mean Firing Rate (Hz)

Figure 2
Figure 3
In vitro

![Bar graph showing mean firing rate (Hz) for non-exercised and exercised conditions.](Image)

- Non-exercised: 58 Hz
- Exercised: 67 Hz

Figure 4
Figure 5