INVITED REVIEW:

(In)activity-Related Neuroplasticity in Brainstem Control of Sympathetic Outflow:
Unraveling Underlying Molecular, Cellular and Anatomical Mechanisms

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Running Head: (In)activity-related neuroplasticity in RVLM

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ABSTRACT

More people die as a result of physical inactivity than any other preventable risk factor including smoking, high cholesterol, and obesity. Cardiovascular disease, the number one cause of death in the United States, tops the list of inactivity-related diseases. Nevertheless, the vast majority of Americans continue to make lifestyle choices that are creating a rapidly growing burden of epidemic size and impact on the US healthcare system. It is imperative that we improve our understanding of the mechanisms by which physical inactivity increases the incidence of cardiovascular disease, and how exercise can prevent or rescue the inactivity phenotype.

The current review summarizes research on changes in the brain that contribute to inactivity-related cardiovascular disease. Specifically, we focus on changes in the rostral ventrolateral medulla (RVLM), a critical brain region for basal and reflex control of sympathetic activity. The RVLM is implicated in elevated sympathetic outflow associated with several cardiovascular diseases including hypertension and heart failure. We hypothesize that changes in the RVLM contribute to chronic cardiovascular disease related to physical inactivity. Data obtained from our translational rodent models of chronic, voluntary exercise and inactivity suggest that functional, anatomical, and molecular neuroplasticity enhances glutamatergic neurotransmission in the RVLM of sedentary animals. Collectively, the evidence presented here suggests that changes in the RVLM resulting from sedentary conditions are deleterious and contribute to cardiovascular diseases that have an increased prevalence in sedentary individuals. The mechanisms by which these changes occur over time and their impact are important areas for future study.
Physical inactivity, defined as a lack of regular exercise, is a well-recognized and independent risk factor for cardiovascular disease and represents an enormous socioeconomic burden on the US healthcare system (5; 20; 32). Current estimates of the total cost of cardiovascular diseases, which can be linked directly or indirectly to physical inactivity, are a staggering $300 billion (32). Although initial observations linking inactivity to increased risk of cardiovascular disease occurred over 60 years ago (67), it is only fairly recently that many cardiovascular diseases such as hypertension, diabetes, and heart failure have been associated with heightened sympathetic nerve activity (SNA) (24; 25; 34; 38; 50). This link has tremendous clinical importance because nearly half of all patients with hypertension do not have their blood pressure under control. As hypertension accounts for over 90% of the 85 million people in the US with cardiovascular disease (32), novel and innovative therapies are needed to control blood pressure in those individuals for whom conventional therapy is ineffective (104). Understanding the mechanisms by which physical inactivity leads to poor cardiovascular health, and how physical activity reduces or reverses cardiovascular disease, are likely to provide new targets for blood pressure-lowering therapies in those unwilling or unable to exercise (70). In addition, new exercise treatments are likely to be individualized for patients who respond to exercise as a therapeutic strategy.

Exercise can decrease or reverse elevations in SNA in disease states such as hypertension and heart failure (26; 109). However, more than a decade ago, Booth and colleagues proposed that inactivity independently affects an individual or experimental
group (6; 7). In fact, this group of investigators was one of the first to designate physically active individuals as the healthy “control” group and sedentary subjects as the treatment or “diseased” group (7). This designation is warranted when one considers that humans evolved as a very active species in order to obtain the food necessary for survival. Only recently in human history has technology eliminated the evolutionary “need” for physical activity and allow survival to a reproductive age. Thus, it may come as no surprise that sedentary individuals are prone to increased resting sympathetic nerve activity (SNA), enhanced baroreflex-mediated sympathoexcitation, and have other markers for sympathetic overactivity and cardiovascular disease (6; 8; 21; 23; 36; 48; 61; 76; 90; 109).

Physical inactivity may contribute to the development of hypertension and other cardiovascular diseases by increasing SNA (35). For example, endurance exercise in hypertensive patients can independently lower indicators of whole body sympathetic activity, systemic vascular resistance, and blood pressure (14; 26). In addition, elevated SNA is a recognized component of the metabolic syndrome. Obese patients with metabolic syndrome are often inactive and have increased norepinephrine spillover and muscle SNA (92; 101). Even in the absence of cardiovascular disease, sympathetic overactivity can have deleterious effects on the cardiovascular system both directly and indirectly (28; 29; 56).

Brain-related cardiovascular disease

Research related to physical inactivity and its effect on SNA has more recently focused on the role of the brain and specifically on certain central nervous system
(CNS) nuclei involved in generation and/or modulation of sympathetic output. Indeed, a number of studies in diseased (43; 46; 84; 85; 89; 103; 109) and non-diseased (58; 64; 65; 73; 74; 78; 80) subjects have implicated brain regions such as the paraventricular nucleus (PVN), the nucleus tractus solitarius (NTS), and the rostral ventrolateral medulla (RVLM) in the development, progression and maintenance of cardiovascular disease. However, few studies have focused on CNS mechanisms by which physical inactivity alone contributes to cardiovascular disease as a major independent risk factor. Our recent work emphasizes this point, reporting that otherwise “normal” sedentary rats show enhanced resting and hypotension-induced increases in splanchnic SNA compared to physically active rats (65; 68; 70). Nonetheless, there is a paucity of data regarding the central mechanisms by which physical activity or inactivity alone affects the sympathetic nervous system independent of other factors and explains our use of the term “(In)activity” in the title of this review article. This review focuses on recent advances in the study of physical inactivity and its impact on control of sympathetic output by the RVLM. The reader is referred to other excellent reviews regarding the effects of exercise on the NTS and PVN in healthy and diseased states (62; 63; 85; 111).

The rostral ventrolateral medulla (RVLM) and sympathetic nervous system regulation

The RVLM is one of the most important brain regions for control of basal and reflex sympathetic activity via bulbospinal neurons that innervate sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord (18; 19; 37-
40). The activity of RVLM neurons is regulated by both excitatory and inhibitory neurotransmitters, primarily glutamate and γ-amino butyric acid (GABA), respectively (18; 40). The RVLM receives a wide variety of inputs, including cardiovascular and exercise-related inputs from both central and peripheral sources (Figure 1) (18; 19; 40). The influence of repeated bouts of exercise (i.e., regular physical activity) and the recurring, often cyclical activation of these exercise-related inputs on the RVLM are poorly understood. Similarly, the effects of an absence of these repetitive inputs (i.e., a sedentary lifestyle) remain unclear.

Independent effect of physical inactivity on sympathoexcitation elicited from the RVLM

The RVLM is not just a key CNS region involved in the normal regulation of sympathetic outflow (97). This region has also been implicated in conditions of augmented SNA associated with cardiovascular diseases (38; 41). Microinjection of the excitatory amino acid glutamate or other glutamate analogs into the RVLM results in large increases in SNA and blood pressure (1; 33; 53; 75; 88; 91). Increased glutamatergic and angiotensinergic signaling in the RVLM is thought to be responsible for elevated blood pressure and/or sympathetic outflow in a number of animal models of cardiovascular disease (103; 107). Importantly, unlike hypertension, the influence of sedentary conditions may not be due to an increase in excitation of RVLM neurons by both glutamate and angiotensin. For example, cardiovascular responses to glutamate microinjections into the RVLM are enhanced in sedentary animals (58; 65; 69) whereas responses to angiotensin II seem to be blunted (4). Since the angiotensin II data were
obtained from sedentary versus swim-trained rats (4), further research will be necessary to determine whether angiotensin II signaling is influenced by other paradigms of physical activity and inactivity. Nonetheless, the RVLM is anatomically and functionally positioned to modulate signals that drive sympathetic outflow in both physiological and pathophysiological states.

As alluded to above, the RVLM plays a major role in regulation of sympathetic output to most vascular beds (38). Although it is still controversial whether specific RVLM neurons differentially regulate specific vascular beds, there is fairly strong evidence, especially in the cat, suggesting that RVLM neurons are topographically segregated according to specific functions (59; 75; 87). In addition, it has been proposed that sympathetic activation in cardiovascular disease states has a specific “signature”; meaning that the classic view of an all-or-none activation of the sympathetic nervous system is likely outdated especially in chronic cardiovascular disease (81; 83). We too have proposed specific enhancement of sympathetic outflows to some but not all vascular beds in sedentary versus active rats (74). Specifically, when comparing sedentary rats to those that exercised voluntarily on running wheels, sympathoexcitatory responses in splanchnic but not lumbar sympathetic nerve activity were enhanced in the sedentary group (65; 74). Previous work from our laboratory supports the hypothesis that this differential effect on specific sympathetic nerves may depend on changes in specific populations of RVLM neurons that regulate different sympathetic nerves. For instance, we have demonstrated activation of different sympathetic nerves when performing glutamate microinjections in subregions of the RVLM (75). In addition, different sympathetic nerves (e.g. renal, lumbar, adrenal)
respond with different degrees of excitation to a variety of perturbations that are known
to increase the activity of RVLM neurons (i.e. hypotension, blockade of GABA
receptors, etc.) (75). Although these multi-nerve recording studies were performed in
non-exercised animals, it is unclear whether the differential sensitivity of sympathetic
nerves is due to the influence of sedentary conditions, or if and how they change in
response to physically active conditions. In light of our recent anatomical observations
(64), it seems reasonable to suggest that differences in the dendritic structure of RVLM
neurons between active and inactive animals occur in some but not all spinally-
projecting RVLM neurons (see “Evidence for structural neuroplasticity” below). Future
experiments will be important for determining how different sympathetic nerves and the
RVLM neurons that regulate their activity change in response to sedentary versus active
conditions. Such studies will provide a better understanding of the overall impact of a
sedentary lifestyle on the development and maintenance of cardiovascular disease.

Focus on splanchnic sympathetic nerve activity.

The RVLM contains neurons that influence sympathetic outflow to the kidney and
skeletal muscles (17; 22; 52; 59). However, more recent investigations on elevated SNA
in cardiovascular disease states including different forms of hypertension have focused
on sympathetic outflow to the splanchnic circulation (43; 45; 82). For example, in
animal models of obesity and angiotensin II/salt-induced hypertension, elevations in
resting splanchnic SNA are associated with elevated blood pressure (43; 45). Using the
obese Zucker rat model, Huber and colleagues provide strong evidence that the
increased splanchnic SNA is maintained by neurotransmission in the RVLM (43). Our
previous study is the only one to our knowledge that has compared splanchnic SNA
after activation of the RVLM in rats that were either inactive or performed voluntary
wheel running for 8-10 weeks (65). This work demonstrated that sedentary conditions
not only resulted in exaggerated splanchnic sympathetic responses to glutamate
microinjected into the RVLM, but also raised resting splanchnic SNA and blood
pressure (65). Collectively, these data support the contention that sympathetic
overactivity to the splanchnic circulation could be an important contributor to
cardiovascular disease in sedentary individuals.

Mechanisms of inactivity-related neuroplasticity in the RVLM

Evidence for structural neuroplasticity. We have suggested that changes at the
level of the RVLM contribute to elevated sympathetic outflow following sedentary
conditions. One mechanism through which this effect could be achieved is via structural
adaptations of RVLM neurons in response to chronic inactivity. Exercise or inactivity-
related neuroplasticity occurs in higher brain centers involved in learning and memory
(27; 106) and in other brainstem regions involved in cardiorespiratory function (78-80).
Because dendritic outgrowth is associated with synapse formation and maturation (30),
increased dendritic branching of cardiovascular RVLM neurons could be an anatomical
basis for enhanced resting splanchnic sympathetic tone and resting blood pressure as
well as enhanced sympathoexcitation in response to activation of RVLM neurons (64;
65). This hypothesis is based on the assumption that sedentary conditions result in an
increase in the number of excitatory synapses, which would increase the influence of
excitatory inputs to the RVLM.
A majority of bulbospinal presympathetic neurons in the RVLM are part of the C1 cell group and by definition express catecholamine synthesizing enzymes, including tyrosine hydroxylase and phenylethanolamine N-methyltransferase (97). The importance of C1 cells in the regulation of resting SNA and various sympathetic reflexes is widely accepted since deactivation of C1 neurons via allatostatin and allatostatin receptor gene transfer in the RVLM decreases resting SNA and blood pressure (57). These neurons are also critical for the full expression of sympathoexcitatory reflexes as demonstrated by several studies using DBH-saporin injected into the spinal cord or into the RVLM (54; 55; 93; 96). We recently examined the structure of bulbospinal C1 neurons in the RVLM that project specifically to the lower thoracic portion of the intermediolateral cell column, where splanchnic sympathetic outflow originates (100). In sedentary versus active (chronic wheel running) rats, we showed that there was a relative increase in a number of parameters related to dendritic complexity (64). These differences included an increase in the length of dendritic branches and an increase in the number of dendritic branch points in sedentary compared to active rats (64). Even more intriguing to us were the differential changes in dendritic branching over the rostrocaudal axis of the RVLM (Figure 2). In sedentary animals, bulbospinal C1 neurons showed more dendritic branching in rostral compared to caudal regions of the RVLM (pale grey bars, Figure 2). In contrast, dendritic branching was consistent along the rostrocaudal axis in physically active animals (dark grey bars, Figure 2). We are unaware of any other experiments that have demonstrated this type of pattern, or change in pattern of dendritic branching in the RVLM over its rostrocaudal extent. Nonetheless, these intriguing findings provide direct anatomical evidence that RVLM
neurons undergo structural neuroplasticity in response to sedentary conditions, active conditions or both (64). Furthermore, the neuroplasticity that occurs within the RVLM of the inactive versus active rats appears to be topographic, with increases in dendritic branching in bulbospinal C1 neurons occurring preferentially in rostral regions of the RVLM.

A major unresolved and fundamental question is which activity state, if not both, affects the structure of RVLM neurons. In other words, does inactivity promote dendritic branching in RVLM neurons? Does chronic exercise reduce branching? Or do both conditions induce opposite changes in branching? Are these phenomena discrete, or are they part of a continuum where the magnitude of change is related to the quality, intermittency and/or duration of neuronal activation via physical activity or inactivity? Cross-sectional studies from Iwamoto and colleagues have suggested that structural neuroplasticity in cardiorespiratory regions of the rat brain is reversible (78) and may depend on age, activity level or both (80). Nonetheless, answers to these questions remain to be firmly established. In our opinion, longitudinal studies examining the time course of development and maintenance of structural changes in RVLM neurons are the next logical step in furthering our understanding of the influence of sedentary versus physically active conditions on brain-related cardiovascular diseases.

Evidence of functional neuroplasticity. As in many other brain regions, the primary excitatory and inhibitory neurotransmitters in the RVLM are glutamate and γ-aminobutyric acid (GABA), respectively (86; 97). The RVLM receives prominent tonic inhibitory input. The primary source of this inhibitory input is the caudal ventrolateral medulla (CVLM) (38; 94; 97). The CVLM receives tonic excitation from the NTS as part
of the baroreceptor reflex (94) and also from other non-barosensitive pathways (15; 18; 95). The actions of GABA in the RVLM are mediated via GABA_A and GABA_B receptors (3; 42); however, activation of GABA_A receptors provides the predominant inhibition in the RVLM (19; 42; 60). GABAergic transmission within the RVLM is important in certain models of hypertension and obesity in which there is a reduction in GABAergic input from CVLM (43; 99).

Work from our laboratory has investigated how GABA modulates glutamatergic excitation of the RVLM in sedentary rats by testing responses to unilateral microinjections of glutamate in the presence or absence of the GABA_A antagonist, bicuculline in anesthetized sedentary or physically active rats (74) (Figure 3). Interestingly, antagonism of GABA receptors produced enhanced pressor responses only in sedentary animals. This finding led us to several important conclusions: 1) pressor responses to glutamate are enhanced in sedentary animals in the absence of GABAergic modulation; 2) GABAergic modulation of glutamatergic excitation in the RVLM is pronounced in sedentary rats but is either masked or non-existent in physically active rats; and 3) despite the lack of GABAergic modulation in active rats, pressor responses were significantly smaller compared to sedentary animals (74). These data show that tonic GABA input is important for suppressing RVLM activity in order to maintain basal blood pressure and SNA in sedentary animals. In addition, changes in the processing of glutamatergic signals could result in enhanced responses even when tonic GABAergic input is removed. Thus, being sedentary affects tonic inhibitory and excitatory signaling mechanisms at the cellular level.
Evidence for molecular neuroplasticity. Molecular alterations in glutamatergic signaling and transmission pathways are likely to underpin the structural and functional alterations in bulbospinal RVLM neurons. Enhanced glutamatergic input augments SNA in other experimental animal models that demonstrate augmented sympathoexcitation (10; 95; 105; 107). Glutamate acts through NMDA (NR1, NR2A, NR2B, NR2C and NR2D subunits) and AMPA (GLUR1, GLUR2 and GLUR3 subunits) receptor subtypes. The inhibitory neurotransmitter GABA and its receptors (GABA_A and GABA_B) also play an important role in controlling the activity of RVLM neurons. Changes in the expression of any of these receptors in a variety of brain regions could mediate enhanced sympathoexcitation in conditions like heart failure and hypertension. For example, studies on the PVN have reported that increased expression of the NR1 subunit of the NMDA glutamate receptor or decreased expression of the GABA_A receptor contributes to elevated SNA in heart failure (47; 51). Similarly, up-regulation of glutamate receptors in the RVLM have been implicated in elevated sympathetic outflow in chronic heart failure (107) and exercise training has been shown to reverse augmented glutamatergic excitation in the RVLM of rats with heart failure (108). These findings suggest that enhanced glutamatergic neurotransmission in the RVLM due to physical inactivity could also result from neuroplasticity in glutamate and GABA receptors in the RVLM. However, heterogeneity in the population of RVLM neurons makes it difficult to investigate receptor neuroplasticity specifically in the spinally projecting neurons and assess their receptor expression. To overcome this limitation we recently combined laser capture microdissection with tract tracing to specifically label and isolate spinally projecting neurons in the RVLM (102) (Figure 4A and 4B). Interestingly, the expression
of the GLUR3 subunit of the AMPA receptor negatively correlated with total running
distance (Figure 4C) and positively correlated with the expression of NR2C subunit of
the NMDA receptor (data not shown). How these changes translate into enhanced
sympathoexcitation upon glutamatergic activation of the RVLM in sedentary compared
to active animals still remains unclear. Nonetheless, these findings suggest that
physical activity modulates excitatory neurotransmission in the RVLM at the receptor
level. In addition to receptor neuroplasticity, an increase in the synthesis and release of
 glutamate could also contribute to enhanced sympathoexcitation in sedentary animals.
These possibilities require further investigation and could involve western blotting for
receptor protein expression levels and electron microscopy that will help us correlate
changes in receptor expression with gross structural alterations that occur in our model.

Potential Mechanisms of RVLM neuroplasticity. Although evidence presented in
this review strongly suggests important inactivity-related neuroplasticity in the RVLM,
the mechanisms by which these changes occur are a critical challenge for future
research if new therapies are to be developed. Numerous studies now point to
alterations in peripheral afferent pathways in sympathoexcitatory disease states
including hypertension and heart failure (31; 110). Many of these pathways, such as
the arterial baroreflex, carotid chemoreflex, and muscle mechano- and metaboreflex are
dysfunctional in disease states and the dysfunction can be improved or corrected by
exercise training (110). Despite the importance of these studies, reports of alterations
in peripheral afferent pathways in response to sedentary conditions alone appear to be
limited to changes in arterial baroreflex function. While unloading of arterial
baroreceptors produces augmented sympathoexcitation in sedentary rabbits and rats
(23; 65; 76), sympathoinhibitory responses to increases in arterial pressure (i.e., loading of arterial baroreceptors) are equivalent in sedentary versus physically active animals (23; 76). Furthermore, because the published data are somewhat contradictory, it is unclear whether arterial baroreceptor and vagal afferent input are altered under sedentary versus physically active conditions (9; 12; 49; 77; 98). Recent deafferentation studies in chronically exercised animals indicate an important role of peripheral chemoreceptor input in contributing to activity-related neuroplasticity in the PVN (11; 16). How these findings in the PVN relate to the neuroplasticity observed in the RVLM is unknown but alterations in PVN function could have downstream effects on neurotransmission in the RVLM. Similarly, the influence of direct and indirect input from upstream nuclei such as the NTS, PVN, and CVLM likely play an important role since there is mounting evidence that some of these areas are influenced by levels of activity and inactivity even in otherwise “normal” animals (66; 71; 71-73). Finally, neuromodulatory or neurotrophic effects from substances including brain-derived neurotrophic factor (BDNF), neuropeptide Y (NPY), and endocannabinoids among others could act in the RVLM and upstream centers to modulate afferent reflexes (2; 13; 44).

**Perspectives**

Physical inactivity independently contributes to the development of cardiovascular disease. The evidence reviewed here establishes a likely role for changes occurring at the level of the RVLM in the increase in cardiovascular disorders that are becoming epidemic in the US. The lack of normal healthy and cyclical
exercise-related input is associated with deleterious changes in the structure and function of RVLM neurons. These changes can result in heightened sympathetic outflow, end organ damage and lead to cardiovascular disease (29). The data presented here provide novel CNS targets for therapies that may slow or prevent the development and/or progression of cardiovascular disease.

Half of patients prescribed three or more antihypertensive medications are still hypertensive (32). Therefore, therapies that target the splanchnic vascular bed or blood pressure regulating neurons in the brainstem may be effective for treating essential hypertension and cardiovascular disease in patients who are unwilling or unable to exercise (83). A better understanding of the mechanisms that cause sympathetic overactivity will allow us to reach the goal of reducing or eliminating the costly consequences of cardiovascular disease.
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Figure 1. Effect of chronic exercise or chronic inactivity on the rostral ventrolateral medulla (RVLM), sympathetic nerve activity (SNA), and cardiovascular (CV) disease. Schematic depicting the RVLM as a key brain region in the integration of exercise-related input including peripheral and central afferents. Chronic exercise (left side, black arrows) versus chronic inactivity (right side, grey arrows) are likely to produce CNS alterations that have opposite effects on blood pressure and CV disease. In the absence of exercise-related inputs (chronic inactivity) changes at the level of the RVLM likely strengthen mechanisms of SNA generation. Although it is possible downstream changes may contribute at the level of spinal cord and ganglia, current evidence indicates that significant neuroplasticity at the level of the RVLM that could explain exaggerated sympathetic nerve responses observed in both sedentary animals and humans. Figure modified from Mueller, 2010 (70).

Figure 2. Inactivity-related structural neuroplasticity in bulbospinal catecholaminergic (C1) RVLM neurons. Upper panels: Examples of reconstructed bulbospinal catecholaminergic RVLM neurons from a physically active rat (chronic wheel running, left panel) and a sedentary rat (right panel). Lower panel: The number of dendritic branch points is plotted as a function of caudal and rostral distance from the caudal pole of the facial nucleus (FN) in sedentary (pale grey bars/arrow) compared to physically active rats (dark grey bars/black arrow). The 500 micrometers immediately caudal to FN0 contains the area traditionally defined as the RVLM. However, our laboratory and other have identified bulbospinal C1 neurons rostral to FN0. The graph
shows that structural neuroplasticity in C1 neurons correlates with the position within the C1 column with rostral but not caudal C1 neurons exhibiting increased branching only in sedentary animals. Figure modified from Mischel et al., 2014 (64).

**Figure 3. Inactivity-related functional neuroplasticity in RVLM regulation of blood pressure.** Mean arterial pressure (MAP) responses to direct activation of RVLM with microinjections of the excitatory amino acid glutamate (Glu, 10 mM, 30 nl) before and after microinjections of the GABA<sub>A</sub> receptor antagonist, bicuculline (2 mM, 60 nl) in sedentary (light grey bars) and physically active (dark grey bars) rats. Direct activation of excitatory amino acid receptors in the RVLM produced increases in MAP that were similar in both groups (Glu Ctrl). However, following blockade of tonic GABA<sub>A</sub> receptor mediated inhibition, the response to glutamate was enhanced in sedentary rats (*, p<0.05), but was unchanged in physically active rats. Pressor responses were also significantly greater in sedentary compared to physically active rats (#, p<0.05). Enhanced responses in sedentary animals returned to control levels consistent with the time course of GABA<sub>A</sub> receptor blockade by bicuculline. These data suggest that endogenous GABA buffers glutamatergic excitation of the RVLM in sedentary but not active rats. Figure modified from Mueller and Mischel, 2012 (74).

**Figure 4. Inactivity-related molecular neuroplasticity in bulbospinal RVLM neurons.** (A) Schematic depiction of the retrograde labeling technique used to identify spinally projecting neurons in the RVLM by microinjections of Fluorogold (FG, 5%) into the intermediolateral (IML) cell column at the T9-T10 level of the spinal cord. FG-
labeled neurons were identified within 500 μm caudal and 150 μm rostral to the facial nucleus (FN). (B) Photomicrograph of FG-labeled neurons that were obtained by laser capture microdissection (LCM) and prepared for polymerase chain reaction (PCR). (C) Relationship between total wheel running distance in physically active rats and fold change in gene expression for the GLUR3 subunit of the AMPA receptor (sedentary rats not shown). Active rats were provided a running wheel in their cage and ran different total distances of their own volition. GLUR3 expression was inversely correlated with running distance in physically active animals (p=0.01). These data suggest that expression of genes encoding glutamate receptor subunits is sensitive to the cumulative level of physical activity in rats. Figure modified from Subramanian et al., 2014 (102).
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Chronic Exercise

Upstream Changes
Higher Centers:
Central Command
Cortex, Hypothalamus, etc.

Brainstem Pathways

Changes at the RVLM:
- SNA Responses to Glutamate
- Inhibitory Modulation by GABA
- Dendritic Branching (C1 neurons)
- Activity-related gene expression

Downstream changes

Spinal Cord
Ganglia

Blood Pressure
CV Disease

Blood Pressure
CV Disease

Chronic Inactivity

Brainstem Pathways

Changes at the RVLM:
- SNA Responses to Glutamate
- Inhibitory Modulation by GABA
- Dendritic Branching (C1 neurons)
- Activity-related gene expression

Downstream changes

Spinal Cord
Ganglia

Blood Pressure
CV Disease

Blood Pressure
CV Disease

Afferent Inputs:
Baroreceptors
Metaboreceptors
Mechanoreceptors

Absence of Exercise-Related Afferent Inputs

Afferent Inputs:
Baroreceptors
Metaboreceptors
Mechanoreceptors
**ΔMAP (mmHg)**

- **Active**
- **Inactive**

<table>
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<tr>
<th>Glu</th>
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*minutes post Bic*

* (*p < 0.05) and # (*p < 0.01) denote significant differences.
**A**

![Diagram](image1.png)

**B**

![Image 2](image2.png)

**C**

Gene Expression (Fold Change) vs. Total Running Distance (km)

- **GLUR3**
- $R^2 = 0.982$
- $p = 0.01$

**Legend:**
- LCM + PCR
- RVLM