(In)activity-related neuroplasticity in brainstem control of sympathetic outflow: unraveling underlying molecular, cellular, and anatomical mechanisms

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Mischel NA, Subramanian M, Dombrowski MD, Llewellyn-Smith IJ, Mueller PJ. (In)activity-related neuroplasticity in brainstem control of sympathetic outflow: unraveling underlying molecular, cellular, and anatomical mechanisms. Am J Physiol Heart Circ Physiol 309: H235–H243, 2015. First published May 8, 2015; doi:10.1152/ajpheart.00929.2014.—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 United States 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.

neurons; sympathetic nervous system; arterial pressure; cardiovascular disease; inactivity

Physical Inactivity Contributes to Cardiovascular Disease Burden in Part Through Increased Sympathetic Nervous System Activity

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 United States 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 United States with cardiovascular disease (32), novel and innovative therapies are needed to control blood pressure in those individuals for whom conventional therapy is ineffective (105). Understanding the mechanisms by which physical inactivity leads to poor cardiovascular health

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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, 110). However, more than a decade ago, Booth and colleagues (6, 7) proposed that inactivity independently affects an individual or experimental group. 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 to obtain the food necessary for survival. Only recently in human history has technology eliminated the evolutionary “need” for physical activity and allowed survival to a reproductive age. Thus it may come as no surprise that sedentary individuals are prone to increased resting SNA, enhanced baroreflex-mediated sympathoexcitation, and have other markers for sympathetic overactivity and cardiovascular disease (6, 8, 21, 23, 36, 48, 61, 76, 91, 110).

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 (93, 102). 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, 90, 104, 110) and nondiseased (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 with 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, 112).

**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-aminobutyric 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 (Fig. 1) (18, 19, 40, 87). 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 output (98). 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, 89, 92). 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 (104, 108). Important, 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 the 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, 88). 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 sedentary
rats are compared to those that exercised voluntarily on running wheels, sympathoexcitatory responses in splanchnic but not lumbar SNA 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 multineurerecordings were performed in nonexercised 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). 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 SNA

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 (43) provide strong evidence that the increased splanchnic SNA is maintained by neurotransmission in the RVLM. 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 wk (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, 107) 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 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 inputs to the RVLM.

A majority of bulboспinal 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 (98). The importance of C1 cells in the regulation of resting SNA has been recently confirmed by selective deactivation of C1 neurons via allatofinin and allatostatin receptor gene transfer in the RVLM, which decreases resting SNA and blood pressure (57). C1 neurons are also critical for the full expression of sympathoexcitatory reflexes as demonstrated by several studies using anti-dopamine β-hydroxylase-saporin injected into the spinal cord or into the RVLM (54, 55, 94, 97). We recently examined the structure of bulboспinal C1 neurons in the RVLM that specifically project to the lower thoracic portion of the intermediolateral cell column, where splanchnic sympathetic outflow originates (101). 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 with active rats (64). Even more intriguing to us were the differential changes in dendritic branching over the rostrocaudal axis of the RVLM (Fig. 2). In sedentary animals, bulboспinal C1 neurons showed more dendritic branching in rostral compared with caudal regions of the RVLM (Fig. 2). In contrast, dendritic branching was consistent along the rostrocaudal axis in physically active animals (Fig. 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 bulboспinal 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 for functional neuroplasticity.** As in many other brain regions, the primary excitatory and inhibitory neurotransmitters in the RVLM are glutamate and GABA, respectively (86, 98). The RVLM receives prominent tonic inhibitory input. The primary source of this inhibitory input is the caudal ventrolateral medulla (CVLM) (38, 95, 98). The CVLM receives tonic excitation from the NTS as part of the baroreceptor
reflex (95) and also from other nonbarosensitive pathways (15, 18, 96). 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, 100).

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) (Fig. 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 nonexistent in physically active rats; and 3) despite the lack of GABAergic modulation in active rats, pressor responses were significantly smaller compared with sedentary animals (74). These data show that tonic GABA input is important for suppressing RVLM activity 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 bulbo-spinal RVLM neurons. Enhanced glutamatergic input augments SNA in other experimental animal models that demonstrate augmented sympathoexcitation (10, 96, 106, 108). Glutamate acts through N-methyl-D-aspartate (NMDA; NR1, NR2A, NR2B, NR2C, and NR2D subunits) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA; glutamate receptor 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, upregulation of glutamate receptors in the RVLM have been implicated in elevated sympathetic outflow in chronic heart failure (108) and exercise training has been shown to reverse augmented glutamatergic excitation in the RVLM of rats with heart failure (109). These findings suggest that enhanced glutamatergic neurotransmission in the RVLM due to physical inactivity could also result from neuromodulation 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 (103) (Fig. 4, A and B). Interestingly, the expression of the GLUR3 subunit of the AMPA receptor negatively correlated with total running distance (Fig. 4C), which also 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 with 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 blot analysis 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, 111). 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.
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 (9, 12, 49, 77, 99) are somewhat contradictory, it is unclear whether arterial baroreceptor and vagal afferent input are altered under sedentary versus physically active conditions.

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, 71–73). Finally, neuromodulatory or neurotrophic effects from substances including brain-derived neurotrophic factor, neuropeptide Y, 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 United States. 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 and 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.
(IN)ACTIVITY-RELATED NEUROPLASTICITY IN RVLM

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Review


