Recruitment pattern of muscle sympathetic nerve activity in chronic stable heart failure patients and in healthy control subjects

Zubin Maslov, Petra

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Petra Zubin Maslov

RECRUITMENT PATTERN OF MUSCLE SYMPATHETIC NERVE ACTIVITY IN CHRONIC STABLE HEART FAILURE PATIENTS AND IN HEALTHY CONTROL SUBJECTS

Doctoral Dissertation
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Split, Croatia 2013.

SVEUČILIŠTE U SPLITU
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Petra Zubin Maslov

OBRAZAC AKTIVACIJE MIŠIĆNOG SIMPATIČKOG ŽIVČANOG SUSTAVA U BOLESNIKA SA KRONIČNIM STABILNIM SRČANIM ZATAJENJEM I U ZDRAVIH KONTROLNIH ISPITANIKA

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I dedicate this PhD thesis
to my family and my husband, Gregori,
for their constant support and unconditional love

CONTENTS

1. LIST OF ABBREVIATIONS

ANS autonomic nervous system

AP action potential

Bf breathing frequency

BP blood pressure

CI cardiac index

CHF chronic heart failure

CPAP continuous positive airway pressure

CVLM caudal ventrolateral medulla

CWT continuous wavelet transform

CO cardiac output

DBP diastolic blood pressure

HF high frequency

HR heart rate

LBNP low body negative pressure

LF low frequency

LVEF left ventricular ejection fraction

MSNA muscle sympathetic nerve activity

NE norepinephrine

NYHA New York Heart Association

OSA obstructive sleep apnea

PCWP pulmonary capillary wedge pressure

Pro – BNP pro – brain natriuretic peptide

PVC premature ventricular contraction

RVLM rostral ventrolateral medulla

SNS sympathetic nervous system

SBP systolic blood pressure

SSNA skin sympathetic nerve activity

SV stroke volume

SVI stroke volume index

TPR total peripheral resistance

VR venous return

V_T tidal volume

2. INTRODUCTION

2.1. Methods used to assess sympathetic nerve activity

The sympathetic nervous system (SNS) plays an important role in regulation of the cardiovascular system. Tonic sympathetic activity is mainly generated by interplay of neurons located in the brain stem nuclei, primarily in the rostral (RVLM) and caudal (CVLM) ventrolateral medulla. These brainstem nuclei are modulated by neurons in the limbic system, hypothalamus and the cortex. The level of SNS activity is determinated by sum of inputs from arterial baroreceptors, cardiopulmonary mechanoreceptors, pulmonary stretch receptors, ergoreceptors and chemoreceptors. Sympathetic activation leads to increased heart rate (HR) through β_1-receptor activation and increased peripheral vasoconstriction through α_1-receptor activation. Thus sympathetic activity exerts a direct effect on the two major parameters that determinate blood pressure (BP), namely total peripheral resistance (TPR) and cardiac output (CO) (1).

Quantification of the SNS activity in humans has shown to be challenging and demanding task. Because of its many functions, a complete assessment of the autonomic nervous system (ANS) is extremely complex. Various procedures have been described as useful tools to quantify autonomic outputs in research as well as in routine clinical evaluation of patients with autonomic dysfunction (Figure 1) (2). Commonly, indirect methods such as measuring BP, HR, skin temperature and conductance have been accepted as representative of SNS activity. One of the routine cardiovascular autonomic tests is the analysis of HR variability in the time-domain. Various challenge maneuvers can be used to activate sympathetic or parasympathetic nervous system and to examine the ANS responses. Some of these maneuvers include: Valsalva maneuver, sustained handgrip test, cold pressor test, tilt table test and active standing.

Valsalva maneuver is a voluntary forced expiration of a subject against a resistance. The subject is asked to blow into special tube to maintain a column of mercury at 40 mmHg for 15 s. At the beginning of maneuver, an increase in transthoracic pressure mechanically leads to transient increase in BP and simultaneous bradycardia (phase I). Due to limited venous return (VR) and low stroke volume (SV), BP decreases with compensatory tachycardia (phase II). When the expiration is stopped (phase III) a further transient fall in BP is observed. In phase IV, probably due to baroreceptors' activation an abrupt rise in BP above the initial values with concomitant bradycardia occurs (3).

Isometric tests, such as a hand-grip test is performed by compressing the dynamometer at approximately one third of the maximum contraction strength for 3 min. Sustained isometric muscle contraction causes a reflex sympathetic activation and concomitant rise in BP and HR. Stimuli from exercising muscle and the central command are responsible for augmentation of SNS activity (4).

The BP response to cold water immersion and mental stress tests may be used to measure SNS activity. Both tests increase HR, BP and muscle sympathetic nerve activity (MSNA) (4).

A provocative tilt test is performed on an automated tilt test table, which allows a consistent slow tilt to 60-80 degrees. During the test there is pooling of blood in the lower extremities that results in reduced cardiac filling pressure, SV and BP. This leads to reflex increase in HR while the increase in SNS activity produces vasoconstriction and a further increase in HR. Tilt-table test is a suitable diagnostic tool for assessment of autonomic regulation during orthostatic challenge and has become a key element in the diagnosis of neurocardiogenic syncope (3).

In orthostatic test (active standing) hemodynamic responses are obtained during assumption of upright from supine position. This is the most frequently performed cardiovascular test of SNS function. Standing from the supine position causes redistribution of blood to sub-diaphragmatic venous systems, which decreases VR and SV. A significant fall in BP is prevented by compensatory tachycardia and vasoconstriction of resistance vessels. In a normal subject, systolic blood pressure (SBP) falls minimally after 1-2 min, diastolic blood pressure (DBP) increases by approximately 10 mmHg and HR increases by 10 beats per minute (4).

Blood levels of epinephrine and norepinephrine (NE) as well as urinary levels of their metabolites directly reflect total body SNS activity. However, these tests are now considered unreliable index of SNS activity because of low sensitivity and inability to quantify regional SNS levels (5).

NE spillover rate method has been used extensively for evaluation of SNS. NE in the plasma reflects the transmitter released by sympathetic nerves and spilled over into the circulation. The NE spillover approach is based on intravenous infusion of small amounts of titrated NE combined with regional venous sampling. The estimate of SNS level is quantified as arteriovenous NE difference across an organ, with correction for extraction of arterial NE, multiplied by the organ plasma flow to provide an index of the neurotransmitter spillover from the neuroeffector junctions. NE spillover rate allows measurements of regional SNS

activity such as NE spillover from coronary sinus or from renal veins. While the technique provides a good estimation of regional level of SNS activity at a particular point in time, it does not allow for continuous recordings of SNS (5) (6).

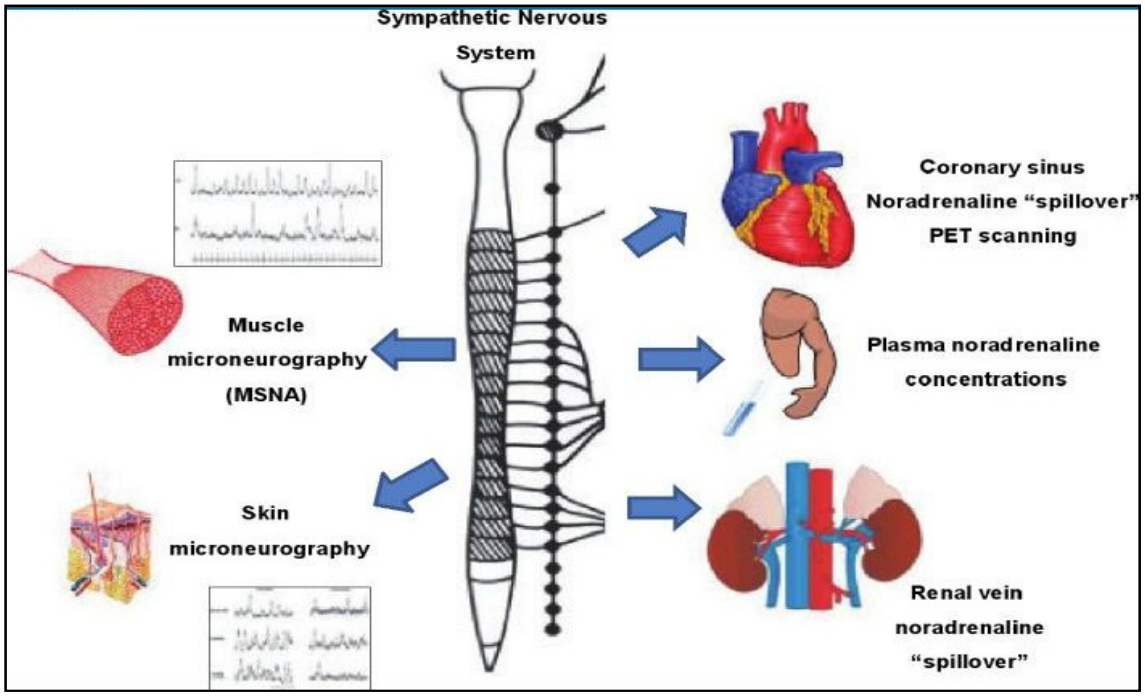

Figure 1. Some of the methods used to measure the sympathetic nerve activity. Reprinted from Ref. No 2 with permission 2012 Blackwell Publishing Ltd.

Spectral analysis of HR variability uses mathematical partitioning to identify cyclic variations of R-R intervals and arterial BP. Power spectrum analysis reveals low-frequency (0.04 – 0.15 Hz; LF) and high frequency (0.15 – 0.4 Hz; HF) components with ANS being the principal determinator of sinus rhythm variability. The HF component is mainly attributed to vagal mechanisms. LF variability in HR is not clearly determined by cardiac sympathetic activity despite uncritical interpretation of the LF variability measurement in this way. Better marker of cardiac sympathetic activity than LF component of HR that can be used from spectral analysis of HR variability is LF/HF ratio (1).

Imaging techniques such as positron emission tomography and single photon emission computed tomography have been used to evaluate the anatomy of sympathetic innervations. Scanning agents that are most widely used are [^{123}I]meta-iodobenzylguanidine (MIBG) and 6-[^{18}F]fluorodopamine and [^{11}C]hydroxyphedrine. These methods have revealed sympathetic denervation in patients with autonomic failure (5).

2.2. Microneurography: technique to evaluate sympathetic nerve activity in health and disease

Postganglionic efferent sympathetic neurons are unmyelinated C-type fibers, and thus cannot be tested directly by conventional neurophysiologic techniques, i.e., nerve conduction studies and electromyography (7). The only method that allows direct recording of SNS activity is microneurography (1). A major advantage of microneurography is its ability to record not only SNS activity at rest, but also to track changes in cardiovascular regulation in response to various stimuli. However, due to its invasiveness and time-consuming nature of the procedure, microneurography is used for research-based evaluation of ANS function. The safety of this technique was confirmed in prospective follow-up study involving hundreds of subjects (1;8).

Microneurography was developed by Hagbarth and Vallbo in 1965-1966 in Sweden, within the Department of Clinical Neurophysiology at the Academic hospital in Uppsala (9). The very first step toward developing of the microneurography was taken by Hagbarth, who inserted a needle into his own ulnar nerve. This group soon showed exciting possibilities of the new method that could monitor impulse traffic, not only in myelinated, but also in unmyelinated postganglionic sympathetic fibers. In many early experiments they noticed a spontaneous activity with sound reminiscent of waves approaching a distant shore or the sound of someone walking on snow (10). These findings were the beginning of new era in SNS research. Since mid 1960s microneurography has been used to study various types of afferent and efferent fibers in peripheral limb nerves.

Peripheral nerves contain several fascicles that are connected to defined skin area or to a muscle. Therefore, by microneurography, it is possible to assess both skin and muscle sympathetic nerve activities (11). The activity of the postganglionic efferent sympathetic fibers that innervate resistance blood vessels within the muscles is known as muscle sympathetic nerve activity (MSNA). The other efferent group of sympathetic fibers innervates blood vessels within the skin and piloerector muscles and their activity is known as skin sympathetic nerve activity (SSNA) (1). In particular interest in the study of cardiovascular diseases is the assessment of MSNA with the use of microneurography. This method has provided new insight into the role of the SNS in physiology of aging and gender differences as well as in pathophysiology of chronic heart failure (CHF), renal failure, hypertension and obstructive sleep apnea (OSA) (12-14).

The peroneal nerve adjacent to the fibular head is often used for MSNA recordings because of its subcutaneous position in the lateral popliteal area. When searching for the peroneal nerve, the fibular head is used as an anatomical landmark, since the nerve passes around the fibular head on its way to innervate skin and muscles of the distal leg. Percutaneous electrical stimulation of short duration (0.2 ms) and small voltage (3-7 V) is used to map the position of the nerve before the microelectrode insertion. Tungsten microelectrode, called active microelectrode, is inserted percutaneously into the peroneal nerve. Another ground microelectrode is placed subcutaneously 2 - 3 cm away from the active microelectrode. Small adjustments of the microelectrode are required to obtain the signal coming specifically from sympathetic fascicules that selectively innervate blood vessels in the distal leg muscles (MSNA). Confirmation that the recorded signal represents MSNA is determined by the absence of skin paresthesia and a signal that increases in response to voluntary apnea but not during arousal to a loud noise (15).

The most common and oldest way of MSNA quantification obtained by microneurography is the integrated multi-unit approach. In multi-unit recordings, sympathetic activity appears as bursts of vasoconstrictor impulses, the outflow of which is under potent arterial baroreflex control (13), (16). Bursts display cardiac rhythmicity and occur during temporary reductions of BP that corresponds to diastolic blood pressure (DBP) (15). In multi-unit analysis, raw, unfiltered, noisy signal is first amplified through the pre-amplifier (100 x) and amplifier (1000 x). Afterwards the signal is band-pass at a bandwidth of 700 - 2000 Hz, a frequency range found to give an optimal signal-to-noise ratio for the multiunit sympathetic discharges in order to expose bursts and remove the noise from the recordings. Filtered signal is then rectified and passed through a laky integrator with time constant of 0.1 s. Described process forms a smooth signal with discharge of sympathetic nerve activity displayed as narrow peaks called “bursts”. Each burst is composed of action potentials (APs) that fire simultaneously from 5 – 20 different sympathetic fibers within the recording area of the active microelectrode (17).

The following criteria must be met for acceptable MSNA signal: 1) signal–to–noise ratio >2:1; 2) spontaneous bursts of neural activity synchronous with arterial pulse; 3) latency from preceding R wave 0.9–1.2 s; 4) tapping or stretching the muscle of the distal leg must elicit afferent mechanoreceptor discharge, whereas stroking the skin does not. The latter ensures that microelectrode records spontaneous activity originating from muscle sympathetic fibers rather than from sensory or skin sympathetic fibers; 5) increase in the burst number and /or amplitude must occur during the end - expiratory apnea but not after startle (15).

The rate of sympathetic nerve discharge obtained by microneurography is quantified as the number of bursts of sympathetic nerve activity per minute *(burst frequency)* or as number of bursts per 100 heart beats *(burst incidence)*. Of interest, sympathetic burst amplitude depends on the number of sympathetic fibers that concomitantly discharge. As a consequence, this index is largely influenced by the proximity of the electrode tip to the active sympathetic fibers (11). In order to allow interindividual comparison, amplitude of each burst must be normalized to the largest burst amplitude in the baseline period. Total MSNA can be calculated as the sum of all normalized amplitudes per minute.

Multi-unit bursts that appear in the integrated neurogram are formed by APs from several sympathetic neurons firing at the same time. Additionally, information regarding individual sympathetic fiber activity is lost within the integrated burst. An important novelty in studying sympathetic nerve discharge was introduced by Macefield and colleagues who started single-unit recordings in mid 1990s (18). The method was originally used to record activity from single motor and sensory neurons. With the use of single unit approach, activity of a single sympathetic neuron can be detected and tracked over time; before, during and after application of various cardiovascular stimuli. This can be achieved with the use of high-impedance microelectrode of 10 Ohms (in comparison with the microelectrode used for the integrated signal recordings – 3 Ohms). High impedance electrode has a small recording area that only receives the activity of the fibers in close proximity of the microelectrode (within 2 μm). Therefore, investigator can successfully isolate a full AP generated from a single sympathetic, vasoconstrictor nerve (19;20). This is achieved by manipulating the electrode until large unitary discharges appear out of the multi-unit bursts (Figure 2).

Criteria for single-unit recordings are: constancy of the shape of unit's AP and correlation with at least one of the cardiovascular parameters (18). With the use of single unit approach it has been discovered that vasoconstrictor sympathetic fibers in the skeletal muscles have the ability to fire up to 7 times per sympathetic burst, yet they tend to fire only once per sympathetic burst. Previous studies revealed the causality of this phenomenon in the fact that sympathetic bursts are just too short to allow prolonged firing (20). Inputs from arterial baroreceptors are responsible peripheral factor that constrain the firing of sympathetic fibers into bursts during diastole. However, cardiac rhythmicity is abolished, but prolonged bursting pattern is preserved if the glossopharyngeal and vagus nerves are blocked with anesthetics. This suggests that intrinsic supraspinal mechanisms shape the sympathetic outflow into a bursting pattern (5).

Given the extremely large rates of data acquisition (20 000 Hz) and resultant files, single-unit recordings can only be performed for 5 minutes, limiting the period of investigation (21). An additional disadvantage of the single unit method is the inability to detect latent, larger population of sympathetic neurons, silent at rest, but recruited as a sympathetic response to physiological stress (chemoreflex or baroreflex activation) (22).

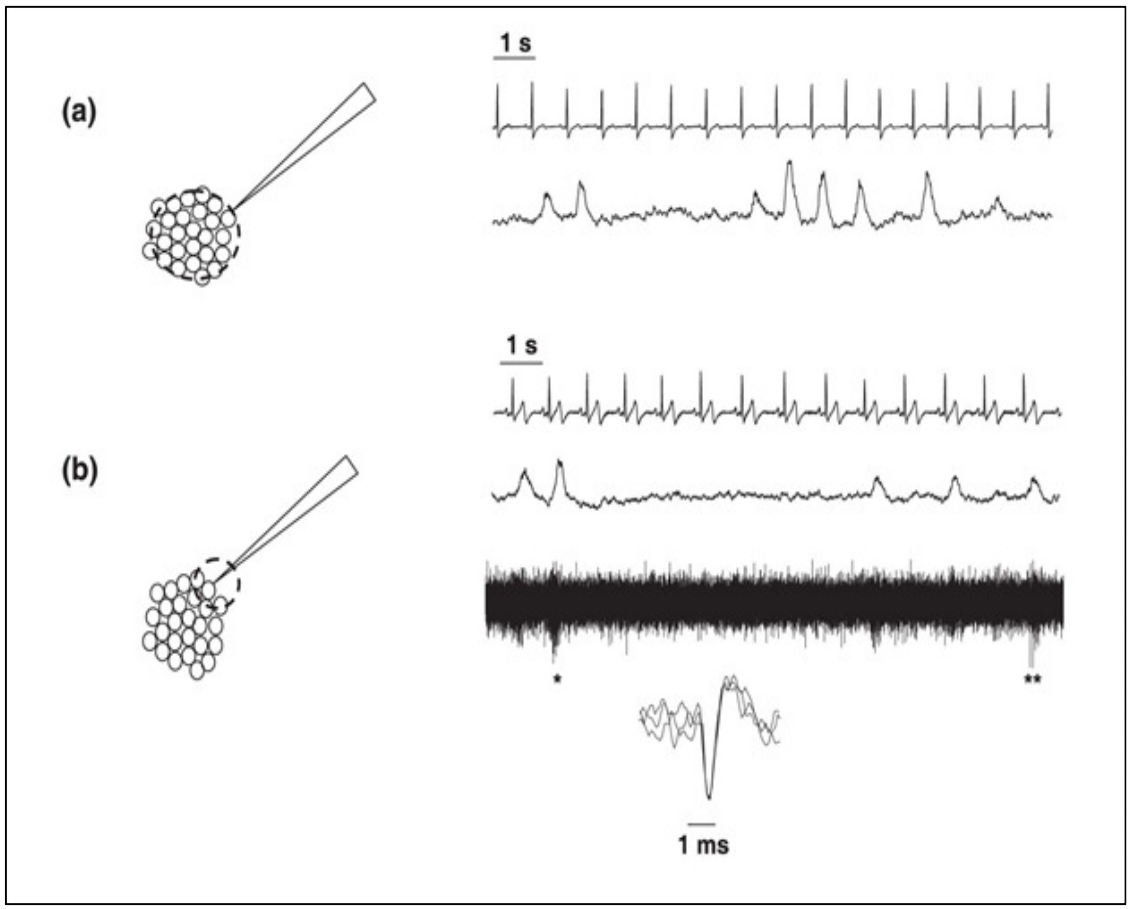

Figure 2. (a) Multi-unit muscle sympathetic nerve activity (MSNA). The low impedance electrode records the activity of a high number of sympathetic fibers (multi-unit MSNA). (b) Single-unit MSNA. The high impedance electrode records the activity of a smaller number of vasoconstrictor fibers. The activity of one single unit can be recorded and appears as larger spikes out of the raw nerve activity signal. Superimposed action potentials (spikes) show uniform morphology, indicating that it is generated by the same sympathetic fiber. Reprinted from Ref. No 21, with permission from 2012 Blackwell Publishing Ltd.

Previous studies of MSNA have shown a large interindividual difference in the number of sympathetic bursts, some people have few and others have many spontaneously occurring bursts of SNS activity. It is likely that this interindividual variability has a genetic

background, and since discharge frequencies in single vasoconstrictor fibers are similar in subjects with few and many bursts, the differences in the number of multi-unit bursts are probably due to a higher number of active fibers in subjects with many bursts (16). Interestingly, individuals with high number of multi-unit bursts do not necessary have higher level of BP than those with few bursts. Possible explanation for the lack of relationship between mean levels of BP and MSNA is a balance between CO and MSNA. This is supported by demonstration of an inverse relationship between the two variables; subjects with high MSNA were found to have low CO and vice versa (12). Level of human SNS activity shows high level of inter-individual variability. Because of this marked difference between individuals, strict normal criteria are difficult to define when comparing a group of subjects. Another characteristic of human SNS activity at rest is its long term intra-individual reproducibility: MSNA remains similar over long period (10 years) with a tendency to increase with age (23).

While traditional integrated MSNA provides general information about changes in vasoconstrictor sympathetic discharge, the integration process loses important neurophysiologic information regarding AP content (i.e. number of AP within the burst, AP morphology and AP frequency). Recently Salmanpour and colleagues have developed a different method to analyze sympathetic multi-unit neurogram that uses continuous wavelet transform (CWT) for AP detection (Figure 3) (17). Major parts in the CWT process are separation of the APs signal from the background noise in recorded raw neurogram and categorization of APs into different "clusters" based on their peak–to–peak amplitude. The AP detection algorithm involves the following steps: 1) Design of a "mother wavelet" (or a frequency template) matched to an average AP waveform constructed from several real raw MSNA signals; 2) Application of the CWT to the filtered MSNA to provide a wavelet coefficient between the signal of interest (i.e. AP) and the mother wavelet (the largest wavelet coefficient occurs in the presence of the APs, but negligible coefficients occur when applied to the noise); 3) Separation of the wavelet coefficients related to APs and those related to noise using wavelet tresholding analysis; 4) Isolation of the largest supratreshold wavelet coefficients provides the exact location of the negative peak for each AP and 5) Separation of the APs from the original filtered MSNA signal using detected location of APs - as a center of predefined window (3.2 ms) (17;24). In this way, the peak-to-peak amplitude of each extracted AP remains unaltered. Extracted APs are then ordered based on the size of the peak-to-peak amplitude and grouped into clusters based on similar peak-to-peak amplitude. Cluster bin width are automatically defined based on Scott's Rule and are identified sequentially on

the basis of the relative size within a given individual for the data set being interrogated. With this approach, the number of total clusters (i.e. groups of APs with similar peak to peak amplitudes) varies by subject. Not all clusters are present in every integrated burst. Therefore the term active cluster relates to those clusters present for a defined condition, such as before / during / after the breath hold (25) or before / during / after application of continuous positive airway pressure (CPAP) (26).

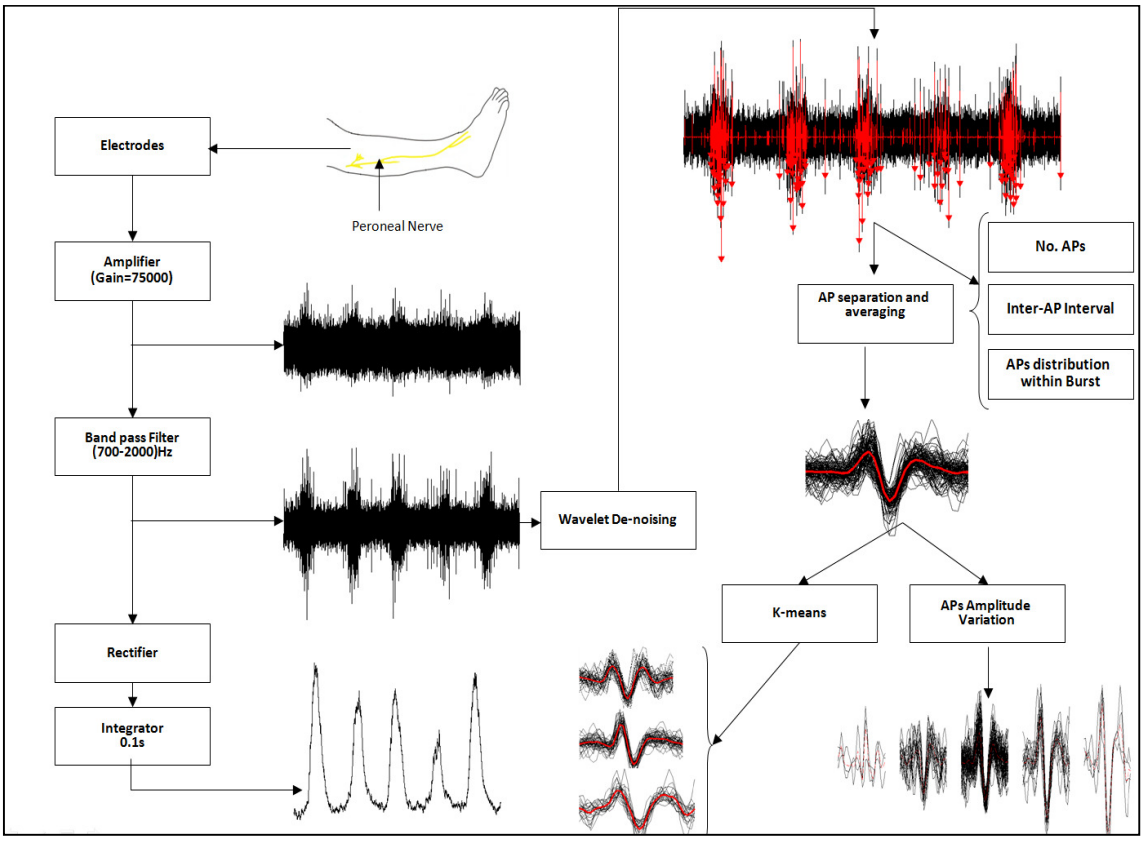

Figure 3. Detection and classification of Raw Action Potential Patterns in Human Muscle Sympathetic Nerve Activity (with courtesy of Professor J. Kevin Shoemaker, Neurovascular Research Laboratory, Western University, London, Ontario, Canada).

CWT based AP detector has provided a methodological advance in MSNA analysis that can be used to study the recruitment pattern of sympathetic neurons under various conditions in health and disease. The advantage of the "AP population detector" technique is the ability to detect the activity of different sympathetic fibers contributing to the burst creation (22;27). The recruitment pattern of skeletal motor neurons during motor tasks is based on neuronal size where smaller motor neurons, with slower conduction velocity are being recruited first and larger, higher threshold neurons are being recruited under increasing load (Henneman's size principle)(28). AP detector analysis has shown that the principle of recruitment based on

neuronal size (e.g. Henneman's size principle) is conserved across excitable neuronal systems: sympathetic augmentation during a physiological stress (e.g. chemoreflex stress) is also accomplished by recruiting groups of larger neurons with larger APs and shorter conduction velocities (22). This interpretation was supported by evidence of 1) an increase in the number of APs within a given burst of sympathetic activity in relation to integrated burst size, 2) the recruitment of otherwise silent clusters of sympathetic neurons as a function of integrated burst size and 3) the increased presence of larger APs (axons) during sympathetic excitation elicited by breath-holding (22).

With the use of the previously mentioned single-unit recording we would not be able to gather information regarding activation of the larger population of neurons that become active only in extreme physiological (or pathophysiological) conditions.

2.3. Sympathetic nervous system overactivity in chronic heart failure

Excessive sympathetic activation under resting conditions accompanies CHF and tends to increase with disease severity (29). This heightened sympathetic drive is recognized as an important marker of poor prognosis in CHF patients (30-32). Sympathetic activity plays an essential role in maintaining BP in acute HF, but excessive sympathetic activity in CHF has deleterious effects on the heart, including cardiac myocyte apoptosis (29) and development of spontaneous depolarization and ventricular arrhythmias (5). It has been shown that CHF patients belonging to New York Heart Association (NYHA) class III or IV have increased urinary catecholamines and their metabolites. Rates of NE spillover from the failing heart to plasma at rest are up to 50 times the normal rate in untreated patients. This level of NE increase corresponds to the NE release in healthy hearts during near maximal exercise (5). Early, mild CHF has selective, cardiac SNS overactivity with normal level of sympathetic activity toward skeletal muscles, kidneys, gastrointestinal system and skin (33). Studies based on direct recordings of MSNA in CHF patients have shown that the noradrenergic activation inversely correlates with measures of left ventricular stroke work, SV and left ventricular ejection fraction (LVEF) (32). With further deterioration of the heart function, sympathetic overdrive extends to other vascular beds such as renal and skeletal (5).

Exact mechanisms of sympathoexcitation in CHF and central nervous system (CNS) circuitry involved still remain to be elucidated. Functional impairment of several types of reflexes was proposed to be an afferent stimulus in development and progression of the

sympathetic overdrive in CHF. Former, prevailing model suggests that in CHF, central, sympathetic outflow remains unopposed by loss of inhibitory influences from altered baroreceptors, cardiopulmonary receptors and/or pulmonary stretch receptors. Because arterial baroreceptor reflex vagal control of the HR is impaired early in heart failure, a parallel reduction in its reflex buffering of sympathetic outflow has also been assumed (32). However, an updated model of sympathoexcitatory mechanisms in CHF has been proposed by Floras at the beginning of 2000s (33). In this updated model, CHF-associated sympathetic overactivity reflects the net balance between compensatory responses to impaired systolic function and individual variation in non-baroreflex mediated sympathoexcitatory mechanisms such as coexisting OSA, obesity and reflexes from exercising muscles (metaboreceptors) (32).

In CHF patients the presence of significant inverse relationship between stroke work index and MSNA suggests that arterial baroreflex retains the capacity to modify efferent sympathetic outflow in a response to changes in CO or arterial BP (32). An examination of hemodynamic and sympathetic responses to premature ventricular contractions (PVC) can be used to test the baroreflex function in CHF patients. As PVC produces a prolonged diastolic period, diminished SV and transient decrease in DBP, it represents an acute physiological stress resulting in baroreceptor-mediated change in MSNA (34). A significant increase in the amplitude and duration of the sympathetic burst has been identified immediately after programmed stimulation-induced PVC (post-PVC) in healthy individuals (35). The prevalence of PVC increases in CHF patients (34;35) and a higher rate of PVCs can contribute to a state of elevated sympathetic discharge both in peripheral tissue (MSNA) and within the heart (36). PVCs are also encountered periodically in healthy people with a prevalence that increases with age (26).

CHF patients can exhibit high level of sympathetic activation with a burst incidence approaching 100% at baseline (21;29). This has led to a question of whether or not sympathetic discharge in CHF patients can be further increased during physiological stress (such as PVC) and if so, is the increase in sympathetic burst due to an increase in AP discharge of already active sympathetic neurons or due to recruitment of additional, larger sympathetic neurons that are silent at rest and “reserved” just for stressful events. Single-unit recordings from the multi-unit bursts of MSNA demonstrate increased firing frequency and firing probability of individual sympathetic fibers in CHF patients during sinus rhythm and also an increased incidence of multiple firings of already active fibers within post-PVC burst enhancement in CHF (37-39).

An additional reflex, that has pulmonary stretch receptors as an afferent component, has been identified as an important factor in the pathophysiology of CHF sympathoexcitation. Naughton and colleagues demonstrated a correlation between the spontaneous pattern of breathing and MSNA in CHF patients (40). Specifically, higher respiratory frequency, and lower tidal volume (V_T) were related to higher MSNA (41). Patients with chronic and prolong heart failure often suffer from pulmonary congestion and thus have lower V_T than healthy individuals. Additionally, they exhibit rapid and shallow breathing to compensate for lower V_T. This pattern of breathing, commonly observed in CHF patients, causes pulmonary stretch receptors to stretch less and results in diminished afferent inhibition of the central sympathetic outflow. Waxing and waning pattern of breathing interrupted with apnea periods, known as Cheyne - Stokes breathing, occurs in approximately 37 % severe CHF patients and has been recognized as a sign of poor prognosis (42). Consequently, respiratory factors should be also considered when evaluating mechanisms responsible for sympathetic nervous system activation in CHF patients.

One of the novel non-pharmacological therapeutic options in therapy of CHF that primarily targets respiratory and autonomic disturbances in CHF is application of CPAP (42-44). An improvement in left ventricular function with long-term CPAP use in CHF patients has been ascribed to increased intrathoracic pressure and reduction in left ventricular afterload by decrease in transmural left ventricular pressure (40). However, the data on CPAP influence on ANS and the role of ANS in CPAP-induced improvement in heart function in CHF patients is still inconsistent. The response of the ANS after short-term CPAP application appears to be different between CHF patients and healthy individuals. In young subjects with normal cardiac filling pressures, a positive end - expiratory pressure induced by CPAP causes reduction in VR, followed by a decrease in CO in accordance with Frank- Starling mechanism. Diminished CO leads to unloading of aortic baroreceptors and a reflexive increase in the MSNA (41). Contrary to healthy subjects, the short-term effects of CPAP in CHF patients are less consistent. Positive end – expiratory pressure in CHF patients with increased cardiac filling pressures may cause a reduction in afterload and result in increased CO without the need for sympathetic outflow augmentation (40). In contrast to the idea that sympathoinhibition should occur if CPAP enhances CI, Heindl et al found a modest increase in multi-unit MSNA in both, CHF patients and healthy subjects (45).

3. GOALS AND HYPOTHESIS

3.1. Study I

Sympathetic overactivity in CHF is well documented by traditional MSNA analysis and is quantified as multi-unit sympathetic bursts. As each burst is made up of several sympathetic neurons firing in synchrony, firing characteristics of individual sympathetic neurons are lost in the integration process. Moreover, the level of multi-unit MSNA in severe CHF patients is nearly maximal at rest (100 bursts per 100 heart beats) and further increases during various stimuli cannot be assessed with traditional multi–unit approach. AP detector CWT technique was used in the Study I to examine AP recruitment properties of the sympathetic fibers in CHF patients and in control subjects during sinus rhythm and during the PVCs.

The first goal of Study I was to identify and compare firing properties of the sympathetic fibers in CHF patients with healthy, age- and gender-matched controls during sinus rhythm.

The second goal was: 1) to explore the recruitment strategies of sympathetic nerve reserve during PVC-induced decrease in BP in CHF patients and 2) to compare obtained results with healthy, age- and gender-matched controls.

The purpose of Study I was to test the following hypothesis:

1. Besides exhibiting higher multi-unit MSNA, stable CHF patients have higher number of APs per sympathetic burst, higher AP firing frequency and higher number of different active clusters of sympathetic neurons than healthy, age- and gender-matched control subjects during sinus rhythm.
2. In stable CHF patients, PVC-induced sympathetic activation is accomplished by the same recruitment mechanisms as in healthy individuals: increase in APs per sympathetic burst, increase in AP firing frequency and recruitment of additional subpopulation of larger sympathetic neurons.
3. The overall baroreceptor-mediated increase in vasoconstrictor sympathetic discharge elicited by PVC is attenuated in CHF patients if compared with healthy, age- and gender-matched control subjects.

3.2. Study II

The goal of Study II was to investigate short - term effects of CPAP on the firing strategies of muscle sympathetic neurons in CHF patients and in healthy, age- and gender-matched controls.

The purpose of Study II was to test the following hypothesis:

Short term application of CPAP in CHF patients and healthy subjects would reflect the CPAP-associated changes in central hemodynamics:

1) CPAP induced CO improvement in CHF patients is associated with baroreceptor - mediated sympathoinhibition.
2) CPAP induced CO decrease in healthy subjects is associated with baroreceptor - mediated sympathoexcitation through an increase in AP firing frequency.

4. SUBJECTS AND METHODS

4.1. Subjects and ethical procedures

CHF patients and healthy age- and gender- matched controls were included in both studies conducted in the laboratory of the Department of Integrative Physiology at School of Medicine, University of Split.

All subjects gave written informed consent to participate in the study that was conducted in accordance with the Declaration of Helsinki and was approved by research ethics board at The University of Split, School of Medicine.

CHF patients were recruited from the Department of Cardiology, Clinic of Internal Medicine at University Hospital of Split and from the Croatian Register of Heart Failure Patients (Croatian Cardiology Society). They were eligible for the study if they met the following criteria: age 20 – 75 years, LVEF < 40%, NYHA class I – III, in stable condition (no rales on auscultation or tibial edema) with no recent (1 month) history of decompensation or hospitalization and on stable therapy (last 3 months).

Exclusion criteria were atrial fibrillation, pacemaker dependence, history of smoking or alcoholism and history of comorbidities (kidney disease, obstructive lung disease, cerebrovascular insult, and severe anemia Hb < 90 g/l).

Control subjects were subjects without heart disease, that were nonsmokers, and none were on regular drug treatment as indicated by brief history and physical examination.

For the purpose of the Study I, CHF subjects were selected on the basis of their PVC occurrence from a larger number of CHF patients.

4.2. Methods used in the Study I and the Study II

Medical history and physical examination were obtained from each subject.

Anthropometric measurements. Height and body weight were measured for each subject and body mass index (BMI) was calculated from obtained parameters (BMI = body mass (kg) / body weight (m)) ^{2}.

Spirometry. All subjects underwent dynamic spirometry test (Quark PFT; Cosmed, Rome, Italy)

Echocardiography. LVEF was determinated using two-dimensional echocardiography (Vivid Q; GE, Milwaukee, WI, USA).

ECG. At the beginning of the protocol, one – channel ECG (ECG; Bioamp, ADInstruments, Castle Hill, Australia) was used to monitor HR activity and to record PVCs.

Hemodynamic parameters. BP was measured continuously with the use of photoplethysmography (Finometer; Finapress Medical Systems, Arnhem, Netherlands). From the continuous BP measurement, the arterial pulse wave was analyzed by an improved method of Wesseling (Modelflow program) which computes changes in the left ventricular SV from the pulsatile systolic area (46). The values of SBP and DBP obtained by photoplethysmographic method were gauged using the mercury sphygmomanometer. CO was calculated as SV times HR.

Blood samples for blood tests were obtained from an antecubital vein from each participant. All blood tests were done in Biochemical laboratory at University Hospital Split. ECLIA (Electrochemiluminiscence immunoassay; analyzer Cobas E601; Roche Diagnostics GMBH, Mannheim, Germany) was used to obtain pro-BNP.

Microneurography. MSNA was measured with the use of tungsten microelectrode inserted into the peroneal nerve. A reference electrode was inserted subcutaneously 1 -3 cm away from the recording site. Small adjustments of the active microelectrode were made until pulse synchronous multi-unit bursts, characteristic of sympathetic neural activity were found. Confirmation that the recorded signal represented MSNA was determinated by the absence of skin parestesia and presence of a signal that increased in response to voluntary apnea but not during arousal to a loud noise. The MSNA signal was amplified 1000 x through a preamplifier and 100 x by a variable gain, isolated amplifier. The MSNA signal was then bandpass, filtered at a bandwidth of 700 – 2000 Hz, sampled at 10 000 Hz (Powerlab/16SP; ADInstruments) and stored in a personal computer for a subsequent analysis using Chart software (version 5.5.6.7.).

AP detector CWT software. APs were detected and extracted from the filtered, raw MSNA signal using AP detector technique based on CWT (APD v2, Aryan Salmanpour, Neurovascular Research Laboratory, School of Kinesiology, Western University, London, Ontario, Canada) as described in the Introduction section.

A pneumatic respiratory belt, located around the chest at the level of the xiphoid process, was coupled to a different pressure transducer (Prignitz Mikrosystemtechnik, Wittenberge, Germany). It was used to monitor chest movements in the protocol of the *Study II*.

Breath by breath analyzer (AMIS2000, Innovision A/S, Odense, Denmark) was used to measure respiratory parameters including V_T and breathing frequency (Bf) in the protocol of the *Study II*. Ventilatory volume (V_E) was computed as V_T times Bf.

A mouthpiece connected to a CPAP device (BiPAP Vision, Respironics, Pittsburg, PA, USA) was used in the protocol of the Study II for CPAP application.

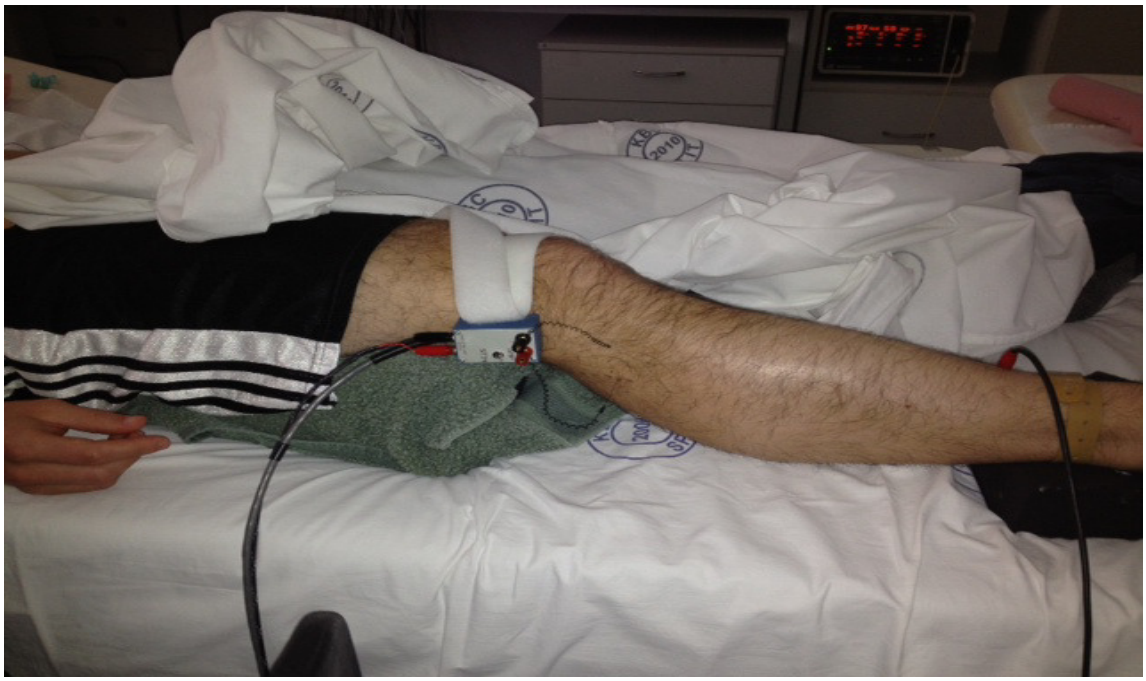

Figure 4. One of the subjects in the Laboratory of Department of Integrative Physiology at School of medicine, University of Split, at the beginning of the protocol, instrumented with microneurography equipment used to record MSNA.

4.3. Protocol used in the Study I

The Study I was conducted in the morning hours over two consecutive days. All participants were asked not to consume caffeine beverages and alcohol for at least 12 hours and not to eat for 2 hours before the experimental day. CHF group was asked to continue their normal medication therapy on the morning of the study.

On the first day of the protocol subjects were informed about the purpose of the study and all procedures and potential risks of the methods used in the Study. A brief history and physical examination were obtained from each subject. Blood samples were withdrawn from the antecubital vein to measure levels of pro-BNP. ECG, spirometry and echocardiography were performed on each subject.

On the second day of the protocol subjects assumed the supine position and were instrumented for hemodynamic and MSNA recordings. They were given 10 min of quiet rest to allow stabilization of hemodynamic parameters. Afterwards, data were collected during a 10 – 15 min period of quiet breathing. During this time enough number of PVCs accompanied with large post–PVC bursts was recorded.

4.4. Protocol used in the Study II

The first day of the Study II was the same as in the Study I, with addition of instructions on how to use the CPAP device. All participants were familiarized with the breathing through a mouthpiece and breathing with positive end-expiratory pressure to minimize potential hemodynamic effects of anxiety on the data.

On the second day of the study, subjects were placed in supine position and after instrumentation were given 10 min of quite rest. After hemodynamic parameters were stabilized, subjects began breathing through a mouthpiece with a pneumatic one – way valve connected to a breath by breath respiratory gas analyzer with nose clip in place. All subjects breathed room air through a mouthpiece for 10 min to obtain baseline values. After baseline recordings, CPAP was applied for 5 min at 5 cmH_2O level and then for 5 min at 10 cmH_2O level. 10 minutes of recovery on room air followed CPAP application.

4.5. Data acquisition and statistical analysis

In both studies, MSNA was quantified in two ways: 1) as an integrated MSNA signal, in which SNS activity was expressed as *burst frequency* (the number of bursts per min) and as *burst incidence* (the number of bursts per 100 heart beats). Integrated bursts of MSNA were identified as exhibiting pulse-synchrony, having SNR of at least 2:1 with respect to a previous period of neural silence. 2) In the AP detection process that uses CWT software, SNS activity was expressed as the number of APs within a sympathetic burst (APs/burst) and as APs fired per second (AP firing frequency). Extracted APs were then ordered based on peak-to-peak amplitude and grouped into different sized clusters. To enhance AP detection with CWT technique, data were selected when signal to noise (SNR) was ≥ 3 (17). The average raw MSNA SNR for CHF group was 3.74±0.2, and for controls it was 4.39±0.5. According to previous analysis (17), we expected that the level of SNR~ 3.74 would produce a 91±11 % correct detection rate for APs.

Study I

In the Study I the frequency of PVCs was different between the subjects. To provide similar level of data from each individual, APs were analyzed for maximum periods of six heartbeats before PVC, during which time at least two sinus rhythm bursts were present. In total, 164 sinus rhythm bursts and 35 post-PVC bursts were selected in 6 CHF patients and 121 sinus rhythm bursts and 35 post-PVC bursts from 6 control subjects. Even though CHF group exhibited higher number of PVCs than controls, a similar number of PVCs was selected in both groups to enable statistical comparison of MSNA and AP parameters. In the data analysis, PVCs were visually detected from the ECG and corresponding BP, MSNA and AP parameters were determinated for each of PVC.

Study II

In the Study II, all parameters were analyzed and average at 6 different points: 1) during the last minute of baseline period, 2) during the first minute (start) and 3) last minute (end) of CPAP at 5 cmH_2O level, 4) during the first minute (start) and 5) last minute (end) of CPAP at 10 cmH_2O level, 6) and during the last 1 minute of recovery.

Results in both studies are expressed as mean ± SD. Level of significance is set at $P<0.05$. Statistical analyses were performed using Statistica 7.0 software (Statsoft, Inc., Tulsa, USA) and SigmaStat 3.11 (Systat Software Inc, San Jose, CA, USA).

In the Study I we used mixed analysis of variance (ANOVA) to assess the effects of burst type (sinus rhythm vs. post-PVC) and group (CHF group vs. control group). Student's two tailed, unpaired t test was used to assess the differences between the groups in terms of the increase of AP parameters and increase of hemodynamic parameters from sinus rhythm to PVC. Distribution analysis was used to assess the proportionate shift in peak to peak amplitude of APs between the sinus rhythm and post-PVC burst in both groups.

In the Study II we used Friedman ANOVA to identify the main effect of time (baseline vs. start and end of each CPAP level) on MSNA, hemodynamic and ventilatory parameters in each group. In case of significance, pair-wise comparisons were assessed using the Wilcoxon test. Baseline anthropometric, hemodynamic, ventilatory and MSNA data between the groups were compared using Mann-Whitney U test. Relationships between CPAP responses in MSNA parameters (burst frequency, burst incidence, AP frequency mean burst area/min and AP/burst; dependant variables) and hemodynamic variables (DBP, SV and CO; independent variables) were analyzed by linear regression. The inter-subject variation was accounted for by multiple regression, with subjects as dummy variables. The remaining, intra-subject relationships were evaluated as the partial correlation coefficients (univariate analysis). To further account for mutual associations of independent variables, the associations of SV and CO were adjusted for DBP and the associations of DBP were adjusted for SV and CO (multivariate analysis).

5. RESULTS

5.1. Study I

The anthropometric and clinical characteristics of the subjects participating in the Study I are listed in the Table 1.

Table 1. Anthropometric and clinical characteristics of the subjects included in the Study I

	CHF patients (N=6)	Controls (N=6)
Age (yr)	62 ± 11	59±5
Gender male/female	4/2	3/3
Height (cm)	174 ± 0.1	174±0.1
Weight (kg)	83 ± 16	95±22
BMI	27 ± 2	31±7
LVEF (%)	33 ± 4	-
LVDd (mm)	76 ± 1	-
NYHA class		
I	2	-
II	3	-
III	1	-
Etiology		
Dilated cardiomyopathy	3	-
Coronary artery disease	2	-
Idiopathic	1	
Drug regimen		
ACEi	5	-
Digitalis	2	-
Diuretics	6	-
β- blockers	5	-
MSNA		
Burst frequency (burst/min)	55*	30
Burst incidence (burst/100hb)	83*	43

Values are mean ± SD. * $p<0.05$ different from controls. BMI indicates body mass index; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic diameter; NYHA, New York Heart Association; ACEi, angiotensin converting enzyme inhibitor; MSNA, muscle sympathetic nerve activity.

Figure 5 shows recordings of spontaneous PVCs in CHF patients and in control subjects. Each PVC was characterized by prolonged diastole and accompanied by a large post-PVC burst. During sinus rhythm, compared with control group, CHF group had higher integrated MSNA burst frequency (30±6 vs. 55±6 bursts/min, $p<0.05$) and higher burst incidence (43±7 vs. 83±7 bursts/100hb; $p<0.05$) than controls. Compared with bursts observed during sinus rhythm, the post-PVC burst integral was greater than bursts that occurred during sinus rhythm by ~365% in CHF patients, ($p<0.05$) and ~ 632% in controls

($p<0.05$). A group (CHF vs. Control) by burst type (sinus rhythm vs. post-PVC) interaction was observed for burst integral; $F(1,140) = 11.28$, $p<0.05$.

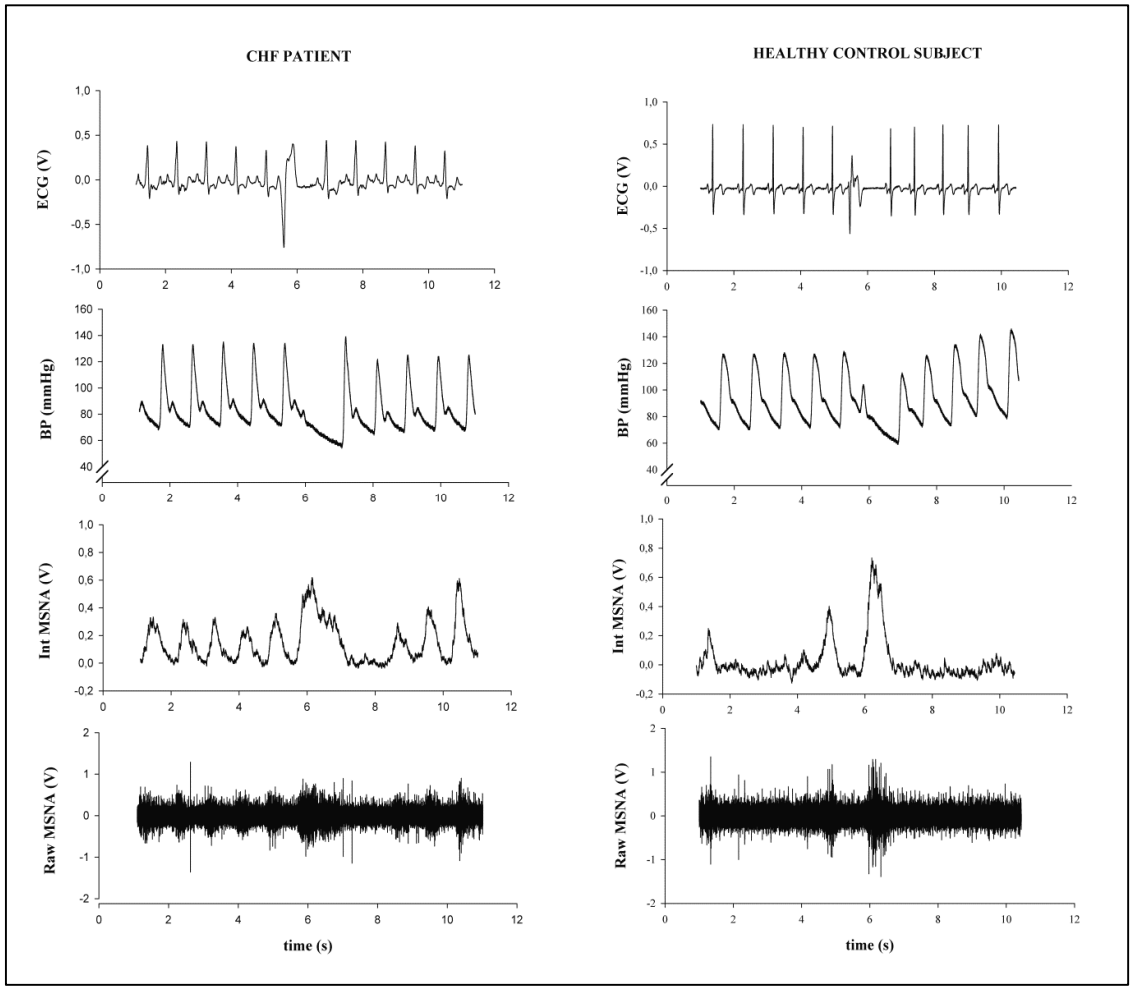

Figure 5. Original examples of premature ventricular contractions (PVCs) in a chronic heart failure (CHF) patient and in the control subject with recording s of ECG, blood pressure (BP), as well as the integrated and raw filtered muscle sympathetic nerve activity (MSNA) neurograms. The PVC triggered a fall in diastolic blood pressure and a large post-PVC burst of MSNA compared with bursts that preceded the PVC in both groups.

Figure 6 illustrates the occurrence of postganglionic sympathetic APs for each AP cluster as a function of burst size between sinus rhythm bursts and the post-PVC burst. This visual representation illustrates the presence of smaller and medium sized APs in sinus rhythm bursts but the higher probability of a large AP subpopulation during the post-PVC burst.

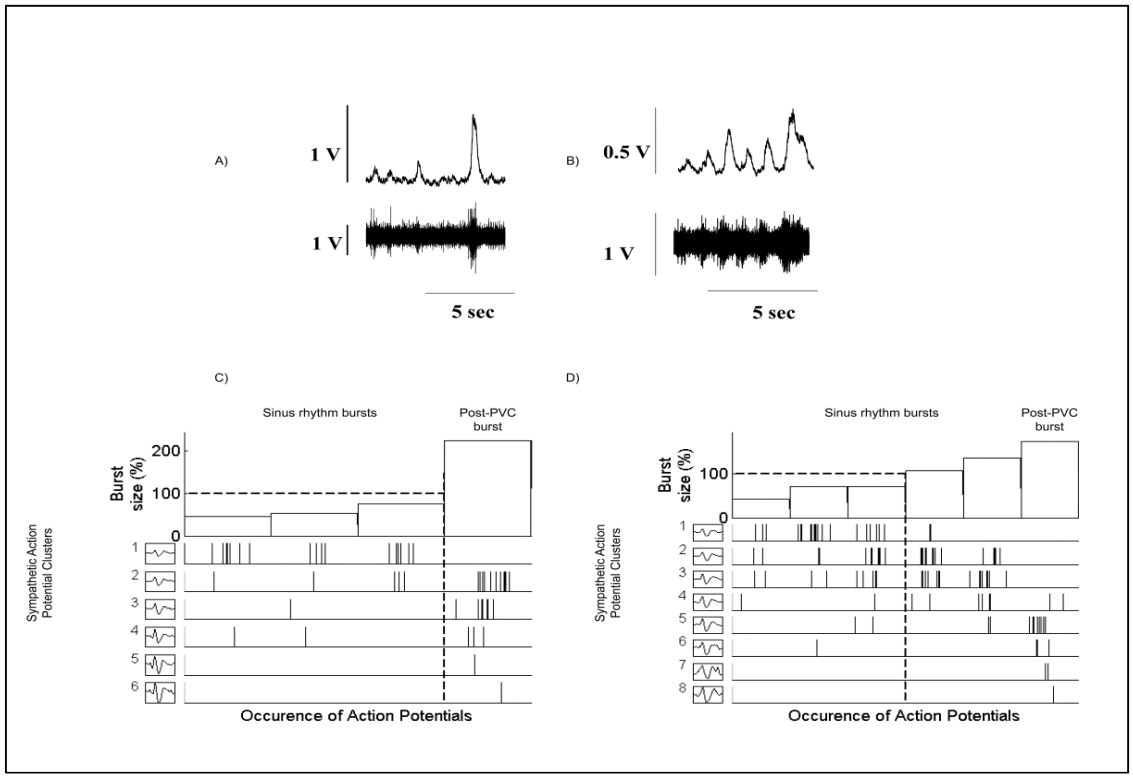

Figure 6. Occurrence of postganglionic sympathetic APs for each AP cluster as a function of burst size between sinus rhythm bursts and the post-PVC burst in control subject (plots A and C) and in chronic heart failure subject (plots B and D). Plots A and B provide the integrated and filtered raw muscle sympathetic nerve activity (MSNA) tracings of the respective sinus rhythm and post-PVC bursts used to display the range of integrated sympathetic bursts ordered by burst size as a percentage of baseline (*C* and *D*, top panels) together with the occurrence of postganglionic sympathetic action potentials as a function of integrated burst size for each action potential cluster (C and D, lower panels). Dashed lines represent the mean of integrated burst sizes (horizontal) and corresponding action potential cluster range (drop lines). In both subjects, clusters of larger amplitude are predominately recruited in the post-PVC burst.

Main effects of group *($p<0.001$)* and burst type *($p<0.001$)* were observed for AP/burst (Fig 7). In a point-wise contrast, CHF group (17±8 APs/burst) had greater AP content per burst during sinus rhythm bursts than controls (9±3 APs/burst; *$p<0.05$*; Fig 7). Also, the sinus rhythm burst AP frequency was greater in CHF (14±8 AP/s) compared with controls (7±2 AP/s; *$p<0.05$; Fig 7*). This contrast formed the basis of an interaction effect between group and burst type for AP frequency ($F(1,140) = 7.642$ *$p<0.01$).* During sinus rhythm bursts, the number of active clusters per burst was greater in CHF patients (5±1 clusters/burst) compared with controls (3±1 clusters/burst; *$p<0.05$*; Fig 7).

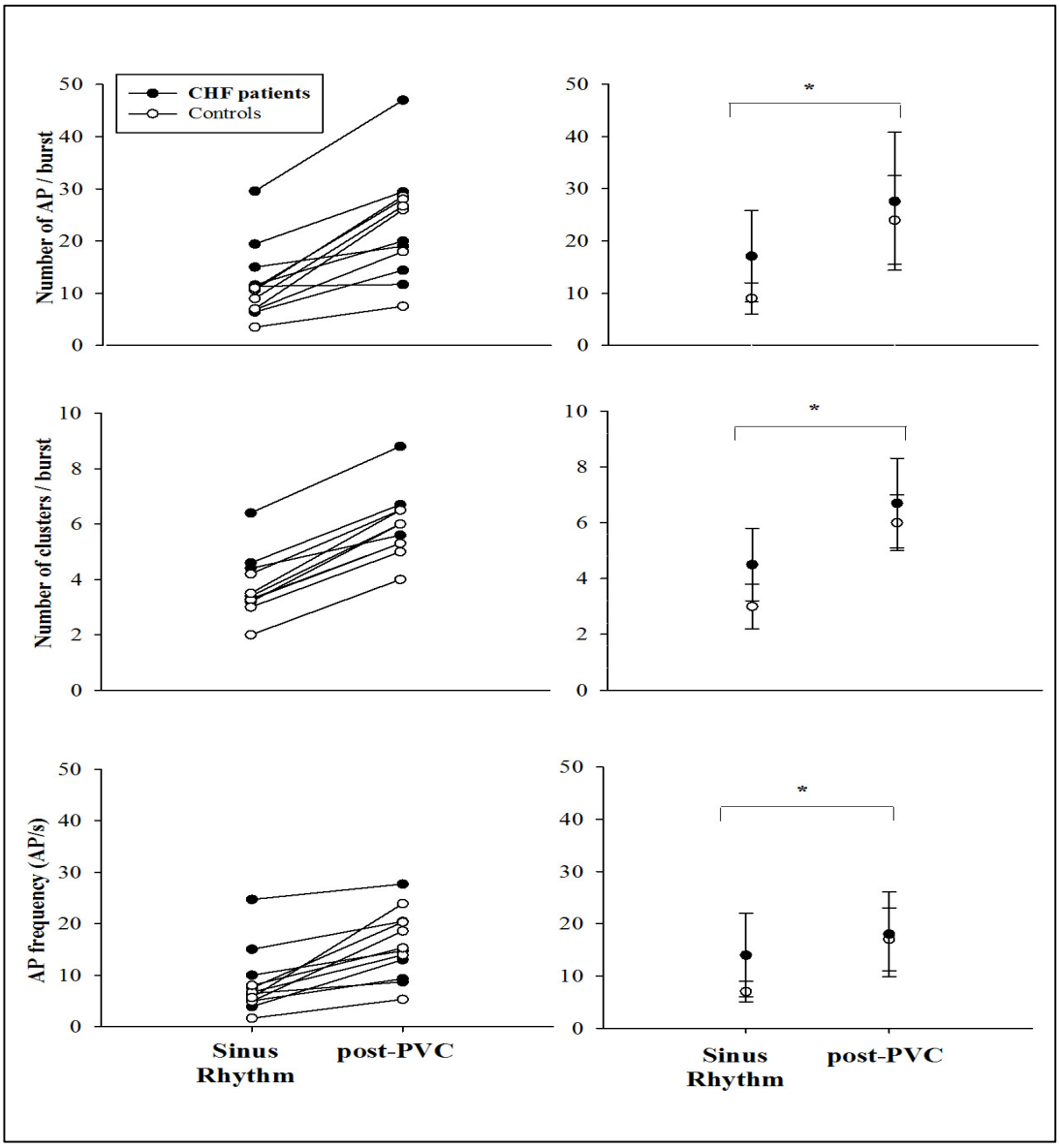

Figure 7. Action potential (AP) characteristics in sinus rhythm and post-PVC bursts in CHF patients (*closed circles*) and in controls (*open circles*). Left panels illustrate data for each individual. Right panels show mean ± standard deviation data.*,$p<0.05$ main effect for burst type.

The increase in AP/burst between sinus rhythm and post-PVC bursts was less in CHF patients (10±8 AP/burst) than in controls (15±7 AP/burst $p<0.01$) (Fig 8). Similarly, the change in AP frequency between sinus rhythm and post-PVC bursts was less in CHF (4±6 AP/s) compared to the control group (10±5 AP/s, $p= 0.01$). The increase in number of active AP clusters on going from sinus rhythm to post-PVC bursts was the same between CHF and Control (Fig 8).

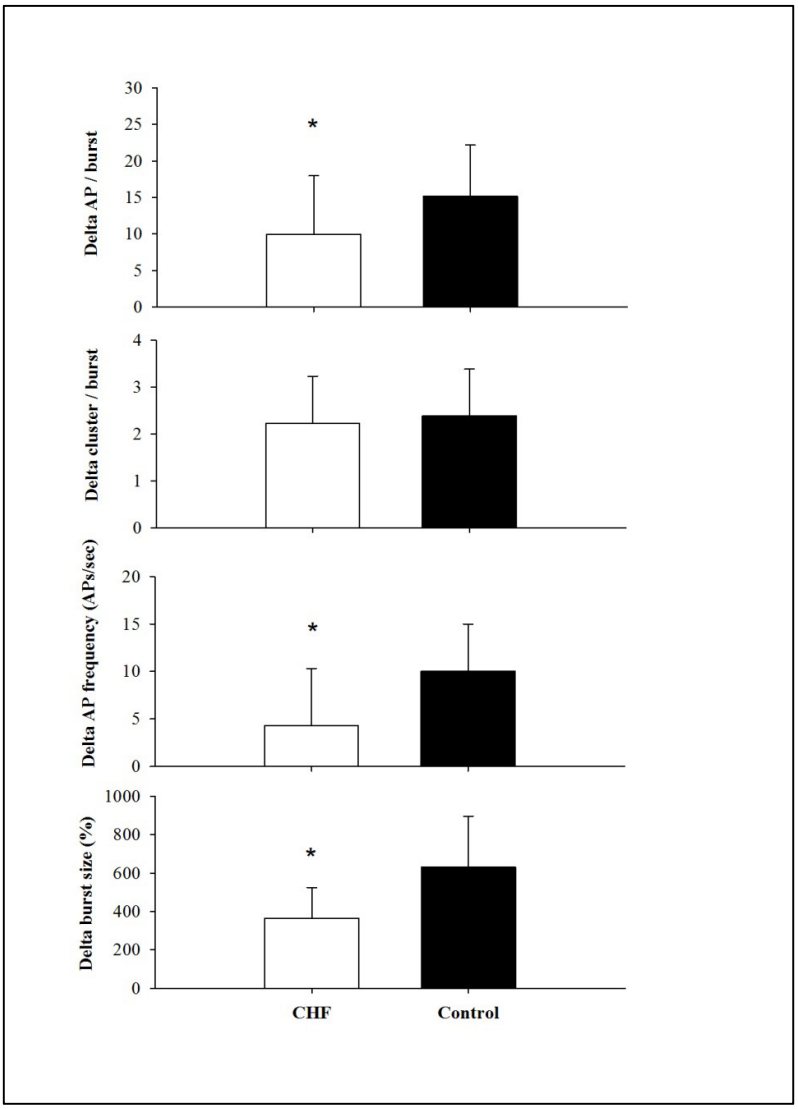

Figure 8. Histograms representing increase in AP variables in CHF patients (open bars) and in controls (solid bars). Delta, difference between AP parameters in post-PVC burst and in sinus rhythm bursts. Values are expressed as means±SD.
* $p<0.05$ compared with controls.

Figure 9 represents the distributions of sinus rhythm bursts and post-PVC bursts in terms of their unique AP clusters (based on their peak-to-peak amplitude). Both groups exhibited a rightward shift in the distribution towards larger APs in the post-PVC burst in comparison to sinus rhythm.

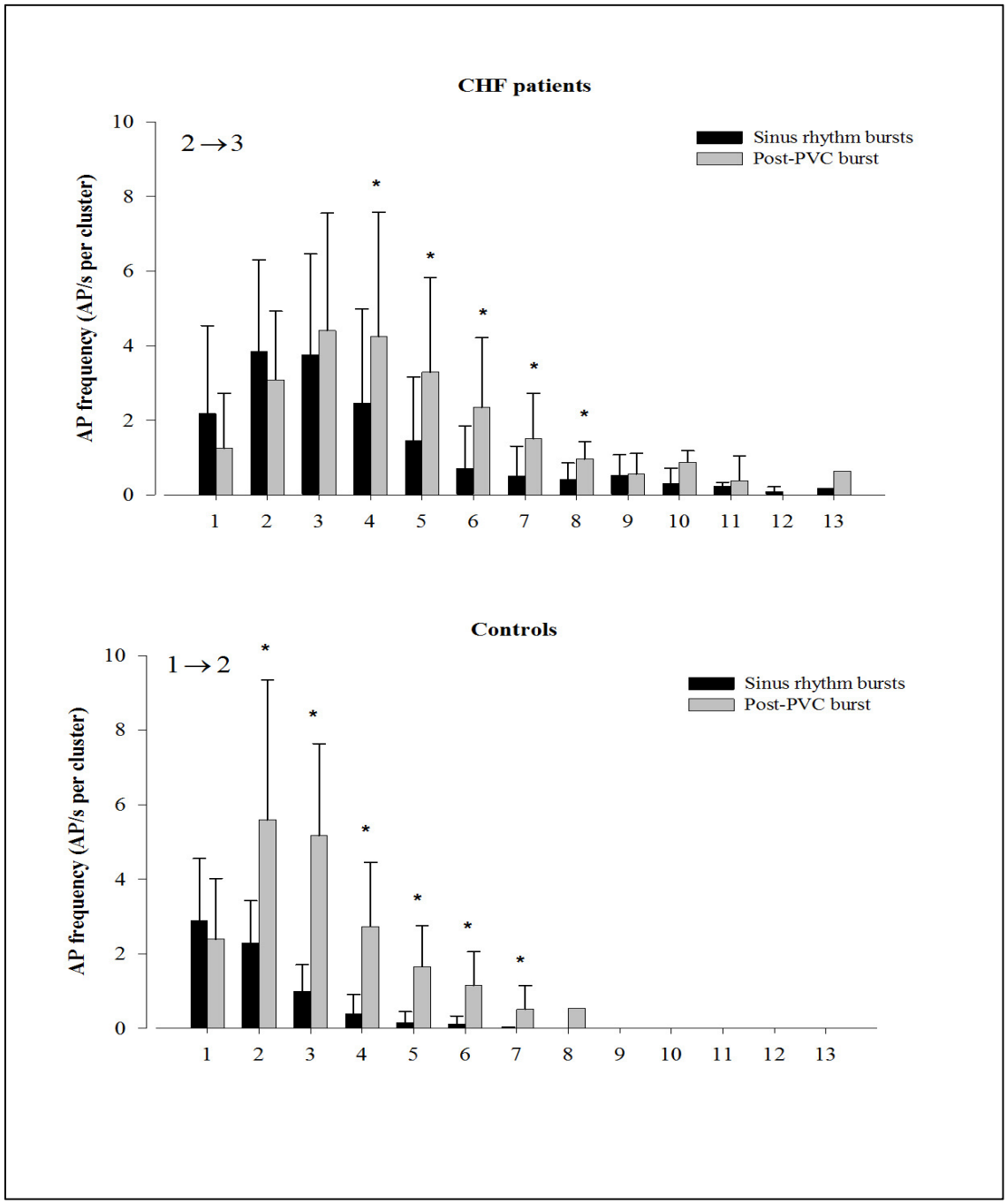

Figure 9. Histograms representing individual differences in AP frequency (AP/s) per cluster of sympathetic fibers between sinus rhythm bursts and post-PVC bursts in CHF patients and in controls. Values are expressed as means±SD. Numbers on graphs indicate changes in Mode in AP clusters from sinus rhythm bursts to post-PVC burst.*, $p<0.05$ compared with sinus rhythm bursts.

Compared with controls (72±8mmHg) CHF patients had a lower sinus rhythm DBP (61±9 mmHg; $p=0.01$). Despite an initially lower DBP in CHF patients, PVCs caused a similar DBP fall in both groups (13±5 vs.14±8 mmHg, CHF vs. controls, respectively). RR

interval was similar between the groups during sinus rhythm (0.85±0.09 sec vs. 0.8±0.05 sec *CHF* vs. controls, respectively) but the PVC-induced RR interval was longer in CHF than in controls (1.29±0.2 sec vs. 1.08±0.2 sec, $p=0.01$, CHF *vs.* controls, respectively) as was the increase in RR interval duration from sinus rhythm to PVC (0.43±0.2 sec vs. 0.28±0.16 sec, $p<0.05$, CHF vs. controls, respectively).

5.2. Study II

The anthropometric and clinical characteristics of the subjects participating in the Study II are listed in the Table 2.

Table 2. Anthropometric and clinical characteristic of the Study II

	CHF patients (N=7)	*Controls (N=8)*
Anthropometrics		
Age (yr)	61 ± 9	53 ± 7
Gender male/female	5 / 2	6 / 2
Height (cm)	173 ± 0.1	180 ± 0.1
Weight (kg)	88 ± 12	78 ± 13
BMI	29 ± 3*	24 ± 3
pro-BNP (pmol/l)	84.7 ± 39	
Spirometry		
VC (% predicted)	89 ± 7*	111 ± 7
FEV_1 (predicted)	87 ± 12*	108 ± 7
Echocardiography		
LVEF (%)	35 ± 4	-
LVDd (mm)	75 ± 9	-
NYHA class		
I	1	-
II	5	-
III	1	-
Etiology		
Dilated cardiomyopathy	3	-
Coronary artery disease	3	-
Idiopathic	1	
Pharmacotherapy		
ACEi	5	-
AT1 receptor blockers	1	-
Diuretics	7	-
Digitalis	1	-
β- blockers	4	-
Nitrates	2	

Values are mean±SD. * *p<0.05* different from controls. BMI indicates body mass index; pro-BNP, pro brain natriuretic peptide; VC, vital capacity; FEV_1, forced expiratory volume in one second; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic diameter; NYHA, New York Heart Association; ACEi, angiotensin converting enzyme inhibitor; AT1 receptor blockers, blockers of type 1 receptors of angiotensin II.

As expected, CHF group had significantly higher integrated MSNA burst frequency and higher burst incidence at baseline (Table 3).

Table 3. Difference in baseline sympathetic parameters between groups.

	CHF patients (N=7)	***Controls (N=8)***
Burst frequency (burst / min)	55 ± 9 *	33 ± 8
Burst incidence (burst / 100 heart beats)	85 ± 11*	48 ± 11
Mean burst area / min	5 ± 1*	2 ± 1
AP frequency (APs / min)	746 ± 366*	263 ± 116
AP incidence (APs / 100 heart beats)	1138 ± 559*	364 ± 164
AP/burst	13 ± 5	8 ± 2
Distinct clusters / burst	5 ± 1*	4 ± 0.7

Values are mean±SD. * $p<0.05$ different from controls.

Furthermore AP detection analysis exposed a three-fold higher AP frequency and AP incidence in the CHF patients when compared to controls *($p<0.05$)*. Compared with Control, total MSNA activity, expressed as mean burst area/min, was greater by 150% in CHF patients *($p<0.05$)*. Except for mean arterial pressure (MAP) and SBP which were by ~14 mmHg and by ~27 mmHg higher in controls than in CHF patients *($p<0.05$)*, the groups did not differ in DBP, HR, SV, CO, V_T, Bf and V_E at baseline (Table 4).

Table 4. Hemodynamic and ventilatory responses to CPAP 5 and 10 cmH_2O.

Hemodynamic and ventilatory parameters		*CHF patients (N=7)*						*Controls (N=8)*					
		Baseline	*CPAP 5 cm H_2O start*	*CPAP 5 cm H_2O end*	*CPAP 10 cm H_2O start*	*CPAP 10 cm H_2O end*	*Recovery*	*Baseline*	*CPAP 5 cm H_2O start*	*CPAP 5 cm H_2O end*	*CPAP 10 cm H_2O start*	*CPAP 10 cm H_2O end*	*Recovery*
MAP (mmHg)	mean	84.1†	82.8	86.4	84.0	83.3	84.4	97.8	100.2	101.8	102.3	102.9	99.7
	SD	12	11	15	12	13	14	8	9	9	7	8	9
SBP (mmHg)	mean	122.5†	135.5*	135.0*	131.6*	130.1*	133.5	148.0	153.5	156.2	158.7	157.5	150.1
	SD	21	26	23	16	15	26	19	23	23	21	20	23
DBP (mmHg)	mean	63.4	66.2	65.4	64.8	63.8	64.8	71.1	74.7*	75.2	77.1*	77.4*	73.2
	SD	8	12	13	13	13	11	7	6	5	4	6	6
SV (ml)	mean	94.5	91.1	90.1	92.1	93.6	95.8	111.0	101.7*	102.1*	96.5*	95*	105.5
	SD	19	22	23	23	25	22	17	14	10	12	12	14
HR (bpm)	mean	64	65	63	68	68	64	67	66	66	65	66	65
	SD	7	8	7	5	6	7	8	7	7	7	12	7
CO (l/min)	mean	6.1	5.9	5.7	6.1	6.3	6.1	7.5	6.7	6.7*	6.2*	6.2*	6.8
	SD	2	1	2	1	2	1	2	1	1	1	1	1
V_T (ml)	mean	640	700	800	951	980	520	825	1274*	1177	1358*	1155*	769
	SD	382	220	550	556	560	392	106	385	407	277	322	116
Bf (bpm)	mean	16	16	16	19	16	19	12	13	15*	16*	16*	12
	SD	6	7	7	9	10	8	3	3	3	4	4	3
V_E (l/min)	mean	8.8	10.1	10.4	14.8	12.7	8.1	10.0	16.7*	16.8*	20.7*	7.8*	9.9
	SD	3	3	5	5	5	4	2	4	5	6	4	3

MAP indicates mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; HR, heart rate; CO, cardiac output; V_T, tidal volume; Bf, breathing frequency; V_E, ventilatory volume. *$p<0.05$ different from baseline, † $P<0.05$ different from controls.

Hemodynamic Response to CPAP. Both levels of CPAP, 5 and 10 cmH_2O, did not influence DBP, HR, SV or CO in the CHF patients, but caused an immediate increase in SBP at 5 cmH_2O that was sustained during the start and end of the 10 cmH_2O level (Table 4). Control group demonstrated an abrupt decrease in SV and CO with 5 cmH_2O of CPAP which was sustained during both measurement points of CPAP 10 cmH_2O ($p<0.05$). In contrast, DBP increased at the beginning of CPAP 5 cmH_2O and then rose again during the 10 cmH_2O of CPAP ($p<0.05$). SaO_2 did not change with CPAP in either group. In both groups, all hemodynamic parameters returned to baseline values during recovery after cessation of CPAP.

Ventilatory Response to CPAP. 5 cm H_2O of CPAP caused a 449 mL augmentation of V_T in control subjects (Table 4) that was maintained through the 10 cmH_2O of CPAP ($p<0.05$). Changes in V_E in healthy subjects followed a similar trend during CPAP. Bf was increased at the end of level 5 cmH_2O of CPAP and continued to be increased during level 10 cm H_2O ($p<0.05$). All ventilatory parameters returned to baseline levels during recovery. In contrast to the control group, ventilatory parameters were not modified by CPAP in the CHF patients, although V_T tended to be increased during 10 cmH_2O of CPAP compared to baseline ($p=0.07$). SaO_2 remained unchanged in both groups during CPAP breathing.

Sympathetic Nervous Response to CPAP. A main effect of group (CHF vs. controls) was observed at baseline for burst frequency, burst incidence, mean burst area/min, AP firing frequency, and AP incidence ($p<0.05$). In the control group, compared to baseline, integrated MSNA burst frequency was increased by ~18 % and burst incidence by ~27 % at the beginning of 10 cmH_2O of CPAP and remained elevated until the end of CPAP ($p<0.05$) (Figure 10).

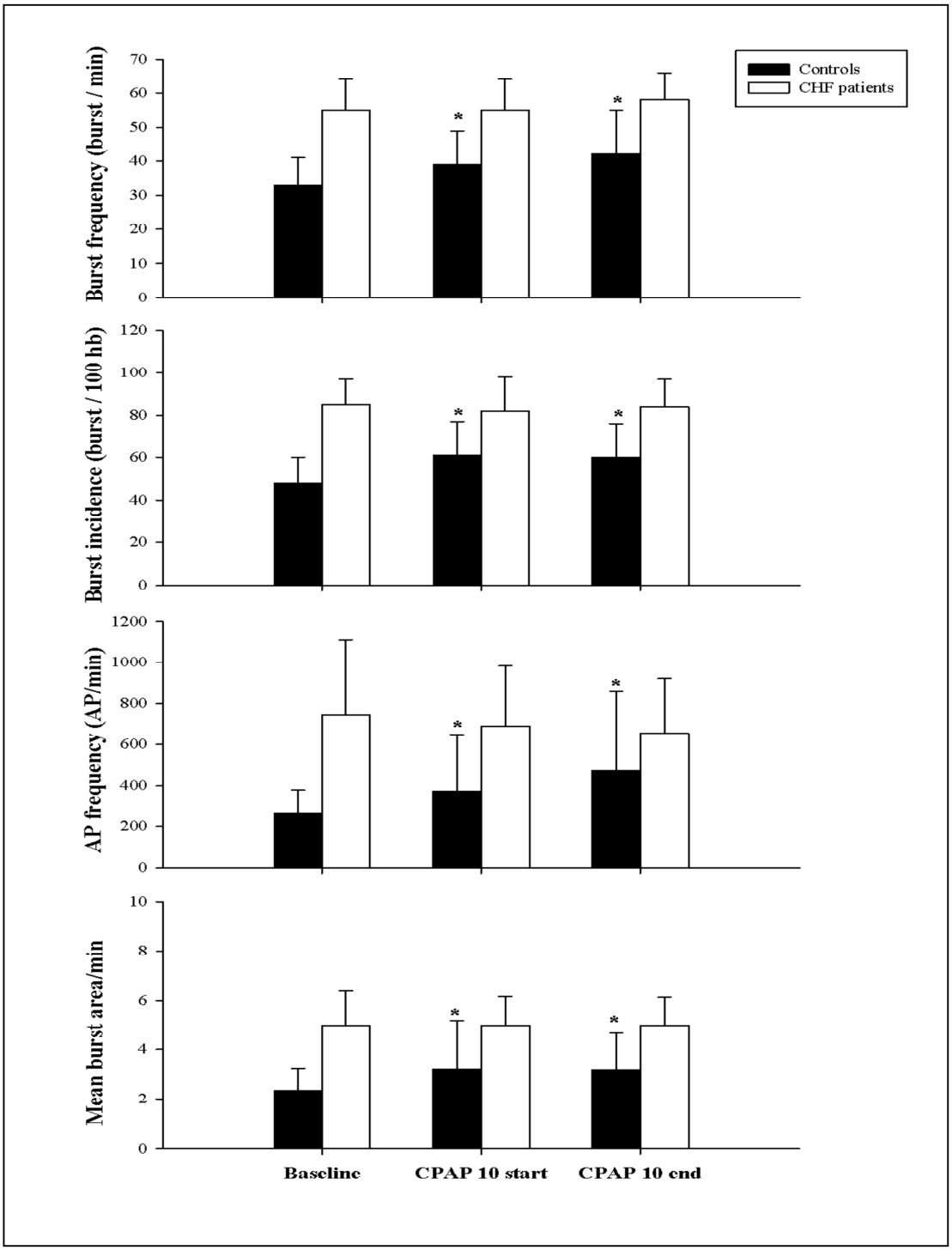

Figure 10. Multi-unit MSNA and action potential (AP) firing frequency during baseline, beginning (start) of the CPAP 10 cm H_2O and at the end of CPAP 10 cm H_2O in healthy subjects *(dark bars)* and in chronic heart failure (CHF) patients *(light bars)*. Values are mean±SD. *, $p<0.05$ compared to controls.

In both groups the overall number of distinct clusters of APs was similar during CPAP as during baseline breathing. Original recordings of the integrated and filtered MSNA data at baseline (panels A) and during CPAP 10 cmH_2O (panels B) are shown for one healthy subject

in Figure 11 and for one CHF patient in Figure 12. The occurrence of individual AP clusters as a function of burst amplitude at baseline (panel C) and during CPAP (panel D) in one healthy middle aged subject is shown in Figure 2 and in one CHF patient is shown in Figure 3. In the healthy subject, AP clusters of sympathetic neurons present at baseline increased firing frequency during CPAP, while the number of different clusters of sympathetic neurons was unaltered with CPAP (6 AP clusters at baseline and 6 during CPAP breathing). As shown in Figure 12, sympathetic pattern remained unaltered in CHF patients while on CPAP.

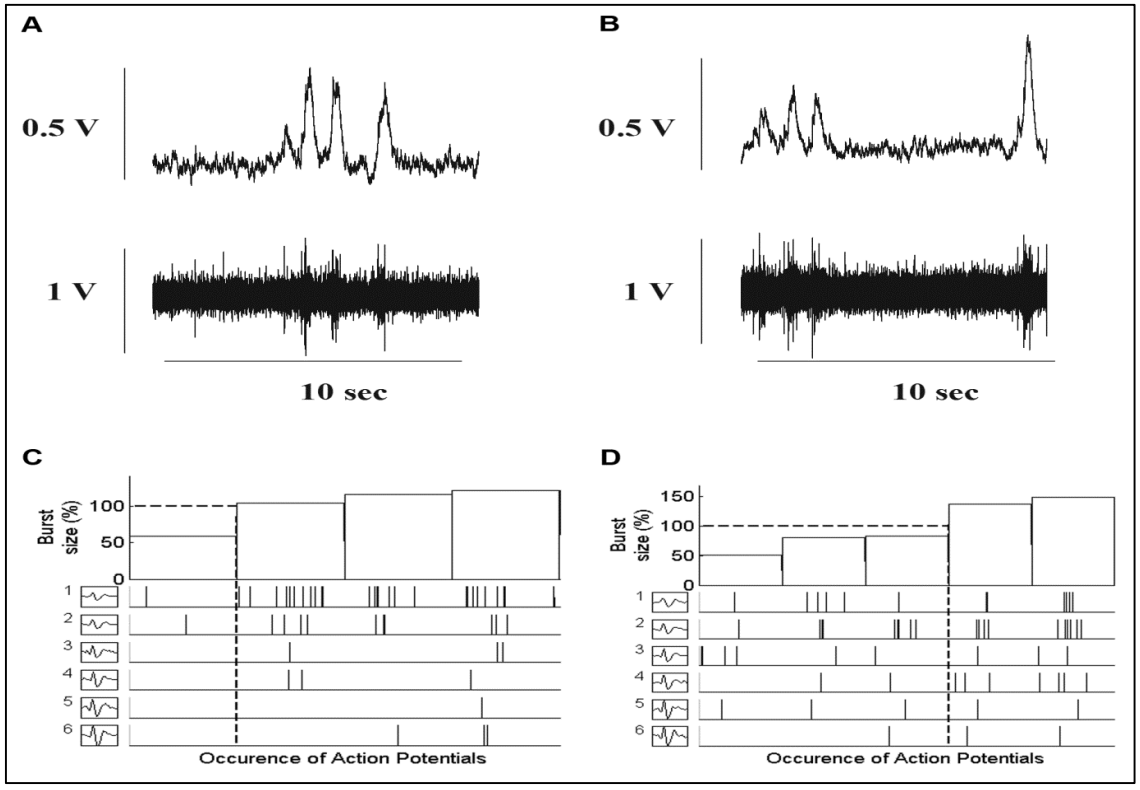

Figure 11. Occurrence of postganglionic sympathetic APs for each AP cluster as a function of burst size between baseline (plots *A* and *C*) and during the CPAP (plots *B* and *D*) in one healthy control subject. Plots A and B provide the integrated and filtered raw muscle sympathetic nerve activity (MSNA) tracings during baseline and CPAP breathing used to display the range of integrated sympathetic bursts ordered by burst size as a percentage of baseline (*C* and *D*, top panels) together with the occurrence of postganglionic sympathetic action potentials as a function of integrated burst size for each action potential cluster (*C* and *D*, lower panels). Dashed lines represent the mean of integrated burst sizes (horizontal) and corresponding action potential cluster range (drop lines). Note the same number of different clusters present at baseline and during the CPAP breathing (6).

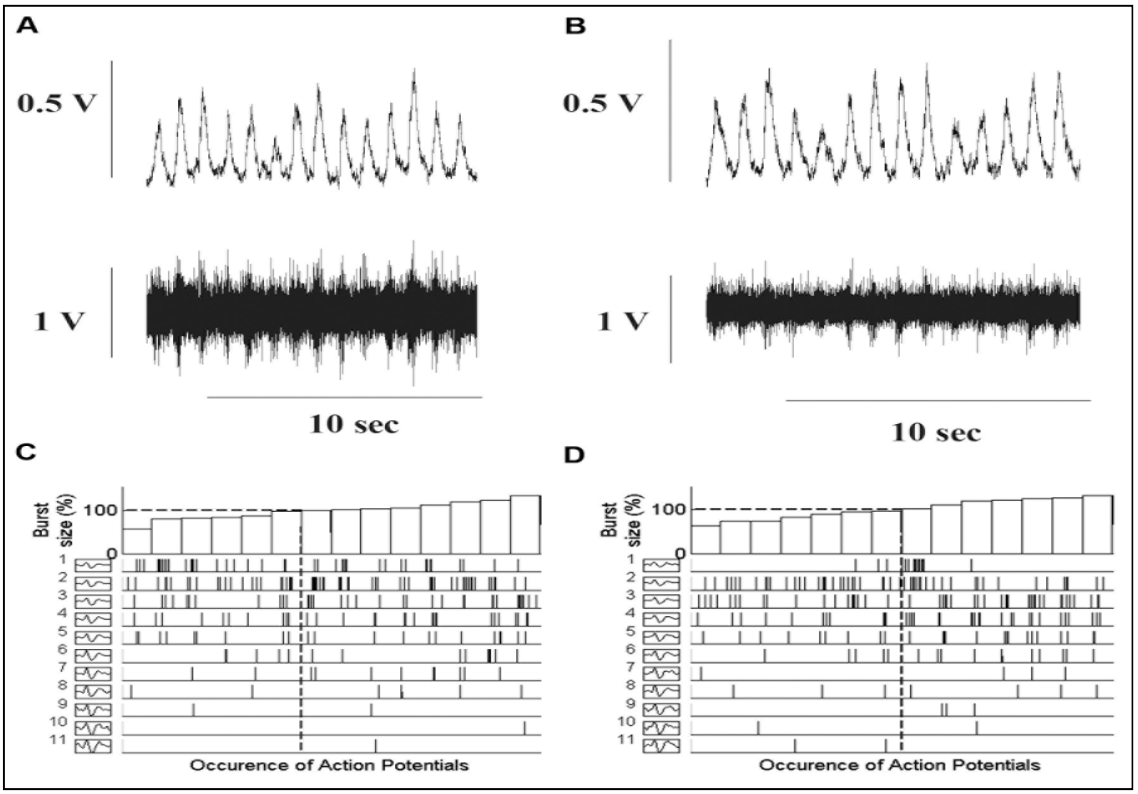

Figure 12. Occurrence of postganglionic sympathetic APs for each AP cluster as a function of burst size between baseline (plots *A* and *C*) and during the CPAP (plots *B* and *D*) in one CHF patient. Plots A and B provide the integrated and filtered raw muscle sympathetic nerve activity (MSNA) tracings during baseline and CPAP breathing used to display the range of integrated sympathetic bursts ordered by burst size as a percentage of baseline (*C* and *D*, top panels) together with the occurrence of postganglionic sympathetic action potentials as a function of integrated burst size for each action potential cluster (*C* and *D*, lower panels). Dashed lines represent the mean of integrated burst sizes (horizontal) and corresponding action potential cluster range (drop lines). Note the same number of different clusters present at baseline and during the CPAP breathing (11).

Association of sympathetic and hemodynamic responses to CPAP. The medium sized linear correlations between sympathetic and hemodynamic responses to CPAP were observed in controls, but not in CHF patients (Table 5); the greatest were between CO and burst incidence (r=-0,68; p=0.01), CO and mean burst area (r= -0,58; p =0.01) and DBP and burst incidence (r=-0,6; p=0.01). Adjustment for mutual association of hemodynamic variables diminished theses associations. Notably, since the heart rate was practically unaffected, to avoid over adjustment, the stroke volume and cardiac output were not mutually controlled for.

Table 5. Linear correlation coefficients (p-values) between muscle sympathetic nerve activity (MSNA) and hemodynamic variables during CPAP performed in healthy individuals.

MSNA parameters	**DBP**		**SV**		**CO**	
	Raw	**Adjusted[#]**	**Raw**	**Adjusted[†]**	**Raw**	**Adjusted[†]**
Burst incidence	-0.6 (0.01)	-0.05 (0.8)	-0.69 (0.02)	-0.43 (0.09)	-0.68 (0.01)	-0.45 (0.07)
Burst frequency	-0.41 (0.09)	-0.16 (0.5)	-0.4 (0.1)	-0.09 (0.7)	-0.38 (0.12)	-0.13 (0.6)
Mean burst area / min	-0.43 (0.79)	-0.11 (0.68)	-0.52 (0.032)	-0.32 (0.22)	-0.58 (0.01)	-0.42 (0.9)
AP frequency	-0.26 (0.3)	-0.17 (0.53)	-0.29 (0.25)	-0.12 (0.64)	-0.36 (0.15)	-0.25 (0.33)
AP/burst	-0.09 (0.71)	-0.05 (0.84)	-0.17 (0.49)	-0.19 (0.46)	-0.29 (0.24)	-0.33 (0.2)

Data are from 7 healthy subjects (dummy variables) during baseline, CPAP 10 cmH_2O start and CPAP 10 cmH_2O end. DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output. [#]; adjusted for stroke volume and cardiac output [†]; adjusted for diastolic blood pressure

6. DISCUSSION

6.1. Study I

A novel finding of the Study I is the fact that despite higher number of APs per burst, higher AP firing frequency and more active clusters of sympathetic neurons during sinus rhythm, CHF patients retain the ability to further increase AP content during post-PVC burst through recruitment of additional neurons. AP content of the post-PVC burst has increased in both groups, but to a greater extent in controls than in CHF patients. However, similar increase in the number of active clusters of sympathetic neurons within post-PVC burst was observed in both groups. These results suggest that sympathetic nervous system reserve in moderate CHF patients is preserved through the recruitment of additional sympathetic neurons.

Although the central mechanisms determining sympathoexcitation in CHF are not known, they probably reflect the net balance and interaction between augmented excitatory and diminished inhibitory influences (33). The lower DBP during sinus rhythm in CHF group may contribute to the greater overall SNA in CHF patients through the baroreflex pathway. Given the critical role of the baroreflex in sympathetic inhibition, the long pause and decay in DBP that follows a PVC should result in increased sympathetic burst amplitude, duration and area (32). As reported earlier (31), augmentation of sympathetic burst size and duration occurs in response to PVC in CHF patients. However, the change in burst size was lower in CHF group compared with controls in the current study largely due to smaller overall AP content, not the ability to recruit larger APs. Yet, the post-PVC fall in DBP was the same in CHF and control participants. Thus, these CHF patients demonstrated an attenuated ability to reflexively increase sympathetic outflow. While this difference cannot be explained mechanistically in the current study, one might investigate the possibility that heightened arterial stiffness in CHF impairs the baroreflex sensitivity to a fall in DBP (47).

Several mechanisms have been described to explain short term sympathoexcitatory responses in humans (20;48). These include an increase in the frequency of sympathetic neurons that are already active producing a higher firing probability across bursts, repeated firing of the same neuron within the same burst, and the recruitment of additional, previously silent neurons(22;25;38). Previously, Macefield and colleagues (18;20;37;38) demonstrated the increased firing probability of particular single neurons in CHF patients, both across and within bursts. These investigators used a single-unit recording method to quantify sympathetic

outflow, a method which assesses the firing frequency of a single sympathetic fiber over time. While this single unit approach suggests whether or not a neuron becomes more or less active, it cannot address the question about latent populations of sympathetic neurons that express a low firing probability and/or become active in response to physiological stress such as the PVC. The approach used in the current study, while unable to track the firing patterns of single axons, emphasizes the patterns of activity in all APs comprising the burst, with information regarding the size of each AP and the appearance of new and larger APs on a burst-by-burst basis. The potential problem of complete overlap and summation of concurrent spikes has been established in this approach (17), being <1% and apparent summated APs are deleted. Thus, the method offers the opportunity to observe the presence of different sizes of APs and their firing probabilities. Large axons have larger APs and this principle has enabled the observations in healthy individuals, that larger bursts often contain larger APs that are not present in smaller bursts and the increased probability of their recruitment during severe chemoreflex (22) or baroreflex (27) stress.

During sinus rhythm, the CHF group had higher total AP frequency compared to controls. Higher AP frequency in CHF patients was caused by higher burst incidence and also by a greater number of APs within a single burst. Moreover, sinus rhythm bursts of MSNA in CHF patients contained a greater number of active APs clusters. Therefore, both a greater frequency of integrated bursts and an increased AP content within each burst, contribute to the heightened baseline sympathetic outflow in CHF. Previous observations that the high number of APs in sinus rhythm bursts of healthy individuals is strongly related to a baroreflex mechanism (24) supports the fact outlined above - that lower DBP during sinus rhythm may contribute to the larger number of APs per burst and active clusters in CHF patients than in controls. In as much as the number of APs determinates the size of an integrated burst, different central mechanisms are proposed to affect burst occurrence or frequency (gating) vs. size (49), these data indicate that CHF patients produce aberrations in both features of sympathetic control, such that more efferent APs per burst and bursts per min are emitted.

CHF patients exhibited lower level of an overall increase in AP content within post-PVC burst than controls when compared to sinus rhythm. One part of an increase in AP content in the post-PVC burst is repeated firing of the same sympathetic neuron (38). The other part of additional APs within the post-PVC burst is activation of larger AP clusters that were not observed frequently during sinus rhythm bursts. Thus, neurons that were normally latent during sinus rhythm bursts were recruited during the post-PVC burst. Importantly, inspection of Figure 9 indicates that heart failure had a larger effect on the ability to increase

APs/burst but not AP clusters. These data indicate that the ability to recruit previously latent neurons was not affected by CHF. Rather, and by inference, the reduced ability to increase the integrated size and AP content of a post-PVC burst might be due to an attenuated ability to enhance further the repeated firing of already-active neurons.

CHF impairs arterial baroreflex regulation of HR but not MSNA burst frequency (33;50). Also, a normal increase in total body norepinephrine spillover was observed in CHF patients during sodium nitropruside infusion (33;51). The fall in DBP following PVC provides an analogous baroreflex hemodynamic stress indicated by the lack of post-PVC burst in one patient in whom DBP did not fall following the PVC (data not shown). However, the additional analysis of burst size and AP content provides information not apparent when the frequency of bursts in the integrated MSNA signal forms the sole basis of baroreflex sensitivity. Certainly, a burst occurs during a post-PVC period if DBP falls. But, the size of the burst represents the number of APs contributing to the integrated signal (52). In this regard, the current data expose an element of potential baroreflex dysregulation of MSNA control in CHF. CHF patients started with higher AP parameters during sinus rhythm bursts and, thereby, a ceiling effect may be expressed that limits the overall increase in total sympathetic outflow for the same fall in DBP.

The CHF patients retain their standard pharmacological treatment during the study, and this practice may interfere with MSNA. We choose this strategy to avoid rebound cardiovascular responses and associated baroreceptor-mediated effects on sympathetic nerve traffic and also to maintain comparative consistency with previous work in this population (34;37;53). Second, microneurography measures peripheral sympathetic outflow toward leg muscles and may not reflect cardiac sympathetic drive. However, cardiac norepinephrine spillover correlates with muscle sympathetic nerve traffic and increases in patients with major ventricular arrhythmias (54).

A clinical significance of the revealing AP recruitment patterns in heart failure lies in correlation between MSNA and cardiac norepinephrine spillover (55) and the mechanistic basis of sudden death after periods of frequent ectopic beats (56). A chronic elevation in sympathetic outflow may be arrhythmogenic, decreasing the threshold for ventricular fibrillation (36;57). Better understanding of sympathetic firing strategies may elucidate the underlying mechanisms of cardiac sudden death that commonly occurs in CHF patients. The current observations that CHF produces aberrations in both integrated burst frequency and in AP recruitment within each burst suggest that central control of these two discharge features are modified in CHF.

6.2. Study II

Study II reported for the first time difference in sympathetic firing strategies between healthy individuals and CHF patients in a response to CPAP application. In healthy middle-aged individuals short-term application of CPAP caused a reduction of SV and CO and corresponding augmentation of MSNA burst incidence and AP firing frequency. However, CPAP did not cause activation of latent, larger subpopulation of sympathetic fibers that are reserved to be active at higher levels of physiological stress. In contrast, short term application of CPAP did not alter MSNA or hemodynamics in CHF patients.

Recordings of multi-unit MSNA after long-term CPAP use in patients with OSA have shown a decrease in awake sympathetic overactivity (42). However, the firing pattern of sympathetic fibers' response to CPAP use is still not clarified. To date, studies of acute effects of CPAP application on SNS activity in CHF patients have solely reported multi-unit MSNA recordings and these authors have come up with different conclusions on CPAP effect on MSNA in CHF patients. While some of those previous studies have reported that acute CPAP use elicited MSNA in CHF patients through unloading of the cardiopulmonary baroreceptors (45) others have shown a selective decrease in cardiac sympathetic activity with positive expiratory airway pressure (58), and Naughton and colleagues found no change in sympathetic activity with CPAP use in CHF population (41).

Information regarding firing strategies of sympathetic neurons during various cardiovascular and respiratory stimuli in CHF patients is also very limited. This is the first study to report the activation pattern of postganglionic sympathetic neurons during CPAP in CHF patients and in healthy middle-aged individuals. An important contribution to our understanding of sympathetic firing strategies in healthy individuals and in CHF patients was made by Macefield and colleagues (18;19) who used previously described single-unit method to record individual sympathetic fiber activity. However, this method cannot record firing activity of all sympathetic fibers contributing to a sympathetic burst and cannot identify latent reserve of sympathetic neurons that may become active in response to physiological stimuli such as hypotension, CPAP or low body negative pressure (LBNP) application.

To examine an impact of CPAP on sympathetic activity in CHF patients and in age- and gender-matched healthy individuals, we used the same method as in the Study I to detect and classify APs (17). This method allowed us to examine the pattern of activity of all sympathetic fibers within each multi-unit sympathetic burst. Moreover the AP detection

software enabled us to identify the appearance of new, larger APs within a given neurogram, reserved to become active only during cardiovascular or respiratory stimuli.

Hemodynamic effects of long-term and short-term use of CPAP have been extensively examined in the previous studies (40;59;60). A diminished VR and consequent decrease in CO are associated with CPAP, as well as with LBNP (61) (62). When applied in young healthy subjects, both conditions cause hemodynamic stress and baroreceptor unloading with increase in multi-unit MSNA (63;64). Results of the Study II demonstrate a linear correlation between sympathetic (burst incidence) and hemodynamic (CO and BP) responses in the group of healthy individuals. These findings are consistent with hypothesis that CPAP-induced fall in CO reflexively triggered the observed sympathetic responses in healthy subjects. The current results support earlier observations (63;64) and further extend the knowledge about CPAP effects on AP firing patterns in health and disease.

One of the questions addressed in the current study was whether or not such hemodynamic stress would be sufficient to recruit additional "subpopulations" of larger and faster-conducting APs. Previously, conditions of large hemodynamic stress such as -80 mmHg lower body suction or prolonged breath-hold demonstrated (22;27) such a reserve of efferent sympathetic axons. However, smaller stressors such as LBNP of -60 mmHg, in young healthy subjects elicited an increase in the multi-unit MSNA as well as AP firing frequency of the sympathetic neurons without recruiting new, additional clusters of sympathetic neurons (27). In the current study, breathing at 10 cm H_2O CPAP caused a decrease in CO by ~18 % and a corresponding rise in MSNA burst frequency which would be considered a neural response to a modest cardiovascular stimulus. In the Study II augmentation in AP firing frequency during CPAP in healthy subjects was mostly due to increased activity of already active clusters of sympathetic neurons without recruitment of new, larger sympathetic neurons. The same number of clusters of sympathetic neurons was active before and during CPAP with higher firing rate during CPAP as shown in Figure 11 C and D. Observations from the above-mentioned studies and from the current study suggest that recruitment of latent subpopulation of larger sympathetic neurons is probably reserved for conditions of higher sympathetic needs like severe orthostatic stress or chemoreflex challenge (22;25;27;65).

In our recent study we have shown that elevated baseline level of sympathetic discharge in CHF patients is achieved by an increase in both parameters: in the number of AP per burst and in the number of different active clusters of sympathetic fibers per burst. In the Study II the AP detection technique demonstrated ~ 3-fold higher AP frequency and AP incidence in

CHF patients' baseline neurograms if compared with controls. Higher AP firing rates were associated with higher multi-unit MSNA as well as higher number of distinct active clusters of sympathetic neurons. CPAP did not alter sympathetic firing activity in CHF group in a manner seen in healthy subjects, as central hemodynamics were not changed during CPAP in CHF group and there was no need to adjust BP.

When administrated nocturnally over a period of one month in CHF patients with OSA, CPAP lowers plasma norepinephrine spillover (66). This sympathoinhibitory response on CPAP use is most likely attributed to attenuation of apnea- related hypoxia and arousal from sleep. In contrast to these effects of long-term CPAP use, an opposite (45) or unchanged (41) effect on sympathetic outflow was reported during short-term CPAP use. The outcomes of the Study II also indicate that acute CPAP has no sympathoexcitatory or inhibitory effect in CHF patients. The likely explanation for unchanged MSNA in CHF patients relates to the unaltered CO in this group. An increased cardiac filling pressure characterizes CHF; thus, short positive pressure breathing with CPAP decreases left ventricular transmural pressure, unloads the inspiratory muscles and reduces myocardial oxygen demand without affecting CO (40;41). In these conditions there is no need for further augmentation of MSNA.

Another potential explanation for unaltered MSNA in CHF patients is that sympathoinhibitory effect of CPAP on pulmonary-stretch receptors (58) and stimulation of pulmonary vagal afferents (67) was counterbalanced with cardiopulmonary baroreceptor unloading and reflex sympathoexcitation (45). While V_T was increased in healthy subjects with CPAP, it remained unaltered in CHF patients. These findings are in line with previous studies using short-term CPAP in CHF patients (41;45;68), showing that brief use of CPAP affects cardiac filling and transmural pressures more than ventilatory parameters. In healthy subjects increased firing of sympathetic neurons during CPAP was likely associated with diminished CO that was not seen in our CHF patients. Therefore hemodynamic effect of short-term CPAP on sympathetic nerve activity seems to override the possible ventilatory sympathoinhibitory effect of increased V_T in healthy individuals.

This study emphasized the acute effects of CPAP on MSNA discharge, using the expected variations in cardiovascular stress between healthy control and CHF patients. Examination of the neurophysiologic basis of sympathoinhibition in CHF patients with prolonged CPAP treatment would require additional research. We decided to follow the same clinical approach regarding pharmacological therapy for the Study II as for the Study I meaning the pharmacological therapy of the CHF patients was preserved to avoid rebound cardiovascular responses and associated baroreceptor-mediated effects on sympathetic nerve

traffic. This allowed us comparative consistency with previous work in this population (37;67;68).

7. CONCLUSIONS

7.1. Study I

Despite chronically elevated sympathetic tone, stable CHF patients have the capacity to further increase sympathetic discharge through recruitment of an additional subpopulation of sympathetic fibers during transient, acute baroreflex unloading. However, CHF patients have shown to have limited ability to increase firing of the sympathetic fibers that are already active at baseline. Former statement is supported by evidence of smaller augmentation of AP firing frequency when compared to healthy controls.

7.2. Study II

A brief application of CPAP in healthy middle-aged individuals increases AP firing frequency of sympathetic neurons. However, there was no recruitment of additional sympathetic fibers that are known to be reserved for high physiological stress circumstances. These findings indicate previously unrecognized effect of CPAP on firing strategies of SNS in healthy middle-aged individuals.

In contrast to healthy individuals, short term application of CPAP in CHF patients had no effect on central hemodynamics that would require modifications in sympathetic outflow.

8. SUMMARY

8.1. Study I

Constant sympathetic overactivity is a well known hallmark of chronic heart failure (CHF) that tends to increase with disease severity. One of the common abnormalities of heart function associated with CHF is occurrence of premature ventricular contractions (PVCs) that tend to transiently decrease blood pressure (BP) and cause reflex increase in sympathetic discharge.

PVCs that often occur in these patients have been already recognized as a pronounced sympathoexcitatory stimulus that elicits formation of larger bursts of muscle sympathetic nerve activity (MSNA). However, firing pattern of activation of postganglionic sympathetic neurons in CHF patients and in healthy individuals is still poorly investigated.

Aim of the Study I in the present Doctoral Dissertation is to evaluate firing properties of postganglionic sympathetic neurons in CHF patients and healthy age- and gender- matched individuals. Second aim is to examine and compare the strategies of SNS activity in CHF patients and in healthy subjects as a response to PVC. PVCs were identified in both groups (CHF and controls) and sympathetic neurograms of sufficient signal-to-noise ratio were obtained using the microneurography.

Neurograms of 6 CHF patients and 6 healthy controls were analyzed using action potential (AP) detection software that enables identification of individual sympathetic APs and their amplitude-size classification into different clusters. During sinus rhythm, CHF patients had greater number of APs per burst, higher AP firing frequency and higher number of active clusters of sympathetic neurons compared to healthy controls. PVCs caused an increase in AP firing frequency and in the number of active clusters. However, compared with controls, an increase in burst integral, AP firing frequency, and APs per burst was less in CHF patients. The PVC-induced increase in active clusters per burst was similar between the groups, suggesting that CHF patients retained the sympathetic reserve through the recruitment of larger APs but not through augmentation of already active sympathetic neurons.

8.2. Study II

Continuous positive airway pressure (CPAP) application is a novel therapy for patients with chronic heart failure (CHF), a condition often related to sleep disordered breathing. Favorable effects of CPAP include correction of respiratory breathing pattern, improvement in left ventricular function and enhanced exercise tolerance. The data on whether the benefits of CPAP application in CHF patients are direct consequence of ANS responses are still inconsistent. Moreover, firing pattern of sympathetic fibers during various respiratory stimuli in health as well as in heart failure remains to be elucidated.

The aim of the Study II is to assess the firing pattern of sympathetic fibers during CPAP application in CHF patients and in healthy age- and gender- matched controls.

Microneurography was used to measure muscle sympathetic nerve activity (MSNA) from 8 healthy middle aged individuals and from 7 CHF patients. The same AP detection software was used to extract action potentials (APs) from the recorded neurograms as for the purpose of the Study I. Extracted APs were quantified as AP firing frequency and classified into different clusters based on the size of their peak-to-peak amplitude. Ventilation and various hemodynamic parameters were measured as well. The protocol included CPAP application for 5 minutes at each level of 5 and 10 cmH_2O.

While on CPAP, stroke volume (SV) and CO (cardiac output) decreased whereas multi-unit MSNA, AP frequency and mean burst area/min increased in healthy middle aged subjects. In contrast, CPAP had no effect on hemodynamics, MSNA or AP parameters. A negative linear correlation was observed between sympathetic and hemodynamic responses to CPAP in control group, but the same was not observed for CHF group.

Consequently, it can be concluded that the impact of CPAP on central hemodynamics in healthy individuals elicited a moderate activation of sympathetic neurons through increased AP firing frequency, whereas in CHF patients both hemodynamics and MSNA remained unaltered.

9. SUMMARY IN CROATIAN (SAŽETAK)

9.1. Study I (Istraživanje I)

Kronično srčano zatajenje je karakterizirano trajno povećanom simpatičkom aktivnošću koja je pokazatelj stupnja težine bolesti. Jedna od poznatih abnormalnosti srčane funkcije u kroničnom srčanom zatajenju je učestala pojava prijevremenih ventrikulskih kontrakcija nakon kojih dolazi do naglog pada dijastoličkog tlaka te posljedično, refleksnog porasta simpatičke aktivnosti. Prijevremene ventrikulske kontrakcije su snažan simpatičko-ekscitacijski podražaj koji uzrokuje pojavu velikih izbijanja mišićne simpatičke aktivnosti mjerene metodom mikroneurografije. Detaljan obrazac aktivacije simpatičkih neurona koji svojim istovremenim okidanjem stvaraju povećana simpatička izbijanja nakon prijevremene ventrikulske kontrakcije, do danas nije u potpunosti poznat.

Prvi cilj Istraživanja I u ovoj doktorskoj disertaciji je istražiti i usporediti obrazac aktivacije post-ganglijskih simpatičkih neurona u bolesnika sa kroničnim,stabilnim srčanim zatajenjem i u zdravih kontrolnih ispitanika, koji po dobi i spolu odgovaraju ispitnoj skupini. Nadalje, drugi cilj ovog Istraživanja je utvrditi strategije odgovora simpatičkog živčanog sustava na nagli pad arterijskog tlaka uslijed prijevremene ventrikulske kontrakcije u bolesnika sa kroničnim, stabilnim srčanim zatajenjem te dobivene rezultate usporediti sa zdravim kontrolnim ispitanicima.

U obje skupine (ispitnoj i kontrolnoj) su prepoznate prijevremene ventrikulske kontrakcije, te su metodom mikroneurografije dobiveni zapisi mišićne simpatičke živčane aktivnosti. Dobiveni mikroneurografski zapisi 6 bolesnika sa kroničnim, stabilnim srčanim zatajenjem i 6 kontrolnih ispitanika su analizirani korištenjem računalne aplikacije koja omogućava identifikaciju pojedinačnih simpatičkih akcijskih potencijala (AP) iz neuralnog zapisa, te ih svrstava prema veličini u različite skupove.

Rezultati Istraživanja I su pokazali da, tijekom sinus ritma, bolesnici sa kroničnim, stabilnim srčanim zatajenjem imaju veći broj AP unutar pojedinog simpatičkog izbijanja, veću frekvenciju okidanja AP i veći broj aktivnih- različito velikih skupova simpatičkih neurona u usporedbi sa kontrolnim ispitanicima. Nadalje, zapažena je razlika između skupina u opsegu simpatičkog odgovora na pad arterijskog tlaka uslijed prijevremene ventrikulske kontrakcije, ali ne i razlika u mehanizmu postizanja povećane simpatičke aktivnosti u tom odgovoru. Prijevremene ventrikulske kontrakcije su uzrokovale porast u frekvenciji okidanja

AP i porast u broju aktivnih skupova simpatičkih neurona u obje skupine. Ipak, ukupan porast u veličini izbijanja, u frekvenciji okidanja AP i u broju AP unutar pojedinog izbijanja bio je manji u bolesnika sa kroničnim, stabilnim srčanim zatajenjem nego u kontrolnoj skupini. Prijevremene ventrikulske kontrakcije su uzrokovale sličan porast u broju različito velikih skupova simpatičkih neurona u obje skupine. Navedeni rezultati govore u prilog činjenici da bolesnici sa kroničnim, stabilnim srčanim zatajenjem imaju očuvan simpatički refleksni odgovor na stres i to kroz regrutaciju većih simpatičkih neurona, ali ne i kroz povećanu aktivnost simpatičkih neuron već aktivnih tijekom sinus ritma.

9.2. Study II (Istraživanje II)

Kronično srčano zatajenje je često povezano sa poremećajima disanja tijekom spavanja kao što su opstruktivna apneja u spavanju i periodično disanje (Cheyne- Stokes). Jedna od novijih ne-farmakoloških metoda liječenja ovih bolesnika je primjena kontinuiranog pozitivnog tlaka preko nazalne maske (engl. *Continuous Positive Airway Pressure, CPAP*) čija dugotrajna upotreba ispravlja patološki obrazac disanja, dovodi do porasta izbačajne frakcije lijeve srčane klijetke i poboljšava rezultate testa opterećenja. Uloga simpatičkog živčanog sustava u fiziologiji poboljšanja stanja ovih bolesnika uslijed primjene CPAP-a - još nije u potpunosti jasna. Nadalje, suprotstavljena su saznanja o utjecaju CPAP-a na obrazac aktivacije simpatičkog živčanog sustava u bolesnika sa kroničnim srčanim zatajenjem i u zdravih ljudi.

Cilj Istraživanja II je utvrditi obrazac aktivacije post-ganglijskih mišićnih simpatičkih neurona tijekom kratkotrajne primjene CPAP-a u bolesnika sa kroničnim, stabilnim srčanim zatajenjem i u zdravih kontrolnih ispitanika koji po dobi i spolu odgovaraju ispitnoj skupini.

Metodom mikroneurografije izmjerili smo mišićnu simpatičku živčanu aktivnost u 7 bolesnika sa kroničnim srčanim zatajenjem i u 8 zdravih ispitanika. Računalna aplikacija korištena u Istraživanju I, koja identificira pojedinačne simpatičke AP iz filtriranog, sirovog zapisa mišićne simpatičke aktivnosti, je korištena i za potrebe Istraživanja II. AP izdvojeni iz snimljenih zapisa simpatičke aktivnosti su kvantificirani kao frekvencija okidanja AP (broj AP u minuti) te su svrstani prema veličini amplitude u različite skupove. Ventilacijski i kardiovaskularni parametri su također mjereni istovremeno sa snimanjem simpatičke aktivnosti. Protokol je uključivao: 1) snimanje svih navedenih parametara tijekom 10 minuta mirnog disanja, 2) primjenu CPAP-a pri tlaku od 5 cm H_2O tijekom 5 minuta, 3) primjenu CPAP-a pri tlaku od 10 cm H_2O tijekom 5 minuta, te 4) snimanje svih navedenih parametara tijekom 10 minuta odmora, mirnog disanja.

U zdravih ispitanika CPAP je uzrokovao pad udarnog volumena i pad srčanog minutnog volumena, te istovremeni porast frekvencije i incidencije izbijanja mišićne simpatičke živčane aktivnosti te porast frekvencije okidanja AP. U bolesnika sa kroničnim, stabilnim srčanim zatajenjem kratkotrajna primjena CPAP-a nije dovela do promjene razine mišićne simpatičke živčane aktivnosti niti je utjecala na mjerene kardiovaskularne i ventilacijske parametre. Utvrđena je negativna korelacija između simpatičkog i kardiovaskularnog odgovora na primjenu CPAP-a u skupini zdravih ispitanika, ali ne i u bolesnika sa kroničnim srčanim zatajenjem.

Navedeno sugerira da u zdravih ispitanika, kratkotrajna primjena CPAP-a prvenstveno utječe na promjene centralnih kardiovaskularnih parametara (pad udarnog volumena i srčanog minutnog volumena) koje refleksno dovode do porasta simpatičke aktivnosti i to preko mehanizma porasta frekvencije okidanja AP simpatičkih neuorna. Identična primjena CPAP-a u bolesnika sa kroničnim srčanim zatajenjem, pak nije uzrokovala promjenu centralnih kardiovaskularnih parametara, pa time ni promjenu razine simpatičke aktivnosti.

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11. CURRICULUM VITAE

Petra Zubin Maslov

Department of Integrative Physiology
School of Medicine, University of Split
Soltanska 2, 21 000 Split, Croatia
petra.zubin@mefst.hr
Phone: +385 21 557 853

Date and place of birth: February 17th 1984, Split, Croatia

EDUCATION

- May 2013 - Passed United States Medical License Examination (USMLE) Step 1with a score of 250
- July 2012 - Participated in a graduate course named Reflex Cardiovascular Control. Final exam passed with a score of 91% at the Western University, London, Ontario, Canada
- December 2009 to Current – Candidate for PhD in Evidence Based Medicine Doctoral Programme at Department of Integrative Physiology, School of Medicine, University of Split
- December 2009 - Croatian Medical License Exam, Ministry of Health of the Republic of Croatia, Zagreb
- September 2002 to September 2008 - Doctor of Medicine, average grade 4.76 (on a scale from 1 to 5), School of Medicine, University of Split
- 1998 to 2002 - Gymnasium "Marko Marulic", Split
- 1990 to 1998 - Elementary school "Split 3", Split

HONORS AND AWARDS

- 2008 - School of Medicine, University of Split Award for extraordinary achievements
- 2004 to 2008 - Community of Split Scholarship for extraordinary students
- 2003 to 2004 - University of Split Undergraduate Scholarship

WORKING EXPERIENCE

- December 2009 to Current - Department of Integrative Physiology, School of Medicine, University of Split
- 2008 to 2009 - Clinical internship for Doctor of Medicine at University Hospital of Split

RESEARCH INTEREST

- Microneurography, Action Potential Analysis, Autonomic Nervous System, Chronic Heart Failure, Sleep Apnea

RESEARCH EXPERIENCE

- 2009 to Current - Research Assistant for Prof. Zeljko Dujic at Department of Integrative Physiology, School of Medicine, University of Split
- May to July 2012 - Neurovascular Research Laboratory, Western University, London, Ontario, Canada. Data analysis for Chronic Heart Failure Study with Prof. Kevin J. Shoemaker

TEACHING EXPERIENCE

- 2009 to Current - Teaching assistant, Course: Physiology at Graduate Degree program in Medicine
- 2009 to Current - Teaching assistant, Course: Physiology at Graduate Degree program in Dental Medicine
- 2009 to 2012 - Teaching assistant, Course: Physiology at Undergraduate program in Physiotherapy
- 2009 to 2012 - Teaching assistant, Course: Physiology at Undergraduate program in Nursery
- 2004 to 2005 - Course demonstrator at Department of Biochemistry
- 2003 to 2004 - Course demonstrator at Department of Anatomy, Histology and Embryology

AFFILIATIONS

- 2011 - Croatian Physiological Society
- 2010 - Croatian Medical Chamber
- 2005 to 2008 - Croatian Medical Students International Committee (CROMSIC, IFMSA)

PUBLICATIONS

- **Zubin Maslov P**, Shoemaker JK, Dujic Z. Firing patterns of muscle sympathetic neurons during apnea in chronic heart failure patients and healthy controls. Auton Neurosci Oct 2013; doi: 10.1016/j.autneu.2013.09.016.
- Bakovic D, Pivac N, **Zubin Maslov P**, Breskovic T, Djamonja G, Dujic Z. Spleen volume changes during adrenergic stimulation with low doses of epinephrine. J Physiol Pharmacol 2013; 65(5).
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- Cross TJ, Kavanagh JJ, Breskovic T, **Zubin Maslov P**, Lojpur M, Johnson BD, Dujic Z. The effects of Involuntary Respiratory Contractions on Cerebral Blood Flow during Maximal Apnoea in Trained Divers. PLoS One, 2013 Jun 26;8(6):e66950.
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- **Maslov PZ**, Breskovic T, Brewer DN, Shoemaker JK, Dujic Z. Recruitment pattern of sympathetic muscle neurons during premature ventricular contractions in heart failure patients and controls. Am J Physiol Regul Integr Comp Physiol. 2012 Dec;303(11):R1157-64.

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- Breskovic T, Uglesic L, **Zubin P**, Kuch B, Kraljevic J, Zanchi J, Ljubkovic M, Sieber A, Dujic Z. Cardiovascular changes during underwater static and dynamic breath-hold dives in trained divers. J App Physiol (1985).2011 Sep;111(3):673-8.

PRESENTATIONS

- **Zubin Maslov P**, Breskovic T, Brewer DN, Shoemaker JK, Dujic Z. Recruitment pattern of sympathetic muscle neurons during premature ventricular contractions in heart failure patients and controls. Congress of Croatian Physiological Society and 2. Regional Congress of the Physiological Societies, Zagreb, Croatia, September 2012
- **Zubin P**, Breskovic T, Uglesic L, Kuch B, Kraljevic J, Zanchi J, Ljubkovic M, Sieber A, Dujic Z. Cardiovascular changes during underwater static and dynamic breath-hold dives in trained divers. Congress of Croatian Physiological Society and 1. Regional Congress of the Physiological Societies Osijek, Croatia, September 2011.