Recording sympathetic nerve activity in conscious humans and other mammals: guidelines and the road to standardization

Emma C. Hart[1], Geoffrey A. Head[2], Jason R. Carter[3], B. Gunnar Wallin[4], Clive N. May[5], Shereen M. Hamza[6], John E. Hall[7], Nisha Charkoudian[8] and John W. Osborn[9]

Running title: Guidelines for measuring sympathetic nerve activity

[1] University of Bristol, School of Physiology, Pharmacology and Neuroscience, Bristol, United Kingdom

[2] Baker IDI Heart and Diabetes Institute, Melbourne, Australia

[3] Michigan Technological University, Houghton, MI, USA

[4] University of Gothenburg, Gothenburg, Sweden

[5] Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia

[6] University of Alberta, Canada

[7] Department of Physiology and Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS, USA

[8] US Army Research Institute of Environmental Medicine, Natick, MA, USA

[9] University of Minnesota, Department of Integrative Biology and Physiology, Medical School, Minneapolis, MN, USA

Corresponding author: Dr. Emma C. Hart

School of Physiology Pharmacology and Neurosciences, Biomedical Sciences Building, University of Bristol, BS8 1TD, UK. *Email*: emma.hart@bristol.ac.uk

Running title: Measuring and interpreting SNA in mammals

Contents outline

1. Abstract

Over the past several decades, studies of the sympathetic nervous system in humans, sheep, rabbits, rats and mice have substantially increased mechanistic understanding of cardiovascular function and dysfunction. Recently, interest in sympathetic neural mechanisms contributing to blood pressure control has grown, due in part to the development of devices or surgical procedures, which treat hypertension by manipulating sympathetic outflow. Studies in animal models have provided important insights into physiological and pathophysiological mechanisms, which are not accessible in human studies. Across species and among laboratories, various approaches have been developed to record, quantify, analyze and interpret sympathetic nerve activity (SNA). In general, SNA demonstrates "bursting" behavior, where groups of action potentials are synchronized and linked to the cardiac cycle via the arterial baroreflex. In humans, it is common to quantify SNA as bursts/minute or bursts/100 heartbeats. This type of quantification can be done in other species, but is only commonly reported in sheep, which have heart rates similar to humans. In rabbits, rats and mice, SNA is often recorded relative to a maximal level elicited in the laboratory to control for differences in electrode position among animals or on different study days. SNA in humans can also be presented as total activity, where normalization to the largest burst is a common approach. The goal of the present paper is to put together a summary of "best practices" in several of the most common experimental models, and to discuss opportunities and challenges relative to the optimal measurement of SNA across species.

Keywords: blood pressure, autonomic nervous system, nerve recording, rat, rabbit, sheep, mouse, human

2. Introduction

Over the past several decades, the importance of sympathetic nerve activity (SNA) in the control of human cardiovascular function has become increasingly clear. Elevations in SNA have important roles in the pathophysiology of essential and renovascular hypertension, as well as chronic kidney disease and congestive heart failure. From a physiological perspective, the sympathetic nervous system is of considerable interest, as it transmits patterns of information embedded in frequencies of nerve firing that provide fine regulation of blood flow, perfusion pressure, cardiac output, and release of hormones such as renin.

From the earliest recordings of postganglionic sympathetic nerves in anesthetized rabbits and decerebrate cats by Adrian, Bronk and Phillips in the 1930's, the distinctive patterns of respiratory and cardiac coupling revealed that adaptation of brain centers controlling SNA occurred at a rapid rate and that SNA was never really "steady" (1). Importantly, different vascular beds receive levels of activity influenced by different afferent and central signals. Thermoregulatory information controls SNA to skin as does the level of oxygen, whereas baroreceptor input appears to have less influence (103). By contrast, muscle, renal, splanchnic and cardiac SNA are strongly influenced by baroreceptors and chemoreceptors (56). Thus recordings of sympathetic nerves were recognized to reveal a dynamic window into the workings of the cardiovascular and thermoregulatory systems.

While many studies extended these findings to other species, including rats and mice, the impact of anesthesia on cardiovascular and autonomic function has limited the usefulness of the technique. Kirchner described the first recordings from an unanesthetized cat by means of chronically implanted electrodes embedded into a

Plexiglas block and noted marked differences in SNA between sleep states, and during emotional stress (64). An improved design came from the work of Schad and Seller who described a method of embedding a spiral Teflon coated platinum wire electrode in a silicone gel (105). Ninomiya developed a cowskin collagen electrode in cats (95). The signal was amplified by a differential amplifier, filtered between 60-600 Hz, rectified and integrated. Higher success rates and longer recordings were achieved by Dorward and colleagues in conscious rabbits with the main difference being that the electrode was a platinum or stainless steel spiral and sutured to the renal artery rather than the muscle (29). A similar electrode design was developed for chronic recordings of cardiac SNA in conscious cats with studies conducted between 4 and 11 days after implantation (112). Techniques have been miniaturized and applied to recordings of SNA in conscious rats and mice (46, 88). In sheep, sharpened insect pins were used as needles to pass through the renal or cardiac nerve sheath to access the nerve fibers and were held in place by glue with leads exteriorized through an incision to be connected later for recording when the animal was conscious (81). A major development in the last decade has been the use of radiotelemetry to record SNA in conscious unrestrained animals in their home cage compared with the laboratory environment. These have been applied in rabbits (5) and rats (87, 129). Particularly striking has been the development of simultaneous recordings from SNA to more than one region in conscious rats (87, 129) and in conscious sheep (98). Additional methods have been developed for recording SNA in conscious mice (46).

In the mid-1960's, Karl-Erik Hagbarth and Åke Vallbo developed the technique of microneurography to record SNA in humans (114). Sympathetic neural recordings were documented from nerves containing muscle afferent (44), muscle efferent (45), skin afferent (113), and skin efferent (23) fibers. A historical perspective of the development

and early progression of microneurography has been previously documented (115), and there are several recent reviews outlining various technical aspects of the approach (19, 71, 126).

As described below, microneurography involves the percutaneous insertion of tungsten microelectrodes in superficial nerves (primarily the peroneal [fibular], median or radial nerves), and the direct recording of neuronal electrical activity. The activity (usually muscle sympathetic nerve activity, MSNA) can be analyzed as single units or as an integrated multiunit recording. The high impedance of the electrode ensures that only active fibers near the electrode tip are recorded. A separate, and somewhat more invasive, method to measure SNA in humans is the norepinephrine spillover technique, which provides excellent estimates of SNA directed to specific vascular beds not accessible to microneurography in humans (i.e. renal, cardiac, cerebral)(31-33). However, norepinephrine spillover does not provide significant temporal resolution of patterns of SNA. The present discussion related to humans will focus primarily on measurement of SNA using microneurography.

Whilst there have been several recent reviews that have independently covered technical aspects of sympathetic recordings in humans (126) and animals (42, 107) the goal of the present discussion is to bring together current state-of-the-art techniques and recommendations for recording and analyzing SNA in several species. We attempt to provide a comprehensive summary of best practices, highlighting parallels across species, as well as technical and interpretational challenges. We recognize that more than one technique to record SNA could provide significant extra information regarding sympathetic mechanisms of disease. An additional aim of this guide is to highlight

differences in the information contained in sympathetic recordings in humans and other species.

3. Measurement of sympathetic nerve activity in humans

3.1. Multi-unit recordings of SNA

3.1.1. *Participant preparation and laboratory conditions*

Before recording SNA in humans, it is recommended that preparation of participants is standardized to allow for reliable comparisons to be made A) between participants, B) pre- and post-intervention (when SNA is measured on different days), and C) among different laboratories recording SNA.

Acute alcohol and caffeine intake both induce increases in MSNA (20, 100), whilst smoking has been shown to change MSNA (93, 94). Therefore, it is recommended that participants refrain from alcohol, caffeine and smoking for a minimum of 12 hours prior to an experiment. Since intense physical activity has mixed effects on resting MSNA following exercise (101), it is also recommended that participants abstain from intense exercise for a minimum of 12 hours prior to any studies. Additionally, a large meal causes increased MSNA, which appears to be sustained ~90 minutes into the post-prandial period (21). Therefore, it is recommended that microneurography studies are not started until at least 3 hours following a meal. Given that the female sex hormones appear to have an effect on resting MSNA in eumenorrheic women (89) and women taking oral contraceptives (50, 90), it is suggested that women of childbearing age are studied during the same hormone phase of their menstrual cycle. Finally, before recording resting MSNA, all participants should be asked to void their bladder (34).

Laboratory conditions should be kept within a tight range to ensure reliability and validity of sympathetic recordings. Variations in ambient temperature can cause changes in

MSNA (35) and skin sympathetic nerve activity (SSNA) (6), thus ambient temperature should be kept between 21-24C. To avoid activation of SNA via environmental disturbances, all recordings should be completed during quiet rest. Background noise should be kept to a minimum, however, the participant should not fall asleep (54).

3.1.2. *Technical aspects of recording*

Microneurography in humans involves insertion of a tungsten microelectrode transcutaneously into a post-ganglionic sympathetic nerve bundle. Typical nerves accessed via microneurography include the peroneal and tibial nerves of the lower leg, and the radial, median, and ulnar nerves of the arm. The tungsten electrode is tapered with a tip diameter of ~5 μm, a shaft diameter of ~100-200 μm, and of varying lengths to allow for a range of depths required to access various peripheral nerves. Each tungsten electrode used to record SNA is epoxy-insulated, except for the electrode tip which remains uninsulated. The epoxy-insulation can be of varying levels, allowing for a range of electrode impedances (range 50 kΩ - 10 MΩ; typically 1-2 MΩ at 1 kHz) that impact the area of the recording. For example, higher electrode impedance results in a smaller recording volume within the nerve fascicle, allowing a more focused recording. This ultimately results in the ability to assess single-fiber recordings in addition to the more traditional multi-fiber recordings (74).

Tungsten is the metal of choice because its electrical (conduction) and mechanical (thin, stiff, and non-brittle) properties are ideal for percutaneous insertion of the nerve. In addition to the active electrode used to record sympathetic activity, a non-insulated electrode is inserted ~1-2 cm from the active electrode to serve as a reference for the electrical signal. The active and reference electrodes are attached to an electrically-isolated preamplifier that amplifies the signal prior to being routed to a main amplifier

where it is amplified further (total gain ~50,000 – 100,000). The signal is typically bandpass filtered at 700 – 2,000 Hz, full-wave rectified, and displayed as a mean voltage neurogram. Most often the signal is integrated at a time constant of 0.1 seconds, but can also be root mean square (RMS) processed. When heart rate is high and approaching 100 beats/ min, it is recommended to use a shorter time constant for integration, to prevent the appearance of fused bursts when measuring MSNA.

Efferent SNA to muscle (MSNA) and skin (SSNA) have several distinct features that make it possible to distinguish between the two signals on the mean voltage neurogram that is displayed in real-time. The more commonly recorded MSNA is characterized by pulse-synchronized burst pattern in which a burst occurs ~1.3 seconds after an electrocardiogram R-wave (22) (Figure 1). Importantly, MSNA burst confirmation should be facilitated by asking the subject to perform a voluntary end-expiratory apnea, which elicits chemoreflex-mediated increases in MSNA. MSNA does *not* increase in response to a sudden startle stimulus such as a yell or loud clap. In contrast, SSNA is not pulse-synchronized, and the burst shape is much more variable. SSNA bursts are often (but not always) longer, broader, and shallower when compared with the sharp, distinct MSNA morphology (Figure 2). Moreover, a sudden startle stimulus (e.g., yell or clap), as well as light stroking of the skin, elicit marked increases in SSNA. It is imperative to perform these auditory and physical confirmation tests prior to proceeding with a MSNA or SSNA experiment to ensure only one signal (MSNA or SSNA) is being recorded. SSNA is under minimal baroreflex control and participates only minimally in blood pressure (BP) regulation (43). Thus when a research question is primarily focused on the sympathetic regulation of BP, MSNA should be recorded; when measuring thermoregulatory control of skin blood flow, SSNA should be recorded (see also cautionary notes below regarding quantification and interpretation of SSNA signals)

(131). Anatomically, the skin and muscle nerve fascicles are in very close proximity to one another and fibers from one type may cross over to the other type of fascicle. Such crossover becomes more common in more proximal recording sites. Because of these anatomical features, it is possible to have both MSNA and SSNA signals mixed within the same neurogram. This can and should be avoided since having both signals interferes with burst detection, quantification and mechanistic interpretation.

3.1.3. Identifying and quantifying multi-unit SNA

***Burst identification*:** Burst identification for MSNA is typically initiated through a variety of commercially available or customized software programs that requires a minimum 3:1 signal-to-noise ratio, where bursts occur ~1.3 seconds following the previous R-wave within a search window of ~0.5 seconds. Peripheral sympathetic conduction velocity is fairly consistent across subjects (115), thus the inter-individual variability of "burst latency" depends on other factors and primarily on subject height. The greater the subject height, the greater the burst latency (37).

First automated burst detection is complete by using a data analysis program (e.g. Spike 2; Cambridge Electronic Designs, WinCPRS, or a custom made software), where a minimum amplitude is set for burst detection (e.g. bursts that are 2+ standard deviations above the noise). The program automatically marks the bursts to be included in the analysis. A trained investigator must then examine the entire signal for unidentified bursts or inaccurately identified bursts. The latency of a multi-unit burst is inherently associated with its size; where a larger burst has a faster latency. This may, in part, be related to the 'size principle' where recruitment of bulbospinal fibers with faster conduction velocities may occur as the intensity of the sympathetic burst becomes greater (116). Therefore, a useful way to determine if bursts have been marked correctly

is using a scatter plot of latency versus burst amplitude. Any bursts outside the standard error should be checked for shape, size and latency to consider whether this is a burst or not. The investigator must also take into consideration any electrical shift of the baseline of the recording as that can interfere with automated MSNA analyses utilizing burst amplitude or area (limitations associated with burst area/amplitude are discussed in a section below). Finally, there are instances where the signal quality degrades or there is increased noise due to tension of the limb of interest (leg or arm); this is in part due to the addition of EMG (electromyography) activity to the neurogram. Such changes are typically the result of discomfort or an induced autonomic stress maneuver (i.e., cold pressor test, mental stress, etc.). Accordingly, there is always a subjective aspect to burst analysis. There is even greater subjectivity with SSNA burst identification (23), where bursts are not pulse-synchronized and have more varied burst morphology than MSNA bursts.

To avoid excessive variability and minimize bias in burst identification, a single investigator should be responsible for burst detection of an entire study, and should remain blinded to the condition or treatment until burst detection is complete. However, in the end there is no substitute for a high quality neurogram that reveals signal-to-noise ratios much larger than the minimum 3:1 ratio for MSNA (often >10:1 ratio for larger bursts), and low baseline noise and definitive boundaries for SSNA analysis.

Quantifying SNA: After having identified a recording site the subject should relax for about 10-15 min. Then, to quantify resting MSNA or SSNA, it is generally recommended to record for >5 mins during quiet supine or semi-supine rest. The resting value is then derived as the average MSNA or SSNA over this time period. Due to the pulse synchronicity of MSNA, the most accepted method to quantify MSNA is calculation of

burst rate over the recorded time period, which is expressed as bursts per minute (burst frequency) and bursts per 100 heartbeats (burst incidence; (18, 39, 48, 109, 117)).

Burst frequency reflects the average SNA that the vascular smooth muscle is exposed to over a specified time period. Burst incidence, on the other hand, reports how often bursts occur in relation to the available number of heart beats. Both ways of quantifying burst rate/occurrence should be reported. An increase of burst frequency may be due to an increased burst incidence or an increased heart rate or a combination of both. However, as White et al. (126) emphasize, changes in burst/100 heart beats should sometimes be interpreted with caution. For example, during an intervention where there is a disproportionately larger increase in heart rate versus the number of bursts, this would indicate that there was a large decrease in MSNA, when the amount of bursts in a minute increased (see White et al. 108 for review). In this case, whether the decreased burst incidence actually reflects a decrease in central sympathetic neural firing is unclear. Regardless, if the number of bursts in a certain time period (e.g., a minute) is increased, then the vasculature is likely exposed to increased norepinephrine during that period of time.

Less frequently used methods of quantifying resting MSNA are based on burst strength. This involves using the area or the amplitude of a burst. Before using these variables to quantify MSNA, the neurogram must be calibrated/ normalized to avoid errors in quantification based on inter-test variations in the mean voltage of the neurogram. For example, in one test session the electrode may be very close to an active bundle of muscle sympathetic fibers, providing a large mean voltage, whereas in a subsequent or separate test session the recording electrode may be further away from the bundle fibers, causing a lower mean voltage. Typically, the largest burst in the neurogram

during a baseline recording is assigned a peak value of 100 or 1000 arbitrary units (AU) and a period where there are no bursts is assigned a value of 0 AU. The mean amplitude of the bursts in the baseline neurogram is then calculated. To use burst area, the start and the end of each burst should be marked, then the area of each burst is calculated using the integral between the two time points. The mean area and total integrated activity (total MSNA) can then be calculated (126). As noted below, however, total MSNA at rest should not be used for comparison between individuals or groups.

Kienbaum et al. (63) evaluated spontaneous baroreflex control of MSNA in two ways: by examining the relationship between BP and the % chance of burst occurrence, and by examining the relationship between BP and the "strength" (area) of a burst. Whereas there was a consistent and strong relationship between burst occurrence and BP, the relationship between burst strength and BP was only significant about 50% of the time. They concluded that there are likely two central sites that control SNA in humans, one controlling whether a burst occurs or not, and the other controlling the strength of the burst. The differential control of burst occurrence and strength of a burst certainly occurs in other mammals such as rats (24), cats (77) and sheep (99). Thus, expressing data as only burst occurrence may decrease the total amount of mechanistic information in a given report.

There are, however, several limitations in using methods of burst strength to quantify resting MSNA. First, this analysis should only be completed on high quality neurograms where signal-noise ratio is high and it is clear that the electrode has not moved from the original site. This analysis should also be avoided when there is interference from motor units. Additionally, measurement of variations in burst strength and total activity should be confined to interventions that are completed during the *same* recording. Thus,

comparing baselines amongst individuals, or within the same individual across different test sessions, should not be done due to unquantifiable differences in electrode position relative to the neurons being measured. However, comparing changes in total MSNA during an acute laboratory stress (i.e., mental stress, cold pressor test, lower body negative pressure, etc.) is appropriate, although conclusions may be influenced by the choice to quantify as absolute or percent-change (39, 126).

Although the present discussion is focused on sympathetic recordings related to BP regulation, it is pertinent to briefly mention how resting SSNA is typically quantified. Following normalization of the neurogram, SSNA is normally expressed as the total area under the mean voltage neurogram (92, 108). A change in SSNA during an intervention is calculated as the % change in the total area of the mean voltage neurogram relative to baseline (92). Because the SSNA signal in humans is irregular and can incorporate numerous nerve types (sudomotor, pilomotor and vasomotor) involved in more than one type of response (startle reflex, thermoregulation etc), it is important to use appropriate caution when collecting, interpreting and reporting data involving SSNA (131). Because of these issues, absolute values for SSNA should not be compared between groups.

Quantifying MSNA and cardiac arrhythmias: Cardiac arrhythmias can lead to difficulties in quantifying MSNA, and it is particularly important to consider in subsets of patients where arrhythmias are prevalent. Currently there are no recommended approaches to dealing with cardiac arrhythmias during MSNA analysis and is an area which needs work, particularly due to the wide range of arrhythmias that can occur. The most common arrhythmia that can affect MSNA quantification is premature ventricular contractions (PVC's), particularly when they occur in patterns leading to bigeminy and trigeminy. The problem with PVC and MSNA quantification is whether to count the PVC

as a heartbeat and the reflex change in BP that they induce. A PVC typically leads to one wide burst occurring, which causes an increase in BP in the next heart beat. When trigeminy or bigeminy occurs the BP can become labile, limiting the number of burst per minute (Figure 1B, C). When comparing pre- and post-intervention recordings, when PVC's are present in one recording only, this can lead to detecting a pseudo-change in MSNA incidence or frequency. When there are a limited number of PVC's present within a recording, we recommend cutting out that section of the recording in the analysis (i.e. the 6-8 cardiac cycles and associated part of the neurogram preceding and following the PVC). When PVCs occur frequently, for example during periods of bigeminy or trigeminy, the change in blood pressure during this time has a powerful influence on burst firing pattern (Figure 1), therefore these recordings may need to be excluded from the overall analysis. Another common arrhythmia is atrial fibrillation. MSNA is difficult to quantify during periods of atrial fibrillation and should not be included in analyses. Performing a resting ECG before starting MSNA acquisition may help identify participants who should not be included in the main data set.

Reproducibility of multi-unit MSNA recordings: Previous studies have shown that the level of resting MSNA (burst incidence) in the supine position is highly reproducible within individuals (36, 39, 41, 109, 127). Importantly, the level of MSNA (burst frequency) recorded in the peroneal nerve is equivalent to that recorded in the radial nerve (102). Additionally, the level of MSNA recorded in the peroneal nerve is proportional to noradrenaline spillover measures of SNA directed to the heart (118) and renal (122) vascular beds during supine rest.

3.1.4. *Success rates and safety*

The microneurographic technique, as well as the associated burst detection and subsequent analyses, requires extensive training from an independent and highly-skilled microneurographer with a minimum of one-year experience (preferably more). In the authors' laboratories ~80% of recordings are successful (the ability to implant, find and keep an adequate recording). Recommended qualifications and safeguards for microneurography have been previously outlined (30). It is typically recommended that repeated measurements from the same nerve should be made at a *minimum* of one month apart. If repeated measurements are needed at an interval less than one month, the opposite limb should be used to make a recording.

3.2. Single unit recordings

3.2.1. *Technical aspects of single unit recordings*

Search procedure: The initial step in single unit recordings is to find a multiunit site, the target zone of which is characterized in a standard way (see above). Next, the experimenter makes minute electrode adjustments and, after each adjustment, carefully inspects the original or discriminated neurogram, at rest and after suitable test maneuvers, with the aim of detecting possible high amplitude sympathetic spikes. Because of the anatomical arrangement with a number of C-fibers lying close together inside Schwann cells, the electrode will never record activity selectively from only one sympathetic fiber. Instead the aim of the search is to find a site in which the action potentials from one fiber will have amplitudes that appear (visually) to be clearly higher than those of other fibers (Figure 3).

Electrode impedance: Due to the anatomical arrangement of the fascicles, the yield of single units in a recording is likely to increase with small selective electrode tips (i.e. high impedance electrodes). On the other hand, electrodes that are too selective have

practical disadvantages (AC-noise, difficulty in finding active fibers) so impedances (before skin penetration) from a few up to ten MΩ are usually considered adequate. To the authors' knowledge, no systematic studies have been conducted to evaluate optimal electrode impedance for single unit recordings. Such studies would have to include impedance data from before skin penetration, in recording sites and at the end of the experiment.

Analysis of spike shape: In a given electrode site all action potentials generated by a single nerve fiber have an identical waveform (usually triphasic with a large negative component for vasomotor but sometimes biphasic for sudomotor neurons, Figure 3). Therefore, the hallmark of a successful single unit recording is that inspection of individual spikes and **superimposition** of all spikes in question demonstrate wave form similarity (72, 74). Activity in other fibers is best recognized by the potentials having different amplitudes in the microneurographic record. Occasionally, activity in more than one single fiber can be identified in the same electrode site; in such cases more than one group of reproducible potentials can be distinguished (each group with its own distinct amplitude). A weakness of the visual superimposition analysis is the low signal-to-noise ratio in single unit recordings. When the identity of a wave form is assessed the noise may occasionally create uncertainty as to whether or not an individual spike should be accepted in a group.

Quantitative analysis of spike shape: To date, the identity of a wave form has almost always been assessed visually in single fiber recordings. Recently, however, a more quantitative analysis incorporated the steepness of the main negative component as well as spike duration to distinguish action potentials from different fibers (119). Such

quantitative potential analyses are likely to increase quality and yield of fibers in the analysis of future single unit studies.

3.2.2. *How to quantify single unit activity*

Since postganglionic sympathetic neurons discharge very irregularly, firing in an individual fiber may be characterized both by mean firing frequency and instantaneous firing frequencies (= inverse of interspike intervals). Rest periods of 3 to 5 min duration are typically analyzed. Since many neurons show a degree of cardiac rhythmicity, it has also proved useful to quantify fiber firing rate by relating it to cardiac interval by using the following parameters: (i) firing probability (= the percentage of cardiac intervals in which a given neuron is active), (ii) number of spikes per cardiac interval (72, 74).

This terminology indicates that differences in the strength of multi-unit sympathetic activity (i.e. the number and the intensity of multiunit bursts) may be brought about in two ways: by differences in (i) the number of active neurons or (ii) the mean firing frequency of the neurons (or a combination of both). A difference in mean firing frequency may, in turn, be due to (a) a difference in firing probability of active neurons or (b) a difference in the number of spikes the neurons discharge within the bursts (or a combination of both). The usefulness of relating firing to cardiac interval is illustrated by the fact that in some diseases sympathetic excitability is altered, the result being a change of the distribution of spikes per cardiac interval (3, 55, 73).

3.2.3. *The information content in single and multi-unit recordings.*

Single unit and multiunit recordings of SNA provide different types of information. Muscle nerves seem to contain only vasoconstrictor fibers and, at recumbent rest, the strength of multiunit activity in leg muscles (expressed as number of multiunit bursts in the mean voltage neurogram) is reproducible from day to day over several years (36). On the other

hand, there are large reproducible inter-individual differences in strength of activity. Since multiunit neurograms from different arm and leg nerves exhibit parallel behavior, it has been suggested that a multiunit record provides a measure of the strength of sympathetic vasoconstrictor activity to all skeletal muscles. There is also evidence that the inter-individual differences in resting MSNA correspond to similar inter-individual differences in resting sympathetic outflows to heart and kidney (67, 118, 122) . Fascicles containing fibers that innervate the skin may contain several types of sympathetic nerve fibers (e.g. vasoconstrictor, sudomotor and piloerector fibers) that often make it difficult to quantify multiunit activity in a meaningful way.

A single unit record, on the other hand, provides information about the activity of an individual sympathetic fiber during a period of 3 - 5 minutes (72, 74). The record tells us about the temporal characteristics of unitary firing, i.e. A) how often a fiber is active, B) if a fiber fires multiple spikes in a burst, C) if the fiber also fires between bursts, D) how firing of a fiber occurs in relation to internal factors, such as the cardiac rhythm, the arterial BP or the respiration, and E) how firing occurs in relation to external stimuli.

On the other hand, in healthy subjects as well as in patients with various disease states, there is still no report on the variability in single unit firing between different segments of prolonged (hours) recordings or over time in repeated recordings in the same person. In addition, with the current type of recording electrodes it will be difficult to sample sufficiently large single unit materials from individual subjects to allow conclusions on possible inter-individual differences of unitary firing. In other words, single unit recordings cannot be used for reliable assessment of an individual´s resting MSNA. Development of new electrode types (e.g. multichannel electrodes) may overcome this limitation.

4. Recording multi-unit SNA in *conscious* animals

In general, recording SNA in humans is limited to nerves innervating skeletal muscle and skin. Therefore, recording SNA to other targets important in cardiovascular regulation must be conducted in experimental animals. Additionally, animal models of various disease states allow for more detailed, invasive analysis of mechanisms than is possible in human patients. This is typically done using a bipolar electrode placed around a nerve bundle, as illustrated for the renal nerve in Figure 4. A voltage difference between the electrodes is detected as action potentials travel along the axons. When measured for a single axon, this generates a single spike above and below the zero voltage differential. However, since the nerve bundle contains many axons, the signal is comprised of multiple spikes, or *units*, the height of which is partially dependent on the spatial relationship between the axon and electrode; axons closest to the electrode generate a larger spike and those further away generate a smaller spike. It is important to note that this multi-unit recording contains both *efferent* (Figure 4 shown in red) and *afferent* axons (Figure 4 shown in blue). The electrical contact between the nerve and electrode is typically insulated in a medical grade silicone solution. The multiunit signal is often quantified by first rectifying the raw signal which *flips* all the unit activity above the zero potential and then integrating the total signal such that the *rectified-integrated* signal is expressed in units of voltage x time^{-1} (Figure 4).

The limitation of this method is that the voltage measured is affected by several variables such as the number of axons in the bundle, their spatial relation to the electrode, the quality of the nerve dissection, and physical differences between electrodes. Moreover, there is not one nerve bundle, but several, and the size and location of these bundles vary among individual animals. As such, not all recordings are

conducted using identical numbers of nerve fibers. Taking all of these variables into consideration, the challenge has been to statistically compare the absolute voltage of nerve activity *between* subjects. To some degree, variability among animals can be reduced by recording from the same nerve bundle (when possible), using the same electrode and amplifier settings, and having the same electrophysiologist conduct all recordings. Details for recording SNA to various targets in several species are described in the following sections.

4.1. Multi-unit recordings of SNA in conscious rabbits

Of all the species that have been used to measure SNA, the rabbit has proved to be particularly suitable because of its relative size which is small compared with dogs or sheep but large enough to provide easy access for surgical implantation of an electrode around the sympathetic nerve. Rabbits are also placid enough to remain quietly in a box for several hours while recordings are made without restraint or stress. Indeed, recordings of the level of SNA are virtually identical in the laboratory compared to the rabbit's home cage (69). Furthermore, the method of electrode implantation described by Dorward and colleagues first in anesthetized rabbits (28) and later in conscious rabbits (27, 29, 65), allowed for recordings to be made over several weeks after giving the rabbit several days recovery during which time much of the confounding effects of anesthesia (29) and the impact of surgery on cardiovascular and circadian rhythms are eliminated (8). Importantly, one of the main advantages of the rabbit is the long duration of viable recordings that can be made (2, 5, 8, 53).

Electrode implantation: SNA in conscious rabbits is most often recorded from the renal nerve, mainly because of the relatively easy access afforded by a retroperitoneal

approach but lumbar SNA has also been recorded (62) and in some cases both sites together (97). The most common electrode is a bipolar design constructed using fine Teflon coated single strand or multi-strand stainless steel wires (29). These are placed around the isolated nerve bundle(s) in a spiral or u-shape. The spiral is preferred for long lasting recordings due to the lesser pressure the electrode makes when coming in contact with the nerve bundle. To isolate the area from the surrounding tissue, the insulation on the wire remains intact except where the nerve contacts the wire and the electrode is fixed to a nearby artery or underlying tissue using sutures or tissue glue to prevent movement between the nerve and electrode. The electrode-nerve system is then embedded in a 2-part silicone elastomer such as Kwik-sil (World Precision Instruments, Sarasota, Florida, USA). A ground lead is either implanted in nearby tissue or inserted acutely under the skin at the time of recording.

The success of the implantation is quite high (80-90%) in the hands of experienced and trained operators with consideration of several factors. The operation is best performed in the shortest time possible which is usually 90-110 minutes but extreme care must be taken not to damage or handle the nerve bundle directly (42). Importantly, the key to success is maintenance of an intact blood supply and venous drainage in the nerve bundle and to separate sufficient length of nerve to allow for a very gradual diversion of the nerve from the artery to its trajectory within the electrode and gel assembly. Minimal gel should be used and the assembly should be firmly fixed to the artery. Thus, practice to develop the skill and speed to perform the operation will greatly improve the success rate.

Signal processing: The raw SNA signal recorded from the whole nerve bundle requires amplification (usually 10-20000 times) using a low noise differential preamplifier and

bandpass filtering (usually between 50-100 Hz and 1-5 kHz) (4, 8, 29, 70). Importantly, the ground lead is buried under the skin, which reduces noise and interference. In most cases, the signal is rectified and integrated either with a resetting integrator (29) or using a resistance-capacitance “leaky integrator” with a 20ms time constant (76). The latter produces a series of peaks (76) the timing of which is related to the pressure pulse (42). A root mean square processor can also be used to emulate the leaky integrator (52). From this data, total SNA can be determined but also the amplitude of the bursts and their frequency (burst to burst) (76). The advantage of this method is that the association of the bursts to the cardiac and respiratory cycles can be determined. Burst height may more reflect recruitment of fibers while burst frequency more closely reflects the synchronous firing of active fibers (75). In addition to the resetting (29, 79) or leaky integrator (76), some groups have also used spike frequency using either hardware or software such as the spike counting setting available in the Matlab data acquisition system (70).

The frequency of data acquisition of sympathetic signals critically depends on which aspect of SNA is to be quantified. For integrated signals a relatively low frequency of acquisition is recommended such as between 500-1000 Hz. However, for spike counting or single unit analysis much higher frequencies such as 20000 Hz are necessary to capture the shape of the unit in order to allow for separation of different individual units (12).

Differentiating between signal and noise: A problem that is common to all multi-unit SNA recordings is that they must be closely scrutinized to eliminate noise and to separate the real SNA from artifacts. While visual inspection of the raw signal is essential, a very useful tool is a high quality audio monitor with sufficient base frequency

response to be able hear the sound of the bursts that are typical of good recordings (42, 107). Sharp clicks or noises are likely to be artifacts as is the hiss of white noise, while a SNA burst is more like the sound of a puff of air into a microphone giving a “boompff” like sound (42, 107). The presence of an electrocardiogram (ECG) artifact can sometimes be seen in both the raw and integrated signals (42) and can be eliminated by increasing the low end of the band pass filter to 300 Hz (107). For the most part the use of the differential amplifier which determines the potential difference between the signals detected at each recording electrode, mostly eliminates undesirable signals such as ECG and the electromyogram (42). The latter is often generated by the rabbit moving in the holding box and these periods in essence need to be eliminated from the analysis during editing. Electrical noise at 50-60 Hz can be eliminated by careful grounding of the animal, notch filtering and adequate electromagnetic shielding of the room.

One of the advantages of the leaky integrator is that the signal between bursts can be used as the zero and as such the background noise is eliminated from the measured signal. This can be confirmed by silencing the real SNA using a pressor agent to raise BP and provide baroreflex inhibition of SNA (42, 107) (Figure 5). As well or alternatively, ganglion blockade is commonly used to estimate the noise level in rabbits (Figure 5) and can show the presence of an ECG artifact in the signals since these are not eliminated by ganglion blockade. However, even short-acting ganglionic blockers such as pentolinium have an action lasting up to 3 hours and their administration may not be practical. In rabbits, use of the nasopharyngeal response to normalize renal SNA (RSNA) produces a marked bradycardia and the noise level may be estimated from the quiet period between the large bursts of SNA (42) (Figure 5). While there is good correlation between this estimate of noise and that obtained during ganglion blockade (42), in some cases SNA between bursts does not return to baseline due to the

properties of the leaky integrator. One must make sure that there is adequate time between the bursts of SNA for the integrated signal to reset before the SNA associated with the diastole increases (Figure 5).

Quantification and normalization: Longitudinal studies permit a within-animal design for different treatments but often between-animal comparisons need to be made that are not conducive to a randomized crossover design. However, SNA expressed in raw units of voltage cannot be compared between animals because the level of integrated SNA is dependent on both the frequency of burst firing and the conditions at the point of contact between electrode and nerve. The proximity of the electrode to the active nerve fibers, infiltration of fluid and inflammatory tissue, and patency of a good blood supply all have a role in determining the size of the signal (10). Thus there is huge variation in the actual microvolt levels that one can measure from individual animals. The coefficient of variation is approximately 150% for RSNA (9) compared with 9% for BP. Even longitudinal studies are plagued by gradual degradation of SNA over time, which in itself can vary between animals.

The solution to the variability among animals has been to try to normalize the values onto an arbitrary but comparable scale. Common methods across many species have been to use either the resting value as 100% (13, 52), the maximum value after baroreceptor unloading (29, 62) or the maximum burst height (used mainly in humans). The most common method in the rabbit is to use the nasopharyngeal reflex to near maximally activate the renal nerve and use this value as 100 normalized units (29). While these methods minimize differences due to the physical conditions of the electrode, they are not good at comparing between different groups of animals (or humans).

Recently, the nasopharyngeal normalizing method was further refined to use a 100ms window positioned at the peak of the sympathetic burst (11) since it more closely resembled the method of using the maximum burst height technique which is used in human MSNA recordings (120). Thus it was possible to compare the nasopharyngeal reflex normalization with the maximum burst height method (11). The latter was effective in eliminating most of the effects of different electrode recording condition differences but could not be used to detect differences between animals. By contrast the nasopharyngeal reflex could be used for comparing groups of animals.

Duration of recordings: In rabbits, a recovery period of at least 6 days is recommended to minimize the effect of anesthesia and surgery on cardiovascular and circadian rhythms. Continued recordings lasting up to 5 weeks after electrode implantation are common (2, 5, 8, 53) but over time the success rate diminishes considerably. There is a time-related decay in signal that is likely due to scarring at the electrode contact combined with death of nerve fibers. Scaling either by the nasopharyngeal reflex, upper baroreflex plateau or even the resting level of activity abolishes the time related decay (9). Guild et al. reported that a single rabbit recording lasted at a relatively stable level over 100 days, but this is unusual (42). Armitage reported 60% of recordings were viable at 4 weeks and 30% at 5 weeks; this is the more common experience (2).

Radiotelemetry in rabbits: Telemetric systems have been developed to allow SNA recording chronically in conscious rabbits (5). Whilst the components of the recording system, such as transmitters and preamplifiers, differ with this system, the stainless steel wire spirals, which surround the nerve, are the same as those in traditional systems (2, 5). The levels of SNA in the home cage recorded by telemetry are closely similar to those in the laboratory (69). The particular advantage of telemetry for SNA is that one can record over 24 hours, allowing for examination of the circadian rhythms or other patterns of activity such as those associated with feeding (14).

4.2. Multi-unit recordings of SNA in conscious sheep

Recording SNA in large animals is technically challenging and few studies have been published. Miki and colleagues successfully recorded acute responses of RSNA to head up tilt and water immersion in conscious dogs (83, 84) in the late 1980s and early 1990s but this work has not continued. The sheep is a docile species that is easier to work with than dogs and has proven to be an ideal species for recording SNA in the conscious state.

Types of electrodes: The sympathetic nerves in sheep have a thicker sheath than nerves in small animals, which makes it difficult to reliably obtain a signal with a large signal to noise ratio using electrodes that wrap around the nerve. Therefore an implantable intra-fascicular electrode was developed which gives a good signal-to-noise ratio and is simpler and quicker to implant than wire electrodes that wrap around the whole nerve. This approach has been successfully used to simultaneously record cardiac and RSNA in conscious sheep (7, 57, 98) with experienced surgeons the success rates of obtaining a recording are ~80% and ~90%, respectively. This type of

electrode has also been employed to record lumbar SNA in sheep but the success rate of these recordings is low (10-20%).

This technique using an intra-fascicular electrode is similar to that used in humans where recording electrodes are inserted into fascicles of the peroneal nerve. In that case, because the nerve innervates both skin and skeletal muscle, the electrode is positioned so that activity to either of these fields is recorded (discussed above). In sheep, it is not possible to pick individual fascicles of the thoracic cardiac nerves due to the small size of the nerves. In the renal nerves, there is no definitive evidence that individual fascicles have functional differences, although this could be the case.

Manufacture and implantation of electrodes: The electrode is constructed from stainless steel entomological pins (0.05 mm diameter) etched to a fine point, glued with marine araldite into the end of Teflon-coated 25-strand silver-coated copper wire (30 cm long) (CZ1174SPC, Cooner Wire, Chatsworth, CA) (124). The exposed tip of the electrode (1.5–2.0 mm in length) is pushed obliquely through the nerve sheath, ensuring that the tip is positioned in the center of the nerve. Up to six electrodes are implanted into the nerve and fixed in place with cyanoacrylate glue. The implantation site is covered with a layer of Kwik-Sil (WPI, Glen Waverly, Victoria, Australia), and the wires are looped and exteriorized through the sutured wound. A stainless steel disc with a ring that protrudes through the skin is implanted subcutaneously and used as a ground.

Cardiac sympathetic nerves: Under general anesthesia, electrodes are implanted in the left cardiothoracic nerves (124). Briefly, an incision is made above the left fourth rib, the periosteum is opened, and the rib is removed. The thorax is opened, held open with a rib retractor and the lungs are held back with wet packs. With the use of a binocular

microscope the thoracic cardiac nerves are identified and the fascia over the nerves is removed. Up to six electrodes are implanted as described above.

Renal sympathetic nerves: Under general anesthesia the left or right renal nerve is exposed via a paracostal, retroperitoneal approach. With the aid of a dissection microscope, the main renal nerve is identified, running along or parallel to the renal artery, and is cleared of surrounding fat. The recording electrodes are inserted into the nerve and held in place with cyanoacrylate glue. The wires are looped and exteriorized through the wound that is sutured in layers.

Recording of SNA in conscious sheep: SNA is recorded in conscious standing sheep at least 4 days after electrode implantation to reduce the effects of surgical stress. It has been shown that heart rate and cardiac SNA (CSNA) decrease to stable levels by the 4th day after surgery (Figure 6). For recording CSNA and RSNA it is important to complete the experiments in conscious sheep, since under isoflurane anesthesia CSNA is dramatically reduced, even though BP is decreased, whereas RSNA is increased (unpublished data). This is similar to the observations in cats in which anesthesia with pentobarbital caused a greater reduction in CSNA than RSNA and anaesthesia with chloralose inhibited CSNA but increased RSNA (78).

Both CSNA and RSNA are recorded differentially between pairs of electrodes, and for each nerve the pair with the best signal-to-noise ratio is selected. With the use of this technique, signal-to-noise ratios between 4:1 and 8:1 are obtained on the fourth day after surgery, and the signal remains usable (>2:1) for an additional 7-14 days. The signal is amplified (x 20,000) and filtered (200-1,000 Hz bandpass), displayed on an

oscilloscope, and passed through an audio amplifier and loudspeaker and recorded on a computer using a micro 1401 interface and Spike 2 software (Cambridge Electronic Design, Cambridge, UK). SNA is recorded at 5000 Hz; this frequency was decided by simultaneously recording RSNA at 2500, 5000, 7500 and 10,000 Hz and counting spikes/min. There was no observed increase in the spikes/min measured at sampling rates above 5000 Hz, so this frequency was chosen for future experiments. In a few cases ECG occurs on the CSNA neurogram and if this is mild it can be removed by increasing the low end of the bandpass filter. Occasionally EMG can been seen and heard on the neurograms, but this cannot usually be filtered out and, as it is usually intermittent occurring when the animal moves, this part of the record is discarded.

Analysis of SNA: The resting heart rate in sheep is ~70 beats/min, with clearly distinguishable bursts of SNA (Figure 7), thus allowing SNA to be analyzed on a beat-to-beat basis, as is done in humans. For each heartbeat, a custom written routine in the analysis program Spike 2 (Cambridge Electronic Design, Cambridge, UK) is used to determine diastolic, systolic, and mean arterial BPs and the number of discriminated spikes above threshold between the following diastolic pressures. The threshold is determined by recording activity during infusion of phenylephrine to raise arterial pressure to a sufficient level to abolish SNA. It is also possible to abolish SNA with a ganglion blocker, but sheep tolerate this less well than phenylephrine and take much longer to recover. In Spike 2, the threshold is adjusted to a level so that spikes from small bursts are counted. The threshold for each animal is checked over a 60-s control recording by an experienced investigator to ensure that each burst crosses the threshold and that spikes from the smallest bursts are counted. The entire record is then checked and inaccurately identified bursts are removed.

These data are used to provide three measures of SNA; 1) total SNA is quantified on a beat-to-beat basis by measurement of the number of spikes above threshold for a set period of time (1-5 min) to determine spikes/s and burst size (spikes/burst), 2) burst incidence (bursts/100 heart beats) and 3) burst frequency (bursts/min). As in other species, studies in sheep have shown differential control of cardiac and RSNA (80) in accord with numerous other studies showing organ specific control of SNA in animals (58, 91). For example, the resting level of RSNA is much higher than CSNA (Figure 7), intracerebroventricular angiotensin II or hypertonic saline stimulated CSNA, but inhibited RSNA (82, 125) and, following hemorrhage, resuscitation with hypertonic saline caused a rapid and dramatic increase in CSNA, but no change in RSNA (40). Such findings emphasize that recordings in one nerve cannot be used as a surrogate for activity in other sympathetic nerves during changes to specific inputs.

4.3. Recording SNA in conscious rats

Without question the rat is the most commonly used species to study the anatomy and physiology of the sympathetic nervous system. However, in a recent report by Kenny and Mosher it was determined that, of the nearly 1300 publications on studies of SNA in rats between 1981 and 2010, more than 1100 were conducted in anesthetized animals (60). Moreover, only 10 (less than 1%) were conducted in conscious rats 3 days or more following electrode implantation (60).

Methods to chronically implant electrodes and record renal, lumbar and splanchnic SNA in conscious rats for periods longer than 3 days have been driven by two laboratories. Miki pioneered long-term recording of SNA in conscious rats using a hardwired system and external amplifiers (85-88, 128, 130). More recently Stocker and Muntzel have utilized wireless telemetric recording of renal, lumbar and splanchnic SNA in conscious

rats and eloquently described methods for standardization of surgical approaches and quantification of SNA in conscious rats (107). Here we summarize key components of the methods developed by these two laboratories.

Electrodes and surgical implantation: The major challenge is the ability to successfully implant the electrode and maintain a stable electrical contact and nerve viability over time. This requires outstanding surgical skills and the proper electrode design and implantation. Electrodes are typically bipolar single or multi-stranded Teflon insulated stainless steel. The basic strategy for electrode implantation is similar to that described above for the rabbit: the nerve is gently dissected; the bipolar hook is placed around the nerve and imbedded in medical grade silicone (Kwik-Sil, WIP, Sarasota, FL). More specific details are described by Stocker and Muntzel (107) and Miki (85-88, 130). Figure 8 shows the basic approach for implantation of lumbar (A), renal (B) and splanchnic (C) electrodes as described by Stocker and Muntzel (107). In all cases, Teflon insulation is stripped away from the tip of the electrode, which is then shaped in to a hook and placed around the nerve. The electrode is then stabilized by suturing it to a nearby structure such as the aorta.

Once the electrode is in place and stabilized, amplification and recording of the signal is possible in one of two ways: exteriorization of the leads and connection to external amplifiers via an electrical commutator (85-88, 128, 130) or wireless radiotelemetric transmission (107). It the case of the latter, the body of the transmitter is placed either in the abdominal cavity or subcutaneously.

Recording and analysis of SNA: Issues related to the quality of the nerve recording, determination of background noise, and quantification and normalization of multi-unit

nerve activity are similar to those discussed above for rabbits and have been discussed in detail previously (107) (85-88, 128, 130). Figure 9 shows an example of RSNA measured in a conscious rat (107). Raw and integrated RSNA are shown before and after the ganglionic blocker hexamethonium (A) or a pressor dose of phenylephrine (C), each of which results in total suppression of RSNA thus allowing determination of background noise. Note the arrows indicating this burst frequency, which can also be quantified as shown in panels B and D (107). As such, SNA can be quantified in several ways depending on the goal of the study. These acute responses are expressed as percent of baseline, absolute voltage of the integrated signal, or normalized to a scale of zero (background noise) to 100 (maximal SNA in response to a stimulus).

Duration of recordings and success rate: As in other species, chronic measurement of SNA in the rat is a challenge since the signal may fade over time due to a variety of factors. Stocker and Muntzel suggest that the 4-6 day recovery period following implantation is the “danger zone” as most investigators have reported that the signal drops to zero in ~40% of cases (107). Interestingly, they report that of the rats that are successfully studied beyond this period of time, 80% will maintain a stable recording for several weeks (107). The single greatest determinant of success rate is the skill of the surgeon who is implanting the electrodes. This fact cannot be overstated. This is likely the explanation for the high rate of success by Miki and Yoshimoto. They have successfully recorded lumbar and RSNA in conscious rats in several studies (85-88, 128, 130). In addition, these investigators are responsible for the only study, in any species, to continuously record SNA from two different nerves (renal and lumbar) over several weeks during the development of hypertension (128). However, these recordings were not measured simultaneously in the same animal. Also, it is important to note this

was accomplished using a standard hardwired system, which points out that although wireless recording may be easier, it is not required.

4.4. Recording RSNA in Conscious Mice

Over the past few decades, interest in the mouse as a scientific animal model has increased significantly and mice have been integrated into almost every facet of biomedical research. This is not surprising considering the similarities in genome and physiological function of the mouse and human as well as the relative ease of targeted manipulation of the mouse genome to investigate complex diseases and physiological functions. Given the integral role of the autonomic nervous system (ANS) in modulating virtually every system in health and disease, evaluation of autonomic tone in experimental mice has been an important goal of physiology research.

The use of mice for physiological studies of the ANS function presents several key challenges, in addition to the obvious difficulties associated with their small size. A major challenge lies in maintaining physiological BP over the course of anesthesia, surgical preparation and nerve recording during experiments that may last several hours (46). In this respect, the cardiovascular system of the laboratory mouse has proven to be exquisitely sensitive to anesthesia and surgery. It is exceedingly difficult to maintain a stable BP, within the physiological range, when mice are exposed to myriad anesthetic agents, in numerous combinations and doses, as well as the surgery required to implant electrodes needed for recording SNA in organs such as the kidneys. In many of the reported studies of RSNA in anesthetized mice, mean arterial pressure has averaged 60-80 mmHg, far below the BP in normal conscious mice which averages 100-120 mmHg, depending on the mouse strain (59, 110). The ideal approach is thus to avoid anesthesia altogether and directly record SNA in conscious, freely moving mice with

appropriate time permitted to recover from surgery, which has, until recently (46, 47), not been previously reported.

Instrumentation & Surgery: The specialized equipment and surgical methods required for instrumenting mice and for recording RSNA following recovery were described by Hamza and Hall (46, 47). Here, we focus on special considerations for successfully applying this technique. It is essential to construct an implantable electrode customized for mice since telemetric technology commercially available for larger species such as the rat, have not yet been miniaturized for the mouse. Multiple-stranded, Teflon coated stainless steel wire appears to be optimal compared to the single-stranded variety since the multiple-stranded wire provides more flexibility and translates into better position of the electrode tips without stretching the delicate renal nerve bundle. Another important benefit is increased comfort of the mouse once the wire leads are tunneled out of the abdomen and exteriorized dorsally. As described for recording RSNA in the rat and rabbit, it is best to strip the Teflon coating only at the very tips of the bipolar electrode, which are in contact with the nerve. This helps to minimize electrical noise as well as potential damping of the signal. The optimal configuration of the electrode consists of three wires, two forming the bipolar leads and the third, positioned underneath these as the signal reference. Grouping these wires together with polyethylene tubing fixes them in a firm position, which increases the ease by which the electrode tip may be slipped underneath the renal nerve bundle.

A left flank retroperitoneal approach, rather than an abdominal incision, is ideal for access to the nerves of left kidney in the mouse and improves surgical recovery. During surgery, it is critical to keep the renal neurovascular bundle moist at all times with warm physiological saline to prevent death (and reduced viability) of the nerves. Once a slip of

renal nerve is identified with the aid of a stereomicroscope, the best approach is to use straight, fine forceps to carefully isolate a length of the nerve by dissecting the connective tissue on either side of the bundle, without pinching, touching or otherwise disturbing the nerve and associated microvessels. The longer the length of nerve that can be isolated, the better for ease of positioning the electrode, although this will vary among animals. Although it may be tempting to isolate multiple slips of renal nerve that can sometimes be observed coursing along the renal vessels, and placing more than one small bundle on the recording electrode, this does not necessarily produce the desired high quality signal. We recommend committing to one optimal renal nerve bundle and leaving any remaining fibers undisturbed. Once the renal nerve is isolated, gently lift the bundle with angled forceps in one hand and gently slide the electrode tip underneath, at no time during this step should any tension be applied to the nerve. Adjust the position of the electrode such that the nerve bundle makes good contact with both wires and ensure that the third (reference/ground) wire is in good contact with the underlying tissue. This last point is important for the mouse since ECG contamination of the RSNA signal can be problematic and we have found that the ground wire in close proximity to the recording electrodes produces a “cleaner” RSNA signal than when a reference electrode is tunneled into nearby muscle.

Once the electrode is in place, it is also essential to slip a small piece of paraffin film underneath the bipolar leads to further isolate them from underlying tissue. Perhaps one of the most important steps in this technique is to completely dry excess moisture from the nerve and surrounding tissue with small absorbent spears before quickly applying a conservative amount of silicone elastomer to the nerve/electrode unit. Failure to completely dry this area before applying the silicone will result in damping of the signal and an inability to decipher RSNA.

Signal Quality & Troubleshooting Tips: At the time of recording, electrode leads, including reference wire should be connected to a preamplifier/differential amplifier unit, amplified 10,000X, filtered between 100 – 1000Hz and recorded at 1000Hz. The preamplifier and home cage of the mouse should be placed within a grounded Faraday cage, which is effective for eliminating electrical noise. Ideal traces should have clear bursts of RSNA with amplitudes visible above the background noise; the determination of which is described immediately below (Figure 10). As in other species, a good method to demonstrate "true" RSNA in the conscious mouse is to intravenously infuse a small bolus dose of phenylephrine to pharmacologically increase arterial BP. This should immediately and dramatically suppress RSNA, with the degree of suppression dependent upon the dose of phenylephrine administered. Phenylephrine injection can also be used to help determine background noise, which can be estimated from the remaining trace upon complete suppression of RSNA. In addition, the post-ganglionic nature of RSNA can be verified by i.v. injection of a bolus of the ganglionic blocker hexamethonium (50μg/g body wt.), although this is best done at the end of the experiment since the effects in the mouse may be long lasting and compromise cardiovascular stability immediately before an experiment.

The technology for recording RSNA by radiotelemetry is, unfortunately, not available for mice. The smallest transmitter currently available weighs 13 g and is recommended for animals weighing over 200 g. Further miniaturization of radiotransmitters would advance this field of research. However, it is possible to implant a telemetric blood pressure recording device in the same mouse for simultaneous recording of RSNA with reliable BP (46).

Time Course & Success Rate: With appropriate training in this technique, all mice survive and thrive following this surgery with no complications. RSNA can generally be recorded successfully in 80% of the mice for up to 72 hours post-op. However, in our experience, RSNA can be recorded for up to 5 days post-surgery in a small subset of mice. Although allowing ample time for surgical recovery is optimal, this must be tempered with the knowledge that the viability of mouse renal nerves is relatively short compared to that observed in larger rodents. Since the ability to reliably record RSNA in this model tends to decrease with time following surgery, an extended post-op recovery period of 8-10 days is not currently possible and we instead recommend a 48 hour recovery period before recording a single experiment in the conscious mice. With further refinement of this technique longer recovery periods may be accommodated in the future without compromising the integrity of the RSNA signal.

Signal Analysis & Data Presentation: RSNA can be recorded online using data acquisition systems that also allow analysis of the trace such as PowerLab and LabChart software (AD Instruments). The Spike Histogram module in this software package can be used to discriminate between spikes of nerve activity and background noise (as estimated by suppression of RSNA, as noted above). RSNA can be presented as spikes/second, although there are inherent problems with this method as described elsewhere in this article. In terms of the mouse, presentation of RSNA as a percentage of baseline minimizes error in evaluating responses both within and between individual animals and groups. Digital integration and rectification of the RSNA signal utilizing LabChart software with Time Constant Decay of 0.1 seconds is also an option, with data presented as μV/s. The latter method may be preferable since it also takes into account-increased amplitude of nerve spikes in addition to frequency.

4.5 Single Unit analysis in animals

In parallel to the increasing use of recordings single units of SNA from skin and muscle in humans, there have been several studies in animals. The latter has been made easier by the ability of the neurophysiologist to physically split the nerve into fine bundles containing a few axons some of which show spontaneous activity. The reason for studying units is that there is increasing evidence that the information differs from the patterns observed in multiunit preparations. For example, in human obesity, greater levels of multiunit activity are recorded compared with control subjects but single unit analysis revealed that the greater activity was due to a recruitment of active units rather than a change to firing frequency of single units (66). By contrast, the effect of hypertension was to increase the firing of already active units (66). In animal studies multi-unit SNA (whole nerve) quantification in rabbits suggests that higher levels are recorded in angiotensin induced hypertensive rabbits compared to normotensive rabbits due to greater burst amplitude (68). However, single unit analysis revealed that this must have been due to recruitment of inactive fibers as the average unit frequency was less in hypertensive compared to normotensive rabbits (12). Thus there is a parallel effect on SNA between the effect of obesity in humans and the effect of hypertension in rabbits. Physiologically, these findings indicate that there are several distinct ways in which the CNS activates the sympathetic nervous system. Thus animal models that show higher SNA may not necessarily reflect neurophysiological mechanisms in humans.

Electrodes and spike search procedure*:* Unlike in humans, all single unit studies in animals (to date) have been performed under anesthesia, which also can quite dramatically alter the patterns of SNA. Nevertheless, several studies have revealed important properties of single postganglionic units. The search for units starts by cutting

the distal end of the nerve bundle, splicing through the nerve sheath and then splitting the nerve bundles into fine filaments and placing them on a monopolar electrode usually bathed in a mineral oil with a reference electrode piercing the nearby muscle (12, 26) or using a bipolar electrode (25, 61, 104). Prior to splitting, whole nerve recordings can be made for comparison to the unanesthetized condition (12). The fibers are spilt until only a few distinguishable spikes are visible in the recording (25).

Analysis of spike shape*:* In order to confirm that the spikes being recorded are indeed from one single neuron, it is usual to compare the shape of the action potential. To do this, data acquisition of the signals is required at a very high frequency such as 20000 Hz which is about 10-20 times the rate required for an integrated multiunit nerve recording. There are various methods to confirm consistency in the shape of the spike such as using a windows discriminator (25) or a computer program to analyze action potentials (15). Recently a new computer method was developed that assigned spikes to templates that were generated using the Iterative Self Organizing Data Analysis technique (ISODATA) (111) which automates the pattern recognition aspect of spike recognition and is necessary when there are many active spikes in the recordings (12) (Figure 11). Such approaches have been used previously but for spike pattern recognition of brain extracellular recordings (106). In both cases high frequency (20000 Hz) data acquisition software was used and programed in the Labview graphic language (12).

Quantification of unit activity*:* The basic information is the distribution of basal firing frequencies in units and their linkage to cardiac and respiratory patterns. Many studies have used interventions to determine whether the units are primarily reflective of vasomotor neurons or have other qualities. The consensus is that the vast majority of

spontaneously active units are indeed cardiovascular vasomotor being inhibited by baroreceptor activity and showing activation to chemoreceptor activation. DiBona and colleagues found mostly spontaneously active vasomotor units but about 10% non-active units responded to thermal stimulation. Similarly, in dogs, renal units showed either baro-dependent and baro-independent activity (61). However, studies in rabbits found only cardiac related vasomotor type units (12, 26). So unless the total number of units in the recording is quantified by electrical stimulation, it is not possible to know the number of inactive units. A proportion of inactive units at rest, however, can be activated by a maneuver, which helps with characterizing these units. Thus the characteristics of active units are easily quantified but not *all* inactive units and hence the issue of recruitment of inactive neurons cannot be easily determined. This is a fundamental difference between whole nerve and unit analysis.

Frequency histograms can be used to show the distribution of firing of a population of units. Cardiac rhythmicity expressed in percentage can be determined using post R wave histograms as calculated from the difference between maximum and minimum unit activity, expressed as a percentage of the mean activity (26). Similarly, respiratory rhythmicity percentage can be determined by post respiratory peak. In animal studies it is also possible to perform full baroreflex curves relating BP with unit firing frequency as well as responsivity to chemoreceptor or other afferent signals (26).

5. Similarities and differences between human and animal recordings of SNA

Similarities and opportunities for inter-species comparison: Much of the rationale for the use of animal models in biomedical research comes from their applicability to human physiology and pathophysiology. It is ultimately because of this relationship that

the more detailed, invasive and/or mechanistic insight that is possible in animals can help guide research and clinical care of human patients. Physiologically, one of the most generalizable aspects of recordings of vascular SNA across mammals is their participation in BP regulation. Sympathoexcitatory and sympathoinhibitory responses to baroreflex, chemoreflex and other stimuli in non-human models provide important insights into human physiology.

In addition to responsiveness to specific stimuli, baseline levels of SNA and their relationships with hemodynamic variables can also provide important information. Over the past decade, the importance of inter-individual variability in SNA, vascular resistance, cardiac output and BP has become increasingly apparent and has provided important insights into how resting BP is determined in men and women (17, 48) (49). In a direct comparison between rats and humans, Charkoudian et al. demonstrated that the coefficient of variability (CV) for cardiac output, total peripheral resistance and arterial pressure was similar between male Sprague-Dawley rats and humans (16). Interestingly, the CV for MAP (0.07) was one-third of that for CO (0.19- 0.21) and TPR (0.22 – 0.24) in both species, supporting the idea that variability in the latter variables supports tighter regulation of BP. These data also provide further support for the rat as a good representation of sympathetic and hemodynamic mechanisms in the human.

In terms of basic neurophysiological control mechanisms, data from both humans and animals support the idea of differential regulation of sympathetic outflow to different regions of the body. In humans, this is shown by the strikingly different patterns of activity and responsiveness between muscle and skin SNA recordings (121, 131). Additionally, in healthy humans, resting MSNA correlates with norepinephrine spillover estimations of renal and cardiac SNA (118, 122). In sheep, cardiac and RSNA exhibit

different patterns of resting tone in healthy animals and, as in humans (51) there is a greater increase in CSNA than RSNA in heart failure (98). In rats, recordings of renal and lumbar SNA have demonstrated differential control at rest and in response to various maneuvers (128, 129).

Differences and challenges with inter-species comparison: Methods for quantification of SNA present perhaps the most significant challenge when attempting to compare SNA reports from animals to those from humans. There are at least two reasons for this. First, in regard to SNA directed to the kidney, it is not possible to directly compare between animal and human studies since it is not feasible to record RSNA in humans outside of a surgical setting. Our current knowledge related to RSNA in humans is based on indirect assessment using renal norepinephrine spillover (33), which provides quantitative, mechanistic information but is invasive and has minimal temporal resolution.

Second, when sympathetic activity to skeletal muscle (MSNA) is recorded in humans (48, 120), the quantification method is quite different than that used in most animals, in part due to much higher heart rates in small animals. In humans, MSNA is often quantified by the frequency of bursts, either as bursts/minute or bursts/100 heartbeats. This type of analysis can also be done in sheep (98, 123) and the concept has been explored for rats and rabbits for lumbar, splanchnic and RSNA (96) (107). Interestingly, calculation of bursts/ 100 heartbeats in rats and rabbits often results in values above 100, indicating that in these species (unlike humans), it is possible to have more than one burst of activity within a cardiac cycle. In humans, each burst of activity represents an individual disinhibition followed by inhibition of SNA by the diastolic and systolic pressure wave of the corresponding individual cardiac cycle. Therefore, the possibility of

more than one burst per cardiac cycle in other species deserves further attention, since it suggests important neurophysiological differences in control mechanisms for neuronal activity across species.

6. Criteria for validating sympathetic nerve activity

As noted in the sections above, there is potential in all recordings of SNA for non-SNA "noise" that can confound interpretation. Therefore, it is vital that each SNA recording is validated as a legitimate SNA signal, to minimize the potential for noise or artifact being interpreted as SNA. Thus, a set of criteria must be met to confirm that SNA is being recorded and that the signal is not artifact or a recording from a different fiber type. Table 1 shows the criteria that should be met in order to validate the signal (at least 4 of the criteria should be met). In short, in all species, the signal should be linked to the cardiac cycle, baroreceptor and chemoreceptor sensitive, inhibited by ganglionic blockade and in the low μV range. In humans, it is essential to ensure that an MSNA recording does not include SSNA by completing maneuvers that activate SSNA, such as arousal (loud noise), stroking the skin (to check for skin afferents), etc.

7. OVERALL SUMMARY AND DIRECTIONS FOR FUTURE WORK

The sympathetic nervous system has a major role in the integrative regulation of cardiovascular function in humans and other mammals. Sympathetic nerve activity can be recorded in several species, including humans, where it is most commonly recorded as MSNA or SSNA. In conscious rats and rabbits, the most common recording is of RSNA. Recordings of RSNA have also been reported in conscious mice, although this is less common and more technically demanding due to their small size. Both cardiac and renal sympathetic activities have been recorded in conscious sheep. The sheep,

because of its larger size and slower heart rate, exhibits patterns of sympathetic neural activity that are more similar to the human.

Over the past several decades, technical advances in our ability to record from sympathetic nerves in several species have opened up unprecedented opportunities for mechanistic insight and facilitated translational research. A similar experience across all forms of SNA recordings has been that the technique must be taught by a person with extensive experience to a pupil who will dedicate enough time to learn, not only the "basics" but also the "subtleties" involved in these complex measurements. In addition, since all forms of sympathetic nerve recordings are technically demanding, it is important to delineate and standardize appropriate methods for collection and interpretation of SNA data.

We are fortunate that the community of cardiovascular and autonomic scientists who work with sympathetic nerve recordings is growing. The implications of our work for increasing scientific insight, advancing clinical pharmacology and medical device development have grown exponentially over the past decade; particularly in the fields of hypertension and heart failure. As the demand for information increases, we must strive to meet that demand with careful and rigorous technology, design and methodology, in order to provide the highest quality data to the biomedical community.

DISCLAIMER

Although Dr. Charkoudian is an employee of the United States government, the views, opinions, and/or findings contained in this article are those of the authors and should not be construed as an official United States Department of the Army position, or decision, unless so designated by other official documentation. Approved for public release; distribution unlimited.

References

1. **Adrian E, Bronk D, and Phillips G.** Discharges in mammalian sympathetic nerves *J Physiol (Lond)* 74: 115-133, 1932.
2. **Armitage JA, Burke SL, Prior LJ, Barzel B, Eikelis N, Lim K, and Head GA.** Rapid onset of renal sympathetic nerve activation in rabbits fed a high-fat diet. *Hypertension* 60: 163-171, 2012.
3. **Ashley C, Burton D, Sverrisdottir YB, Sander M, McKenzie DK, and Macefield VG.** Firing probability and mean firing rates of human muscle vasoconstrictor neurones are elevated during chronic asphyxia. *J Physiol* 588: 701-712, 2010.
4. **Barrett CJ, Guild SJ, Ramchandra R, and Malpas SC.** Baroreceptor denervation prevents sympathoinhibition during angiotensin II-induced hypertension. *Hypertension* 46: 168-172, 2005.
5. **Barrett CJ, Ramchandra R, Guild SJ, Lala A, Budgett DM, and Malpas SC.** What sets the long-term level of renal sympathetic nerve activity - a role for angiotensin II and baroreflexes? *Circ Res* 92: 1330-1336, 2003.
6. **Bini G, Hagbarth KE, Hynninen P, and Wallin BG.** Thermoregulatory and rhythm-generating mechanisms governing the sudomotor and vasoconstrictor outflow in human cutaneous nerves. *J Physiol* 306: 537-552, 1980.
7. **Booth LC, Bennet L, Guild SJ, Barrett CJ, May CN, Gunn AJ, and Malpas SC.** Maturation-related changes in the pattern of renal sympathetic nerve activity from fetal life to adulthood. *Exp Physiol* 96: 85-93, 2011.
8. **Burke SL, Evans RG, and Head GA.** Effects of chronic sympatho-inhibition on reflex control of renal blood flow and plasma renin activity in renovascular hypertension. *Brit J Pharmacol* 159: 438-448, 2010.
9. **Burke SL and Head GA.** Method for in vivo calibration of renal sympathetic nerve activity in rabbits. *J Neurosci Meth* 127: 63-74, 2003.
10. **Burke SL, Lambert E, and Head GA.** New approaches to quantifying sympathetic nerve activity *Curr Hypertens Rep* 13: 249-257, 2011.
11. **Burke SL, Lim K, Moretti J-L, and Head GA.** Comparison of sympathetic nerve activity normalization procedures in conscious rabbits *Am J Physiol Heart Circ Physiol* 310: H1222-H1232, 2016.
12. **Burke SL, Lukoshkova EV, and Head GA.** Characteristics of renal sympathetic nerve single units in rabbits with angiotensin-induced hypertension. *Exp Physiol* 101: 50-66, 2016.
13. **Burke SL, Malpas SC, and Head GA.** Effect of rilmenidine on cardiovascular responses to stress in the conscious rabbit. *J Auton Nerv Syst* 72: 177-186, 1998.
14. **Burke SL, Prior LJ, Lukoshkova EV, Lim K, Barzel B, Davern P, Armitage JA, and Head GA.** Reduced preprandial dipping accounts for rapid elevation of blood pressure and renal sympathetic nerve activity in rabbits fed a high-fat diet *Chronobiology International* 30: 726-738, 2013.
15. **Capowski JJ.** The spike program: a computer system for analysis of neurophysiological action potentials. In: *Computer Technology in Neuroscience*, edited by Brown PB. Washington: Hemisphere Publishing, 1976, p. 237-251.

16. **Charkoudian N, Gusman E, Joyner MJ, Wallin BG, and Osborn J.** Integrative mechanisms of blood pressure regulation in humans and rats: cross-species similarities. *Am J Physiol Regul Integr Comp Physiol* 298: R755-759, 2010.
17. **Charkoudian N, Joyner MJ, Barnes SA, Johnson CP, Eisenach JH, Dietz NM, and Wallin BG.** Relationship between muscle sympathetic nerve activity and systemic hemodynamics during nitric oxide synthase inhibition in humans. *Am J Physiol Heart Circ Physiol* 291: H1378-1383, 2006.
18. **Charkoudian N, Joyner MJ, Johnson CP, Eisenach JH, Dietz NM, and Wallin BG.** Balance between cardiac output and sympathetic nerve activity in resting humans: role in arterial pressure regulation. *J Physiol* 568: 315-321, 2005.
19. **Charkoudian N and Wallin BG.** Sympathetic neural activity to the cardiovascular system: integrator of systemic physiology and interindividual characteristics. *Compr Physiol* 4: 825-850, 2014.
20. **Corti R, Binggeli C, Sudano I, Spieker L, Hänseler E, Ruschitzka F, Chaplin WF, Lüscher TF, and Noll G.** Coffee acutely increases sympathetic nerve activity and blood pressure independently of caffeine content: role of habitual versus nonhabitual drinking. *Circulation* 106: 2935-2940, 2002.
21. **Cox HS, Kaye DM, Thompson JM, Turner AG, Jennings GL, Itsiopoulos C, and Esler MD.** Regional sympathetic nervous activation after a large meal in humans. *Clin Sci (Lond)* 89: 145-154, 1995.
22. **Delius W, Hagbarth KE, Hongell A, and Wallin BG.** General characteristics of sympathetic activity in human muscle nerves. *Acta Physiol Scand* 84: 65-81, 1972.
23. **Delius W, Hagbarth KE, Hongell A, and Wallin BG.** Manoeuvres affecting sympathetic outflow in human skin nerves. *Acta Physiol Scand* 84: 177-186, 1972.
24. **DiBona GF and Jones SY.** Reflex effects on components of synchronized renal sympathetic nerve activity. *Am J Physiol* 275: F441-446, 1998.
25. **DiBona GF, Sawin LL, and Jones SY.** Differentiated sympathetic neural control of the kidney. *Am J Physiol Regul Integr Comp Physiol* 271: R84-R90, 1996.
26. **Dorward PK, Burke SL, Jänig W, and Cassell J.** Reflex responses to baroreceptor, chemoreceptor and nociceptor inputs in single renal sympathetic neurones in the rabbit and the effects of anaesthesia on them. *J Auton Nerv Syst* 18: 39-54, 1987.
27. **Dorward PK, Flaim M, and Ludbrook J.** Blockade of cardiac nerves by intrapericardial local anaesthetics in the conscious rabbit *Australian Journal of Experimental Biology and Medical Science* 61: 219-230, 1983.
28. **Dorward PK and Korner PI.** Effect of d,l-propranolol on renal sympathetic baroreflex properties and aortic baroreceptor activity. *Eur J Pharmacol* 52: 61-71, 1978.
29. **Dorward PK, Riedel W, Burke SL, Gipps J, and Korner PI.** The renal sympathetic baroreflex in the rabbit. Arterial and cardiac baroreceptor influences, resetting, and effect of anesthesia. *Circ Res* 57: 618-633, 1985.
30. **Eckberg DL, Wallin BG, Fagius J, Lundberg L, and Torebjork HE.** Prospective study of symptoms after human microneurography. *Acta Physiol Scand* 137: 567-569, 1989.
31. **Esler M, Jackman G, Bobik A, Kelleher D, Jennings G, Leonard P, Skews H, and Korner P.** Determination of norepinephrine apparent release rate and clearance in humans. *Life Sci* 25: 1461-1470, 1979.

32. **Esler M, Jennings G, Korner P, Blombery P, Sacharias N, and Leonard P.** Measurement of total and organ-specific norepinephrine kinetics in humans. *Am J Physiol* 247: E21-28, 1984.
33. **Esler MD, Jennings GL, Johns J, Burke F, Little PJ, and Leonard P.** Estimation of 'total' renal, cardiac and splanchnic sympathetic nervous tone in essential hypertension from measurements of noradrenaline release. *J Hypertens Suppl* 2: S123-125, 1984.
34. **Fagius J and Karhuvaara S.** Sympathetic activity and blood pressure increases with bladder distension in humans. *Hypertension* 14: 511-517, 1989.
35. **Fagius J and Kay R.** Low ambient temperature increases baroreflex-governed sympathetic outflow to muscle vessels in humans. *Acta Physiol Scand* 142: 201-209, 1991.
36. **Fagius J and Wallin BG.** Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. *Clin Auton Res* 3: 201-205, 1993.
37. **Fagius J and Wallin BG.** Sympathetic reflex latencies and conduction velocities in normal man. *J Neurol Sci* 47: 433-448, 1980.
38. **Fagius J, Wallin BG, Sundlof G, Nerhed C, and Englesson S.** Sympathetic outflow in man after anaesthesia of the glossopharyngeal and vagus nerves. *Brain* 108 (Pt 2): 423-438, 1985.
39. **Fonkoue IT and Carter JR.** Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol* 309: R1380-1386, 2015.
40. **Frithiof R, Ramchandra R, Hood SG, and May CN.** Hypertonic sodium resuscitation after hemorrhage improves hemodynamic function by stimulating cardiac, but not renal, sympathetic nerve activity. *Am J Physiol Heart Circ Physiol* 300: H685-692, 2011.
41. **Grassi G, Bolla G, Seravalle G, Turri C, Lanfranchi A, and Mancia G.** Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man. *Clin Sci (Lond)* 92: 285-289, 1997.
42. **Guild SJ, Barrett CJ, McBryde FD, Van Vliet BN, Head GA, Burke SL, and Malpas SC.** Quantifying sympathetic nerve activity; problems and pitfalls, the need for standardization. *Exp Physiol* 95: 41-50, 2010.
43. **Hagbarth KE, Hallin RG, Hongell A, Torebjörk HE, and Wallin BG.** General characteristics of sympathetic activity in human skin nerves. *Acta Physiol Scand* 84: 164-176, 1972.
44. **Hagbarth KE and Vallbo AB.** Discharge characteristics of human muscle afferents during muscle stretch and contraction. *Exp Neurol* 22: 674-694, 1968.
45. **Hagbarth KE and Vallbo AB.** Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta Physiol Scand* 74: 96-108, 1968.
46. **Hamza SM and Hall JE.** Direct recording of renal sympathetic nerve activity in unrestrained, conscious mice. *Hypertension* 60: 856-864, 2012.
47. **Hamza SM, Hall, J.E.** Novel method for simultaneously recording renal sympathetic nerve activity and blood pressure with intravenous infusion in conscious,unrestrained mice. *Journal of Visualized Experiments (JoVE)* In Press, 2016.

48. **Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach JH, and Joyner MJ.** Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension* 53: 571-576, 2009.
49. **Hart EC, Joyner MJ, Wallin BG, and Charkoudian N.** Sex, ageing and resting blood pressure: gaining insights from the integrated balance of neural and haemodynamic factors. *J Physiol* 590: 2069-2079, 2012.
50. **Harvey RE, Hart EC, Charkoudian N, Curry TB, Carter JR, Fu Q, Minson CT, Joyner MJ, and Barnes JN.** Oral Contraceptive Use, Muscle Sympathetic Nerve Activity, and Systemic Hemodynamics in Young Women. *Hypertension* 66: 590-597, 2015.
51. **Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA, and Korner PI.** Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 73: 615-621, 1986.
52. **Hasser EM, Nelson DO, Haywood JR, and Bishop VS.** Inhibition of renal sympathetic nervous activity by area postrema stimulation in rabbits. *Am J Physiol Heart Circ Physiol* 253: H91-H99, 1987.
53. **Head GA and Burke SL.** Sympathetic responses to stress and rilmenidine in 2K1C rabbits : evidence of enhanced nonvascular effector mechanism. *Hypertension* 43: 636-642, 2004.
54. **Hornyak M, Cejnar M, Elam M, Matousek M, and Wallin BG.** Sympathetic muscle nerve activity during sleep in man. *Brain* 114 (Pt 3): 1281-1295, 1991.
55. **Ikeda T, Murai H, Kaneko S, Usui S, Kobayashi D, Nakano M, Ikeda K, Takashima S, Kato T, Okajima M, Furusho H, and Takamura M.** Augmented single-unit muscle sympathetic nerve activity in heart failure with chronic atrial fibrillation. *J Physiol* 590: 509-518, 2012.
56. **Iriki M and Korner PI.** Central nervous interactions between chemoreceptor and baroreceptor control mechanisms. In: *J Auton Nerv Syst*, edited by Brooks CM, Koizumi K and Sato A. Amsterdam: Elsevier, 1979, p. 415-426.
57. **Jardine DL, Charles CJ, Melton IC, May CN, Forrester MD, Frampton CM, Bennett SI, and Ikram H.** Continual recordings of cardiac sympathetic nerve activity in conscious sheep. *Am J Physiol Heart Circ Physiol* 282: H93-99, 2002.
58. **Jänig W and McLachlan EM.** Specialized functional pathways are the building blocks of the autonomic nervous system. *J Auton Nerv Syst* 41: 3-13., 1992.
59. **Kass DA, Hare JM, and Georgakopoulos D.** Murine cardiac function: a cautionary tail. *Circulation research* 82: 519-522, 1998.
60. **Kenney MJ and Mosher LJ.** Translational physiology and SND recordings in humans and rats: A glimpse of the recent past with an eye on the future. *Auton Neurosci* 176: 5-10, 2013.
61. **Kidd C, Linden RJ, and Scott EM.** Reflex responses of single renal sympathetic fibres to stimulation of atrial receptors and carotid baro-and chemoreceptors. *Quarterly Journal of Experimental Physiology* 66: 311-320, 1981.
62. **Kiel JW and Bishop VS.** Effect of fasting and refeeding on mesenteric autoregulation in conscious rabbits. *The American journal of physiology* 262: H1407-1414, 1992.

63. **Kienbaum P, Karlssonn T, Sverrisdottir YB, Elam M, and Wallin BG.** Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol* 531: 861-869, 2001.
64. **Kirchner F.** Correlations between changes of activity of the renal sympathetic nerve and behavioural events in unrestrained cats. *Basic Res Cardiol* 69: 243-256, 1974.
65. **Korner PI, Dorward PK, Blombery PA, and Frean GJ.** Central nervous beta-adrenoceptors and their role in the cardiovascular action of propranolol in rabbits. *Circ Res (Suppl I)* 46: I-26-I-32, 1980.
66. **Lambert E, Straznicky N, Schlaich M, Esler M, Dawood T, Hotchkin E, and Lambert G.** Differing pattern of sympathoexcitation in normal-weight and obesity-related hypertension. *Hypertension* 50: 862-868, 2007.
67. **Lambert EA, Schlaich MP, Dawood T, Sari C, Chopra R, Barton DA, Kaye DM, Elam M, Esler MD, and Lambert GW.** Single-unit muscle sympathetic nervous activity and its relation to cardiac noradrenaline spillover. *J Physiol* 589: 2597-2605, 2011.
68. **Lim K, Burke S, Moretti J-L, and Head G.** Differential activation of renal sympathetic burst amplitude and frequency during hypoxia and stress in angiotensin hypertension. *Exp Physiol* 100: 1132-1144, 2015.
69. **Lim K, Burke SL, Armitage JA, and Head GA.** Comparison of blood pressure and sympathetic activity of rabbits in their home cage and the laboratory environment *Exp Physiol* 97: 1263-1271, 2012.
70. **Liu JL and Zucker IH.** Regulation of sympathetic nerve activity in heart failure - A role for nitric oxide and angiotensin II. *Circ Res* 84: 417-423, 1999.
71. **Macefield VG.** Sympathetic microneurography. *Handb Clin Neurol* 117: 353-364, 2013.
72. **Macefield VG, Elam M, and Wallin BG.** Firing properties of single postganglionic sympathetic neurones recorded in awake human subjects. *Auton Neurosci* 95: 146-159, 2002.
73. **Macefield VG, Rundqvist B, Sverrisdottir YB, Wallin BG, and Elam M.** Firing properties of single muscle vasoconstrictor neurons in the sympathoexcitation associated with congestive heart failure. *Circulation* 100: 1708-1713, 1999.
74. **Macefield VG, Wallin BG, and Vallbo AB.** The discharge behaviour of single vasoconstrictor motoneurones in human muscle nerves. *J Physiol* 481 (Pt 3): 799-809, 1994.
75. **Malpas SC, Bendle RD, Head GA, and Ricketts JH.** Frequency and amplitude of sympathetic discharges by baroreflexes during hypoxia in conscious rabbits. *Am J Physiol Heart Circ Physiol* 271: H2563-H2574, 1996.
76. **Malpas SC and Ninomiya I.** A new approach to analysis of synchronized sympathetic nerve activity. *Am J Physiol Heart Circ Physiol* 263: H1311-H1317, 1992.
77. **Malpas SC and Ninomiya I.** Effect of chemoreceptor stimulation on the periodicity of renal sympathetic nerve activity in anesthetized cats. *J Auton Nerv Syst* 37: 19-28, 1992.
78. **Matsukawa K, Ninomiya I, and Nishiura N.** Effects of anesthesia on cardiac and renal sympathetic nerve activities and plasma catecholamines. *Am J Physiol* 265: R792-797, 1993.

79. **Matsumura K, Abe I, Fukuhara M, Tominaga M, Tsuchihashi T, Kobayashi K, and Fujishima M.** Naloxone augments sympathetic outflow induced by centrally administered endothelin in conscious rabbits. *Am J Physiol Regul Integr Comp Physiol* 266: R1403-R1410, 1994.
80. **May CN, Frithiof R, Hood SG, McAllen RM, McKinley MJ, and Ramchandra R.** Specific control of sympathetic nerve activity to the mammalian heart and kidney. *Exp Physiol* 95: 34-40, 2010.
81. **May CN and McAllen RM.** Baroreceptor-independent renal nerve inhibition by intracerebroventricular angiotensin II in conscious sheep. *Am J Physiol Regul Integr Comp Physiol* 42: R560-R567, 1997.
82. **May CN, McAllen RM, and McKinley MJ.** Renal nerve inhibition by central NaCl and ANG II is abolished by lesions of the lamina terminalis. *Am J Physiol Regul Integr Comp Physiol* 279: R1827-1833, 2000.
83. **Miki K, Hayashida Y, Sagawa S, and Shiraki K.** Renal sympathetic nerve activity and natriuresis during water immersion in conscious dogs. *Am J Physiol* 256: R299-305, 1989.
84. **Miki K, Hayashida Y, Tajima F, Iwamoto J, and Shiraki K.** Renal sympathetic nerve activity and renal responses during head-up tilt in conscious dogs. *Am J Physiol* 257: R337-343, 1989.
85. **Miki K, Kosho A, and Hayashida Y.** Method for continuous measurements of renal sympathetic nerve activity and cardiovascular function during exercise in rats. *Exp Physiol* 87(1): 33-39, 2002.
86. **Miki K, Oda M, Kamijyo N, Kawahara K, and Yoshimoto M.** Lumbar sympathetic nerve activity and hindquarter blood flow during REM sleep in rats. *Journal of Physiology* 557: 261-271, 2004.
87. **Miki K and Yoshimoto M.** Differential effects of behaviour on sympathetic outflow during sleep and exercise. [Review] [26 refs]. *Experimental Physiology* 90: 155-158, 2005.
88. **Miki K, Yoshimoto M, and Tanimizu M.** Acute shifts of baroreflex control of renal sympathetic nerve activity induced by treadmill exercise in rats. *Journal of Physiology* 548: 313-322, 2003.
89. **Minson CT, Halliwill JR, Young TM, and Joyner MJ.** Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* 101: 862-868., 2000.
90. **Minson CT, Halliwill JR, Young TM, and Joyner MJ.** Sympathetic activity and baroreflex sensitivity in young women taking oral contraceptives. *Circulation* 102: 1473-1476., 2000.
91. **Morrison SF.** Differential control of sympathetic outflow. *Am J Physiol Regul Integr Comp Physiol* 281: R683-698., 2001.
92. **Muller MD, Sauder CL, and Ray CA.** Mental Stress Elicits Sustained and Reproducible Increases in Skin Sympathetic Nerve Activity. *Physiol Rep* 1, 2013.
93. **Narkiewicz K, van de Borne PJ, Hausberg M, Cooley RL, Winniford MD, Davison DE, and Somers VK.** Cigarette smoking increases sympathetic outflow in humans. *Circulation* 98: 528-534, 1998.

94. **Niedermaier ON, Smith ML, Beightol LA, Zukowska-Grojec Z, Goldstein DS, and Eckberg DL.** Influence of cigarette smoking on human autonomic function. *Circulation* 88: 562-571, 1993.
95. **Ninomiya I, Yonezawa Y, and Wilson MF.** Implantable electrode for recording nerve signals in awake animals. *J Appl Physiol* 41: 111-114, 1976.
96. **Pellegrino PR, Schiller AM, Haack KK, and Zucker IH.** Central Angiotensin-II Increases Blood Pressure and Sympathetic Outflow via Rho Kinase Activation in Conscious Rabbits. *Hypertension* 68: 1271-1280, 2016.
97. **Ramchandra R, Barrett CJ, Guild SJ, and Malpas SC.** Evidence of differential control of renal and lumbar sympathetic nerve activity in conscious rabbits. *Am J Physiol Regul Integr Comp Physiol* 290: R701-708, 2006.
98. **Ramchandra R, Hood SG, Denton DA, Woods RL, McKinley MJ, McAllen RM, and May CN.** Basis for the preferential activation of cardiac sympathetic nerve activity in heart failure. *Proc Natl Acad Sci U S A* 106: 924-928, 2009.
99. **Ramchandra R, Hood SG, Frithiof R, and May CN.** Discharge properties of cardiac and renal sympathetic nerves and their impaired responses to changes in blood volume in heart failure. *Am J Physiol Regul Integr Comp Physiol* 297: R665-674, 2009.
100. **Randin D, Vollenweider P, Tappy L, Jéquier E, Nicod P, and Scherrer U.** Suppression of alcohol-induced hypertension by dexamethasone. *N Engl J Med* 332: 1733-1737, 1995.
101. **Ray CA and Hume KM.** Sympathetic neural adaptations to exercise training in humans: insights from microneurography. *Med Sci Sports Exerc* 30: 387-391, 1998.
102. **Rea RF and Wallin BG.** Sympathetic nerve activity in arm and leg muscles during lower body negative pressure in humans. *J Appl Physiol (1985)* 66: 2778-2781, 1989.
103. **Riedel W, Iriki M, and Simon E.** Regional differentiation of sympathetic activity during peripheral heating and cooling in anesthetized rabbits. *Pflugers Archive* 332: 239-247, 1972.
104. **Rogenes PR.** Single-unit and multiunit analyses of renorenal reflexes elicited by stimulation of renal chemoreceptors in the rat *J Auton Nerv Syst* 6: 143-156, 1982.
105. **Schad H and Seller H.** A method for recording autonomic nerve activity in unanesthetized freely moving cats. *Brain Res* 100: 425-430, 1975.
106. **Stewart CM, Newlands SD, and Perachio AA.** Spike detection, characterization, and discrimination using feature analysis software written in LabVIEW. *Comput Methods Programs Biomed* 76: 239-251, 2004.
107. **Stocker SD and Muntzel MS.** Recording sympathetic activity chronically in rats: surgery techniques, assessment of nerve activity, and quantification. *AmJ Physiol* 305: H1407-H1416, 2013.
108. **Strom NA, Meuchel LW, Mundy DW, Sawyer JR, Roberts SK, Kingsley-Berg SM, and Charkoudian N.** Cutaneous sympathetic neural responses to body cooling in type 2 diabetes mellitus. *Auton Neurosci* 159: 15-19, 2011.
109. **Sundlof G and Wallin BG.** The variability of muscle nerve sympathetic activity in resting recumbent man. *J Physiol* 272: 383-397., 1977.
110. **Tanida M, Hayata A, Shintani N, Yamamoto N, Kurata Y, Shibamoto T, Morgan DA, Rahmouni K, and Hashimoto H.** Central PACAP mediates the

sympathetic effects of leptin in a tissue-specific manner. *Neuroscience* 238: 297-304, 2013.
111. **Tou JT and Gonzales RC.** *Pattern recognition principles. "ISODATA algorithm" in Chapter 3, Pattern classification by distance functions*. Reading, MA: Addison-Wesley, 1974.
112. **Tsuchimochi H, Matsukawa K, Komine H, and Murata J.** Direct measurement of cardiac sympathetic efferent nerve activity during dynamic exercise. *Am J Physiol Heart Circ Physiol* 283: H1896-H1906, 2002.
113. **Vallbo AB and Hagbarth KE.** Activity from skin mechanoreceptors recorded percutaneously in awake human subjects. *Exp Neurol* 21: 270-289, 1968.
114. **Vallbo AB and Hagbarth KE.** Impulses recorded with micro-electrodes in human muscle nerves during stimulation of mechanoreceptors and voluntary contractions. *Electroencephalography and clinical neurophysiology* 23: 392, 1967.
115. **Vallbo AB, Hagbarth KE, and Wallin BG.** Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol* 96: 1262-1269, 2004.
116. **Wallin BG, Burke D, and Gandevia S.** Coupling between variations in strength and baroreflex latency of sympathetic discharges in human muscle nerves. *J Physiol* 474: 331-338, 1994.
117. **Wallin BG and Charkoudian N.** Sympathetic neural control of integrated cardiovascular function: insights from measurement of human sympathetic nerve activity. *Muscle Nerve* 36: 595-614, 2007.
118. **Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, and Jennings G.** Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol* 453: 45-58, 1992.
119. **Wallin BG, Karlsson T, Pegenius G, Karlsson AK, Macefield VG, and Elam M.** Sympathetic single axonal discharge after spinal cord injury in humans: activity at rest and after bladder stimulation. *Spinal Cord* 52: 434-438, 2014.
120. **Wallin BG and Sundlof G.** A quantitative study of muscle nerve sympathetic activity in resting normotensive and hypertensive subjects. *Hypertension* 1: 67-77, 1979.
121. **Wallin BG, Sundlof G, and Delius W.** The effect of carotid sinus nerve stimulation on muscle and skin nerve sympathetic activity in man. *Pflugers Arch* 358: 101-110., 1975.
122. **Wallin BG, Thompson JM, Jennings GL, and Esler MD.** Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. *J Physiol* 491 (Pt 3): 881-887, 1996.
123. **Watson AM, Hood SG, Ramchandra R, McAllen RM, and May CN.** Increased cardiac sympathetic nerve activity in heart failure is not due to desensitization of the arterial baroreflex. *Am J Physiol Heart Circ Physiol* 293: H798-804, 2007.
124. **Watson AM, Mogulkoc R, McAllen RM, and May CN.** Stimulation of cardiac sympathetic nerve activity by central angiotensinergic mechanisms in conscious sheep. *Am J Physiol Regul Integr Comp Physiol* 286: R1051-1056, 2004.
125. **Watson AMD, Mogulkoc R, McAllen RM, and May CN.** Stimulation of cardiac sympathetic nerve activity by central angiotensinergic mechanisms in conscious sheep. *Am J Physiol Regul Integr Comp Physiol* 286: R1051-1056, 2004.

126. **White DW, Shoemaker JK, and Raven PB.** Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci* 193: 12-21, 2015.
127. **Yamada Y, Miyajima E, Tochikubo O, Matsukawa T, and Ishii M.** Age-related changes in muscle sympathetic nerve activity in essential hypertension. *Hypertension* 13: 870-877, 1989.
128. **Yoshimoto M, Miki K, Fink GD, King A, and Osborn JW.** Chronic angiotensin II infusion causes differential responses in regional sympathetic nerve activity in rats. *Hypertension* 55: 644-651, 2010.
129. **Yoshimoto M, Nagata K, and Miki K.** Differential control of renal and lumbar sympathetic nerve activity during freezing behavior in conscious rats. *Am J Physiol Regul Integr Comp Physiol* 299: R1114-1120, 2010.
130. **Yoshimoto M, Sakagami T, Nagura S, and Miki K.** Relationship between renal sympathetic nerve activity and renal blood flow during natural behavior in rats. *American Journal of Physiology Regulatory Integrative & Comparative Physiology* 286, 2004.
131. **Young CN, Keller DM, Crandall CG, and Fadel PJ.** Comparing resting skin sympathetic nerve activity between groups: caution needed. *J Appl Physiol (1985)* 106: 1751-1752; author reply 1753, 2009.

Table 1: Criteria for validation of recordings of sympathetic nerve activity. At least 4 of these criteria should be met. Examples of typical tests to check the criteria are shown for both animals and humans.

Criteria	**Humans**	**Animals**
1. Pulse synchronous	A) Clear pulse (cardiac cycle) synchronous bursts with a latency of ~1.3s from the R-wave inhibiting burst	A) Usually pulse related in all species
2. Not activated by sensory stimuli (to exclude SSNA in humans)	A) Arousal stimuli, e.g. shout, clap or bang. B) Skin afferents are not stimulated upon tactile touch	Not routinely assessed
3. Baroreceptor sensitive	A) Linked to spontaneous falls and rises in BP B) Valsalva maneuver C) Inhibited during phenylephrine D) Activated during sodium nitroprusside E) Activated during head-up tilt	A) Inhibited during phenylephrine B) Activated during sodium nitroprusside

4. Chemoreceptor sensitive	A) End expiratory breath hold (hypercapnia) B) Hypoxia	A) Sodium cyanide injection
5. Ganglionic blockade	(Rarely done, and not essential) Inhibition with: A) systemic trimethaphan	Inhibition with: A) Pentolinium tartrate B) Hexamethonium
6. Low raw signal voltage	~50 micro V	~50 micro V
7. Other	A) (Rarely done) Inhibition with systemic clonidine	A) Excitation via the nasopharyngeal reflex (mostly in rabbits)

FIGURE LEGENDS

Figure 1: A microneurography recording of muscle sympathetic nerve activity (MSNA) in a male (A) and female participant (B, C). **A** shows a poor quality recording with very poor signal to noise ratio. **B** shows a good quality recording during normal sinus rhythm, the arrow indicates the r-wave that is linked to the inhibition of the burst (baroreflex latency = 1.2s for that burst). **C** illustrates MSNA during the development of spontaneous bigeminy in the same female participant (in the same recording session). During normal sinus rhythm (B) MSNA was ~42 bursts/min, compared to 24 bursts/min during spontaneous bigeminy (C).

Figure 2: Temporal patterns of muscle sympathetic (MSNA) and skin SNA (SSNA) before, during and 30 minutes after temporary baroreceptor deafferentation, completed by bilateral local anesthesia of glossopharyngeal and vagus nerves in the neck. Note that cardiac rhythmicity in MSNA gradually disappears (during bilateral block) and returns after anesthesia (together with associated changes of blood pressure and heart rate). Numbers below blood pressure (BP) record indicate seconds after bilateral anesthesia. ECG; electrocardiogram. Data from Fagius et al. (38).

Figure 3. *Left:* Recording from a muscle vasoconstrictor fiber in the peroneal nerve. **A**) One spike per multiunit burst. **B)** Individual and **C**) superimposed action potentials. From Macefield et al (74) *Right*: Recording from a sudomotor nerve fiber in the peroneal nerve of awake subject exposed to whole body heating that evoked sweating. Asterisks indicate spikes that were superimposed in the insert. From Macefield et al (72).

Figure 4. Representation of the standard method for recording sympathetic nerve activity in a multi-fiber preparation in a rat. A bipolar hook electrode is placed around the nerve and the voltage difference between the two electrodes is amplified, then rectified and finally integrated. Recording from an intact nerve includes both afferent (shown in blue) and efferent (shown in red) nerve activity.

Figure 5. Example recordings of blood pressure (upper), raw nerve (middle) and integrated nerve activity (lower) from a conscious rabbit. The signal can be optimized if background noise is subtracted. Noise values can be estimated from the inter burst interval during resting (far left), during the nasopharyngeal reflex (left), during phenylephrine (right) and/or during ganglion blockade with pentolinium (far right). The value of 6-8 for noise, measured over 100ms, is approximately 2% of the maximum signal produced during nasopharyngeal stimulation (left).

Figure 6. Mean arterial pressure (MAP), heart rate (HR) and cardiac sympathetic nerve activity (CSNA) burst incidence post-operation (the day of surgery was day 0). Eleven normal sheep were used in total, but data from every sheep were not available for each day. Numbers are as follows: day 1 n=4; day 2 n=5; day 3 n=7; day 4 n=7; day 5 n= 6; day 6 n=5; day 10 n=3. *Unpublished data* from Watson AM (2005), PhD thesis, University of Melbourne.

Figure 7. A recording of arterial pressure (AP), central venous pressure (CVP) and neurograms and spike frequency of cardiac and renal SNA (RSNA) in a conscious sheep showing bursts of SNA synchronised with the cardiac cycle and a higher resting burst incidence of RSNA than CSNA.

Figure 8. Schematic figure showing the basic approach for implantation of lumbar (A), renal (B) and splanchnic (C) electrodes as described by Stocker and Muntzel (107). In all cases, Teflon insulation is stripped away from the tip of the electrode, which is then shaped in to a hook and placed around the nerve. The electrode is then stabilized by suturing it to a nearby structure such as the aorta.

Figure 9. Example of renal SNA (RSNA) measured in a conscious rat (107). Raw and integrated renal SNA are shown before and after the ganglionic blocker hexamethonium (A) or a pressor dose of phenylephrine (C), each of which results in total suppression of RSNA thus allowing determination of background noise. Note the cessation of sympathetic bursts (denoted by the arrows) after hexamethonium (A) or phenylephrine (C). Note as well the indicators for burst frequency, which can also be quantified in response to hexamethonium (B) and phenylephrine (D). From Stocker and Muntzel (107).

Figure 10. Representative trace demonstrating physiological blood pressure and RSNA (raw and integrated) in a conscious mouse 48 hours following surgery (46).

Figure 11. A: Example of burst of unit activity (left), Section of record magnified (middle panel) to show waveforms of individual units. The horizontal lines indicate the thresholds for selecting the spikes. B: Right panels: Two templates generated from one filament. Heavy black lines represent the averages of all spikes selected to generate templates 1 or 2; n is the number of spikes used to generate that template.