Analysis of sympathetic neural discharge in rats and humans

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Neural signals convey information through two different modalities: intensity and discharge pattern. The intensity code is based on the number of action potentials per unit time, which is then easily translated into neurotransmitter release. This kind of information may be assessed simply by counting the number of spikes or bursts over a time unit. However, the discharge pattern is a further, efficient means of neural information transfer. Rhythmic patterns (i.e. oscillations) can support highly structured, temporal codes based on correlation and synchronization. It is therefore clear that applying frequency domain analysis to sympathetic activity recorded in animals and humans may provide additional information about the neural control of the circulation. Over the last century, data obtained by the analysis of sympathetic activity in experimental animals, and recently also in humans, have provided fundamental contributions to our understanding of the physiological mechanisms involved in the neural control of circulation, as well as how these are altered in cardiovascular and non-cardiovascular diseases. The aim of this paper is to address some aspects related to the recording, analysis and interpretation of sympathetic activity in rats and humans, with special emphasis on analysis in the frequency domain.

Keywords: autonomic nervous system; baroreflex mechanisms; central oscillators; spectral analysis

1. A brief historical introduction

Adrian et al. (1932) first reported the presence of oscillatory activity in sympathetic nerve fibre recordings. Four years later, Bronk et al. (1936) recorded multifibre cardiac sympathetic nerve activity (SNA) in anaesthetized cats and...
described the synchronized nature of this activity and its relationship with the cardiac cycle. These authors were able to modulate SNA by different manoeuvres and considered these rhythmic discharges to be the simple consequences of phasic input from arterial baroreceptors. In the 1960s and 1970s, several studies described multi- and single-fibre SNAs. The presence in SNA of oscillatory components faster than the cardiac cycle (Green & Heffron 1967) and the high coherence of neural bursts in different vascular beds (Gebber et al. 1995) suggested that oscillations of SNA have a complex origin, not only determined by the phasic input from arterial baroreceptors. These observations were initially carried out in animals, but later substantially confirmed in humans when the technique to record peripheral muscle and skin SNAs was developed (Delius et al. 1972).

Subsequent studies in the 1980s underlined the important role played by some brainstem cell groups in the genesis of SNA oscillations (Dampney 1994). Gebber et al. (1995) hypothesized that coupled brainstem generators cause synchrony between the rhythmic nerve discharges of sympathetic nerves supplying different end organs. Nevertheless, the neural circuits responsible for SNA oscillations and their precise localization(s) are not yet well understood. Rhythmic oscillations at frequencies from 10 to 0.1 Hz are detectable in normal SNA. The 10 Hz rhythm in SNA, identified by Cohen & Gootman (1970) and extensively studied by Barman & Gebber (1992), was coherent with the activity in a proportion of neurons in the rostral ventrolateral medulla. The presence of rhythmic oscillations at low (approx. 0.1 Hz in humans and 0.4 Hz in rats) and high (synchronous with respiratory rate) frequencies in both sympathetic and parasympathetic fibres, coherent with the oscillations present in heart rate and systolic arterial pressure variabilities, represents a new element in the debate about the origins of SNA oscillations. These observations were reported both in conscious animal studies (Brown et al. 1994) and in human neural recordings of peroneal sympathetic fibres (Pagani et al. 1997). Later, Malliani (2000) suggested that ‘two basic disturbances like vasomotion and respiration might be transformed by the wisdom of the body into a code providing information to the most various central structures about the state of excitation–inhibition balance characterizing the various patterns of the organism’. The observation that both low- and high-frequency oscillation components appear to be widely distributed among sympathetic post-ganglionic (Montano et al. 1992) as well as medullary neurons involved in the regulation of cardiovascular function may be interpreted in this context (Montano et al. 1996).

2. Sympathetic neural discharge in rats

(a) General considerations about data collection

One of the main functions of cardiovascular sympathetic efferents is to mediate rapid circulatory adjustments during natural behaviours and exposure to aversive stimuli. For this simple reason, the most informative studies on the sympathetic control of the circulation have to be carried out on unanaesthetized, preferably unrestrained, animals. Furthermore, these kinds of studies avoid the well-documented confounding influence of anaesthesia on cardiovascular autonomic reflexes (Shimokawa et al. 1998). One limitation of the studies on conscious animals, however, is that it is not yet technically feasible to record action potentials from...
single sympathetic axons (Thorén & Ricksten 1979). Instead, multifibre recordings are easily performed because sympathetic axons are grouped into fascicles and multiple units fire fairly synchronously. Whole-nerve activity recordings have been obtained from various sympathetic nerves in conscious sheep, dogs, cats, rabbits and rats. SNA recordings in conscious mice have not yet been reported.

The rat has been, and still is, the most commonly used species in cardiovascular research. In conscious rats, multifibre recordings of SNA have been obtained from the branches of the splanchnic (Ricksten et al. 1984; Stauss et al. 1995), renal (Lundin et al. 1984; Koepke & DiBona 1985; Barrès et al. 1992; Miki et al. 2002), adrenal (Unger et al. 1984) and lumbar (Thornton et al. 1989; Miki et al. 2004) nerves. The renal nerve has usually been preferred mainly because it is, with little doubt, of purely post-ganglionic nature (Scislo et al. 1998). The lumbar sympathetic chain contains axons of both pre- and post-ganglionic sympathetic neurons. However, this is probably inconsequential because first the proportion of pre-ganglionic axons is low (less than 10%; Scislo et al. 1998; Kanbar et al. 2008), and second it is quite likely that pre-ganglionic signals are transmitted through sympathetic ganglia with little modification (McLachlan 2003). The splanchnic nerve contains post-ganglionic axons supplying the splanchnic circulation as well as glands and muscle in the gut. These nerve populations subserve quite different functions and thus are differentially regulated (Morrison 2001). Furthermore, depending on the recording site, the splanchnic nerve may or may not contain axons supplying the adrenal medulla. It is of note that some functional heterogeneity is also present in renal and lumbar SNA recordings. The renal nerve contains axons of neurons that innervate blood vessels, tubules and juxtaglomerular cells in the kidney (DiBona 2000). However, as far as the baroreflex control of SNA is concerned, renal SNA (RSNA) behaves as a homogeneous population, because it is almost completely abolished during electrical stimulation of the aortic depressor nerve (Petiot et al. 2001). The lumbar chain contains mostly axons of neurons supplying skeletal muscle and skin of the hindlimb. The predominance of muscle over cutaneous SNA in resting lumbar SNA is suggested by its strong barosensitivity: muscle SNA is strongly pulse modulated by the arterial baroreceptor reflex, whereas skin SNA is not (Häbler et al. 1994). Finally, a common limitation to all three SNAs is that, when the nerve is not cut distal from the recording electrode, a contribution from the activity of afferent nerves cannot be ruled out. In the case of the renal nerve, this contribution is probably negligible as RSNA recorded after acute ganglionic blockade is not consistently higher than post-mortem activity (Bertram et al. 2005).

Usually, whole-nerve SNA is bandpass filtered, full-wave rectified and integrated before being sampled for off-line analyses. The continuously increasing computing power and storage capacity now make it possible to record SNA for rather long periods (several hours) at high sampling rates (up to several kHz), and thus to analyse fluctuations with widely varying kinetics.

(b) Sympathetic neural discharge in rats: time domain analysis

The simplest way of analysing fluctuations of SNA is to calculate mean values over a given duration and to plot these values as a function of time and/or experimental condition. When the duration of each segment is sufficiently long
(a few minutes), SNA changes can be referred to as tonic changes. It should be mentioned, however, that changes in whole-nerve integrated SNA are paralleled by changes in the overall indices of variability of SNA such as variance or total spectral power (Bertram et al. 2000). This observation simply reflects the redundancy existing between mean levels and variance estimates of SNA, because this nerve signal is essentially constituted by intermittent bursts of varying amplitude and frequency (McAllen & Malpas 1997; Malpas 1998).

For example, tonic increases in RSNA have been reported to occur during non-hypotensive haemorrhage (Scrogin et al. 1998), air-jet stress (Lundin et al. 1984; Koepke & DiBona 1985; Kanbar et al. 2007a), treadmill exercise (Miki et al. 2002) and grooming (Miki & Yoshimoto 2005). During rapid eye movement sleep, RSNA decreases while lumbar SNA increases (Miki & Yoshimoto 2005).

As recently reviewed by Barman & Kenney (2007), a disadvantage of time domain analysis is that it does not provide any information about the relative contribution to changes in the mean SNA level of the many rhythmic components contained within the SNA signal. For the same reason, frequency domain methods have been preferred to time domain methods for assessing relationships between two SNAs or between one SNA and a cardiovascular variable. It should be mentioned, however, that one important study used a non-parametric cross-correlation function to show that fluctuations in RSNA were not directly responsible for arterial pressure lability after sinoaortic baroreceptor denervation in rats (Barrè`s et al. 1992).

(c) Sympathetic neural discharge in rats: frequency domain analysis

Population activity within any one sympathetic nerve is more or less synchronized, i.e. individual sympathetic fibres tend to fire synchronously so that whole-nerve activity appears in the form of bursts. Spectral analysis is the method of choice for identifying and quantifying rhythms in SNA. Early studies performed on anaesthetized animals focused on fast rhythms, especially the rhythm synchronous with the heart beat, and disregarded slower fluctuations (for review see Malpas 1998). It is only recently, especially owing to recordings in conscious rats, that the importance of slow and very slow fluctuations of SNA has been recognized (Burgess et al. 1999; Chapuis et al. 2004). The most commonly used spectral methods are based on fast Fourier transform (FFT) algorithms. When applied to recordings of sufficiently long duration, these methods can provide a quantitative assessment of SNA fluctuations over a wide range of frequencies (see below). Based on their characteristic frequencies, the SNA fluctuations can be separated into five categories.

(i) The cardiac-related oscillations of SNA

As mentioned above, the SNA of conscious rats shows a rhythmicity at the frequency of the heart beat. This oscillation is seen in lumbar (Kanbar et al. 2008) and splanchnic (Persson et al. 1992) SNA recordings, but is especially prominent in recordings from the renal nerve (DiBona & Jones 1995; Kanbar et al. 2007b). It has been shown that the cardiac-related oscillation of RSNA is abolished after sinoaortic baroreceptor denervation, both acutely in anaesthetized rats (Petiot et al. 2001) and chronically in conscious rats (Kunitake & Kannan 2000). Moreover, intermediate reductions in the amplitude of this
oscillation are observed after chronic, partial (aortic) baroreceptor denervation (Kanbar et al. 2007b). These simple observations indicate that the arterial baroreceptor reflex plays a major role in the generation of this oscillation, either by synchronizing pre-existing desynchronized oscillations or by periodically interrupting a randomly generated activity (McAllen & Malpas 1997). In conscious rats, the amplitude of the cardiac-related oscillation of RSNA follows a bell-shaped curve in response to drug-induced changes in arterial pressure. Such behaviour is well predicted by a simple linear model where pulse-synchronous baroreceptor activity periodically inhibits a steady level of SNA, with the only assumption being that baroreceptor activity occurs above a threshold value of arterial pressure (Bertram et al. 2005). Regarding the function of these oscillations, it has been proposed that they might serve to set the mean level of vasoconstrictor tone. This is because the low-pass filter properties of the resistance vasculature prevent fast SNA oscillations (more than 1 Hz in rats) being translated into oscillations of vascular tone and arterial pressure. These fast oscillations, rather, produce ‘tetanic’ contractions of vascular smooth muscle cells and thereby contribute vasoconstrictor tone. However, the importance of the cardiac-related oscillation is probably overstated, because this oscillation accounts for only a small fraction of the HF power of RSNA (see below). Furthermore, sinoaortic baroreceptor-denervated rats that lack cardiac-related oscillations have a normal or augmented sympathetic vasoconstrictor tone (Zhang et al. 1995).

(ii) The respiratory-related oscillations of SNA

In rats, oscillations of SNA synchronous with respiratory movements (between 0.8 and 2 Hz) have been consistently observed (Persson et al. 1992; Kunitake & Kannan 2000), and are sometimes referred to as high-frequency (HF) oscillations. It has been proposed that they have a mixed origin: first, a central coupling between respiratory neurons and neurons of autonomic pathways; and second, a baroreflex response (Pilowsky 1995; Häbler et al. 1996). Chronic sinoaortic baroreceptor denervation has been reported either to diminish (Kunitake & Kannan 2000) or to leave unchanged (Julien et al. 2003) the respiratory oscillations of RSNA in conscious rats. During infusion of vasoactive drugs, the amplitude of these oscillations is reflexly modulated (Bertram et al. 2005). As spectral power in the respiratory band contributes approximately 20 per cent of total spectral power (when computed from 0.03 to 25 Hz) of RSNA in conscious rats, baroreflex-mediated changes in the amplitude of respiratory oscillations of RSNA significantly contribute to baroreflex-mediated changes in total spectral power and mean RSNA.

(iii) The low-frequency oscillations of SNA

The so-called low-frequency (LF) oscillations of SNA are observed at the same frequency as vasomotor waves of arterial pressure (often referred to as Mayer waves), i.e. approximately 0.4 Hz in rats (Julien 2006). These oscillations are present in renal (Brown et al. 1994) and lumbar (R. Kanbar 2008, personal communication) SNAs, and much less clearly in splanchnic SNA (Persson et al. 1992; Stauss et al. 1995). There is strong coherence between fluctuations of arterial pressure and renal or lumbar SNA at this frequency. Arterial pressure Mayer waves
are not seen in ganglion-blocked rats (Cerutti et al. 1994) or in chronically sympathectomized rats (Julien et al. 1995), which points to the direct role of SNA in producing LF arterial pressure oscillations. On the other hand, the LF oscillations of RSNA and arterial pressure are abolished after sinoaortic baroreceptor denervation (Kunitake & Kannan 2000; Julien et al. 2003), which demonstrates that both oscillations need the feedback from arterial pressure to be present. The most likely explanation for the LF oscillations of SNA is that, just as any negative feedback loop containing a fixed time delay, the sympathetic limb of the arterial baroreceptor reflex tends to become unstable at a characteristic frequency named the resonance frequency of the loop. It has indeed been shown that in rats the resonance frequency of the sympathetic baroreflex loop is close to the actual frequency of Mayer waves (Bertram et al. 1998). The fixed time delay in the loop is attributable to afferent, central and efferent neural conduction times (approx. 100 ms together) plus the latency of the vascular response (approx. 500 ms) (Bertram et al. 2000; Petiot et al. 2001; Julien et al. 2003; Chapuis et al. 2004; Oréa et al. 2007). At the resonance frequency, addition of the further time delay due to the low-pass filter properties of the vascular smooth muscle response causes the baroreflex effect on blood pressure to be delayed by exactly one half-period, resulting in positive feedback and potential instability in the loop. The amplitude of LF oscillations of SNA will then, at least partly, depend on the gain of the baroreflex system at its resonance frequency (Ringwood & Malpas 2001; Chapuis et al. 2004).

Interestingly, Mayer waves and accompanying LF oscillations of RSNA are enhanced during emotional stress (Barrès et al. 2004), which increases mean RSNA, and, conversely, the oscillations are blunted during sedation with pentobarbital (Cheng et al. 2004), which decreases mean RSNA. During drug-induced baroreceptor loading and unloading, the amplitude of LF oscillations of RSNA shows biphasic changes: reaching a maximum at approximately $-20 \text{mmHg}$ and then decreasing at larger AP reductions (Bertram et al. 2005). These data point to a complex relationship between the mean level of RSNA and the amplitude of LF oscillations of RSNA.

(iv) The very low-frequency fluctuations of SNA

When recorded over several hours in conscious rats, RSNA presents slow, non-periodic fluctuations that produce a $1/f$ trend in the power spectrum (Burgess et al. 1999; Chapuis et al. 2004). Surprisingly, RSNA is only weakly related to arterial pressure in the very low-frequency (VLF, $<0.15 \text{Hz}$) band as indicated by the low coherence values computed between the two variables. One explanation for this lack of coupling is the interference of perturbations affecting independently either variable. Sinoaortic baroreceptor denervation strongly increases the VLF power of arterial pressure, but does not alter the VLF power of RSNA and does not increase coherence between the two variables in the band (Julien et al. 2003; Chapuis et al. 2004). These observations indicate that central nervous structures can generate slow fluctuations of RSNA in the absence of baroreflex influences. They also suggest that, under normal conditions, baroreflex-mediated changes in SNA limit VLF fluctuations of arterial pressure, e.g. those produced by myogenic responses originating in regional circulations (Létienne et al. 1998). Therefore, slow RSNA fluctuations comprise both baroreflex- and non-baroreflex-mediated RSNA variations (Burgess et al. 2003).
The very high-frequency fluctuations of SNA

Fast fluctuations of SNA, except those synchronous with the heart beat, have been consistently ignored and considered as background noise. However, in rats, the spectral power of post-mortem RSNA in the very high-frequency (VHF, 2.5–25 Hz) band amounts to less than 3 per cent of the VHF power measured in the conscious state (Bertram et al. 2005), thus demonstrating that these fast and very fast fluctuations of RSNA are not measurement noise. VHF power contributes nearly 70 per cent of the total spectral power and, within this band, RSNA spectral power at the heart rate accounts for not more than 10–12 per cent. VHF power is extremely barosensitive and, thus, accounts for the major part of the baroreflex-mediated changes in mean RSNA level (Bertram et al. 2005). Furthermore, it is likely that VHF power is present up to frequencies much higher than 25 Hz (B. Chapuis & C. Julien 2008, unpublished observations). It may therefore be produced by unitary discharges. This raises the intriguing, and as yet unexplored, possibility that asynchronous spikes might work just as well as synchronous spikes in generating sympathetic vasoconstrictor tone.

3. Sympathetic nerve activity recordings in humans

The technique for intraneural recording of sympathetic discharge activity in humans (microneurography) was developed in 1969, by Vallbo, Wallin and colleagues at the University of Uppsala, Sweden. Since then, several studies based on the recording of the activity of groups of sympathetic neurons (multi-unit recording) or on the discharge pattern of a single sympathetic fibre (single-unit recording) have been published.

(a) Technical aspects

Details of the microneurography technique have been extensively reported elsewhere (Sundlof & Wallin 1977; Vallbo et al. 1979, 2004). Briefly, multi- and single-unit recordings of post-ganglionic SNA are obtained in humans by inserting unipolar tungsten microelectrodes into peripheral nerves. The raw neural signal is fed to a pre-amplifier (1000-fold amplification), filtered by a bandpass filter (bandwidth between 700 and 2000 Hz), rectified, amplified (100-fold) and integrated (time constant, 0.1 s) by a nerve traffic analyser system.

Peripheral nerves contain several fascicles that are connected either to defined skin area or to a muscle. Therefore, by microneurography, it is possible to assess both skin and muscle sympathetic nerve activities (Mosqueda-Garcia 1996).

Muscle sympathetic fibres discharge spontaneously in synchronized bursts coupled with the cardiac cycle and blood pressure fluctuations (Delius et al. 1972). Carotid sinus (Furlan et al. 2000) or aortic (Sanders et al. 1988) baroreflex loading is associated with inhibition of muscle sympathetic nerve activity (MSNA), whereas pharmacological-induced decrease in arterial pressure by nitroprusside, leading to arterial baroreceptor unloading, is attended by an enhancement of MSNA (Mosqueda-Garcia 1996; Pagani et al. 1997). Baroreceptor denervation results in a marked increase in neural sympathetic discharge and in a loss of its rhythmicity (Delius et al. 1972; Jordan et al. 1997; Furlan et al. 2001).
In the integrated neurogram of MSNA, the indices of sympathetic discharge activity are burst incidence (bursts/100 heart beats) and frequency (bursts min\(^{-1}\)). Total sympathetic activity is quantified by the number of bursts multiplied by mean burst area. Of interest, sympathetic burst amplitude depends on the number of sympathetic fibres that concomitantly discharge. As a consequence, this index is largely influenced by the proximity of the electrode tip to the active fibres. To overcome this limitation, single burst amplitude can be normalized by the highest burst amplitude recorded or, as recently reported, by the mean amplitude observed during controlled breathing (Barbic et al. 2008).

(b) Baroreceptor mechanisms and MSNA

In physiological conditions, MSNA and heart rate undergo a continuous modulation by arterial aortic and carotid baroreflex mechanisms in order to buffer modifications of blood pressure.

During standing, the arterial baroreceptor and cardiopulmonary afferent unloading initiates a reflex neural sympathetic excitation and a concomitant vagal withdrawal, which produce arterial and venous vasoconstriction, an increase in heart rate and cardiac inotropism aimed at maintaining blood pressure and cerebral perfusion.

Figure 1 depicts beat-by-beat invasive arterial pressure, muscle SNA and heart rate recordings obtained in a healthy volunteer during a 75° head-up tilt. The relationship among different signals is provided by baroreflex mechanisms. Note that post-ganglionic SNA is organized after a discharge pattern with a period of 10 s mimicking analogous spontaneous fluctuations of heart rate and arterial pressure. Therefore, the rhythmic oscillations of a variable, i.e. arterial pressure, are mirrored by analogous modifications in the discharge pattern of its neural control (i.e. post-ganglionic SNA, MSNA; Furlan et al. 2000).

(c) Laterality of muscle sympathetic nerve activity

In humans, the development of a variable pressure neck chamber method for bilateral stimulation (Eckberg & Sleight 1992) has furnished the opportunity to study the carotid component of arterial baroreflex activity and its modulatory effects on heart rate, MSNA and their variability. When the carotid baroreflex area was stimulated by a 0.1 Hz sinusoidal suction, with pressure oscillations ranging from 0 to \(-60\) mmHg, no asymmetry in the neural sympathetic discharge response could be elicited. Indeed, right and left carotid baroreflex activation resulted in similar changes in MSNA burst rate and frequency, and in the power of the 0.1 Hz component of MSNA variability (Furlan et al. 2003).

A different approach to address the problem of laterality is based on the concomitant recording of sympathetic traffic from both right and left peroneal nerves.

Figure 2 shows the neurograms of concomitant recordings of right and left peroneal MSNA and their power spectra during spontaneous breathing at rest, obtained in right-handed subjects. The burst amplitude was normalized by the average burst amplitude during controlled breathing at 0.25 Hz (figure 2a(i), b(i)). Note that, in spite of the similar burst rate, the resulting normalized burst amplitude was higher in the right MSNA recording compared with the left one. In addition, the sympathetic discharge activity pattern was alike in the right and left neurograms, as suggested by the similar MSNA variability power spectra.
Therefore, in humans, the use of normalized burst amplitude may disclose a laterality of efferent post-ganglionic sympathetic activity that remains hidden when conventional burst rate and MSNA variability indices are used. Presently, the potential role of handedness in producing laterality in the MSNA normalized burst strength is unclear.

4. Spectral analysis of muscle sympathetic nerve activity in humans

(a) Technical aspects

Traditional analysis of the MSNA signal is based on visual computation of the rate (bursts min$^{-1}$) and amplitude of the bursts. Conversely, a neural time series better suited for spectral analysis is provided through the integration of the continuous MSNA signal according to

$$MSNA(i) = 1/t(i) \int MSNA(t) \, dt,$$

Figure 1. Relationship among (a) heart rate (beats min$^{-1}$), (b) beat-by-beat invasive arterial pressure (mmHg) and (c) MSNA in a healthy subject during a gravitational stimulus. Traces refer to a 30 s period starting at the fifth minute of the gravitational stimulus obtained during a 20 min lasting tilt manoeuvre.

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where each integral was performed over the time window delimited by the \(i\)th cardiac cycle of period \(t(i)\). This series of variability measures of MSNA is synchronous with the other variability signals, such as tachogram and systogram on a beat-by-beat basis.

The time series obtained are then analyzed by means of FFT or autoregressive (AR) techniques, these latter automatically providing the number, centre frequency and power of each relevant component. Power is expressed in both absolute and normalized units (nu), which are obtained by dividing the power of each spectral component by the total variance from which the VLF component has been subtracted, and multiplying this value by 100 (Porta et al. 2009). This normalization of MSNA spectral components minimizes the drawback of this approach, which does not provide an absolute calibration of nerve activity.

(b) Studies in healthy subjects

The presence of rhythmic components in MSNA variability with a frequency slower than the heart rate, within the ranges of the low (LF) and high (HF) frequencies, was first reported by Eckberg et al. (1985) and Saul et al. (1990). However, these and other investigators (Kingwell et al. 1994) considered only the relationship between the absolute values of LF and HF oscillations and the average value of sympathetic activity in static conditions, in keeping with the more traditional view holding ‘intensity’ as the exclusive modality of neural coding. In this regard, it is important to underline that the absolute values of MSNA oscillations may only be valid when considering dynamic responses to

\[ \text{Figure 2. Integrated neurograms of concomitant (a) right and (b) left peroneal MSNA during spontaneous breathing: (i) recordings and (ii) relative power spectra. In both neurograms, the MSNA burst amplitude was normalized by the average burst amplitude during controlled breathing at 15 breaths per min. PSD} = \text{power spectral density.} \]
stimuli as static values lack of definite physical value, as the MSNA signal varies according to electrode position, the number of active single units, electronic amplification, filtering and electrode impedance. To avoid this inconvenience, a normalization procedure (calculation of LF- and HF-normalized units or LF/HF ratio) should be considered (Montano et al. 2001).

Pagani et al. (1997) observed that the MSNA spectral profile displays clear-cut LF and HF oscillations that are highly coherent with the corresponding blood pressure and R–R interval spectral components. Moreover, during sympathetic activation induced by IV nitroprusside, an increase in predominance of the LF oscillation in the variability of MSNA, R–R and arterial pressure was observed, while, conversely, sympathetic inhibition and vagal activation induced by IV phenylephrine were associated with a predominance of the HF components in all variability signals (Pagani et al. 1997). The absolute values of MSNA spectral components correlated significantly with measures of nerve activity such as burst frequency and amplitude. In addition, attendant changes in MSNA (assessed by its amplitude or normalized power of spectral components rather than bursts min$^{-1}$) were paralleled by similar changes of the LF and HF components of heart rate variability (HRV) when expressed in normalized units or as LF/HF ratio, being suggestive of common central mechanisms governing both sympathetic and parasympathetic cardiovascular modulations (Pagani et al. 1997). These data, of course, are limited to the small range of arterial pressure changes tested in this study. However, they seem to suggest that the two main oscillatory rhythms are markers of neural excitation (LF) and inhibition (HF), and that in healthy subjects they are modulated in a reciprocal manner. However, this holds true within physiological changes, but in the conditions characterized by co-activation of sympathetic and parasympathetic systems, this behaviour may be lost (Paton et al. 2005).

Similar results were obtained in a subsequent study (Furlan et al. 2000), in which a gravitational (i.e. a tilt manoeuvre) rather than a pharmacological stimulus was used to increase sympathetic activity in spontaneously breathing humans. They showed that tilting induced an increase in the absolute values of the LF and HF spectral components of MSNA as well as of the VLF component. However, normalization, accounting also for the change in VLF, indicated a significant increase in the LF oscillations and decrease in the HF oscillations of MSNA (Furlan et al. 2000). Cooke et al. (1999), when examining the changes in the integrated absolute areas of the LF and HF components of MSNA during graded tilt, found an increase in both components. However, the area of very low frequency (VLF) also increased markedly with increasing tilt angle, thus affecting the fractional distribution of power, which would have been offset by the use of normalization. Moreover, the subjects were on paced breathing during the graded tilt protocol (Cooke et al. 1999), and since respiration is a most effective modulator of sympathetic rhythmicity (Eckberg et al. 1985; Van De Borne et al. 2001), this could be another reason why these investigators obtained different results.

An additional observation supporting the hypothesis of the central common autonomic oscillatory network comes from a study by Montano et al. (1998), evaluating the effects of low and high doses of atropine on MSNA and cardiovascular variabilities. Muscarinic blockade induced by high-dose atropine led to the near abolition of HRV; however, the LF and HF components of MSNA
remains clearly apparent, reflecting the vagotonic effect of the drug and thus allowing one to appreciate that muscarinic blockade alters the relationship between autonomic drive and target function (Porta et al. 2000). Conversely, low-dose atropine, which enhances vagal drive without impeding muscarinic transmission, was associated with a shift of power towards HF in both heart rate and MSNA variabilities (Montano et al. 1998). It is noteworthy that MSNA, expressed as bursts over time, was not modified by low-dose atropine, unlike the oscillatory components (Montano et al. 1998).

This apparent dissociation between gross average activity and spectral profile of MSNA was even more evident in other studies. Van De Borne et al. (2001) reported that hypercapnic hyperventilation was associated with increases in heart rate and MSNA. In normal conditions, this would be expected to be associated with a relative enhancement of the LF powers of heart rate and MSNA, while it was, instead, observed as an increase in the HF spectral components and a decrease in the LF components in both signals (Van De Borne et al. 2001). Thus, changes in ventilation may result in dissociation between R–R and MSNA and may also disrupt the interactions between LF power and sympathetic drive. This has to be taken carefully into account when dealing with experimental protocols involving paced breathing or when interpreting HRV spectral data without having ventilation monitored.

Similarly, Cogliati et al. (2004), assessing the effects of acute β-blockade on cardiovascular and MSNA variability in healthy humans, reported that atenolol administration was associated with an expected bradycardia but with an unexpected increase in MSNA and its HF oscillatory power. As the coherence value between the HF oscillation of MSNA and respiration was significantly increased after atenolol, they hypothesized that these changes were related to an increased respiratory coupling. As it has been reported in humans (Stauss et al. 1998; Stauss 2007) that the gain of the transfer function between sympathetic activity and skin arterial blood vessels reaches its maximum at approximately 0.1 Hz, i.e. in the LF range, with progressive decay, a relative shift of spectral power towards an HF predominance might imply reduced effectiveness of neural vasoconstrictor activity. These mechanisms could play a protective role in pathological conditions characterized by an increased sympathetic activity such as the acute phase of myocardial infarction (Cogliati et al. 2004).

(c) Some considerations on spectral analysis of skin sympathetic nerve activity in humans

Skin sympathetic nerve activity (SSNA) differs markedly from MSNA in terms of control mechanisms and function. Indeed, whereas MSNA is constituted by vasoconstrictor fibres, mostly regulated by baroreceptor and other related cardiovascular reflex mechanisms, SSNA contains vasomotor and sudomotor fibres responding to mental and thermal stimuli (Mano 1998). Regional changes in SSNA in response to heat stress are not secondary to baroreflex regulation of blood pressure, because arterial and cardiopulmonary baroreceptor denervation does not alter vasomotor responses (Rowell 1983). A study by Grassi et al. (1998) reported that the alerting reaction associated with an increase in blood pressure was characterized by MSNA inhibition and SSNA excitation. It has also been reported recently that SSNA recorded from the peroneal nerve contains a
vasodilatory component synchronous with sudomotor nerve activity (Sugenoya et al. 1998). Thus, whereas MSNA subserves a primary role in the regulation of cardiovascular variables, SSNA function is mainly related to thermoregulation. To add complexity, Kamiya et al. (2008) reported the incomplete dependence of skin blood flow on vasoconstrictor SSNA, confirming that non-neural mechanisms control cutaneous circulation even at normothermic rest.

Cogliati et al. (2000) were the first to describe the detection of clear LF and HF rhythms in the variability of discharge of SSNA, similar to the LF and HF oscillations observed in MSNA variability and coherent with oscillations in R–R interval and systolic pressure. These findings were later confirmed by Cui et al. (2006). Although SSNA does not contribute to R–R interval or blood pressure, the LF and HF components of SSNA are similar to and coherent with the LF and HF oscillations in R–R interval and blood pressure. Thus, common central mechanisms may contribute to the similarities in morphology and regulation of MSNA and SSNA.

5. Conclusions

In neural networks, information is coded simultaneously based on two different modalities (Gerstner et al. 1997): intensity (i.e. the amplitude strength of the signal or ‘tonic’ activity) and discharge pattern (i.e. the oscillation of the signal or phasic activity). Sympathetic activity in rats and humans is characterized by three major common rhythmicities, such as the VLF, LF and HF components. Moreover, in conscious rats, VHF frequencies have been recently described. While there is agreement between investigators regarding the importance of spectral analysis of sympathetic discharge variability for the evaluation of neural control of circulation, certain differences remain regarding some aspects of methodology and interpretation of findings. In this regard, we have to take into account that physiological measures, and particularly indices derived from spectral analysis, are affected by experimental bias and errors. Moreover, the information content of the rhythmic components may be spread across several drive inputs (baroreflexes, chemoreflexes, sympathetic excitatory afferent reflexes, central oscillators, ventilation, central command, etc.) making it difficult to disentangle the contribution of each single determinant to the overall complexity, at least in closed-loop conditions. Open-loop experiments have demonstrated that, in rats, (i) the arterial baroreceptor reflex is directly involved in the production of cardiac-related oscillations of SNA and oscillations associated with vasomotor waves (LF or Mayer waves), (ii) respiratory SNA oscillations have a mixed central and baroreflex origin, (iii) VLF oscillations derive from the combined and probably time-varying influence of several mechanisms, and (iv) the origin of VHF oscillations is still unsettled.

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