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REVIEW | *50 Years of Microneurography: Insights into Neural Mechanisms in Humans*

Physiological and pathophysiological firing properties of single postganglionic sympathetic neurons in humans

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Macefield VG, Wallin BG. Physiological and pathophysiological firing properties of single postganglionic sympathetic neurons in humans. *J Neurophysiol* 119: 944–956, 2018. First published November 15, 2017; doi:10.1152/jn.00004.2017.—It has long been known from microneurographic recordings in human subjects that the activity of postganglionic sympathetic axons occurs as spontaneous bursts, with muscle sympathetic nerve activity (MSNA) exhibiting strong cardiac rhythmicity via the baroreflex and skin sympathetic nerve activity showing much weaker cardiac modulation. Here we review the firing properties of single sympathetic neurons, obtained using highly selective microelectrodes. Individual vasoconstrictor neurons supplying muscle or skin, or sudomotor neurons supplying sweat glands, always discharge with a low firing probability (~30%) and at very low frequencies (~0.5 Hz). Moreover, they usually fire only once per cardiac interval but can fire greater than four times within a burst. Modeling has shown that this pattern can best be explained by individual neurons being driven by, on average, two preganglionic inputs. Unitary recordings of muscle vasoconstrictor neurons have been made in several pathophysiological states, including heart failure, hypertension, obstructive sleep apnea, bronchiectasis, chronic obstructive pulmonary disease, depression, and panic disorder. The augmented MSNA in each of these diseases features an increase in firing probability and discharge frequency of individual muscle vasoconstrictor neurons above that seen in healthy subjects, yet firing rates rarely exceed 1 Hz. However, unlike patients with heart failure, all patients with respiratory disease or panic disorder, and patients with hyperhidrosis, exhibited an increase in multiple within-burst firing, which emphasizes the different modes by which the sympathetic nervous system grades its output in pathophysiological states of high sympathetic nerve activity.

microneurography; single-unit; sympathetic nervous system; sympathoexcitation

INTRODUCTION

Microneurography, in which an insulated tungsten microelectrode is inserted through the skin of an awake human subject into an accessible peripheral nerve, or even an accessible cranial nerve, has provided a wealth of information on how the sympathetic nervous system controls blood flow, blood pressure, and sweat release, both in health and disease. Much of what we have learned has come from multiunit recordings of action potentials generated by postganglionic sympathetic axons supplying muscle [muscle sympathetic nerve activity (MSNA)] or skin [skin sympathetic nerve activity (SSNA)]. MSNA, which represents the activity of vasoconstrictor neurons supplying vascular beds in skeletal muscle, plays an important role in the regulation of blood pressure. As such, MSNA is strongly influenced by the arterial and cardiopulmonary baroreceptors. Bursts of MSNA are briefer than bursts of SSNA, owing to the tight cardiac rhythmicity of MSNA; the carotid arterial baroreceptors provide the dominant source of temporal coupling of MSNA to the cardiac cycle (Fatouleh and Macefield 2013; Wallin et al. 1975; Wallin and Eckberg 1982). When baroreceptor afferents are blocked, by bilateral anesthesia of the glossophyaryngeal and vagus nerves, MSNA loses its normal cardiac entrainment and assumes much of the character of SSNA (Fagius et al. 1985). In addition to this strong

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coupling to the cardiac rhythm, MSNA is also modulated by respiration: bursts of MSNA are larger in expiration, becoming smaller in midinspiration (Eckberg et al. 1985; Hagbarth and Vallbo 1968; Macefield and Wallin 1995; Seals et al. 1990, 1993). SSNA, which primarily subserves thermoregulation, also exhibits respiratory modulation, although this is sometimes weak (Bini et al. 1980; Delius et al. 1972a, 1972b; Hagbarth et al. 1972).

Despite the significant contributions of multiunit recordings of MSNA and SSNA to our understanding of the operation of the sympathetic nervous system, the information content is fairly coarse (Macefield 2013). This is because the traditional methods of analysis simply rely on counting the number of bursts per minute (burst frequency) or per 100 heartbeats (burst incidence); occasionally total activity (typically, the cumulative burst amplitude calculated over one minute) is also calculated. However, while changes in burst amplitude can indicate differences in sympathetic drive during a maneuver, and the relative distribution of burst amplitudes can provide information on overall burst strength that can be compared across recordings (Sundlöf and Wallin 1977; Sverrisdóttir et al. 1998, 2000), absolute burst amplitudes cannot be compared across individuals. This is because the amplitude of a burst is determined by how close the tip of the microelectrode is to the sympathetic axons, which typically tend to travel within a fascicle of the nerve as groups of 2–44 axons, occasionally individually (Tompkins et al. 2013). MSNA burst incidence varies widely across individuals and cannot be predicted from either blood pressure or heart rate but has been shown to be remarkably consistent in a given individual over time (Fagius and Wallin 1993; Sundlöf and Wallin 1977). It is clear that there is a strong genetic component determining the amount of MSNA, with burst incidence being similar in homozygous twins but differing widely in heterozygous twins (Wallin et al. 1993).

More recent work, using wavelet deconstruction techniques to isolate unitary action potentials from a burst of impulses (Brychta et al. 2007; Salmanpour et al. 2010), has shown that the number of neurons firing in a spontaneous burst of MSNA at rest ranges from 3 to 40 within a single recording site; on average, ~12 neurons are active in a typical multiunit burst of MSNA (Salmanpour et al. 2011a; Salmanpour and Shoemaker 2012; Steinback et al. 2010). Wavelet analysis has also shown that large sympathetic bursts, which we know from standard multiunit recordings have shorter latencies from the R wave to which they are coupled (Wallin et al. 1994), are associated with recruitment of larger neurons with faster conduction velocities (Salmanpour et al. 2011a; Salmanpour and Shoemaker 2012; Steinback et al. 2010). Moreover, it has been shown that maneuvers that cause a sustained increase in MSNA, such as the Valsalva maneuver or an end-tidal or inspiratory-capacity apnea, increase the number of neurons active within a burst (Breskovic et al. 2011; Salmanpour et al. 2011b). However, while wavelet analysis of multiunit recordings has revealed large variability in the number of postganglionic neurons active in a sympathetic burst and shown that recruitment plays a major role in increasing burst amplitude, it cannot provide information on how often a given neuron fires within a burst.

METHODOLOGY OF SINGLE-UNIT RECORDING

Since the first recordings of sympathetic nerve activity were made in the mid-1960s, there have been tantalizing examples of recordings in which large spikes appeared within a burst, leading to the speculation that it would be possible to record spikes from single unmyelinated axons. The first unitary recordings of sympathetic nerve activity were published by Hallin and Torebjörk (1974). These investigators analyzed the firing properties of eight units recorded from cutaneous fascicles of the common peroneal and median nerves, although their identity as cutaneous vasoconstrictor or sudomotor was unknown. Nevertheless, this study showed that while a burst of impulses may contain many spikes, individual units exhibited low levels of activity at rest and fired only one to seven times, even during large arousal bursts. However, a systematic study of single sympathetic neurons did not occur until 20 yr later, when we embarked on a project to characterize the discharge behavior of vasoconstrictor neurons supplying blood vessels in muscle or skin and sudomotor neurons supplying sweat glands. We, and others, have since gone on to examine how firing properties of single neurons change with disease, with most studies having looked specifically at the firing properties of individual muscle vasoconstrictor neurons (Lambert et al. 2012).

Single-unit recordings allow the measurement of firing probability (the unitary equivalent of burst incidence), the mean firing rate of individual neurons within a burst, and, importantly, how often a unit fires within a burst. Although technically challenging, requiring rigorous criteria for accepting a recording as unitary, the approach has revealed some interesting features in the firing properties of individual postganglionic neurons. The aim of this review is to highlight what has been learned from recording from single sympathetic neurons in awake human subjects and how such recordings provide information on how the sympathetic nervous system adjusts its output in different situations.

The approach used is similar to that used to record multiunit activity, the only difference being that the microelectrode has to have a much higher electrical impedance and hence a more focal recording area to selectively record the action potentials generated by an individual axon. Small manual adjustments of the microelectrode are made until a site is found in which large spikes from a putative single axon stand out from the noise. Two examples of unitary recordings from a single muscle vasoconstrictor neuron are shown in Fig. 1. The unit illustrated in Fig. 1*A* was firing spontaneously, while that in Fig. 1*B* is shown during a maximal inspiratory breath hold, a maneuver that causes a sustained increase in MSNA. In both recordings, the superimposed action potentials show a consistent morphology, supporting the conclusion that the spikes originated from a single axon. The spikes are typically triphasic with a dominant negativity, which is consistent with their origin from unmyelinated axons, although aberrant waveforms may also occur, at least in pathological states such as spinal cord injury (Wallin et al. 2014). Smaller action potentials can also be seen in the nerve recordings; these are generated by axons further away from the tip of the microelectrode, and it is this "far-field" activity that is largely responsible for the population discharge seen in the integrated nerve signal.

ANALYSIS OF SINGLE-UNIT DATA

Detailed offline analysis is required to ensure that the recording is, in all likelihood, unitary. As with all single-unit recordings, superimposition of the spikes is a necessary requirement to be able to ascertain whether a recording is likely to be from a single axon, although the caveat always remains that the spikes could have been generated by another axon from which the generated spikes have an identical morphology. However, given their low firing probabilities (see below), we believe this to be very rare. Indeed, as suggested in Fig. 1, most

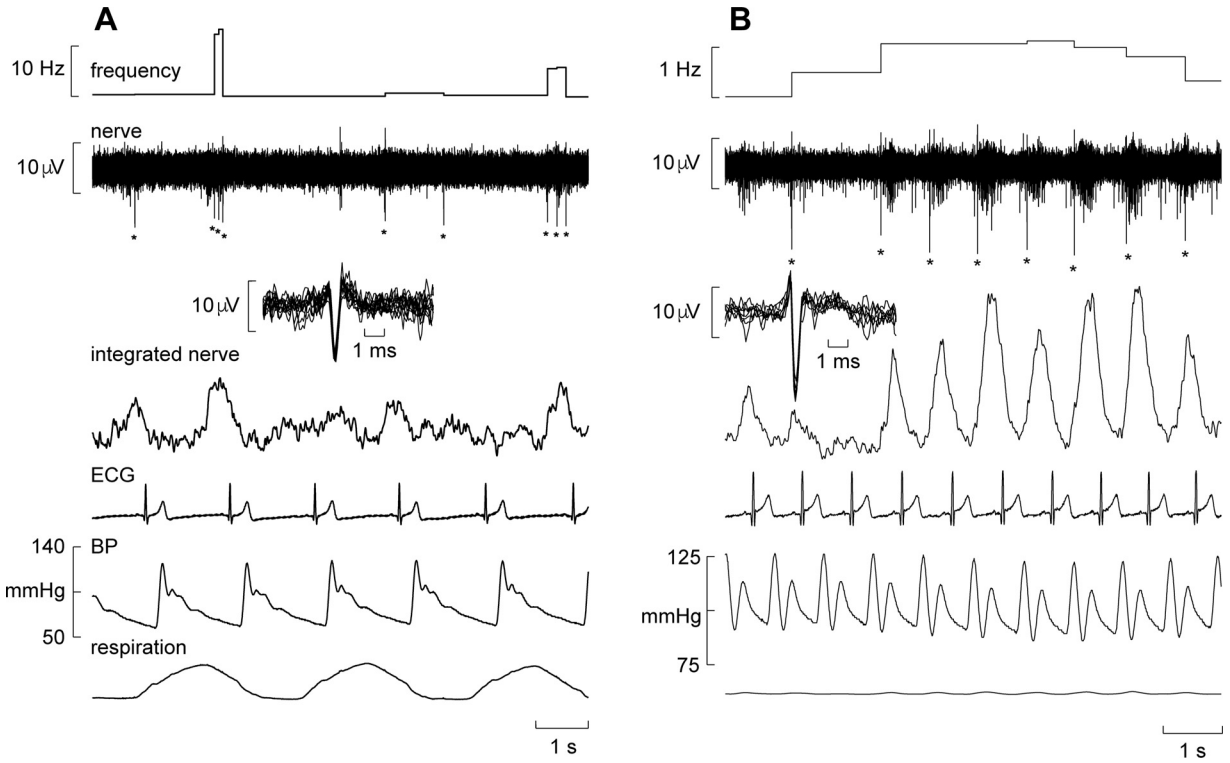

Fig. 1. Single-unit recordings from two muscle vasoconstrictor neurons. *A*: spontaneously active unit firing at rest. *B*: another unit, also spontaneously active at rest, shown during an inspiratory-capacity apnea. Superimposed spikes are shown for the spikes indicated by asterisks. Modified from Macefield and Wallin (1999a) (© John Wiley & Sons).

sympathetic neurons fire only once per cardiac interval but occasionally do fire more than once. Figure 2*A* shows on an expanded time base a single burst of MSNA in which a single muscle vasoconstrictor neuron fires twice. This same neuron is shown generating four spikes in Fig. 2*B*, with another smaller, putative unit also firing four times in the illustrated burst. Individual spikes from the large and small unit are shown in Fig. 2, *C* and *D*, respectively, with the superimposed spikes of the large unit being shown in Fig. 2*E*. Due to the amplitude variability, the smaller spikes were, however, not considered to be unitary. The spike morphologies illustrated in Figs. 1 and 2 are very similar to those shown in Fig. 2, *F–G*, which were recorded from a single postganglionic sympathetic axon supplying the rat tail artery. By comparing the amplitude distributions of spikes generated by individual neurons, it was shown that slight variations in spike amplitude in a unitary recording can be explained by the underlying fluctuations in background noise (Macefield and Wallin 1999a, 1999b). Since spikes generated by different axons often have similar waveforms, we have recently used three waveform indices (spike amplitude, spike duration, and slope of the fast phase of the action potential) to quantify action potential shapes (Wallin et al. 2014). With subsequent statistical analyses, the method proved useful for separating safely between spikes from different axons recorded in a given electrode site. In addition to increasing the quality of the analysis, the method may also increase the yield of acceptable units in a given electrode site.

The advantage of performing a single-unit recording is that, once the electrode tip is close to an active axon, the firing behavior of that neuron can be described in a quantal fashion: it is either active (i.e., it has a quantal value of 1) or silent (i.e., it has a quantal value of 0), and the overall firing probability can therefore be calculated. This, together with information on discharge frequency and the number of spikes a unit generates within a burst, allows one to compare this information across individuals and across groups of healthy subjects or groups of clinical patients. When starting our single-unit studies, we introduced a method of analysis that aimed to standardize the extraction of information from a single-unit recording. From each unitary recording, the following parameters should be obtained: *1*) the firing probability (percentage of cardiac intervals in which a given neuron is active, *2*) the number of spikes per cardiac interval and *3*) the mean instantaneous frequency (calculated as the inverse of the mean interspike interval). However, not all investigators have adopted this approach (see Greenwood et al. 1998, 1999). Nevertheless, according to this analytical paradigm, differences in the intensity of multiunit sympathetic activity (i.e., the number and the intensity of multiunit bursts) may be achieved by differences in *1*) the number of active neurons or *2*) the mean firing frequency of the neurons, or a combination of both mechanisms. A difference in mean firing frequency may be brought about by *1*) a difference in firing probability of neurons active in a burst or *2*) a difference in the number of times the neuron fires within the burst, or a combination of both.

COMMON FIRING PROPERTIES OF MUSCLE AND CUTANEOUS SYMPATHETIC NEURONS IN HEALTH

Our first systematic study examined the firing properties of muscle vasoconstrictor neurons in young healthy subjects (Macefield et al. 1994). Their patterns of activity can be predicted from the behavioral criteria used to establish a

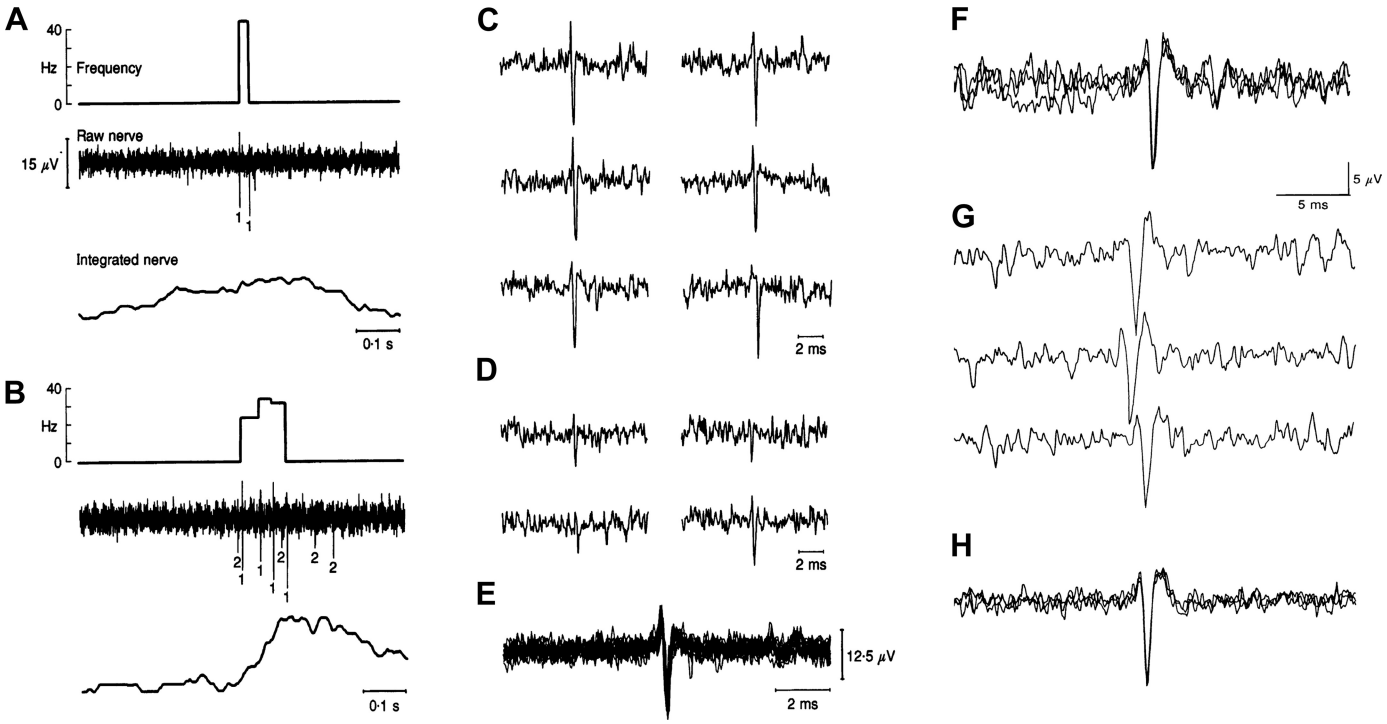

Fig. 2. *A* and *B*: recording of 2 bursts of muscle sympathetic nerve activity (MSNA) in which a large unit (*unit 1*) was discriminated from a smaller unit (*unit 2*). *C*: the 2 spikes from the burst in *A* and the 4 in *B*, shown on an expanded timescale. *D*: the 4 spikes from *B* (*unit 2*) were not considered to originate from a single unit. *E*: 20 superimposed spikes of the large unit. Reproduced from Macefield et al. (1994) (© John Wiley & Sons). *F–H*: recordings from a single sympathetic neuron supplying the rat tail artery, reproduced with permission from Johnson and Gilbey (1996) (© John Wiley & Sons). *F*: consistent spikes shapes and latencies in response to electrical stimulation of the tail. *G*: consistent spike shape but variable latencies in response to electrical stimulation of the sympathetic chain. *H*: superimposed spikes from the unit shown in *F* and *G*.

multiunit recording as being of MSNA: a tight temporal coupling to the R wave of the ECG and an increase in firing probability with an increase in cardiac interval or a decrease in diastolic pressure. In addition, individual muscle vasoconstrictor neurons display respiratory modulation (Macefield et al. 1994). We subsequently characterized the discharge behavior of single units recorded from cutaneous fascicles, but because some of these neurons supply primarily blood vessels and some supply sweat glands, we had to increase skin sympathetic nerve activity selectively, either toward the blood vessels or toward sweat glands. Whole body cooling was used to bias SSNA toward cutaneous vasoconstrictor activity (Macefield and Wallin 1999b), while whole body warming was used to bias SSNA toward sweating (Macefield and Wallin 1996). Thus it is important to note that these recordings were obtained in states of increased sympathetic drive to the target tissues, in which even minor external disturbances had to be avoided to prevent the coactivation of cutaneous vasoconstrictor and sudomotor neurons that occurs in response to arousal. By contrast, the unitary recordings from muscle vasoconstrictor neurons were made at rest in supine subjects, in whom the muscle vasoconstrictor drive is relatively low.

Interestingly, regardless of the type of neuron, muscle vasoconstrictor, cutaneous vasoconstrictor, or sudomotor, the firing properties are remarkably similar: a low-level intermittent discharge, typically at ~0.5 Hz, dominated by solitary spikes. Cutaneous vasoconstrictor and sudomotor neurons also exhibit respiratory modulation and possesses a degree of cardiac rhythmicity (Macefield and Wallin 1996, 1999b), albeit being weaker than for the muscle vasoconstrictor neurons. Nevertheless, the cardiac interval provides a convenient method of dividing a recording into small intervals, allowing one to determine whether an individual neuron is active or quiescent. As noted above, the percentage of cardiac intervals in which a neuron fires one or more spikes is referred to as the firing probability; it does vary widely across units but is generally $<$40% in healthy subjects. Curiously, it does not differ significantly across different types of postganglionic sympathetic neuron.

Figure 3 shows the firing distributions for the three classes of sympathetic neuron studied; the open columns represent those cardiac intervals in which the neurons were silent. In Fig. 3, *D–F*, these intervals have been excluded to show the distribution of single and multiple spikes in those cardiac intervals in which the neurons were active. The distributions are similar for all types of neuron: each class of neuron generated single spikes in ~70% of all cardiac intervals in which it was active. Individual neurons could fire up to seven times within a cardiac interval but this was rare; the majority fired at most two to four spikes per cardiac interval. Therefore, a multiunit burst of sympathetic nerve activity is primarily composed of many neurons generating a single spike, rather than a few neurons firing many spikes, a conclusion supported by wavelet analysis of multiunit recordings (Salmanpour et al. 2011; Salmanpour and Shoemaker 2012; Steinbeck et al. 2010).

Another common feature of human sympathetic neurons is the wide distribution of instantaneous firing rates. While mean discharge frequencies were low, occasionally high instantaneous frequencies could be generated. Short interspike intervals of $<$20 ms were unusual, occurring in 2–4% of all interspike intervals, and usually consisted of two to three

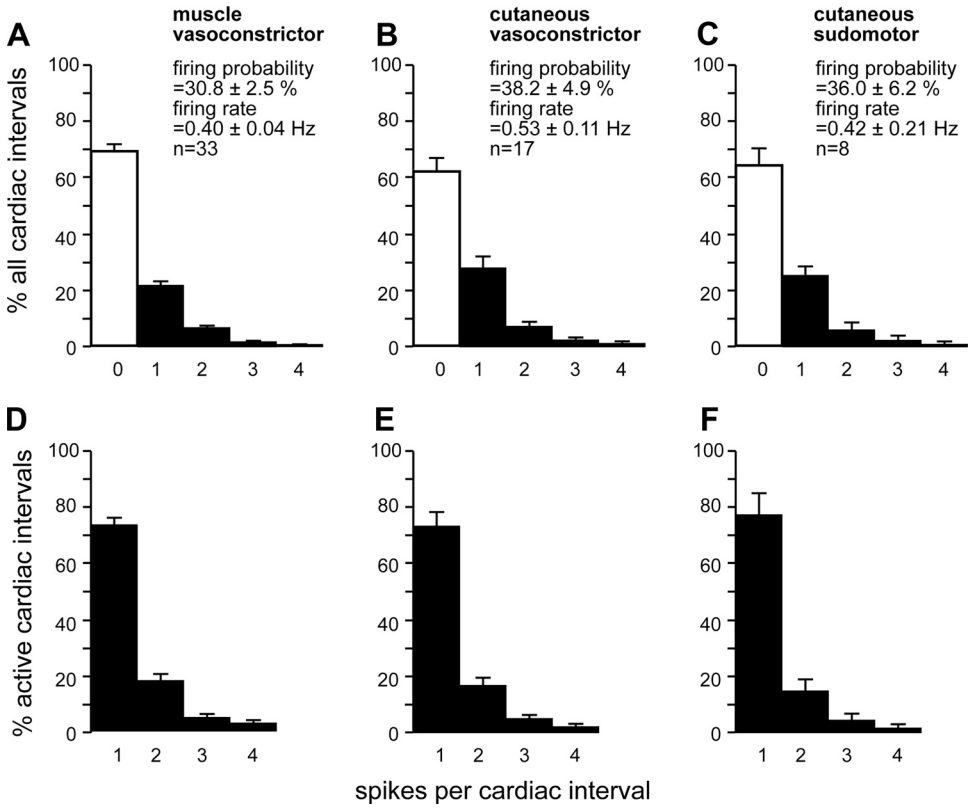

Fig. 3. *A–C*: percentage of cardiac intervals in which populations of single muscle vasoconstrictor neurons (*A*), cutaneous vasoconstrictor neurons (*B*), and sudomotor neurons (*C*) are quiescent (white columns) or fire 1, 2, 3, or 4 spikes (black columns) per cardiac interval. *D–F*: same data for vasoconstrictor neurons (*D*), cutaneous vasoconstrictor neurons (*E*), and sudomotor neurons (*F*) after excluding those cardiac intervals in which a unit was silent. Modified from Macefield et al. (2002) with permission from Elsevier.

spikes. Interestingly, they were not necessarily associated with large bursts, occurring in an essentially random fashion, and were exhibited by all types of sympathetic neurons studied. Figure 4*A* shows an example of a high-frequency triplet generated by a single cutaneous vasoconstrictor neuron. An example of a high-frequency doublet produced by a single muscle vasoconstrictor neuron is shown in Fig. 4*B*. Figure 4*C* illustrates a potential mechanism by which these intermittent short interspike intervals may be produced, through the chance occurrence of action potentials generated by two preganglionic neurons firing almost synchronously. It is postulated that this is but an emergent property of the organization of the sympathetic nervous system, in which largely independent drives in two preganglionic neurons drive a single postganglionic neuron.

MODELING THE FIRING PROPERTIES OF HUMAN SYMPATHETIC NEURONS

Why human postganglionic neurons should primarily fire only once per burst, regardless of their type and at different levels of sympathetic drive, is not known. However, it may simply be an emergent property of the way in which postganglionic sympathetic neurons are driven. A recent modeling study supports this possibility (Macefield 2011). Model spike trains were generated by one-, two-, or three-model preganglionic neurons, with each model neuron converging onto a single postganglionic neuron, as represented schematically in Fig. 4*C*. Figure 5 shows the resulting spike trains produced by converging inputs from two (Fig. 5*A*)- or three (Fig. 5*B*)-model preganglionic neurons onto a model postganglionic neuron. Each preganglionic input fired independently, with a mean interval distribution ranging from 1,000 to 3,000 ms for different trains, and with a standard deviation of each train between 0.5 and 2.0 $\times$ the mean interval. Some of these data are shown in Table 1. To closely mimic the firing of a real postganglionic neuron required that the model neuron be driven by, on average, two preganglionic neurons firing at a mean frequency of 0.4 Hz (means $\pm$ SD interspike interval 2,500 $\pm$ 5,000 ms). As shown in Table 1, the mean firing rate of this artificial neuron was 0.22 Hz, which is similar to that of muscle vasoconstrictor neurons in healthy subjects with high levels of MSNA (0.33 Hz, Table 2) although somewhat lower than that of subjects with low levels of MSNA (0.49 Hz, Table 2). Importantly, the distribution of spikes per cardiac interval (which was set at 1 s, equivalent to a heart rate of 60 beats/min) was similar between the model data and the real data. None of the modeled patterns of preganglionic neurons firing at firing rates $>$0.4 Hz fitted the actual data.

The modeling data can also explain the discharge properties of cutaneous vasoconstrictor neurons, recorded during cold-induced cutaneous vasoconstriction (Macefield and Wallin 1999b), and sudomotor neurons, recorded during heat-induced sweating (Macefield and Wallin 1996), because we know that the firing patterns of these neurons, which were recorded in states of elevated sympathetic drive, are similar to those of muscle vasoconstrictor neurons recorded at rest. Of course, individual neurons do vary in how often they fire (firing probability): some neurons have a low and some a high firing probability. Recently, studies using wavelet decomposition techniques to extract the firing of individual spikes in multiunit recordings have also led to similar observations (Salmanpour et al. 2011; Salmanpour and Shoemaker 2012; Steinbeck et al. 2010). Although some of the modeled postganglionic neurons do fire with a pattern that closely reflects the input from a single preganglionic neuron, as shown in Table 1, none of them

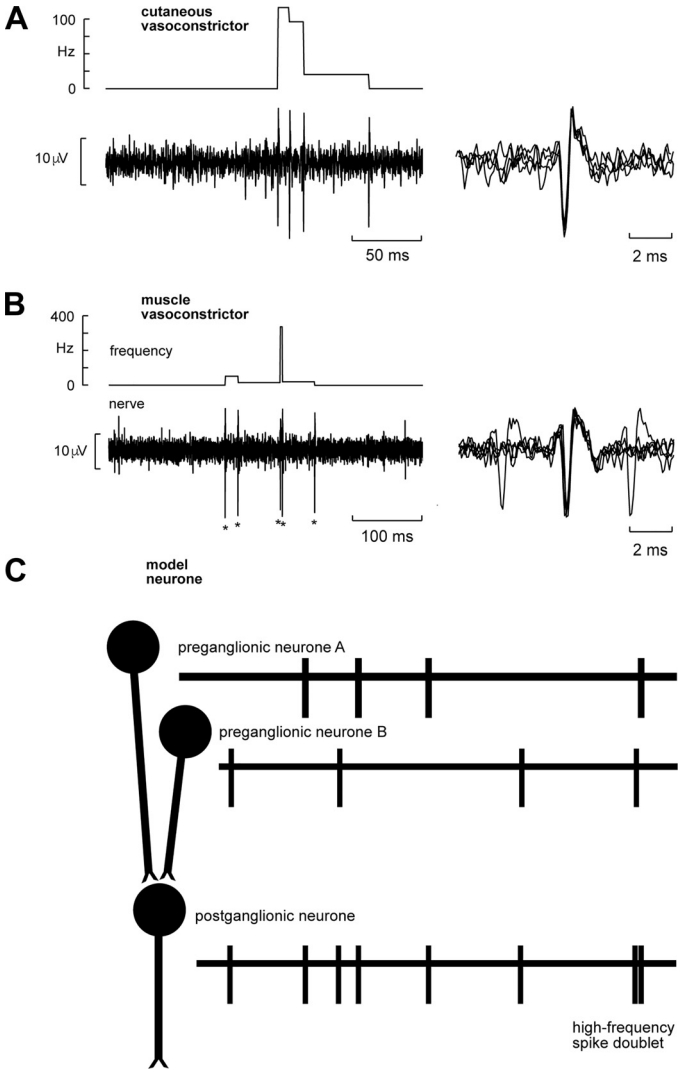

Fig. 4. Examples of high-frequency discharges recorded from a single cutaneous vasoconstrictor neuron (*A*) and a single muscle vasoconstrictor neuron (*B*); superimposed spikes are shown on the right. *C*: schematic representation of a mechanism by which the inputs from 2 active preganglionic neurons combine to generate intermittent high-frequency spike doublets at the postganglionic neuron. Modified from Macefield et al. (2002) with permission from Elsevier.

fired with a pattern that reflected the combined inputs from three preganglionic neurons. Therefore, it is fair to conclude that each postganglionic sympathetic neuron is driven by, at most, two preganglionic neurons.

Interestingly, in the superior cervical ganglion of the rat, some postganglionic neurons have been shown to be driven by two preganglionic neurons with "strong" synapses, and sometimes by three, but most receive excitatory drive from only one preganglionic neuron (Li and Horn 2006; McLachlan et al. 1997, 1998; Rimmer and Horn 2010). Likewise, postganglionic neurons in a lumbar sympathetic ganglion in the rat are mostly driven by one "primary" preganglionic neuron, but inputs from two (and occasionally three) "secondary" preganglionic neurons can sometimes contribute (Bratton et al. 2010). Moreover, when neurons in the paravertebral sympathetic ganglia are partially denervated, there is a preferential increase in the number of "strong" synapses after several weeks. Despite this the properties of the postganglionic neurons following reinnervation are no different from those studied before the partial denervation (Ireland 1999; McLachlan 2007). Notwithstanding the likely effects of general anesthesia on neuronal properties, it seems that the overall synaptic organization of preganglionic and postganglionic neurons in awake humans is similar to that in the anesthetized rat. Moreover, the discharge frequencies are similar to those of single postganglionic axons in anaesthetized experimental animals, which fire at ~1 Hz. Although the identities of the target tissues were unknown, postganglionic neurons in the superior cervical ganglion of the rat discharge spontaneously with a mean (±SE) frequency of 1.8 ± 0.4 Hz (McLachlan et al. 1997). Vasoconstrictor neurons directed to the kidney or the spleen in the cat fire from 1.2 ± 0.2 Hz (Meckler and Weaver 1988), while cutaneous vasoconstrictor neurons supplying the rat tail artery fire from 0.81 ± 0.04 Hz (Johnson and Gilbey 1996) to 1.12 ± 0.09 Hz. However, it has been pointed out that firing rates are clearly shifted toward lower values, the median frequency being 0.9 Hz (Häbler et al. 1999; Meckler and Weaver 1988).

FIRING PROPERTIES OF MUSCLE SYMPATHETIC NEURONS DURING ACUTE INCREASES IN DRIVE IN HEALTHY SUBJECTS

The propensity to fire only once per burst was also found during a maneuver that causes sustained increases in MSNA, an inspiratory-capacity apnea. As illustrated in Fig. 1*B*, large bursts of MSNA were generated in most cardiac intervals during the maximal inspiratory breath hold, yet the individual neuron primarily only fired once per burst. On average the mean firing rate of individual muscle vasoconstrictor neurons was 0.33 ± 0.04 (SE) Hz at rest, increasing to 1.04 ± 0.14 Hz during the apnea (Macefield and Wallin 1999a); this was due to the increase in firing probability (from a resting value to 32.5 ± 6.2 to 56.3 ± 3.1% during the apnea) and an increased probability of multiple firing. The percentage of cardiac intervals in which only one spike was produced decreased from 85.1 ± 4.5% at rest to 61.3 ± 5.6% during the breath hold. Because of this, the percentage of cardiac intervals in which a unit generated two spikes increased from 11.5 ± 2.7 to 26.7 ± 2.2%; the proportion of cardiac intervals in which a unit generated three or four spikes also increased during the apnea, although this did not reach statistical significance.

Murai et al. (2006) also found increases in firing rate and multiple firing of individual muscle vasoconstrictor neurons during the Valsalva maneuver but not during isometric handgrip exercise, despite the large increases in MSNA in both maneuvers. This suggests that the increase in burst amplitude during static handgrip exercise was brought about primarily by the recruitment of additional neurons. As noted above, recruitment of additional neurons has been documented using wavelet analysis of multiunit recordings during the Valsalva maneuver and during both inspiratory-capacity and end-expiratory apneas (Breskovic et al. 2011; Salmanpour et al. 2011). In an elegant study, lower body negative pressure, which causes a sustained increase in MSNA because of unloading of the cardiopulmonary baroreceptors, was shown to increase the firing probability and mean firing rate of individual muscle vasoconstrictor neurons but not the proportion of multiple spikes per cardiac interval (Millar et al. 2013). Lower body positive pressure caused opposite changes, but, curiously, a subpopulation of muscle vasoconstrictor neurons responded in a

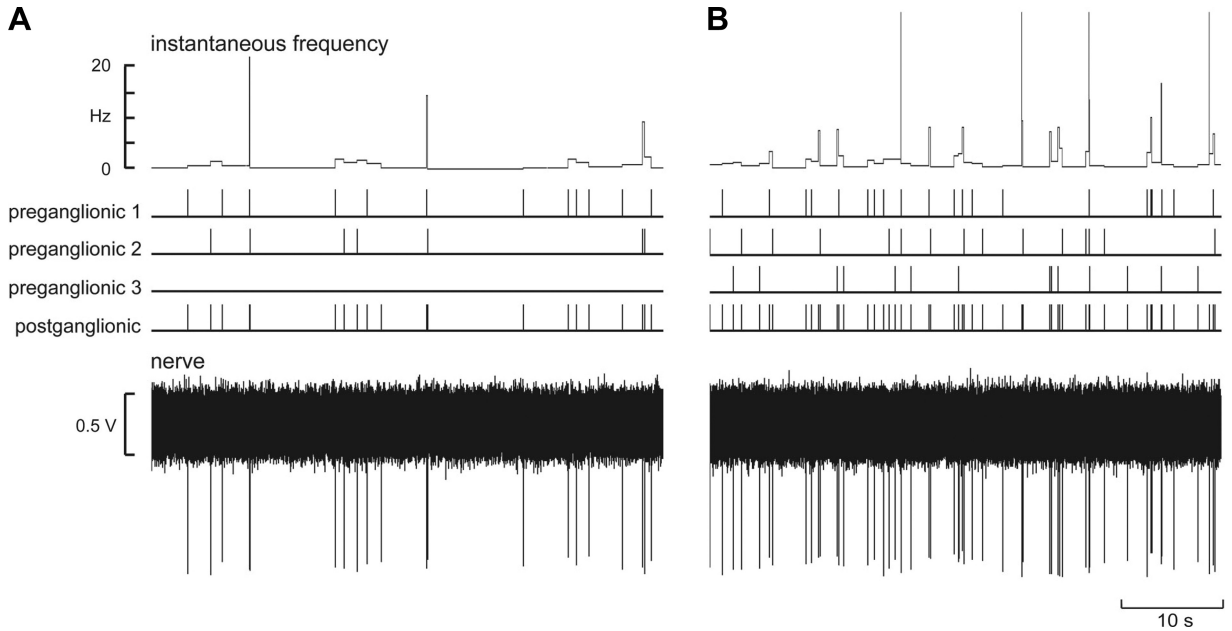

Fig. 5. Spike trains from 2 (*A*) or 3 (*B*) "preganglionic neurons" that converged onto a model "postganglionic neuron." The *bottom trace* shows the emulated nerve recording, in which spikes are superimposed on an underlying background noise. The mean interspike interval for each preganglionic neuron was 2,500 ± 5,000 (SD) ms. Reproduced from Macefield (2011).

paradoxical fashion to unloading or loading of the cardiopulmonary baroreceptors. Therefore, while such a paradoxical pattern was not apparent in multiunit recordings, it only became apparent when analyzing the firing properties of single muscle vasoconstrictor neurons.

FIRING PROPERTIES OF MUSCLE SYMPATHETIC NEURONS IN CHRONIC HEART FAILURE AND RESPIRATORY DISEASE

Multiunit recordings have found augmented MSNA in many cardiovascular diseases. In this context, it is worth remember-

Table 1. *Firing patterns of model postganglionic neurons driven by one-, two-, or three-model preganglionic neurons with differing interspike interval distributions*

Model Data Mean ISI ± SD	0 Spikes	1 Spike	2 Spikes	3 Spikes	4 Spikes	Firing Probability	Mean Frequencies
1,500 ± 750 ms							
Unit 1	42.4	52.6	4.9	0.1	0	57.6	0.52
Units 1 + 2	17.4	41.8	34.0	6.2	0.6	82.6	1.21
Units 1 + 2 + 3	6.8	27.6	38.2	21.0	6.0	93.2	1.96
1,500 ± 1,500 ms							
Unit 1	57.1	37.1	5.4	0.4	0	42.9	0.36
Units 1 + 2	30.0	43.5	21.1	4.9	0.5	70.0	0.77
Units 1 + 2 + 3	15.6	36.9	31.7	11. 9	3.5	84.4	1.25
1,500 ± 3,000 ms							
Unit 1*	70.1	27.6	2.2	0	0	29.9	0.23
Units 1 + 2	49.0	38.9	10.1	1.8	0.1	51.0	0.43
Units 1 + 2 + 3	34.2	41.2	18.6	5.3	0.6	65.8	0.66
2,500 ± 1,250 ms							
Unit 1	46.9	44.6	7.1	1.1	0.1	53.1	0.43
Units 1 + 2	23.6	40.0	25.7	8.6	1.7	76.4	0.89
Units 1 + 2 + 3	11.7	28.9	31.7	18.1	6.8	88.3	1.46
2,500 ± 2,500 ms							
Unit 1*	70.0	27.5	2.5	0	0	30.0	0.24
Units 1 + 2	49.9	37.9	10.9	1.2	0.1	50.1	0.43
Units 1 + 2 + 3	34.7	41.2	19.5	4.1	0.6	65.3	0.65
2,500 ± 5,000 ms							
Unit 1	83.2	15.7	1.1	0	0	16.8	0.11
Units 1 + 2†	69.5	25.6	4.3	0.5	0.1	30.5	0.22
Units 1 + 2 + 3	57.2	32.8	8.6	1.1	0.4	42.8	0.32
Actual data from muscle vasoconstrictor neurons (n = 33)	66.6	21.2	6.4	1.7	0.7	33.4	0.40

Mean data on the percentage of cardiac intervals in which units were silent (0 spikes) or generated 1, 2, 3, or 4 spikes, firing probability, and mean firing rate. Data obtained from real muscle vasoconstrictor neurons are shown in the *bottom row* (data pooled from Macefield et al., 1994; Macefield and Wallin 1999b). Modified from Macefield (2011). ISI, interspike interval. *For these ISI distributions the firing pattern could be partially explained by a postganglionic neuron being driven by one preganglionic neuron. †For this ISI distribution the firing pattern closely matches that of the real sympathetic neurons.

Table 2. *Firing properties of muscle vasoconstrictor neurons in healthy subjects with low or high resting levels of MSNA, during an acute increase in MSNA caused by a maximal inspiratory breath hold, and in patients with bronchiectasis, obstructive sleep apnea, chronic obstructive pulmonary disease, or chronic heart failure*

Muscle Vasoconstrictor Neurons	Units (*n*)	Burst Incidence, %	Firing Probability, %	Mean Frequency, Hz	One Spike, %	Two Spikes, %	Three Spikes, %	Four Spikes %
Healthy: low MSNA at rest	14	21.0 ± 2.2*	25.3 ± 3.0*	0.49 ± 0.06*	65.9 ± 5.5	18.9 ± 2.6	7.1 ± 2.2	6.0 ± 2.4*
Healthy: high MSNA at rest	19	74.9 ± 0.5	34.9 ± 3.6	0.33 ± 0.04	77.6 ± 3.8	18.1 ± 2.9	3.6 ± 1.1	0.5 ± 0.3
Healthy: acute increase in MSNA	9	100*	56.3 ± 3.1*	1.04 ± 0.14*	61.3 ± 5.6*	26.7 ± 2.2*	9.5 ± 3.4*	2.0 ± 1.0
Bronchiectasis	6	77.6 ± 4.3	37.8 ± 6.8	0.72 ± 0.17*	60.0 ± 6.2*	23.2 ± 2.6*	10.6 ± 1.9*	1.5 ± 0.7
Obstructive sleep apnea	12	77.2 ± 5.2	50.7 ± 4.4*	0.96 ± 0.11*	58.7 ± 2.8*	27.3 ± 1.3*	9.7 ± 1.5*	2.9 ± 0.7
Chronic obstructive pulmonary disease	17	85.1 ± 1.9*	52.2 ± 4.9*	0.92 ± 0.12*	63.4 ± 3.3*	24.8 ± 2.0*	8.7 ± 1.0*	2.2 ± 0.6
Chronic heart failure	16	88.1 ± 4.7*	55.1 ± 5.1*	0.98 ± 0.22*	70.6 ± 5.8	18.2 ± 2.4	7.3 ± 2.6*	3.0 ± 1.6

Values are means ± SE. Data are shown for muscle vasoconstrictor neurons in healthy subjects with low (Macefield et al. 1994) or high (Macefield and Wallin 1999) resting levels of muscle sympathetic nerve activity (MSNA), during an acute increase in MSNA caused by a maximal inspiratory breath-hold (Macefield and Wallin 1999), and in patients with bronchiectasis (Ashley et al. 2010), obstructive sleep apnea (Elam et al. 2002), chronic obstructive pulmonary disease (Ashley et al. 2010), or chronic heart failure (Macefield et al. 1999). *Significantly different ($P < 0.05$) from healthy subjects with high levels of resting MSNA. $P < 0.05$. Modified from Macefield (2012).

ing that on an individual basis high multiunit MSNA does not necessarily mean the presence of an underlying disease: many healthy subjects have high levels of resting MSNA (Sundlöf and Wallin 1977). A disease with increased multiunit MSNA is chronic cardiac failure (Leimbach et al. 1986; Macefield et al. 1999; Rundqvist et al. 1997) and, given the coupling between the respiratory and cardiovascular systems, it is perhaps not surprising that multiunit MSNA is increased also in respiratory diseases, such as obstructive sleep apnea (Carlson et al. 1993; Elam et al. 2002; Hamaoka et al. 2016; Narkiewicz et al. 1999), chronic obstructive pulmonary disease (Ashley et al. 2010; Heindl et al. 2001), and bronchiectasis (Ashley et al. 2010).

A comparison of single unit characteristics in these diseases illustrates the strength of single-unit analysis: since the recording measures whether a spike is generated or not, increases in firing probability, mean firing rate, or multiple firing are likely to reflect underlying pathology in a more detailed way than the finding of high multiunit activity does. In healthy subjects with high resting levels of MSNA, individual muscle vasoconstrictor neurons are known to have low firing probabilities, low firing rates, and a low incidence of multiple firing (Macefield and Wallin 1999a). Conversely, as we will see, pathophysiological increases in MSNA are associated with at least one of these features: a high firing probability, a high firing rate, and a high incidence of multiple firing. Moreover, the relative contributions of each of these patterns may differ in different pathologies. At present, however, we have no knowledge of the mechanisms underlying such selective alterations of firing characteristics.

Unitary recordings from muscle vasoconstrictor neurons have been made in chronic heart failure (Elam and Macefield 2001; Macefield et al. 1999), obstructive sleep apnea (Elam et al. 2002), chronic obstructive pulmonary disease (Ashley et al. 2010), and bronchiectasis (Ashley et al. 2010). Similar high multiunit burst incidences were seen in all conditions (77–88%), but there were differences in single unit firing. In chronic obstructive pulmonary disease, obstructive sleep apnea and chronic heart failure mean firing rates were markedly elevated (0.92–0.98 Hz) and also firing probabilities (51–55%) were high. However, in patients with bronchiectasis, mean firing rate was lower than in the other diseases (0.72 Hz) as was firing probability (38%, not different from that of healthy subjects with high levels of MSNA at rest, 35%).

The distributions of spikes generated in each cardiac interval are also shown in Table 2. It is apparent that there is no difference in spike distribution between chronic heart failure patients and controls, an observation confirmed by Murai et al. (2009, 2012). Unlike chronic heart failure, patients with chronic obstructive pulmonary disease, obstructive sleep apnea, and bronchiectasis also showed a shift toward multiple single unit firing.

Even if there is no difference in spike distribution between chronic heart failure patients and controls, Murai et al. (2006, 2009) noted that static-handgrip exercise caused an increase in multiple firing in chronic heart failure but not in controls. A more recent study also showed that lower body positive pressure, rather than inhibiting the firing of single muscle vasoconstrictor neurons, actually caused a paradoxical increase in the majority of the neurons in patients with heart failure, the authors suggesting that this provides evidence of an augmented cardiopulmonary reflex in heart failure (Millar et al. 2015). This fits with what this group also found in healthy subjects, referred to above (Millar et al. 2013).

It is interesting that, compared with healthy controls, there was no difference in spike distribution in chronic heart failure, despite the very marked sympathoexcitation; this suggests that the increase in MSNA was due to an increase in firing probability and, especially, recruitment of previously silent muscle vasoconstrictor neurons. Why there was no increase in multiple firing we do not know, but it had previously been found that multiple firing can occur during the prolonged cardiac intervals following ventricular ectopic beats in chronic heart failure (Elam and Macefield 2001). An example is shown in Fig. 6. Note that, when in sinus rhythm, the neuron fires only once per cardiac interval, but fires twice following each ectopic beat. Mean data are provided in Table 2. An earlier suggestion was that this is simply because there is more time available for multiple spikes to be generated in the prolonged bursts (Macefield and Elam 2004), but an overall increase in excitatory input from the preganglionic neurons could also be responsible for the change in firing pattern.

Recently, Ikeda et al. (2012) showed that heart failure patients with atrial fibrillation had a significantly greater incidence of multiple firing (48 ± 8%) than patients in sinus rhythm (26 ± 3%). This was due to a reduction in the number of solitary spikes from 74 ± 3 to 56 ± 5% and an increase in percentage of cardiac intervals in which the neurons generated

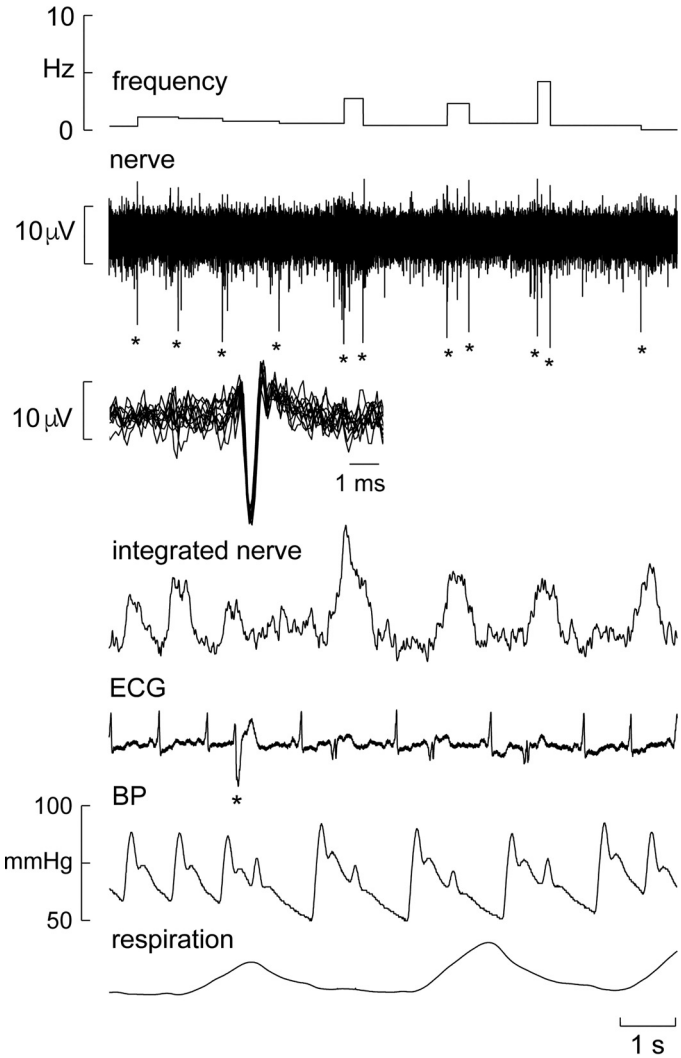

Fig. 6. Recording from a single muscle vasoconstrictor neuron in a patient with severe heart failure, showing a sequence with alternating premature beats. This unit generally fired only once per cardiac interval (indicated by asterisks) at regular rhythm but fired multiple spikes in the hypotensive intervals (i.e., in the large multiunit burst) following premature beats. Reproduced from Elam and Macefield (2001).

two (18 ± 2 to 25 ± 6%), three (5 ± 1 to 13 ± 3%), or four (1 ± 1 to 6 ± 3%) spikes. Moreover, because of the increase in multiple firing, mean discharge frequencies were also higher in atrial fibrillation (Ikeda et al. 2012). This group also showed that single-unit recordings provided a more sensitive measure of the increase in muscle sympathetic outflow in obstructive sleep apnea; while MSNA burst frequency showed a strong correlation to the clinical severity as measured by the apnea-hypopnea index, the correlation to single-unit spike frequency was much tighter. Moreover, there was a greater reduction in solitary firing and a greater shift toward multiple firing of individual neurons in patients with the most severe obstructive sleep apnea (Hamaoka et al. 2016).

It is very likely that an increase in multiple firing will have significant physiological consequences. For instance, it is known that electrical stimulation of sympathetic nerves to blood vessels causes a greater vasoconstriction when delivered in bursts, rather than as a continuous train (Andersson 1983; Nilsson et al. 1985; Polenov et al. 1991). Furthermore, electrical stimulation of human sudomotor neurons leads to a greater release of sweat when the stimuli are delivered in irregular patterns (Kunimoto et al. 1991, 1992). Lambert et al. (2011b) recently showed that cardiac norepinephrine release is higher in periods when muscle vasoconstrictor neurons (and, one would expect, also cardiac sympathetic neurons) fire multiple spikes This supports the idea that multiple firing in itself causes an increase in norepinephrine release. Of relevance to this finding is our recent study in people with spinal cord injury. Although multiunit sympathetic outflow below the spinal lesion was essentially absent, individual neurons presented a very low level spontaneous discharge but could be made to generate up to 12 spikes during the solitary bursts evoked by brief pressure on the bladder (Wallin et al. 2014). Such discharges may contribute both to the high norepinephrine release in the leg (Karlsson et al. 1998), and to the well-known exaggerated vascular response reported after bladder stimuli in patients with chronic high spinal cord lesions, although it is well known that changes in the vasculature also occur following spinal cord injury (McLachlan 2007).

FIRING PROPERTIES OF MUSCLE SYMPATHETIC NEURONS IN HYPERTENSION

It is well established that most forms of human hypertension are neurogenic. On a group level (but not necessarily on the individual level), MSNA is increased in many different forms of high blood pressure: essential hypertension (Grassi et al. 1998; Schlaich et al. 2004), pregnancy-induced hypertension (Fischer et al. 2004; Schobel et al. 1996), renovascular hypertension (Johansson et al. 1999; Miyajima et al. 1991), and chronic kidney disease (Hausberg et al. 2002; Schlaich et al. 2009; Tuncel et al. 2002). In the late 1990s David Mary's group in Leeds, UK, undertook an extensive series of experiments in which they performed single-unit recordings from muscle vasoconstrictor neurons in patients with high blood pressure (Mary and Soker 2003). Unfortunately, they did not count the number of spikes generated per cardiac interval and presented firing rates only as spikes per minute. However, they did find an increase in single-unit firing in patients with essential hypertension: the number of spikes per 100 heartbeats increased from 29 ± 2 (SE) in normotensive subjects to 43 ± 5 in borderline and 63 ± 6 in stage 1 essential hypertension (Greenwood et al. 1999). Recalculating firing rates from their impulses/minute data resulted in mean discharge frequencies of 0.32 ± 0.03, 0.45 ± 0.05, and 0.75 ± 0.07 Hz, respectively. A later study on patients with essential hypertension and left-ventricular cardiac failure showed that single muscle vasoconstrictor neurons generated 76 ± 7 spikes per 100 heartbeats, compared with 52 ± 3 in patients with hypertension alone (Greenwood et al. 2001). In fitting with the lower burst frequency in women, individual muscle vasoconstrictor neurons had lower firing rates in female than male hypertensives (Hogarth et al. 2007). This team also examined pregnancy-induced hypertension: single-unit firing increased from 45 ± 12 impulses per 100 heartbeats in pregnant women with normal blood pressure to 172 ± 30 in women with pregnancy-induced hypertension, the recalculated mean firing rates increasing from 0.60 ± 0.14 and 2.25 ± 0.35 Hz, respectively (Greenwood et al. 1998).

Metabolic syndrome, a consequence of type 2 diabetes, is characterized by high blood pressure, and obese patients with type 2 diabetes have significantly higher firing rates than weight-matched controls without diabetes (78 ± 5 vs. 48 ± 3 spikes per 100 heartbeats; Huggett et al. 2005). Firing properties are different in hypertensive patients who are obese or have normal weight (Lambert et al. 2007). Moreover, controlled weight loss has been shown to reduce mean firing rates from 59 ± 10 to 32 ± 4 spikes per 100 heartbeats, firing probability from 34 ± 5 to 23 ± 3%, and the incidence of multiple firing from 14 ± 4 to 6 ± 1% (Lambert et al. 2007, 2011a). Recently, this group found that patients with drug-resistant hypertension showed a reduction in mean firing rates (43 ± 5 to 27 ± 3 spikes per 100 heartbeats), together with reductions in firing probability (30 ± 2 to 22 ± 2%) and incidence of multiple firing (8 ± 1 to 4 ± 1%), following radiofrequency renal nerve ablation (Hering et al. 2013).

FIRING PROPERTIES OF MUSCLE SYMPATHETIC NEURONS IN PSYCHIATRIC DISEASE

MSNA and cardiovascular risk is increased in major depressive disorder and alleviated following treatment with selective serotonin reuptake inhibitors (Barton et al. 2007). Conversely, patients with anxiety disorder have an elevated cardiovascular risk but multiunit MSNA is not elevated (Lambert et al. 2006, 2008, 2010). However, single unit recordings have revealed that obese women with anxiety have a higher incidence of multiple firing (Lambert et al. 2010). Moreover, despite the mean burst frequencies being similar in panic disorder and controls (26 ± 3 vs. 28 ± 3%), and mean firing rates of individual muscle vasoconstrictor neurons also being similar (0.38 ± 0.09 vs. 0.22 ± 0.03 Hz), firing probability is elevated (45 ± 5 vs. 32 ± 3%) in panic disorder; moreover, the incidence of multiple firing is higher (Lambert et al. 2006).

FIRING PROPERTIES OF CUTANEOUS SYMPATHETIC NEURONS IN DISEASE

The primary role of skin sympathetic nerve activity is to control heat loss from the body through its actions on sweat glands, blood vessels and (phylogenetically) hairs. SSNA also increases during brief arousal stimuli (Hagbarth et al. 1972) or by states of elevated emotional engagement (Brown et al. 2012). Palmar-plantar hyperhidrosis, i.e., excessive emotionally triggered sweating from the palms and the soles of the feet, is a pure sudomotor disturbance; there is no involvement of cutaneous vasomotor or other sympathetic systems. It has been shown to be due to an increase in sudomotor outflow, as demonstrated by recordings from the tibial and median nerves (Iwase et al. 1997; Macefield et al. 2008). In single-unit recordings from sudomotor neurons supplying the fingers of patients with hyperhidrosis, these units had low firing probabilities (30.0 ± 6.7%) and a tendency to fire only once per burst at rest (Macefield et al. 2008). Interestingly, compared with sudomotor neurons supplying the hairy skin of the foot, active during heat-induced sweating, there was no difference in mean firing rates (0.77 ± 0.33 vs. 0.72 ± 0.21 Hz). However, there was a shift toward multiple firing in hyperhidrosis. Again, this shift in multiple firing may have functional outcomes, as overall firing variability will increase if the number of cardiac intervals with more than one spike is higher than when only solitary spikes are generated. Of relevance here is that, as noted above Kunimoto et al. (1991, 1992) demonstrated that sweat release produced by intraneural stimulation of sudomotor axons was higher when the trains of stimuli were composed of irregular frequencies than trains of regular frequencies, an effect that was most pronounced at low stimulation frequencies. Sweat release was maximal with irregular stimulation at 0.49 Hz (Kunimoto et al. 1992), which is close to the firing rates of sudomotor neurons in hyperhydrosis (0.77 Hz) and heat-induced sweating (0.72 Hz). This suggests that the shift toward multiple firing may lead to an increase in the release of neurotransmitter.

CONCLUSIONS

As with studies in experimental animals (Jänig 1985), single-unit recordings from postganglionic sympathetic neurons in awake human subjects, although technically challenging, have increased our understanding of how the human sympathetic nervous system grades its output, both in health and in disease. Because unitary recordings are quantal in nature, a neuron either fires or does not, so the firing properties of individual neurons can be compared across subjects, as well as across different diseases. Therefore, by providing evidence of increases in either firing probability, mean firing rate, or multiple firing it is possible to determine whether an elevated level of sympathetic nerve activity reflects an increase in central sympathetic drive that may be being driven by some underlying pathology, given that pathophysiological increases in sympathetic outflow appear to be associated with at least one of the following: high firing probabilities, high firing rates, or a high incidence of multiple firing. Single unit recordings, coupled with the means of extracting information on the recruitment of neurons from multiunit recordings of sympathetic nerve activity, offer a level of information that, until this approach was developed, was only available from reduced, anesthetized experimental animals. Accordingly, we suggest that the continued application of single-unit analysis of the firing properties of sympathetic nerve activity in health and disease will promise to provide insights into how the sympathetic nervous system operates to maintain homeostasis in physiological and pathophysiological states. However, the challenge of obtaining unitary recordings remains, with the quality of the microelectrode being of paramount importance: it is essential that high-impedance (>5 MΩ) electrodes be used. Moreover, it is imperative that the unitary identity of a recording is confirmed through superimposition of spikes. While there will be some variation in amplitude when the spikes are examined on a contracted time base, and this variation can be accounted for by the fact that the spike generated by a single axon "surfs on the waves" of the underlying noise, when the spikes are superimposed this variation in spike amplitude and morphology essentially disappears. Finally, the development of improved template-matching techniques for extracting spikes from oligounitary recordings will no doubt increase the yield of data, which is currently very low.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

V.G.M. and B.G.W. conceived and designed research; V.G.M. and B.G.W. performed experiments; V.G.M. analyzed data; V.G.M. and B.G.W. interpreted results of experiments; V.G.M. prepared figures; V.G.M. drafted manuscript; V.G.M. and B.G.W. edited and revised manuscript; V.G.M. and B.G.W. approved final version of manuscript.

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