Analysis of Firing Correlations Between Sympathetic Premotor Neuron Pairs in Anesthetized Cats

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McAllen, R. M., D. Trevaks, and A. M. Allen. Analysis of firing correlations between sympathetic premotor neuron pairs in anesthetized cats. J Neurophysiol 85: 1697–1708, 2001. The activity of sympathetic premotor neurons in the rostral ventrolateral medulla (subretrofacial nucleus) supports sympathetic vasomotor tone, but the factors that drive these premotor neurons’ activity have not been determined. This study examines whether either direct interconnections between subretrofacial neurons or synchronizing common inputs to them are important for generating their tonic activity. Simultaneous extracellular single-unit recordings were made from 32 pairs of sympathetic premotor neurons in the subretrofacial nucleus of chloralose-anesthetized cats. Paired spike trains were either separated by spike shape from a single-electrode recording (14 pairs) or recorded from two electrodes less than 250 μm apart (18 pairs). All neurons were inhibited by carotid baroreceptor stimulation and most had a spinal axon proven by antidromic stimulation from the spinal cord. Autocorrelation, inter-spike interval, and cardiac cycle-triggered histograms were constructed from the spontaneous activity of each neuron, and cross-correlation histograms covering several time scales were generated for each neuron pair. No significant peaks or troughs were found in short-term cross-correlation histograms (2 ms bins, ± 100 ms range), providing no support for important local synaptic interactions. On an intermediate time scale (20 ms bins, ± 1 s range), cross-correlation revealed two patterns indicating shared, synchronizing inputs. Repeating peaks and troughs (19/32 pairs) were due to the two neurons’ common cardiac rhythmicity, of presumed baroreceptor origin. Single, zero time-spanning peaks of 40–180 ms width were seen in 5/32 cases. Calculations based on the prevalence and strength of these synchronizing inputs indicate that most of the ensemble spike activity of the subretrofacial neuron population is derived from asynchronous sources (be they intrinsic or extrinsic). If synchronizing sources such as neuronal oscillators were responsible for more than a minor part of the drive, they would be multiple, dispersed, and weak.

Introduction

Understanding the mechanisms responsible for generating sympathetic vasomotor tone represents an essential step in our attempts to define the role of the CNS in the regulation of cardiovascular function. It is known that vasomotor tone depends critically on supraspinal drive, and work over the last two decades has highlighted the role of premotor neurons in the subretrofacial nucleus [synonymous with the rostral ventrolateral medulla (RVLM)] as the major relay for that drive (Dampney 1994; Dampney and Moon 1980; Guyenet 1990; McAllen 1986). But the mechanisms responsible for the generation of the tonic activity of the premotor neurons remain uncertain.

Recent intracellular recordings from rat RVLM sympathetic premotor neurons in vivo demonstrate that under normal experimental conditions, action potentials in sympathetic premotor neurons invariably arise from depolarizing events with the characteristics of excitatory synaptic inputs (Lipski et al. 1996). The observation, in the rat, that blockade of excitatory amino acid receptors or synaptic transmission in the RVLM does not affect sympathetic activity or blood pressure, raises some question about the nature of this synaptic driving input in this species (Sun and Guyenet 1987; Trzebski and Baradziej 1992). However, bilateral microinjections of the synaptic blocker, cobalt chloride, into the RVLM of cats markedly reduce both tonic sympathetic nerve activity and blood pressure (Seller et al. 1990). Similarly in rabbits, bilateral microinjections of the excitatory amino acid receptor antagonist, kynurenic acid, into the RVLM induce a decrease in blood pressure (Blessing and Nalivaiko 2000). Thus while this area is not entirely resolved, much of the activity of sympathetic premotor neurons may be dependent on excitatory synaptic inputs under basal conditions in anesthetized animals.

Currently there is little information regarding the nature of the driving inputs to sympathetic premotor neurons. We reasoned that there were two main possibilities. First the sympathetic premotor neurons may themselves form part of the generator network, in which case the premotor neurons must directly or indirectly influence each other—because interconnections define a network. Second, an antecedent source or sources could drive the premotor neurons. In that case, the activity of premotor neurons might be synchronized by common inputs from those driving sources, especially if they are rhythmic, as suggested by Gebber, Barman and colleagues (see Gebber 1980, 1990). Alternatively the driving inputs might come from multiple, asynchronous sources, causing little synchrony between premotor neurons.

To examine these possibilities, we made simultaneous extracellular recordings from pairs of sympathetic premotor neurons in the subretrofacial nucleus, where the RVLM premotor neurons in the cat are concentrated (McAllen 1986; Polson et al. 1992). We used cross-correlation analysis to detect synchrony between their spike trains on both short (millisecond) and medium (tens of milliseconds to seconds) time scales to describe the degree of synchrony quantitatively in terms of spikes per second (Nordstrom et al. 1992). These data have

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enabled us to estimate the spiking synchrony of the subretrofacial population as a whole. We asked the following questions: do subretrofacial neurons interact synaptically as part of a local network? Do they receive common inputs from antecedent sources that synchronize their activity? If so, how important are those influences for these neurons’ overall activity?

Preliminary accounts of part of this work have appeared in abstract form (Allen and McAllen 1994; McAllen and Allen 1995).

METHODS

Preparation

All experiments were performed in accordance with the Australian National Health and Medical Research Council code of practice for the care and use of animals for scientific purposes and were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute. Experiments were performed on 17 cats of either sex (4.1 ± 0.2 (SE) kg), anesthetized with alpha chloralose (70 mg/kg iv) given after 11 mg/kg im in ketamine hydrochloride. Supplementary doses of chloralose (7–20 mg/kg iv) or pentobarbitone (12–18 mg iv) were given if the cats showed any signs of inadequate anesthesia. Corneal and withdrawal reflexes were tested at least every 20–30 min. During recording periods, animals were paralyzed with bolus doses of pancuronium bromide (2 mg iv). Paralysis was induced after the completion of surgery and was allowed to wear off at intervals when the level of anesthesia was tested conventionally by corneal and withdrawal reflexes. At other times, anesthesia was monitored by reference to blood pressure and the meiotic state of the pupils.

All animals were given a tracheotomy and ventilated artificially with oxygen-enriched air, maintaining end-tidal CO2 levels near 4%.

Rectal temperature was maintained near 38°C by a servo-controlled heating blanket. The right femoral artery and vein were cannulated for later analysis of renal nerve activity. The right common carotid artery was reversibly occluded to reduce brain movement associated with ventilation, and the dura was opened over the medulla.

The subretrofacial nucleus was defined as the region immediately caudal to the facial nucleus where microinjections of 1–5 nl of sodium glutamate (0.1 M) produced a brisk rise in blood pressure. At this site, small patches were opened in the pia mater through which either one or two glass-insulated tungsten microelectrodes were inserted by independent, hydraulic microdrives. In experiments where two electrodes were inserted, the second drive was inserted caudally and aimed rostrally at an angle of 26° such that the electrodes converged to record from sites within 250 µm of each other. Signals from each electrode were recorded differentially with respect to a metal ring, which was held against the medullary surface around the recording site to stabilize recordings. Spike potentials were amplified and filtered (band-pass: 300–3,000 Hz) and recorded along with blood pressure, sinus pressure, and a voice/event channel on magnetic tape for later analysis. Discriminated spikes (see next section), blood pressure, and an event marker were also recorded with a computer-based analysis system (Spike2, CED, Cambridge, UK). This program was used to generate autocorrelation, cross-correlation, and cardiac cycle-triggered histograms of unit activity as well as spike-triggered averages and frequency spectra of renal nerve activity.

Spike discrimination

Spike potentials were monitored on a variable persistence storage oscilloscope. Units were discriminated on-line by their spike shape using a custom-built, two-channel, time + amplitude window discriminator. The discriminator output pulse was used to trigger the stimulator for antidromic activation during the collision test. In all cases, the discrimination process was repeated off-line from tape recordings. Great care was taken first to eliminate the effects of electrical or mechanical artifacts, which occasionally interfered with both channels. To achieve this, close monitoring of spike discrimination was required throughout.

In the cases where paired recordings were discriminated from a single electrode, extra precautions were needed to avoid misleading artifacts. First, it was critical to check that one neuron’s spike was never counted simultaneously by two discriminator channels. Second, in cases where one neuron fired doublet or triplet spikes, which always became progressively smaller and wider during the burst, great care was necessary to identify them as such rather than as action potentials of another neuron. Third, in cases where the amplitude of the second spike was relatively low, it was important to check that the discrimination included no false-negative or false-positive counts due to “waveriding.” Following a large spike, the afterpotentials and the settling time of a filtered signal will include positive and negative swings in baseline potential, on which small spikes may sum to reach, or fail to reach, detection threshold. Such a mechanism could erroneously produce cross-correlation patterns suggestive of short-term neuronal interaction. Close scrutiny was needed to ensure that the desired spikes were all detected and that undesired spikes were not.

Independent evidence for the adequacy of unit discrimination was sought in all cases by plotting the autocorrelogram of each unit (see following text) and assessing its postspike refractory period. If a significant number of spikes appeared in this trough (more than 5% of the mean spike count at times distant from the refractory period), the record was reanalyzed from tape and the discrimination process repeated. A spike-shape analysis program (Discovery, Datawave, Longmont, CO) was used to discriminate spikes in a few difficult cases. Units that failed to pass these tests were eliminated from further analysis.

Analysis

Discriminated spike signals were analyzed off-line from tape, using the Spike2 program to generate auto- and cross-correlation histograms

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(cross-correlograms) of subretrofacial nucleus neuron activity. For cross-correlograms, one spike train was arbitrarily chosen as the trigger and the other as the response. (The opposite choice merely gives the same histogram, reversed in the time axis.) Cross-correlograms were plotted for every unit pair using 2, 20, and 200 ms bins as well as other bin sizes where this helped to clarify particular features. Autocorrelograms and inter-spike interval histograms were also generated for each unit to check the adequacy of unit discrimination and to examine the firing characteristics of individual neurons. Arterial pulse-triggered correlograms (20 ms bins) were generated for every unit by cross-correlating subretrofacial nucleus neuron spikes with the times of peak systolic blood pressure.

Histogram peaks and troughs were detected initially by inspection and were classified as single or recurring. In cases where a putative single peak was superimposed on a slower periodic wave, judgement had to be used to determine the appropriate control value for comparison (Kirkwood 1979). Significance was tested by two methods. First, single-bin counts were considered significantly different from the mean count if their value had a probability of less than 0.1%, as calculated from the mean bin count on the basis of a Poisson process (Abeles 1982; Graham and Duffin 1981). This P value allowed for the null hypothesis being tested simultaneously over approximately 50 bins: inspection of the distribution of bin counts at times remote from the trigger, and thus presumably due to random fluctuations, confirmed that this choice was appropriate. To test the significance of peaks (or troughs) that were spread over several adjacent bins, the cumulative sum (CUSUM) test (Davey et al. 1986; Imamura and Onoda 1983) was then applied. In this case, the CUSUM of the counts in consecutive bins was required to deviate robustly (in a manner not critically dependent on the choice of binwidth, or starting point) beyond the 1% probability value for five consecutive bins to be considered significant (McAllen and May 1994). Finally, in cases where the histogram showed an obvious periodicity, neither method was considered applicable, but the influence on the neuron pair’s synchrony of a common cardiac periodicity (presumably from baroreceptors) was analyzed graphically by plotting two dimensional cross-correlation matrices (see following text).

A custom-written program within Spike2 was used to generate two-dimensional cross-correlation matrices. The occurrence times of response spikes were plotted in 20 ms bins with respect to both the cardiac cycle (abscissa) and the trigger spike (ordinate). The counts in each 20 ms × 20 ms square were color coded and presented as quintiles between the minimum and the maximum values in the matrix (blue, green, red, yellow, white, in ascending order). The process was repeated for each neuron pair, with the “trigger” and “response” spike trains interchanged.

In cases where a significant peak was present in the cross-correlation histogram, its magnitude was expressed as a percentage, comparing the total number of excess counts in the peak (above the mean baseline value) with the geometric mean of the total spike counts of the two neurons (Nordstrom et al. 1992).

Histology

At the end of experiments, direct anodal current (approximately 2 μA for 1 min) was passed through one recording electrode. The electrode was removed, and 50 nl of 2% pontamine blue dye was pressure injected into the same spot to mark the area and help later histological identification. The cat was killed with an overdose of pentobarbitone, and the medulla was removed and immersed overnight in 4% paraformaldehyde. Forty-micrometer transverse frozen sections were cut of the region containing the mark. Marked sites were located microscopically and traced from the X30 projected image on a microfilm reader. Recording sites were reconstructed from depth measurements in the marked tracts.

RESULTS

Paired extracellular single-unit recordings were made from one or two electrodes with tips located within the immediate vicinity of the left subretrofacial nucleus. Additionally, every neuron considered here showed spontaneous activity that was abruptly inhibited on inflation of a carotid blind sac preparation (Fig. 1A). Once two such unit recordings were isolated, attempts were made to determine whether either neuron possessed a spinal axon by looking for an antidromic response to electrical stimulation of the dorsolateral funiculus in the third to fourth cervical segments (Fig. 1B). Standard tests, including the collision test, were then performed to confirm the presence of a spinally projecting axon (Lipski 1981). In all cases, at least one neuron of every pair was demonstrated to possess a spinal axon. Both neurons were proven bulbospinal in the cases of 14/32 pairs, and in a further five cases, the second unit showed a constant latency response to spinal stimulation although formal collision tests were not concluded.

In two animals, whole nerve activity was also recorded from the left (ipsilateral) renal sympathetic nerve. This showed the well-described pattern of large, synchronized bursts whose association with the arterial pulse was probabilistic rather than precise (cf. Gebber 1980, 1990). Spectral analysis confirmed this by showing that in both cases, less than 10% of the power within the 2–15 Hz range was at the cardiac frequency (data not shown). A positive spike-triggered average in renal nerve discharge (cf. Barman and Gebber 1997) could be demonstrated with two of the four subretrofacial neurons recorded at the same time, the peak renal nerve responses occurring 150 and 190 ms after the medullary neuron spikes, respectively (data not shown).

Neuronal characteristics

The basic properties of the neuron population studied are illustrated in Fig. 1. Their firing rates and conduction velocities were as previously established for this population of sympathetic premotor neurons in cats (Barman and Gebber 1985; McAllen 1986). Autocorrelation and inter-spike interval histograms were generated to reveal details of these neurons’ firing patterns (and to confirm single unit discrimination—see methods). Inter-spike interval histograms were usually unimodal with a leftward skew (Fig. 2A) although 7/55 neurons showed a second peak due to the presence of doublet (and in 1 case, triplet) spikes separated by 4–20 ms. All but two neurons’ modal firing intervals were shorter than the cardiac cycle (Fig. 2B), and none was locked 1:1 with the pulse. Firing patterns were classified broadly as regular (where obvious peaks reflecting the modal inter-spike interval appeared in the autocorrelogram: 22/55 neurons, 15 proven bulbospinal; Fig. 2Ai) or irregular (where no such peaks were evident: 25/55 neurons, 16 proven bulbospinal). The remaining eight neurons (6 proven bulbospinal; Fig. 2Aii) showed less distinct peaks in their autocorrelograms and were classified as intermediate. To look for any preferred firing intervals that might be expressed by this neuronal population, their modal inter-spike intervals (including multiple peaks) were plotted in a population histogram (Fig. 2B). Within the range of 50–500 ms, no preferred interval was apparent.

Arterial pulse-triggered correlograms were also plotted from the spontaneous activity of all neurons. Although all neurons...
were shown by carotid sinus inflation to be barosensitive, a
clear cardiac periodicity was found in the discharge of only
41/55 neurons, even though arterial pressure was maintained
within the appropriate range (Figs. 1A and 3). Among those
neurons where such a relation was present, its strength varied
considerably (Figs. 3 and 5).

Cross-correlation

The spontaneous activity of 32 subretrofacial nucleus neuron
pairs was recorded for periods of between 3 and 30 min (range
of individual neuron spike counts 457–20,305; 4,958 ± 436;
mean ± SE). Spike trains of 14 pairs were discriminated by

FIG. 1. Overview of the characteristics of the neurons studied. Neurons were selected on the basis of the following criteria. A: inhibition of activity by baroreceptor stimulation. Traces (from top) show the ongoing activity of 2 neurons recorded simultaneously from 2 electrodes and the arterial pressure recorded from the carotid sinus. Note that both neurons were abruptly silenced when carotid sinus pressure was increased to more than 200 mmHg. B: presence of an axon projecting to the spinal cord (records are taken from the same 2 neurons as in A). Each trace shows 2–5 superimposed sweeps. Electrical stimuli were delivered to the cervical spinal cord (at ↑), at a precise time interval after a spontaneous spike. Both neurons responded with a constant latency spike (*) unless the delay between spontaneous spike and spinal stimulus was less than the critical interval for collision (bottom traces). C: location in the immediate vicinity of the subretrofacial nucleus. Marked recording sites are shown on 2 coronal sections of the cat medulla oblongata taken close to the rostral pole of the inferior olivary complex (IO; back section) and approximately 750 μm caudal (front section). Note the clustering of recording sites within, and adjacent to, the subretrofacial nucleus (unlabeled outline). RM, nucleus raphe magnus; NA, nucleus ambiguus (compact division, also known as retrofacial nucleus); V, spinal nucleus of trigeminal nerve. D: conduction velocities of neurons studied. E: the spontaneous firing rates of neurons studied: ■, data from neurons with a proven spinal axon; ◇, data from neurons for which a spinal axon was not proven.
spike shape from a single-electrode recording; 18 pairs were recorded from two separate electrodes positioned within 250 μm of each other. From these data, cross-correlograms were plotted for every unit pair, using 2, 20, and 200 ms time bins (Figs. 3 and 4).

**SHORT TIME SCALE INTERACTIONS (PEAKS OR TROUGHS OF DURATION LESS THAN 20 ms).** Evidence was sought for excitatory or inhibitory interactions on the millisecond time scale, as may be caused by local feedback or common inputs (Feldman et al. 1980; Kirkwood 1979; Perkel et al. 1967). A methodological limitation of single electrode recordings is the inability to discriminate two spike shapes at once, which imposes a central “dead time” of 1–2 ms duration on their cross-correlogram. Very short-term interactions between those 14 neuron pairs could therefore have been missed. That aside, no significant peaks or troughs were detected in the cross-correlograms plotted on this scale, using data from either one- or two-electrode recordings (Figs. 3 and 4).

**INTERMEDIATE TIME SCALE INTERACTIONS (20 ms BINS, 50 TO 300 ms PEAKS).** The most common patterns observed in cross-correlograms plotted on this time scale were either a flat histogram (8/32) or a small ripple with a periodicity similar to the cardiac interval (19/32) (Figs. 3C and 4, A and B). In 5/32 cases, however, a significant peak was observed spanning the origin (Fig. 4C). In two cases, the relation was strong, such that the synchronous firing amounted to 13.0 and 13.6% of the two neurons’ mean activity. These two cases were found in the same animal and included one common neuron (all 3 neurons were proven bulbospinal). Three other neuron pairs showed significant peaks on this time scale that were much weaker (synchronous activity amounting to 7.7, 3.6, and 2.8% of mean activity in each case). When expressed as a percentage of the individual neurons’ spike activity, the extra spikes attributable to synchrony amounted to $8.7 \pm 2.4\%$ (range 2–26%, $n = 10$) of their mean activity.

**SYNCHRONY OF CARDIAC- AND NONCARDIAC-RELATED ORIGINS.** Synchrony between neurons on the 10 to 100 ms time scale could be due either to the known common influence of arterial baroreceptors or to common influences of other, unknown sources. Rhythmic influences would be expected to produce a repeating pattern on the cross correlogram (Moore et al. 1970). To clarify the origin of these patterns, every neuron’s activity...
was plotted in the form of a two-dimensional matrix cross-correlation histogram. In this display, each spike occurrence was plotted on two axes: on the abscissa in its time relation to trigger spikes fired by the companion neuron (as in a basic cross-correlogram) and in its time relation to the cardiac cycle on the ordinate (as in a pulse-triggered correlogram; Fig. 5). Correlations due to common inputs with a cardiac periodicity then show up as lines of increased count in the direction of the two axes’ common time vector (diagonally, bottom left to top right, Fig. 5, C and D). This was the case for all 19 neuron pairs whose basic cross-correlation showed the “repeating ripple” pattern noted in the preceding text. Horizontal lines recurring at specific times in the cardiac cycle axis reflect the cardiac periodicity of the “response” neuron (Fig. 5, B and C). Any synchrony between the unit pair that is independent of the cardiac cycle will show up as a vertical line (Fig. 5 D). All five of the unit pairs that had central peaks in the intermediate time-scale cross-correlogram showed this latter type of correlation, indicating synchrony due to common, nonbaroreceptor inputs. No other unit pairs showed this pattern.

**FIG. 3.** Example of the analysis performed on the spike trains of neuron pairs. Autocorrelograms (A; 2 ms bins; 8,273 and 9,260 trigger spikes for neurons 1 and 2, respectively) and pulse-triggered correlograms (B; 20 ms bins; 3,876 trigger pulses) were plotted for each neuron. B, bottom trace: the arterial pulse average, triggered from the same systolic trigger times as the histogram. Cross-correlograms (C) for the 2 units were generated on 3 different time-scales, using 2 (left), 20 (right), and 200 ms bins (see Fig. 4). - - -, 0.1% confidence levels (see METHODS for details and justification). The corresponding cumulative sum (CUSUM) plots are shown above, together with lines indicating 1% confidence levels (for details, see text).
LONG TIME SCALE INTERACTIONS (PEAKS OF MORE THAN 1 s DURATION). Where 200 ms binwidths were employed, most neuron pairs showed a flat cross-correlation function (Fig. 4D). In 3/32 pairs, however, repetitive peaks of 4 to 6 s cycle period showed up in their cross- and autocorrelograms (Fig. 4E). It was presumed to reflect synchrony due to common inputs related to the central respiratory cycle, which are known to affect the firing patterns of subretrofacial neurons (McAllen 1987). No respiratory-related trigger signal was recorded in these experiments, however, so this idea was not directly tested.

Reversible baroreceptor denervation

Five neuron pairs were recorded under conditions in which phasic baroreceptor inputs could be removed by maintaining the carotid sinus at constant pressure after section of the contralateral sinus nerve and both vago-aortic trunks. In these cases, the baroreceptor-related activity in the unit pulse histogram and in the cross-correlograms was absent or very small (data not shown). No other synchronizing influences became evident under these conditions other than the presumed respiratory-like synchrony with a period of several seconds, which became stronger in three of these five cases (Fig. 4E).

This study provides new information on the firing properties of the main bulbospinal neuron group that supports basal sympathetic vasomotor tone (Dampney 1994; Guyenet 1990; Kumada et al. 1990; Lipski et al. 1996). In cats, these cells are concentrated in the subretrofacial nucleus (McAllen 1986; Polson et al. 1992). These sympathetic premotor neurons were selected by established criteria: by their location, by their inhibitory response to baroreceptor activation, and in most cases by the possession of an axonal projection to the spinal cord with a conduction velocity in the appropriate range (Coote and Macleod 1984 and cf. Barman and Gebber 1985; Brown and Guyenet 1985; Guyenet 1990; Kanjhan et al. 1995; Kumada et al. 1990; Lipski et al. 1996; McAllen 1986). We deliberately did not select them with regard to a temporal correlation between their activity and subsequent bursts in sympathetic nerve activity (e.g., Barman and Gebber 1997) because we wished our sample to be representative of this premotor neuron population as a whole.

The purpose of this study was to examine the correlations between the firing patterns of pairs of sympathetic premotor neurons so as to shed light on how their tonic activity, and thus vasomotor drive, may be generated. This approach has been used with good effect to analyze respiratory drive...
pathways (e.g., Sears and Stagg 1976; Vaughan and Kirkwood 1997). We sought evidence both for local synaptic interactions, which might indicate that subretrofacial neurons participate in a local generator network, and for rhythmic, synchronizing inputs that might suggest that the subretrofacial nucleus transmits the output from a dynamically coupled network oscillator of the type proposed to generate sympathetic drive (Gebber 1990).

**FIG. 5.** Examples of 2-dimensional matrix cross-correlograms. These displays were generated to distinguish graphically between synchronizing influences from cardiac-related sources and those from cardiac-unrelated sources on the activity of subretrofacial neuron pairs. They were constructed by plotting the “response” neuron’s spikes in their time relation to the trigger spike [as in a conventional cross-correlogram, abscissa; 5,795 (A), 8,365 (B), 2,960 (C), and 9,515 (D) trigger spikes] and in their time relation to the arterial pulse [as in a conventional pulse-triggered correlogram, ordinate; 20,304 (A), 4,991 (B), 3,348 (C), and 5,296 (D) pulse triggers]. The corresponding conventional histograms are each displayed outside the relevant axis. Spike occurrences within the matrix were binned in 20 ms × 20 ms squares, whose final count was color-coded. The colors represent quintiles of the range of counts present within the matrix; in ascending order: blue, green, red, yellow, and white. Four examples are shown (A–D), representing the range of observations. A shows the most common result: no discernible pattern of correlation. Correlations attributable to synchronization by cardiac-related inputs (i.e., baroreceptors) are visible as a bottom left-to-top right diagonal pattern, corresponding to the 2 axes’ common time vector, a pattern that may be discerned in B–D. When the response neuron’s activity is strongly modulated by the cardiac cycle, this shows as a series of horizontal bands (e.g., in B and C). When the 2 neurons are synchronized by a common influence independent of the cardiac cycle, this shows up as a vertical band (as in D). This example shows the 2nd of the 2 strong cross-correlation peaks seen in this study.
Evidence for synaptic interactions between subretrofacial neurons

If subretrofacial neurons are themselves constituent parts of a brain stem oscillator circuit, they should show evidence of interactions of a strength and time course appropriate to support oscillatory behavior. Such interconnections have been inferred for other neuron populations, most clearly where their cross-correlogram shows a sharp (typically 0.5 to 2 ms wide) paracentral peak centered 1–5 ms before or after the trigger time (Abeles 1982; Feldman et al. 1980; Kirkwood 1979; Perkel et al. 1967). This pattern is generally interpreted as evidence that one neuron excites the other after a short delay (or possibly that both are excited, at slightly different latencies, by a common input). They have been found commonly for pairs of adjacent respiratory or reticular neurons (Feldman and Speck 1983; Feldman et al. 1980; Long and Duffin 1984; Schulz et al. 1985). They were also found to be present in a substantial proportion of ventrolateral medullary neuron pairs, selected on the basis of their “sympathetic-related” activity (Barman et al. 1982; Gebber et al. 1987).

No significant short time scale correlations of that type were found here. This sharp discrepancy with the findings of two apparently similar studies (Barman et al. 1982; Gebber et al. 1987) is surprising, especially since the paracentral cross-correlogram peaks identified in their studies were prevalent, prominent, and obvious. If such strong interactions had been present between the neurons studied here, they would not have been missed. It seems unlikely that the animals’ physiological states differed grossly enough to explain such different findings. In both cases, blood pressure was well maintained, and sympathetic vasomotor nerve discharge showed the typical bursting pattern. But we believe that a likely explanation for the difference is that the neuronal populations sampled were not the same. Gebber and colleagues (Barman et al. 1982; Gebber et al. 1987) selected medullary neurons on the basis of “sympathetic-related activity,” whereas ours were selected by the generally accepted criteria for sympathetic premotor neurons (see preceding text). While sympathetic-related activity is a property shown by a proportion of premotor neurons (Barman and Gebber 1985, 1997; also limited data from this study), it is also shown by propriobulbar and other neuronal populations as well (Barman and Gebber 1982, 1997; Varner et al. 1988). Since spinal axons were not verified in the neuron pairs showing short time scale correlations (Barman et al. 1982; Gebber et al. 1987), it is possible that they were not premotor neurons. On the basis of our sample of identified sympathetic premotor neurons, we are forced to conclude that such short-term interactions are not common or obvious in this neuronal population.

Several factors limit this negative conclusion. First, the connections might be weak, and require longer data samples for detection. Second, the synaptic responses might have been dispersed over time (e.g., if they were mediated by non-amino acid neurotransmitters), again causing the response to fall below detection levels (Kirkwood 1979; Moore et al. 1970). Third, their interconnections could have been rare or selective and thus been missed by our sample. Such interconnections have been reported to be prevalent, for example, between adjacent medullary respiratory neurons in cats (Feldman et al. 1980; Long and Duffin 1984), but rarer (10 vs. ≥40%) between pairs separated by 2–4 mm (Vachon and Duffin 1978). But we found no evidence for interactions of this type between subretrofacial neuron pairs, whether recorded from one electrode or from two separated by up to 250 μm.

Evidence for common synchronizing inputs

Our analysis focuses on the evidence for synchronizing influences other than those attributable to known sources such as arterial baroreceptors. This is first because we are interested in defining the nature of excitatory drive to subretrofacial neurons. The baroreceptor-derived input is inhibitory, mediated by GABAergic inhibitory synapses (Sun and Guyenet 1985) on sympathetic premotor neurons themselves (Dembowsky and McAllen 1990; Lipski et al. 1996). Shared inhibitory as well as excitatory inputs can cause cross-correlation peaks (Moore et al. 1970). A second reason is that the postulated oscillators driving sympathetic nerve activity are able to function independently of baroreceptor signals (Gebber 1980, 1990; Koosis 1995; Koosis et al. 1990; Taylor and Gebber 1975). Whether the proposed oscillator circuits are wholly or partly independent of baroreceptor signals, or even if they drift between those two states, their influence should be detectable as a baroreceptor-independent source of synchrony. Baroreceptor-independent synchrony should therefore be detectable in the subretrofacial neuron population if such oscillator circuits drive them. Respiratory-related drive to subretrofacial neurons has been studied elsewhere (Haselton and Guyenet 1989; McAllen 1987) and appears to be relatively minor unless respiratory drive is artificially raised. Moreover, its periodicity is an order of magnitude below the oscillation frequencies proposed to drive sympathetic tone (Gebber 1990).

The following calculation uses our measures of the prevalence and strength of synchrony between subretrofacial neuron pairs to estimate the upper limit of its effect on the overall activity of this premotor neuron population. Certain simplifying assumptions are made but, as discussed in Effects of simplifying assumptions, their effects are likely to be small or to result in an overestimate of the synchronous drive to subretrofacial neurons.

Calculation of the effect of synchronizing inputs on subretrofacial neuron population activity

If we consider there to be N independent (i.e., uncoupled over the time of the test) synchronizing sources, each of which gives synaptic connections to a proportion P of the subretrofacial neuron population, the probability that a randomly selected neuron pair receives any one common input is then

\[ \sum_{i}^{N} p^i \]

Making the simplifying assumption that P is equal for each input, this becomes

\[ N \cdot P^2 \]

which from our measurements = 5/32 or 0.156.

The probability that any single subretrofacial neuron receives such an input would then be
The strength of synchronizing inputs ($S$) has also been measured for the five coupled pairs and accounts for a mean of 8.7% of each neuron’s activity. We may use this to estimate the contribution that synchronizing inputs could make to the spike activity of the whole subretrofacial population. Their proportional contribution would be

$$SP = \sqrt{(0.156N)} = 0.395\sqrt{N}$$

From these figures, if a single synchronizing source were responsible, its calculated contribution to the spike activity of the subretrofacial population as a whole would be 3.4%. If multiple independent synchronizing sources were responsible, 216 of them would be required to account for 50% of the spike activity of the subretrofacial neuron pool.

Effects of simplifying assumptions

The effects of the following assumptions need to be considered. 1) Equal values for $P$: if $P$ values were inhomogeneous, the summed probability of finding a correlated subretrofacial neuron pair (0.156) would be achieved from fewer independent sources (lower $N$). 2) Independence of sources: any coupling of activity between sources (e.g., coupled oscillators) would again effectively reduce the estimated value of $N$. In both cases this would tend to lower our estimate of the contribution of synchronizing sources to subretrofacial population activity. 3) Random sampling of subretrofacial neuron pairs: selecting neurons close together may have increased the chance of finding a correlation. If so, compensation for this effect would again cause us to lower our estimate of the synchronous contribution to subretrofacial neuron population activity. 4) Attribution of synchrony solely to excitatory inputs: any contribution to synchrony from shared inhibitory inputs would also lower the estimate of the contribution of common excitatory inputs.

On the other hand, it is possible that some neuron pairs that we considered to show no synchrony did in fact do so at a level below our detection threshold (ca. 2%). If so, this would increase our estimate of the contribution of synchronous inputs to subretrofacial neuron population activity although the low strength of such inputs would lessen the error. The additional effect of missing one such neuron pair, showing 2% of synchronized spikes due to a single common input, may be calculated as follows.

The probability of this input reaching two subretrofacial neurons ($P^2$) may be taken as 1 in 32 (0.031), and so its probability of reaching one subretrofacial neuron ($P$) would be 0.177. The effect on the subretrofacial population activity would then be $0.177 \times 0.02 = 0.0035$ (i.e., 0.35%). The estimated number of independent sources needed to account for 50% of subretrofacial neuron population activity would then be reduced from 216 to 209. These effects would be greater, however, if we postulate that more than one source was responsible.

Finally, if more than one common synchronizing input was needed to give a detectable level of spike synchrony between subretrofacial neuron pairs, this would imply a higher value of $P$ but a lower value of $S$. In the case where two common inputs were required for detection, the probability of finding these would be

$$SP^2(N - 1).P^2 = (NP^3)^2$$

which (as before) = 0.156

The probability of one subretrofacial neuron receiving one such input would then be

$$0.63\sqrt{N}$$

The mean synaptic strength of each of these inputs would now be halved, so the calculated effect of this on subretrofacial neuron population activity would be

$$0.5*0.087*0.63\sqrt{N} = 0.0273\sqrt{N}$$

This case therefore further reduces the estimate of the synchronous contribution to subretrofacial neuron population activity and increases to 335 the calculated number of independent sources needed to account for 50% of it.

Inferences from calculation

Common, synchronizing inputs to subretrofacial neurons are likely to account for only a minor percentage of the spiking activity of this neuron population. The calculations are compatible with synchronizing (including oscillatory inputs) providing a greater share of the drive to subretrofacial neurons only if those inputs are multiple, dispersed and weak. The assumptions made are unlikely to have affected these conclusions.

Neurons with sympathetic-related activity

One question that arises from the calculations above is how they relate to the finding of Barman and Gebber (1997) that around 25% of the neurons in this region of the ventrolateral medulla show sympathetic related activity. This feature is demonstrated by a positive spike-triggered average (a time-locked wave of averaged sympathetic nerve activity at least 3 times the amplitude of one generated by a “dummy” trigger sequence) (Barman and Gebber 1985). Presumably two subretrofacial neurons that have sympathetic-related activity should also show some enhanced tendency to fire in time with each other. The present data are entirely consistent with such a view. If one neuron in four shows this type of activity, then two randomly selected neuron pairs in a sampled population of 32 would be expected to share that feature. In fact we found five correlated pairs in our sample of 32. The higher than expected number could have been a consequence either of nonrandom selection (we sampled subretrofacial neurons obeying set criteria and located close to each other) or perhaps the existence of yet other sources of synchrony.

With regard to the strength of those correlations, however, it is not possible to compare our measurements with predictions from spike-triggered averages of sympathetic activity. The analog averages of sympathetic nerve activity used by Barman and Gebber (e.g., 1985, 1997) are not calibrated in a manner that can be related to ongoing activity or its zero level. This sensitive technique may, in fact, pick up quite weak correlations.
Subretrofacial neuron ensemble activity and sympathetic drive

The nature of the ensemble of activity conveyed to the spinal cord by the subretrofacial neuron population will be determined not only by the between-neuron correlated activity discussed in the preceding text but also by the spike patterns of individual neurons. For this reason and because they had not previously been documented for this neural population in cats, we studied the firing characteristics of subretrofacial neurons.

About half of the subretrofacial neurons identified in this study fired with a regular discharge, and their mean firing rates (regular and irregular groups) were in the range of 1–14 Hz. They evidently fire more slowly than the analogous, fast-firing neurons in the rat, whose regular discharge is in the 20–40 Hz range (Guyenet 1990). But in either case, it is not easy to understand how more than a small percentage of the spike activity of regularly firing neurons could be seconded to the task of transmitting oscillations in the 2–10 Hz range to the spinal cord. Such a task would best be served by neurons that fired irregularly at rates comparable to the frequencies concerned. The bulbospinal neurons that Barman and Geber (1985, 1997) selected on the basis of sympathetic-related activity did indeed show such characteristics. It seems clear, however, that conventional selection criteria used in the present study have identified a broader neural population, with a more diverse range of firing patterns and generally faster firing rates.

One further possibility we considered is that if subretrofacial neurons shared a common, limited range of preferred firing frequencies, they might imprint that frequency band on sympathetic nerve discharge. Their modal firing intervals covered a broad range, however, without any obvious clustering around a particular value. They were nearly all shorter than both the heart period and the reciprocal of the major (2–6 Hz) frequency component of sympathetic nerve discharge (Geber 1990). This possibility therefore seems unlikely to be a major factor.

While we can make estimates from the present data about the ensemble activity that the subretrofacial neuron population sends to the cord, we cannot determine how this is translated into pre- and postganglionic neuron activity. But the low level of synchrony between subretrofacial neurons contrasts starkly with the strongly synchronous activity seen in sympathetic nerves (McAllen and Malpas 1997). One is led to consider the possibility that while tonic vasomotor drive is largely transmitted by subretrofacial neurons (Dampney 1994), the rhythmic component of sympathetic nerve activity may have other origins.

Conclusion

Subretrofacial neurons, whose activity supports most basal vasomotor tone, are not interconnected in the same way as (e.g.) certain respiratory premotor neurons. Common synchronizing, possibly oscillatory, inputs have some influence on the activity of this neural population but are unlikely to provide the dominant source of excitatory drive that maintains these cells’ tonic discharge, except in the case that they embody several hundred independent sources. Overall it appears that the sources of drive that maintain subretrofacial neuron activity, be they synaptic (Lipski et al. 1996) or due to intrinsic cellular properties (Guyenet 1990), are asynchronous or highly dispersed.

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