Repeated Sequences of Interspike Intervals in Baroresponsive Respiratory Related Neuronal Assemblies of the Cat Brain Stem

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Chang, E. Y., K. F. Morris, R. Shannon, and B. G. Lindsey. Repeated sequences of interspike intervals in baroresponsive respiratory related neuronal assemblies of the cat brain stem. J Neurophysiol 84: 1136–1148, 2000. Many neurons exhibit spontaneous activity in the absence of any specific experimental perturbation. Patterns of distributed synchrony embedded in such activity have been detected in the brain stem, suggesting that it represents more than “baseline” firing rates subject only to being regulated up or down. This work tested the hypothesis that nonrandom sequences of impulses recur in baroresponsive respiratory-related brain stem neurons that are elements of correlational neuronal assemblies. In 15 Dial-urethan anesthetized vagotomized adult cats, neuronal impulses were monitored with microelectrode arrays in the ventral respiratory group, nucleus tractus solitarius, and medullary raphe nuclei. Efferent phrenic nerve activity was recorded. Spike trains were analyzed with cycle-triggered histograms and tested for respiratory-modulated firing rates. Baroreceptors were stimulated by unilateral pressure changes in the carotid sinus or occlusion of the descending aorta; changes in firing rates were assessed with peristimulus time and cumulative sum histograms. Cross-correlation analysis was used to test for nonrandom temporal relationships between spike trains. Favorable patterns of interspike interval sequences were detected in 31 of 58 single spike trains; 18 of the neurons with significant sequences also had short-time scale correlations with other simultaneously recorded cells. The number of distributed patterns exceeded that expected under the null hypothesis in 12 of 14 data sets composed of 4–11 simultaneously recorded spike trains. The data support the hypothesis that baroresponsive brain stem neurons operate in transiently configured coordinated assemblies and suggest that single neuron patterns may be fragments of distributed impulse sequences. The results further encourage the search for coding functions of spike patterns in the respiratory network.

I N T R O D U C T I O N

Many brain stem neurons exhibit “spontaneous” activity in the absence of any specific experimental perturbation (Barman and Gebber 1992; Mason 1997). Neurons with these properties include baroresponsive cells, and neurons that, while functionally associated with the respiratory network as indicated by correlation analysis, may have little or no respiratory modulation of their individual firing rates (Li et al. 1999; Lindsey et al. 1992b, 1998). Patterns of distributed synchrony embedded in such “background” activity have been detected, suggesting that it represents more than the baseline firing rates of brain stem neurons subject only to up or down rate modulation (Lindsey et al. 1997).

There is also a growing body of evidence for repeated sequences of interspike intervals not detected by traditional measures of firing rate (Abeles et al. 1993; Dayhoff and Gerstein 1983b; Ku and Wang 1991; Lestienne and Strehler 1987; Prut et al. 1998; Villa et al. 1999). This advance followed the development of appropriate tools (Abeles and Gerstein 1988; Dayhoff and Gerstein 1983a; Frostig et al. 1990; Tetko and Villa 1997), and together with the synchrony data, motivated the present work. The initial aim was to test the hypothesis that nonrandom sequences of impulses recur in cardiorespiratory-related brain stem neurons that are elements of correlational neuronal assemblies. The detection of such “favored” patterns in single brain stem neurons led to a search for patterns of impulses distributed among multiple spike trains. The presence of such patterns would provide additional evidence for cooperation among baroresponsive respiratory-related neurons proposed to have roles in the regulation of breathing (Arata et al. 2000; Lindsey et al. 1998). Preliminary accounts of these results have been published (Chang et al. 1995; Lindsey et al. 1995).

M E T H O D S

Many of the materials and methods have been described in detail elsewhere (Lindsey et al. 1998). All experiments were performed under protocols approved by the University of South Florida’s Animal Care and Use Committee. Data were obtained from 15 adult cats of either sex (2.0–5.7 kg). Animals were initially anesthetized with sodium thiopental (22.0 mg kg$^{-1}$ iv); anesthesia was maintained with Dial-urethan (allobarbital; Ciba, 60.0 mg kg$^{-1}$; urethan, 240 mg kg$^{-1}$). Blood pressure and respiration were monitored continuously. Animals were given additional Dial-urethan if there was an increase in blood pressure or respiration in response to periodic noxious stimuli (toe pinch). Animals received dexamethasone (2.0 mg/kg) and atropine (0.5 mg kg$^{-1}$). Arterial blood pressure, PO$_2$, PCO$_2$, pH, [HCO$_3$] and end-tidal CO$_2$ were monitored and maintained within normal limits. Core body temperature was maintained at 38.0 ± 0.5°C.

The vago-sympathetic nerve trunks were isolated within the neck between 1 and 4 cm caudal to the carotid sinus and sectioned to eliminate vagal afferent feedback from lung receptors. The influence of aortic baroreceptors via the vagus nerve was also eliminated. The left C5 phrenic rootlet was isolated, desheathed and cut for subsequent

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were ventilated with 100% O₂ to counteract the hypoxia due to
expose the brain stem. In most experiments, a unilateral or bilateral
performed, and the caudal portion of the cerebellum was aspirated to
least 10 consecutive preceding control cycles (Lindsey et al. 1998).
measurements departed more than 2 SD from the average value of at
stimulation were assessed from measures of integrated efferent
inserted into each data file. Two previously described statistical meth-
(Lindsey et al. 1998). The times of onset of the inspiratory and
sure, and a stimulus marker were digitized with 12-bit precision at 20
pulses and entered into a laboratory computer. Multiunit phrenic nerve
from each tape were converted to transistor-transistor logic (TTL)
data entry and analysis
were applied during each recording. Trials were separated by 3- to
series of three to six inflations, each having a duration of approximately 30 s,
embolectomy catheter or by unilateral injection of arterial blood into
sections were prepared for histological examination to verify the
Neuron recording
Neuronal impulses were monitored extracellularly with planar ar-
arrays of six to eight individual tungsten microelectrodes (3–5 MΩ)
were labeled with a number and abbreviations that denoted the site at which their spike
were recorded: RM, rostral midline in the region of n. raphe
caudal midline in the region of nucleus raphe obscurus; CV,
and N, nucleus tractus solitarius or NTS. Signals were amplified,
filtered (100–5,000 Hz band-pass), and recorded on either two or
16-channel FM instrumentation recorders together with phrenic
nerve activity, a stimulus marker, systemic blood pressure, and, in
some experiments, carotid blood pressure. Common synchronization
pulses (5 Hz) were recorded on each tape.
Stimulus protocols
Signals were recorded for at least 15 min prior to the onset of
baroreceptor stimulation by occlusion of the descending aorta with
an embolectomy catheter or by unilateral injection of arterial blood into
the carotid sinus through a catheter connected to a pressure transducer
following occlusion of the lingual and common carotid arteries. Series
of three to six inflations, each having a duration of approximately 30 s,
were applied during each recording. Trials were separated by 3- to
5-min intervals to allow a return to baseline values.

Data entry and analysis
Single neuron action potentials and synchronized timing pulses
from each tape were converted to transistor-transistor logic (TTL)
pulses and entered into a laboratory computer. Multiunit phrenic nerve
activity was amplified and fed into a resistor-capacitor “leaky” inte-
grator with a time constant of 200 ms. The resulting analog signal and
those corresponding to systemic blood pressure, carotid blood pres-
sure, and a stimulus marker were digitized with 12-bit precision at 20
Hz. Data files were merged and first analyzed as detailed elsewhere
(Lindsey et al. 1998). The times of onset of the inspiratory and
expiratory phases were derived from the phrenic nerve signal and
inserted into each data file. Two previously described statistical meth-
ods were used to decide whether each neuron had a respiratory-
modulated firing rate (Morris et al. 1996; Orem and Dick 1983).
Changes in the peak amplitude of integrated phrenic motoneuron activity and respiratory phase durations associated with baroreceptor
stimulation were assessed from measures of integrated efferent
phrenic nerve activity. A change was considered significant when measurements departed more than 2 SD from the average value of at
least 10 consecutive preceding control cycles (Lindsey et al. 1998).

Spike trains were evaluated for responses to baroreceptor stimulation with peristimulus time histogram and cumulative sum histograms
(Davey et al. 1986). An additional method was used to evaluate spike
trains for firing rate changes in respiratory cycles during baroreceptor
stimulation. The null hypothesis was that peak and average firing rates
were not different from control. To reject the null hypothesis, these
parameters, averaged over at least three stimulus trials, had to be
significantly different from the mean of control cycles just preceding
each of the stimuli (P < 0.05, Student’s t-test).

Cycle-triggered histograms were used to classify neurons with
significant respiratory modulation as inspiratory (I) or expiratory (E)
neurons according to the phase during which they were more active.
Neurons with peak firing rates in the first half of the phase were
categorized as decrementing (DEC) neurons. Neurons with peak firing
rates in the second half of the phase were classified as augmenting
(AUG) neurons. Phase spanning neurons were classified first by the
phase with peak average activity and then by the phase transition of
greater activity, e.g., I-EI. Respiratory-modulated neurons with patterns
to be considered as “Others” (OTH). Neurons that were not respiratory modulated were designated NRM. Cardiac cycle-triggered histograms were calculated in some experiments.

Cross-correlograms were calculated for each pair of simultaneously
recorded spike trains and evaluated for significant features (Perkel et al.
1967b). A detectability index (DI, equal to the ratio of the maxi-
num amplitude of departure from background, D, to the background,
divided by the standard deviation of the correlogram noise) was used
to test significance (Aertsen and Gerstein 1985). Values > 2 were
considered significant. If this criterion was met, the D/background
ratio was used as an indicator of the visibility or strength, S, of the
correlation.

All spike trains were screened with autocorrelograms (Perkel et al.
1967a) to aid interpretation of cross-correlograms (Moore et al. 1970)
and to ensure that none of the data sets analyzed had a degree of
autocorrelation suggestive of rapid variations in firing rates that could
result in an underestimation of the numbers of expected patterns (see
Abeles and Gerstein 1988).

Detection of favored patterns in single spike trains
Favored patterns in the spike trains of single neurons were detected
with two methods developed by Dayhoff and Gerstein (1983a). In
the quantized Monte Carlo method, all interspike intervals were first
converted to integer values. A minimum temporal resolution or bin
value was defined for each run, and each interval was assigned an
integer value based on the minimum bin value. For example, if the
minimum bin value was 2.0 ms, then intervals >0.0 ms and <2.0 ms
were assigned the value “1,” intervals ≥2.0 ms and <4.0 were set to
“2;” etc. Repeated sequences of interspike intervals or “words” with
little temporal jitter from one occurrence to the next are more likely
to be detected using small bin values. Words with more temporal vari-
ability from one occurrence to the next are better detected using larger
bin values. Because one has no a priori knowledge of what type of
words, if any, will be found, various bin values should be used. In this
study, at least five different temporal resolutions were used in sequen-
tial pattern searches of each spike train; values of 2.0, 4.0, 8.0, 16.0,
and 32.0 ms, or 2.5, 5.0, 10.0, 25.0, and 50.0 ms, were used most
frequently.

Each spike train was searched for all excessively recurring se-
quences of “quantized” interspike intervals consisting of from 2 to 10
intervals. The original quantized spike train was searched; all words
occurring two or more times were tallied. The quantized interspike
intervals were then randomly shuffled, and the shuffled train was
searched for recurring words. This process was repeated 99 times. The
numbers of occurrences of words of each length detected two or more
times in the original train were then compared with the numbers of
words of each length occurring two or more times in all the shuffled
trains. If words of a particular length and repetition value had a greater
frequency of occurrence in the original train than in any of the shuffled trains, the null hypothesis that they were all generated by a nonrandom process: some sets had a higher than chance probability of having been generated by a random process. The

template matching method can be used in searches for specific nonrandom sequences.

The template match algorithm requires that a specific sequence of interspike intervals in milliseconds be defined together with a “wobble” factor that determined the amount of temporal jitter or noise allowed for each interval in the sequence. For example, if a wobble factor of 3.0 ms is used, then the interval between each spike in the sequence and the first spike in the sequence can vary by ±1.5 ms inclusively and be counted as a matching interval. The program also permits the user to allow up to two extra or missing spikes in a sequence and still have a word match the template, if all the remaining intervals fall within the template’s wobble time windows. The “extra” and “missing” spike options can be used separately or together in a search; neither option was used in this study.

The template matching method was used to search a spike train for multiple occurrences of a specific sequence of interspike intervals and to then search 99 shuffled versions of the same train for the same word. If the number of occurrences in the original train was greater than the maximum found in all shuffled trains, then the word pattern was reported to recur excessively.

In this work, the template method was used to search the spike train for sequences that were similar to a excessively recurring quantized patterns detected by the Monte Carlo method and to evaluate them for significance. As discussed in Dayhoff and Gerstein (1983a), the quantized Monte Carlo and template matching methods are complementary. The two methods treat pattern variability differently. Consequently, a pattern detected with one method may not be detected with the other.

Detection of favored patterns in multiple spike trains

The method of Abeles and Gerstein (1988) was used to search for repeated patterns of action potential sequences distributed among several simultaneously recorded spike trains. The relative times of the spikes within each iteration of the pattern had to be exactly the same; however, events within and between patterns were allowed. The number of spikes that constituted the pattern defined its “complexity.” Patterns of either 4 or 5 spikes that fit within a 500-ms time window and occurred at least twice were counted. The total number of patterns of each complexity were compared with 99% significance levels derived from the ad hoc probability calculation described in the APPENDIX of Abeles and Gerstein (1988). Observed numbers of patterns with a particular spike sequence order were also evaluated for significance with calculated 99% confidence limits.

RESULTS

The spike trains evaluated in this work were drawn from a database containing information on 707 neurons collected dur-
ing experiments done in collaboration with A. Arata and Y. M. Hernandez; changes in average firing rates of these neurons during pressure stimulation of baroreceptors and results of correlational analyses have been reported (Arata et al. 2000; Lindsey et al. 1998). In this parent database, 385 of 387 (99%) medullary raphe neurons had firing probabilities greater than zero throughout the respiratory cycle; 168 of 320 (52%) of the ventrolateral medullary neurons were also classified as “tonic.”

A subset of the tonic neurons represented in the database were evaluated in the present study on interspike interval patterns in baroresponsive neurons. The aim was to search for repeating impulse sequences that could potentially influence pattern-sensitive synaptic mechanisms (Erulkar TABLE 1. Properties of neurons screened for favored patterns of interspike interval sequences with the Monte Carlo and template matching methods

<table>
<thead>
<tr>
<th>Medullary Recording Site</th>
<th>Respiratory Modulation</th>
<th>Patterns</th>
<th>No Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Ventrolateral</td>
<td>Inspiratory</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Expiratory</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>NRM</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Midline</td>
<td>Inspiratory</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Expiratory</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>NRM</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sub-totals</td>
<td></td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary of recording sites, respiratory-modulated discharge patterns, and responses to baroreceptor stimulation. Patterns were detected in spike train data samples recorded during control conditions prior to baroreceptor stimulation protocols. Neurons not tested (NT) either ceased activity or lost signal due to movement before the onset of stimulation. The mean number of impulses in trains with detected patterns was 2,511 ± 2,126 (mean ± SD). Data sets in which no patterns were detected had a mean of 1,178 ± 1,353 spikes. Response abbreviations: ↑, increased firing rate; ↓, decreased firing rate; →, no change in firing rate; NRM, not respiratory modulated.
I, inspiratory; DEC, decrementing; AUG, augmenting; NRM, not respiratory modulated; NT, not tested.

The right column indicates figures that correspond to the particular spike train. See text for details. Response abbreviations as in Table 1. RM, rostral midline in the region of n. raphe magnus; CM, caudal midline in the region of nucleus raphe obscurus; CV, caudal ventrolateral medulla; E-, expiratory; I, inspiratory; DEC, decrementing; AUG, augmenting; NRM, not respiratory modulated; NT, not tested.

Patterns in single spike trains

Figure 1A shows one favored pattern, represented in a sequence detected with a quantization value of 25.0 ms. The Monte Carlo search report (Fig. 1B) that included this pattern lists the numbers of patterns or “words” of a particular length that occurred K or more times. The third column (Count) gives the number of different patterns detected, each of the particular length and frequency of occurrence indicated in the first and second columns, respectively. For example, the highlighted row indicates that seven different patterns, each with five intervals, occurred at least four times. The null hypothesis was that the seven different patterns were not more than expected by chance in a spike train with the same interspike intervals, but with those intervals randomly shuffled. To test the hypothesis, the pattern search was repeated in 100 shuffled data sets. Five patterns of the same class were detected, leading to rejection of the null hypothesis.

As noted in METHODS, if no patterns of length five and four iterations had been detected in the shuffled trains, then all of the listed patterns would have been considered significant. Because this condition was not true for this case, the list of the original seven patterns shown in Fig. 1C does not indicate which patterns can be considered nonrandom under the Monte Carlo test. The template method was used to search for specific interspike interval sequences that occurred more than expected under the null hypothesis.

This template method is illustrated in Fig. 2. The top trace shows a simulated template spike sequence. Another sequence with similar interspike intervals is considered a match if the spikes fall within the specified time windows indicated by the shaded regions (e.g., 2nd trace). This procedure allows for some wobble in the times of occurrence. In the present work, the wobble was ±1/2 the quantization bin value. The method also allows matches if the sequence includes an extra or missing spike. This option was not used in this study. One hundred shuffled trains that retained one copy of the template were also examined. If each had fewer matches than the original data, then the template was considered significant.

One of the interspike intervals sequences in the quantized pattern 4-3-6-4-3 (Fig. 1C) was used as a template. The times when matches to the template were detected are shown in Fig. 2.

Ten examples of quantized patterns and related information from 7 animals. Each set of patterns labeled with an asterisk (*) or dagger (†) was recorded in the same animal. The right column indicates figures that correspond to the particular spike train. See text for details. Response abbreviations as in Table 1. RM, rostral midline in the region of n. raphe magnus; CM, caudal midline in the region of nucleus raphe obscurus; CV, caudal ventrolateral medulla; E-, expiratory; I, inspiratory; DEC, decrementing; AUG, augmenting; NRM, not respiratory modulated; NT, not tested.

Table 2. Properties of favored patterns in single spike trains detected with Monte Carlo and template matching methods

<table>
<thead>
<tr>
<th>Location</th>
<th>Respiratory Modulation</th>
<th>Baroreceptor Response</th>
<th>Number of Intervals</th>
<th>Number of Repetitions</th>
<th>Number in 100 Shuffled Trains</th>
<th>Maximum Number of Quantized Patterns</th>
<th>Examples of Quantized Patterns</th>
<th>Binwidth, ms</th>
<th>Shortest Interval, ms</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM</td>
<td>E-DEC</td>
<td>†</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>4-3-6-4-3*</td>
<td>25.0</td>
<td>51.5</td>
<td>1 and 3</td>
</tr>
<tr>
<td>CM</td>
<td>I-AUG</td>
<td>†</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>6-10-7†</td>
<td>16.0</td>
<td>82.5</td>
<td>6</td>
</tr>
<tr>
<td>CM</td>
<td>NRM</td>
<td>†</td>
<td>6</td>
<td>29</td>
<td>12</td>
<td>9</td>
<td>2-1-2-1-2-2</td>
<td>50.0</td>
<td>3.0</td>
<td>2</td>
</tr>
<tr>
<td>CV</td>
<td>E-DEC</td>
<td>NT</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>6-6-5-7-7</td>
<td>8.0</td>
<td>35.0</td>
<td>5</td>
</tr>
<tr>
<td>CM</td>
<td>NRM</td>
<td>NT</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>6-6-7-6-7-6</td>
<td>8.0</td>
<td>40.0</td>
<td>4</td>
</tr>
<tr>
<td>CM</td>
<td>I-AUG</td>
<td>†</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4-4-3-4-4</td>
<td>32.0</td>
<td>75.0</td>
<td>5 and 6A</td>
</tr>
<tr>
<td>RM</td>
<td>I-AUG</td>
<td>†</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>37</td>
<td>3-3-2-4-2-2-2-4*</td>
<td>50.0</td>
<td>63.0</td>
<td>6B</td>
</tr>
<tr>
<td>RM</td>
<td>E-DEC</td>
<td>†</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>6-5-7-10†</td>
<td>25.0</td>
<td>110.5</td>
<td>6C</td>
</tr>
<tr>
<td>RM</td>
<td>NRM</td>
<td>NT</td>
<td>6</td>
<td>7</td>
<td>18</td>
<td>13</td>
<td>3-3-2-2-3-3</td>
<td>25.0</td>
<td>42.5</td>
<td>6D</td>
</tr>
</tbody>
</table>
3A. The top trace shows a firing rate histogram of spontaneous activity in a raphe obscurus neuron that responded to baroreceptor stimulation with an increase in firing rate (not shown). The bottom trace shows when the matching sequences occurred. The 16 matches exceeded the number expected under the null hypothesis. The thicker lines indicate close or overlapping occurrences of the favored pattern. Two of the words that included an overlapping region are detailed in Fig. 3B. Note that the second sequence had a different quantized value because of the allowed wobble. The possibility of several occurrences of a pattern having overlapping fragments that are elements of a larger pattern was recognized by Dayhoff and Gerstein (1983a). Another example is described below and shown in Fig. 5.

Favored patterns of interspike interval sequences were detected in 31 of 58 spike trains recorded in 12 cats. Table 1 is a summary of the evaluated properties of the screened neurons. Unless otherwise noted, the reported patterns were detected in spike train data samples recorded during control conditions prior to baroreceptor stimulation protocols.

Each train was searched for patterns after intervals were transformed to a sequence of integers that were multiples of at least five different minimum quantization bin values. For a particular quantization bin value, the algorithms searched for all patterns composed of 2–10 interspike intervals. The length of the longest significant sequence found in each of 31 spike trains with detected favored patterns ranged from 2 to 10 intervals (Fig. 4).

Ten examples of quantized interspike interval patterns detected in seven different animals are detailed in Table 2. Both the binwidths (temporal resolution) of the listed quantized patterns and the shortest interspike intervals found with the template matching method are included to give an indication of the absolute time scale of the patterns. The template matching method was used to identify specific sequences.

All words of a particular length and repetition value were reported as significant when there were no occurrences in any of the shuffled data sets of words with those values. One such sequence is shown in Table 2, fourth row from the bottom. Template matching revealed that this sequence recurred three times as overlapping fragments of a longer word composed of 30 interspike intervals (Fig. 5A). This sequence and a second (underlined regions of the firing rate histogram) were found during periods when blood pressure was elevated (Fig. 5B). The inset shows spike times on an expanded time scale for a segment of the data that included the long word. The interspike intervals of the detected sequence were relatively uniform as compared with more variable firing rates in other portions of the spike train. This pattern was detected in a data sample selected from a larger set of spike train data after a search with the same parameters failed to detect favored patterns in data acquired before baroreceptor stimulation. The perturbations of blood pressure were sufficient to reduce significantly the amplitude of integrated phrenic motoneuron activity (Fig. 5B, arrows, bottom trace).

The results suggest that favored patterns were not simply a consequence of firing rate changes locked to the respiratory cycle or cardiac cycles. Significant sequences were detected in nine single neurons with no respiratory modulation of their rates; the firing rates of seven changed during baroreceptor stimulation. Repeating patterns were not detected in nine other neurons with respiratory modulated firing rates, including five baroresponsive cells. Cardiac cycle-triggered histograms calculated for four of the neurons represented in Table 2 (bottom 4 rows) are shown in Fig. 6, together with first-order interspike intervals and autocorrelograms. The interval between the peaks in the cardiac cycle-triggered histogram of the neuron represented in Fig. 6C was 310 ms. The interspike intervals of the favored pattern for this neuron (Table 2, row 8), or of two other neurons from the same animal (Table 2, row 2), were not simple multiples or fractions of that inter-peak interval. The other three spike trains represented in Fig. 6 had no obvious
modulation of their average firing rate that was time locked to the cardiac cycle. One neuron did have a regular periodic discharge; the primary peak in the first-order interspike interval histogram included events ranging from 40 to 95 ms, with the peak at 57.5 ms (Fig. 6D). The intervals represented in the quantized favored pattern 3-3-2-2-3-3 for this neuron shown in Table 2 (bottom row) were within this range. This number of occurrences of this pattern with slightly longer intervals separated by slightly shorter intervals was greater than expected by chance.

Impulses in 18 of the 31 neurons with detected favored patterns were correlated on a short-time scale with other simultaneously recorded spike trains. Examples of primary correlogram features indicative of paucisynaptic interactions are shown in Fig. 7, A–C. Correlogram features, respiratory-related discharge patterns, and firing rate changes during baroreceptor stimulation are graphically enumerated in labeled circles that represent neurons (Fig. 7, D–L). Circles with wider borders correspond to neurons with detected favored patterns.

**Patterns in multiple spike trains**

Our implementations of the pattern detection algorithms were tested with searches for “known” patterns inserted into independent spike trains generated with the network simulation program SYSTM11 (MacGregor 1987). Figure 8A shows an
FIG. 7.  A–C: cross correlograms with representative primary features. Labels on each panel include the recording sites of reference and target neurons and the respiratory modulation of firing rates as defined in the text. Circled numbers correspond to primary correlogram features in the graphical summary of all correlations for this group of neurons in D. Firing rates (spikes s\(^{-1}\)) refer to the largest bin in the corresponding plot. A: correlogram with offset trough; lag 17.5 ms; half-width 70.0 ms; DI (detectability index) 4.2; S (strength), 0.33; 1,965 reference spikes, 1,965 target spikes. The half-width is the duration of contiguous bins with a difference from background at least half that of the bin with the largest difference. B: correlogram with central peak and offset trough; peak: half-width, 35.0 ms; DI, 15.2; S, 1.13; trough: lag, 52.5 ms; half-width, 17.5 ms; DI, 3.9; S, 0.20; 3,380 reference spikes, 1,965 target spikes. C: correlogram with central peak and offset trough; peak: half-width, 37.0 ms; DI, 4.5; S, 0.26; trough: lag, 37.0 ms; half-width, 55.5 ms; DI, 3.9; S, 0.22; 3,150 reference spikes, 3,372 target spikes. D–L: graphical summaries of significant primary correlogram features for pairs of neurons that included at least 1 neuron with favored patterns. Correlogram features and changes in firing rates during baroreceptor stimulation were as indicated in the key at the bottom right. Circles with wider borders represent neurons in which favored patterns were detected. Recording site abbreviations: RM, rostral midline in the region of n. raphe magnus; CM, caudal midline in the region of nucleus raphe obscurus; CV, caudal ventrolateral medulla; RV, rostral ventrolateral medulla. See text for further details.
example of the data used in the validation process for the detection of patterns distributed among several spike trains. The top panel displays the firing times of 10 simulated neurons. The three arrows indicate the times at which copies of a distributed pattern were inserted into 5 of the 10 spike trains. The panel in the bottom left of Fig. 8A details one of the pattern; constituent spikes are marked by arrows. Spike trains were re-ordered in this panel to show the order of insertion. The search report for the example of the validation process indicated detection of the added sequences (Fig. 8B). The numbers of detected sequences that repeated a significant number of times matched the number inserted. Five different patterns of complexity 4 each occurred three times. These patterns represented the five possible combinations of complexity 4 for the one inserted pattern of complexity 5. For example, if the pattern A-B-C-D-E (representing a sequence of 5 spikes in 5 different neurons) were inserted three times, then each of the following patterns would also have three occurrences: A-B-C-D, A-B-C-E, A-B-D-E, A-C-D-E, and B-C-D-E. A utility program confirmed that the pattern of complexity 5, which occurred three times, matched the times of the inserted patterns.

Data sets composed of 4–11 spike trains recorded simultaneously during control periods were screened for repeated
patterns of interspike interval sequences. The search window was set to detect patterns with a total time span of $\leq 500$ ms. Patterns could include more than one spike from the same neuron. The minimum complexity was set to 4. Because of the computational expense of the ad hoc method, the maximum complexity was set to 5. Prior to searches for distributed patterns, all spike trains were screened with autocorrelograms. Firing rate histograms (Fig. 9A) and autocorrelograms (Fig. 9B) from one set of neurons (Table 3, group 7) are illustrated. None of the data sets analyzed had a degree of autocorrelation suggestive of rapid variations in firing rates or bursts that could result in an underestimation of the numbers of expected patterns (see Fig. 1B in Abeles and Gerstein 1988). Neither did any of the neurons have a periodic firing pattern such as that shown in Fig. 6D.

The number of patterns composed of 4 or 5 spikes exceed the number expected under the null hypothesis (99% confidence limits) in 12 of 14 data sets from 10 animals (Table 3). An example of a search report for one sample (group 2, Table 3) is shown in Fig. 10A. For complexities 4 and 5, the numbers of patterns that occurred twice exceeded the values expected for independent spike trains. The temporal resolution for all searches was set to either 0.5 or 1.0 ms because the objective was to screen for relatively precise iterations of temporal patterns. However, time bin resolutions up to 4.0 ms were also used to scan one data set (Table 3, group 10b). With these less stringent match criteria, patterns that repeated up to four times were detected (Fig. 10B).

When significant numbers of patterns were detected in a set of spike trains, the next step was to count the occurrences of specific sequences of spikes that fit within the selected time window. To identify subsets of the patterns that were most
likely to be nonrandom, the observed number of patterns with a particular spike order was compared against the calculated number that defined the 99% confidence limit. All 12 of the data sets with more spatiotemporal patterns than expected under the null hypothesis included significant subsets of spike sequences distributed among spike trains recorded at 2 or more sites (Table 3). For example, a search of the data set represented in Fig. 10 and Table 3, group 2, detected 80 distinct patterns with spike order 1-1-2-4 that occurred twice. One specific sequence of two spikes in neuron 1 followed by spikes in neurons 2 and 4 is represented in Fig. 11A.

Spike trains in this data set were also evaluated with the Monte Carlo and template matching methods for the detection of patterns in single neurons as described in the preceding section of RESULTS. For example, the distributed pattern sequence 1-1-2-4 (Fig. 11A) detected with the ad hoc method included a fragment of a significant template pattern in neuron 1 (boxed area around spike code 1 in Fig. 10A). The template and matching patterns are shown in Fig. 11B. Overall, data from six of seven animals screened for both single and multineuron patterns had favored sequences in single spike trains and significant subcategories of distributed patterns that included intervals in those impulse trains.

**DISCUSSION**

The results of this study confirmed the hypothesis that nonrandom sequences of interspike intervals recur in barorespon-

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**TABLE 3. Summary of results of searches for patterns of spike train distributed among simultaneously recorded neurons**

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Baroreceptor Stimulation</th>
<th>Respiratory Modulation</th>
<th>I</th>
<th>E</th>
<th>N</th>
<th>n</th>
<th>Recording Sites</th>
<th>Bin, ms</th>
<th>Significantly More Patterns*</th>
<th>Maximum Significant Complexity</th>
<th>Maximum Repetitions (Complexity)</th>
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<tbody>
<tr>
<td>1</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>CV, NTS</td>
<td>0.5</td>
<td>5</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>↑, ↓, →, →</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>RM, CM, RV</td>
<td>0.5</td>
<td>5</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>↑, ↓, →</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>RM, RV</td>
<td>1.0</td>
<td>4</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>RM</td>
<td>1.0</td>
<td>4</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>RM, CM</td>
<td>1.0</td>
<td>5</td>
<td>2 (5)</td>
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<tr>
<td>6</td>
<td>↑, →</td>
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<td></td>
<td></td>
<td>11</td>
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<td>4</td>
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</tr>
<tr>
<td>8a</td>
<td>↑, →</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>CM, RV, CV, NTS</td>
<td>0.5</td>
<td>5</td>
<td>2 (5)</td>
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</tr>
<tr>
<td>8b</td>
<td>↑, →</td>
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<td></td>
<td></td>
<td></td>
<td>8</td>
<td>CM, RV, CV, NTS</td>
<td>0.5</td>
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<tr>
<td>9a</td>
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<td></td>
<td></td>
<td>5</td>
<td>RM, CM</td>
<td>1.0</td>
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</tr>
<tr>
<td>9b</td>
<td>↑, ↓, →</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>RM, CM</td>
<td>1.0</td>
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</tr>
<tr>
<td>10a</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td>5</td>
<td>RM, CM</td>
<td>4.0</td>
<td>5</td>
<td>4 (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑, →</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>RM, CM</td>
<td>1.0</td>
<td>5</td>
<td>2 (5)</td>
<td></td>
</tr>
</tbody>
</table>

*n* is number of neurons. Response (arrow) abbreviations as in legend for Table 1. NTS, nucleus of the solitary tract; N, not respiratory modulated (NRM); NT, not tested; RV, rostral ventrolateral medulla; other abbreviations, see Table 2. * Significantly more patterns than expected (99% confidence limits).

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**FIG. 11.** A: fragment of favored word embedded within a distributed pattern in a significant subcategory. The 1st 2 spikes in the train from neuron 1 were elements of a distributed pattern (see spikes enclosed in solid line rectangles and marked with arrows) in the significant sequence category 1-1-2-4. B: dashed line marks fragment of a template pattern that was matched a significant number of times. This fragment corresponded to the spike data from neuron 1 represented in A.
DISTRIBUTED SPIKE PATTERNS

Fig. 12. Simple circuit with recurrent inhibition (top) suggested by correlational data; other inputs to the circuit are assumed. This arrangement represents one example of network relationships that could, given the indicated synaptic actions and appropriate delays, generate both single neuron spike sequences (a) and distributed patterns (b) similar to those detected in this study. Arrows indicate site of transient activity increase (top) and illustrative patterns in simulated spike train data (bottom).

SKE SPIKE PATTERNS

The patterns detected in this study could represent “markers” or fragments of markers of a network repeatedly engaged in similar operations. The possibility of larger patterns identified from overlapping words was predicted by Dayhoff and Gerstein (1983a), who suggested that such patterns could reflect, for example, a second iteration of a process that starts before the previous one is completed.

Some patterns were composed of relatively regular interspike intervals, a property of some types of brain stem neurons recorded in vivo (e.g., Barman and Gebber 1992; Mason 1997). Regular patterns could also reflect moments when the firing rate limit of the neuron is transiently constrained by refractoriness, although this cannot be the sole explanation, given the variations in rate apparent at other times in spike trains with such patterns (e.g., Fig. 5).

Another possibility is that the detected spike patterns represent “signatures” of neuronal relationships. For example, a circuit that promotes synchrony by recurrent inhibition (Fig. 12) could contribute to the generation of regular intervals in individual spike trains and distributed firing patterns and correlations such as those reported here and elsewhere (Lindsey et al. 1992a, 1994). Spike timing relationships in multiple channels also have potential “coding” properties (reviewed in Fetz 1997).

This work focused on the detection of relatively precise temporal patterns. The extra and missing spike options of the template method were not used in this study. Searches for both single neuron patterns and distributed patterns always included small bin resolutions of 0.5–2.0 ms. Searches were not exhaustive. Selected quantization bin values used to screen each spike train were small subsets of possible values, and, because of the computational expense, the range of successive interspike intervals screened for patterns was limited. The failure to detect favored sequences of interspike intervals should not be construed as evidence that patterns did not exist.

The results of supplementary screening with “coarser” temporal resolutions did indicate the presence of more variable patterns. Previous work unmasked overlapping subsets of neurons represented in different patterns of distributed synchrony when the template resolution was changed (Lindsey et al. 1997). That earlier observation suggested that multiple information streams are conveyed concurrently by fluctuations in the synchrony of ongoing activity.

We emphasize that the potential “information content” in the relative firing times of single and multiple neurons must be evaluated with caution. The influence of action potentials may change as a function of many variables, including history-dependent mechanisms that influence transmitter release and postsynaptic mechanisms (Erulkar 1983; Segundo et al. 1963; Zucker 1999), branch point filtering of spike sequences in axons (Baranes-Shahrabany et al. 1984), and concurrent activities in parallel channels (Lindsey and Brown 1982). In this regard, multi-array recording technologies offer an efficient approach for simultaneously monitoring many neurons subject to shared stimulus, history, and state-dependent conditions.

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