Pulmonary stretch receptor spike time precision increases with lung inflation amplitude and airway smooth muscle tension

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Chen Y, Marchenko V, Rogers RF. Pulmonary stretch receptor spike time precision increases with lung inflation amplitude and airway smooth muscle tension. J Neurophysiol 105: 2590–2600, 2011. First published March 16, 2011; doi:10.1152/jn.00514.2010.—Slowly adapting pulmonary stretch receptors (SARs) respond to different lung inflation volumes with distinct spike counts and patterns, conveying information regarding the rate and depth of breathing to the cardiovascular and respiratory control systems. Previous studies demonstrated that SARs respond to repetitions of the same lung inflation faithfully, suggesting the possibility of modeling an SAR’s discrete response pattern to a stimulus using a statistically based method. Urinary-anesthetized rabbit SAR spike trains were recorded in response to repeated 9-, 12-, and 15-ml lung inflations, and used to construct model spike trains using K-means clustering. Analysis of the statistics of the responses to different lung inflation volumes revealed that SARs fire with more temporal precision in response to larger lung inflations, because the standard deviations of real spikes clustered around the modeled spike times of responses to 15-ml stimuli were smaller than those produced by 12 or 9 ml, even at the same absolute firing frequencies. This implied that the mechanical coupling of SAR endings with pulmonary tissue is critical in determining spike time reliability. To test this, we collected SAR responses during bronchial constriction, compared them with those produced by the same SARs under normal airway resistance, and found that their firing reliability improved during bronchial constriction. These results suggest that airway distension and mechanical coupling of the receptor endings with the airway wall (partially determined by smooth muscle tone) are important determinants of SAR spike time reliability.

Hering-Breuer reflex; mechanoreceptors; spike trains; stimulus encoding; response models

METHODS OF MODELING OR DESCRIBING response features and reliability fall into two general categories. The first focuses on describing firing features with explicit mathematical functions, such as Gaussian (Fellous et al. 2004; Schreiber et al. 2003) or exponential (van Rossum 2001) kernels (see Nawrot et al. 1999 for other kernels). These functions are relatively simple, closed forms used to represent neuronal response patterns via interspike interval (ISI) statistics. In addition, the Poisson distribution has been widely used in modeling firing events of neurons, especially those in the visual system (Gabbiani and Koch 1998; Warzecha and Egelhaaf 1999). The second category focuses on the statistical features of spike timing (Foffani and Moxon 2004; Osan et al. 2007) and ISIs (Berger et al. 1990; Christen et al. 2004; Moore et al. 1966; Rapp et al. 1994). These studies have demonstrated, for example, that feline visual cortical neuron ISI distributions change consistently and reproducibly with different stimuli (Berger et al. 1990) and that motion-sensitive neurons in the fly visual system generate reproducible spike patterns, in terms of both timing precision and count (de Ruyter van Steveninck et al. 1997; Warzecha and Egelhaaf 1999), as do retinal ganglion cells of the salamander and rabbit (Berry et al. 1997).

Slowly adapting pulmonary stretch receptors (SARs) respond to different lung inflation volumes with spike trains containing different spike counts (Rogers et al. 2001) and temporal patterns (Chen et al. 2010) that carry information regarding the rate and depth of breathing to the cardiovascular and respiratory control systems (Kubin et al. 2006; Sant’Ambrogio and Widdicombe 2001; Scheglele 2003; Scheglele and Green 2001). Although the firing reliability of neurons in other systems has been studied (e.g., Abeles and Gerstein 1988; de Ruyter van Steveninck et al. 1997; Fellous et al. 2004; Gabbiani and Koch 1998; Martins et al. 2009; Reich et al. 1997; Schreiber et al. 2003; van Rossum 2001; Victor and Purpura 1996; Warzecha and Egelhaaf 1999), SAR firing precision has not. Thus our goal was to evaluate the firing precision of SAR responses to naturalistic stimuli.

Our observation of the reproducibility of spike times in SAR responses to the same stimulus (Chen et al. 2007, 2010) confirms that lung distension information may be carried by the temporal pattern of spikes and suggests that responses to the same stimulus can be modeled by a series of discrete events. Using K-means clustering, we constructed models and then examined SAR spike time reliability under both normal airway resistance and bronchial constriction conditions. The relationship between SAR spike time reliability and stimulus amplitude, as well as between reliability and smooth muscle tension, is quantified and discussed.

METHODS

This study focuses on modeling and analysis of the variability in SAR spike timing produced in response to repeated lung inflations. Data were collected from 15 adult male New Zealand White rabbits (1.9–3.0 kg). All protocols and procedures were approved by the University of Delaware’s Institutional Animal Care and Use Committee and conformed to the standards set in the Animal Welfare Act.

Surgical preparation and recording methods. The surgical preparation and recording methods have been described previously in detail (Chen et al. 2008, 2009, 2010; Rogers et al. 2001) and are only summarized in this report. Rabbits were anesthetized with urethane (1.6 g/kg iv) and intubated, and one femoral artery and vein were cannulated for blood pressure measurement and drug/saline injection, respectively. Via a lateral approach, one nodose ganglion was exposed by retraction and/or removal of overlying tissue. Extracellular spikes generated by SARs during mechanical ventilation (Harvard Bioscience, Holliston, MA) were recorded using a tungsten electrode (Z = ~1–3 MΩ at 1 kHz; FHC, Bowdoinham, ME). Microelectrodes were...
inserted into the nodose ganglion without removal of the connective tissue capsule. The lead wire was coiled into a spring and connected to a headstage amplifier that was held in a micromanipulator, which was mounted on a stereotaxic frame and used to make fine adjustment of the electrode tip position (see Averill et al. 1984; Rogers et al. 2001). SARs were identified by their faithful responses to tracheal pressure (TP) waveforms, and individual SAR recordings were verified by the presence of a unitary waveform in the spike-triggered average of the ipsilateral vagus nerve before the recorded spike time (Marchenko and Rogers 2007; Rogers et al. 2001). TP was recorded at the inspiratory sidearm of the Y-shaped trachea tube and used as a measure of lung distension. TP, SAR extracellular potentials, blood pressure, and expiratory CO₂ signals were collected at 10,000 samples/s (PowerLab 16 and Chart version 5.3; ADInstruments, Colorado Springs, CO). The animals were paralyzed (100 µg·kg⁻¹·h⁻¹ vecuronium bromide intravenously in 3.3 ml·kg⁻¹·h⁻¹ physiological saline) and artificially ventilated (0.5 duty cycle). SARs were further classified as low or high threshold, depending on whether they did or did not fire action potentials between lung inflations, respectively.

**Stimulus protocol.** Continuous recording of individual SARs was maintained for >1 h, during which lungs were inflated with three different volumes (9, 12, and 15 ml), each presented for >20 min at 40 inflations/min. These inflation volumes were chosen because they produce responses in the linear encoding range for SARs (Rogers et al. 2001) and have been used in previous studies of the temporal patterns of SAR responses (Chen et al. 2007, 2010). Data collection commenced after an 8- to 10-min “buffer time” following a switch to a new volume and continued for at least 10 min at that volume. Thus at least 400 responses to each stimulus amplitude (i.e., lung inflation volume) were collected.

During recordings under the normal airway resistance condition, animals were ventilated with O₂-enriched air. To determine whether bronchial constriction causes alterations in spike time variability, we applied methylcholine aerosol (7–10 mg/ml; <5-µm particles) to the airways using a nebulizer (model 3004 TechnoNeb desktop nebulizer; Technology and Health, Miami, FL). Methylcholine, a muscarinic cholinergic receptor agonist, mediates bronchial constriction via pulmonary smooth muscle contraction following its binding to M₃ receptors (Eglen et al. 1996; Mitzner et al. 1992), including in humans (Ding et al. 1987). While the lungs were inflated at 9 ml, the aerosol was gradually added to the ventilation air so that a maximum TP increase of 20–30% was reached within 2–4 min, after which aerosol flow was reduced to maintain a stable TP peak value. Only two volumes, 9 and 12 ml, were used as stimuli in the bronchial constriction condition, because SAR activity became low rate and constant frequency during 15-ml inflations under bronchoconstriction, indicating possible damage/dissociation between SAR endings and pulmonary tissue. These responses lacked any information regarding lung distension, so they were not included in the present data set. Responses under the normal respiratory condition of 9 and 12 ml, respectively, were first recorded for >5 min as controls, with a 5-min buffer time following the switch from one to another. The ventilator volume was then set to 9 ml and the methylcholine introduced. The same SAR’s activity was then recorded in response to 9- and 12-ml inflations (>2 min each) at the same rate of 40 breaths/min. During data acquisition under bronchial constriction at a given inflation volume, peak TP amplitudes were within ±3%, which was within the TP amplitude variability range before methylcholine administration.

**Spike detection and response alignment.** Spike detection was performed off-line at 0.1-ms resolution with Spike2 (version 4.22; Cambridge Electronic Design, Cambridge, UK) using a simple threshold function. Pattern analysis and classification computations were performed using custom-written functions in MATLAB (version 6.5; The MathWorks, Natick, MA). Some postanalysis was performed using Excel (version 10; Microsoft Redmond, WA) and GraphPad (version 5; GraphPad Software, San Diego, CA). Responses were aligned using the peaks in the TP stimulus waveform. This alignment strategy was chosen based on our experience that it produces the smallest TP-spike train waveform alignment errors. TP peaks of every respiratory cycle for the same stimulus (e.g., 9 ml) were first identified as reference points, and their corresponding spike trains were shifted accordingly.

**Model construction.** Although all the responses from the same SAR to a given stimulus may not contain the same number of spikes, in practice the spike count distribution heavily favored one value over all others. In the present study, between 60 and 90% of SAR spike trains in a response to the same stimulus contained the same number of spikes per lung inflation, with other spike numbers distributed on both sides of the mode decreasing rapidly to zero within 1 or 2 spikes, as shown in Fig. 1. This implies that it is reasonable to reduce a group of real spike trains to a single representative model spike train that contains the modal spike number (20 in Fig. 1). In this representation, the standard deviation (SD) of the SAR response spike times around each model spike time may be used as a measure of the relative consistency of SAR responses to a given lung inflation stimulus. One model spike train containing the modal number of spikes from the set of responses to each of the three (normal airway resistance condition) or two stimuli (bronchial constriction condition) was created for each SAR.

To create each model, we used a K-means clustering algorithm (Ball and Hall 1967; MacQueen 1967) to associate all spikes in all response spike trains to the appropriate spikes in the model spike train. K-means, extended K-means, and fuzzy K-means algorithms have been used to cluster spike trains (Fellous et al. 2004; Zouridakis and Tam 2000), and in the present study we used the former to cluster spike train responses to the same stimulus into a single model. The basic steps for K-means clustering of spikes are outlined below.

1) K (k in Eq. 1), the number of clustering centroids (i.e., the spike count of a modeled spike train), was obtained from the modal spike count in the set of responses to a given inflation volume (see above). The distance, G, was defined as the sum of the squares of time difference between the model spikes and the real spikes

\[
g = \sum_{j=1}^{k} \sum_{i=1}^{n} ||x_{ij} - c_{j}||^2
\]

where \(x_{ij} - c_{j}\) is the time difference between a real spike, \(x_{ij}\), and the appropriate model spike, \(c_{j}\). G is therefore a measure of the total distance of the n real spikes from their respective model spike times (centroids). The initial time for modeled spikes was set to the first spike train with K spikes for that SAR. Thus, after one iteration, \(G = 0\).

2) After all the distances between each modeled spike and its associated real spikes from all responses were measured, the objective function was calculated.

3) New centroids were computed by averaging all spike times of each spike in that cluster and moving the centroid to that time, thereby minimizing G.

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Fig. 1. Response spike count histogram for a low-threshold slowly adapting pulmonary stretch receptor (SAR) in response to a repeated lung inflation stimulus. This was the largest variability in spike count for any neuron in this study.

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4) Steps 2 and 3 were repeated until the centroids remain unchanged, resulting in a model spike train with the minimum total distance to all response spikes. Using this procedure, we generated a model spike train to represent the response to each lung inflation volume for each SAR. The SD of real spike times (calculated from the appropriate spike from all responses) within each cluster, as well as over all clusters (see below), was used as a measure of spike time reliability. Individual SAR SDs were calculated in absolute units (ms). Responses with more than K spikes were used in model construction, but the spike farthest from the cluster centroid was discarded if two spikes were close to the same cluster. For simplicity, responses with less than K spikes (typically <10%) were not used so that SDs within the same model could be directly compared by virtue of having the same number of observations. When SDs within the same SAR in response to different stimuli were compared, SDs were averaged.

To determine the relationship between inflation volume and spike time reliability across all SARs, we normalized SDs between 0 (minimum SD) and 1 (maximum SD) over all inflation categories within each SAR data set. These normalized SDs were averaged over all clusters within each individual model (i.e., lung inflation volume), creating three “average” SDs for each SAR (i.e., 1 each for the 9-, 12-, and 15-ml response models), which were used to compare response reliability between different inflation volumes within each SAR. Normalized, average SDs were also averaged over all SARs within each model category to compare 9- vs. 12- vs. 15-ml spike time reliability across all SARs. In addition to TP, its time derivative (dTP/dt) was also calculated and used to assess spike time consistency. T-test statistics were used to compare data with the same number of observations and known normal distributions (e.g., within-model data), whereas the Welch’s t-test was used to compare data with different numbers of observations or unequal variances (e.g., across inflation volumes within the same SAR).

Finally, to evaluate whether increased firing frequency causes decreases in spike time variability, we analyzed instantaneous firing frequency vs. SD, within each SAR, as follows. Only SDs within the same 5-Hz firing rate band were compared, thereby obviating the need for normalization by firing rate, since similar rates were limited by approximately equal theoretical SD maxima. For each observation of a two- or three-way comparison, the appropriate bin (category) in the histogram of all possible relationships was incremented. For two-way comparisons, the categories were 9 > 12 and 12 > 9, 12 > 15, 15 > 12, or 15 > 9 and 9 > 15 ml. The categories for three-way comparisons were 9 > 12 > 15, 9 > 15 > 12, 12 > 15 > 9, 12 > 9 > 15, 15 > 12 > 9, and 15 > 9 > 12 ml. For each SAR, each histogram bin was expressed as a percentage, and χ² tests (with associated P values) were calculated using the uniform distribution as the “expected” values (see below). To provide equal weighting to the distributions for each SAR, we averaged percentages of the same observation (e.g., 9 > 15 > 12 ml) over all SARs to generate a “global average percentage” for each rank order observation. If firing frequency is the primary determinant of SD of spike times (rather than, say, lung inflation volume), then there should be no preferred rank order (i.e., a uniform distribution in the histogram) at the same firing rate, for either a given SAR or over all SARs. However, if lung inflation volume is the major determinant of the relative size of spike time variability (SD), then at the same firing frequency, a statistically significantly nonuniform distribution in the rank order histogram should be apparent, as determined by its χ² statistic and P value.

RESULTS

Recordings from 19 SARs (10 high threshold and 9 low threshold), each of which were presented with 3 different inflation volumes under normal airway resistance, were analyzed. Recordings from 9 SARs (5 high threshold and 4 low threshold) were analyzed at 2 inflation volumes, under both normal and bronchial constriction conditions. Under normal conditions, peak expiratory CO₂ averaged 5.2 ± 0.21, 4.7 ± 0.18, and 3.6 ± 0.19% (SD) during 9-, 12-, and 15-ml inflations, respectively. Changes in expiratory CO₂ were not recorded during bronchoconstriction.

Stimulus amplitude vs. spike time consistency. Figure 2 provides 3 raster plots consisting of 100 randomly chosen responses from a high-threshold SAR, as well as the corresponding TP stimuli, for each of the 3 lung inflation amplitudes. The spike times in responses to 15-ml inflations appear more consistent than those in response to 12 ml, which appear more consistent than those in response to 9 ml. Figure 3 shows the results of the K-means clustering of the same SAR in Fig. 2. The model spike times (solid points) are superimposed on the spike probability histogram (shaded; 0.1-ms bins). It is clear from Fig. 3 that the 15 ml-evoked response spike times are clustered more tightly around the model spikes than those evoked by the 12-ml stimulus, which are tighter than those evoked by the 9-ml stimulus.

Figure 4 demonstrates the relationship between the SD of the spikes around the modeled spike times over the course of each stimulus. Like the receptor in Fig. 4, nearly all SAR had progressively smaller SDs as lung inflation volume increased and demonstrated an inverse (or negative) relationship between SD and TP within the lung inflation stimulus. Figure 4 also shows that the magnitude of this latter effect on SD, in absolute terms (ms), is greatest for 9-ml stimuli (note left y-axis values). In 7 of 10 high-threshold and 9 of 9 low-threshold SARs, the average SD of 9-ml inflation was the largest, whereas that of 15-ml inflation was smallest. As Fig. 4 also illustrates, the SD trend fluctuates near the TP peak, regardless of inflation volume. Eight of 10 high-threshold and 9 of 9 low-threshold SARs demonstrated these same fluctuations corresponding to TP peaks in response to at least 1 stimulus amplitude. Figure 5 shows the relationship of spike time SD vs. dTP/dt for the same SAR as in Fig. 4 and illustrates the overall finding that although SDs increase with the absolute value of dTP/dt, they also briefly increase around the peak (dTP/dt = 0) of the lung inflation stimulus.

To test whether spike time reliability simply reflects an internal state of the SAR as it fires at different rates (i.e., a “preferred” firing frequency), rather than in response to different stimulus features, we compared SD vs. firing frequency for the same SAR across inflation volumes. Figure 6A illustrates an example for one high-threshold SAR. As with all neurons, its instantaneous firing frequency range in response to all three stimuli produced significant overlap. In addition to reaffirming the inverse relationship between lung inflation volume and average SD (see above), this analysis shows that larger lung inflation stimuli produce more reliable spike times compared with smaller lung inflations even at the same absolute firing frequency. For most SARs, the SD rank order of 9 > 12 > 15 ml was the dominant one, including that of the SAR analyzed in Fig. 6Aa. Figure 6Ab reveals that for this neuron, the distribution in rank order of SDs for clusters occurring within a firing rate of 5 Hz of each other strongly favored the 9 > 12 > 15-ml relationship (among all 3-way possibilities), and the distribution of bins in the histogram significantly differed (P = 3.87 × 10⁻⁹⁴) from the uniform distribution expected if inflation volume had no effect on spike time variability (shaded line). In this case, the SD vs. firing rate data were clustered
such that not enough two-way comparisons were available for statistical significance. Figure 6B shows the average percentage, over all SARs, for each rank order of three-way comparisons of SDs. For three-way comparisons, the dominant rank order was $9 \rightarrow 12 \rightarrow 15$ ml, and the only other one occurring at an average rate above that predicted by a uniform distribution ($16.67\%$; shaded line) was $12 \rightarrow 9 \rightarrow 15$ ml, whereas all other orders occurred with less frequency. Again, the distribution differed from the uniform distribution in a highly significant manner, as indicated by the $\chi^2$ test ($P = 1.27 \times 10^{-24}$). Figure 6C provides a frequency histogram for dominant rank order for three-way comparisons for all 19 SARs, of which 13 were $9 \rightarrow 12 \rightarrow 15$ ml and 4 were $12 \rightarrow 9 \rightarrow 15$ ml, and this too varied significantly from that expected if lung inflation played no role in SD ($P = 1.46 \times 10^{-7}$). Thus the most frequently
observed departure from the rule relating smaller SDs to larger lung inflation volumes was between the responses to 9 vs. 12 ml. In two-way comparisons (i.e., cases where only 2 data points fell with 5 Hz of each other), the SDs of clusters in response to 12-ml inflations were more often larger than those responding to 9-ml inflations ($P = 5.73 \times 10^{-7}$), although only five SARs contributed to this data set. For the other

Fig. 4. Standard deviation (SD) of spike responses during lung inflation stimuli. SDs (data points, left y-axes) for the spike times associated with each model spike are shown in response to 9 (top), 12 (middle), and 15 ml (bottom). The TP waveforms (solid line, right y-axes) are shown for reference. Note that left y-axis scales differ in each plot. Time axis label (bottom) applies to all plots.

Fig. 5. SD vs. the rate of change in TP (dTP/dt) during lung inflation stimuli. SDs (points, right y-axes) for spike times associated with each cluster are shown in response to 9 (top), 12 (middle), and 15 ml (bottom). The concomitant dTP/dt waveforms (solid line, left y-axes) are shown for each lung inflation stimulus. Note that the y-axis scales are different for each plot. Time axis label (bottom) applies to all plots.
two-way comparisons, the rule held: 9 > 15 ml ($P = 1.52 \times 10^{-23}$) and 12 > 15 ml ($P = 2.20 \times 10^{-15}$).

Figure 7 shows the typical relationship between TP, dTP/dr, and SD of spike times for two typical high-threshold (A and B), two typical low-threshold (C and D), and one atypical high-threshold SAR (E). These Hanoi-like representations illustrate the observation that higher TP values and slower rates of change produced lower SDs in spike times, with TP exerting much more influence over absolute spike time reliability than dTP/dr. Over all SARs, 15-ml inflations evoked responses with statistically significantly lower averaged spike time variability (i.e., normalized, averaged SDs; see METHODS for details) than both 12-ml ($P < 0.05$ for 16/19 SARs) and 9-ml ($P < 0.0001$ for 17/19 SARs) inflations (F). Although responses to the 12-ml inflations generally produced lower SDs than responses to 9 ml ($P < 0.05$ for 12/19 SARs), averaged over all SARs these differences were not statistically significant (see Fig. 7 legend). The differences in average SDs between high- and low-threshold SARs at the same inflation volume were not statistically significant ($P = 0.223$, 0.354, and 0.221 for 9-, 12-, and 15-ml inflations, respectively).

Bronchial constriction. To evaluate the effect of increased SAR ending/pulmonary tissue mechanical coupling, we applied the same analysis methods to the data collected under control vs. elevated airway smooth muscle tone. Figure 8, A and B, provides an example of the typical alterations in TP waveforms (9-ml inflations) and corresponding spike train responses caused by bronchial constriction. Bronchial constriction invariably altered both the amplitude and time course of the TP waveform, even as the ventilator parameters (e.g., duty cycle, time course) remained unchanged. A direct comparison is shown in Fig. 8C. Phase advance in the TP peak was evident in all cases. As shown, the responses under two conditions were also markedly different. Because the airway conducting walls in which the SAR endings lie are less compliant during bronchial constriction, the mechanical threshold for the SAR during lung inflation increases, and its activity persists through lung deflation and below its elevated mechanical threshold. Under this condition, the highest firing rate does not correspond to the TP peak, but instead occurs during “deflation.” These phenomena were observed in all nine SARs analyzed. Figure 9 shows the typical changes in SAR spike time variability caused by bronchial constriction for one neuron. As shown, bronchial constriction caused a decrease in SD as a function of TP and dTP/dr in response to either 9 or 12-ml lung inflation volumes. This trend was evident over all SARs, and was statistically significant for both stimuli ($P = 0.015$ and 0.009 for 9- and 12-ml inflations, respectively).

DISCUSSION

Our previous study demonstrated that SARs are more likely to fire action potentials at specific times during the lung inflation (Chen et al. 2008). A more recent study validated that the specific temporal patterns of spikes produced by SARs in response to repeated stimuli carry information not present in the spike count alone (Chen et al. 2010). Together, these investigations suggested that it would be possible to summarize an SAR’s response by using a modeled spike train and to assess response reliability based on deviation from the model, which forms the basis of the analysis in the present study.

Principal findings. The novel findings of the present study are that 1) the timing of spike trains produced by SARs becomes increasingly consistent as lung distension increases, both within a single inflation and between inflations of different depths; 2) this increase in consistency is not caused by limitations imposed by an increase in firing rate; and
3) increased constriction of pulmonary smooth muscle also increases spike time consistency, suggesting a relationship between firing consistency and the physical coupling of the receptor ending and airway wall. We found a fundamental relationship between inflation volume and the consistency of the SAR spike times (e.g., Fig. 3). The improvement in spike time reliability may have resulted from increased reliability in reaching spiking threshold caused by integration of depolarization of the sensory endings, even functionally separable endings (Yu and Zhang 2004).

Because larger inflation volumes evoke more spikes over the course of an inflation period (i.e., higher firing rates), it is possible that decreased SDs associated with each model spike were simply the by-product of smaller ISIs. However, a comparison of spike time SDs at the same instantaneous firing thresholds (i.e., the same model spike ISIs) produced by different stimuli demonstrated that this was not the case (Fig. 6). Our analysis suggests that stimulus amplitude, and not absolute firing frequency, determines the spike time consistency of SAR responses and that exceptions to the SD order of 9 > 12 > 15 ml are in the form of 12 > 9 > 15 ml. The lung inflation volumes we used did not cause any SAR to produce a saturated firing response (Chen et al. 2007; Rogers et al. 2001), thereby minimizing SDs via a maximum firing frequency. Thus we conclude that increased spike time reliability in response to these dynamic stimuli is determined by stimulus variables (e.g., slightly larger dTP/dt), rather than by intrinsic firing properties of SARs. Nonetheless, changes in intrinsic biophysical characteristics including, for example, direct CO2 modulation of mechanosensitive ion channel conductances, cannot be definitively ruled out, although this effect has not been reported.

Our analysis shows that lung distension amplitude causes changes in spike time variability independent of firing rate (Fig. 6). Changes in expiratory CO2 levels affect the firing frequency of rabbit SARs, as noted by Mustafa and Purves (1972). According to their findings, the effects on the firing rates of the SARs in this study would be rather modest given the dynamic range in this variable in our experiments (~3.6–5.2%). CO2 increases such as those we report when changing

![Fig. 7. SDs as a function of 2 stimulus variables. A and B: SDs of spikes produced in response to 9-ml (blue), 12-ml (red), and 15-ml stimuli (green) for 2 typical high-threshold SARs. C and D: SD vs. TP and dTP/dt for 2 low-threshold SARs. E: SD vs. TP and dTP/dt for an atypical low-threshold SAR. Note the different z-axis (SD) scales for different plots. F: normalized, averaged SDs (+SDs) over all, only high threshold (high thresh), or only low threshold SARs (low thresh). Bar colors designate the same stimuli as in scatter plots. P values for pairwise comparisons: 1, 9.99 x 10^-7; 2, 0.0971; 3, 8.00 x 10^-2; 4, 0.00255; 5, 0.4013; 6, 0.0191; 7, 5.41 x 10^-4; 8, 0.0758; 9, 2.82 x 10^-1.'
smooth muscle contraction caused by either CO2 or direct M3 receptor activation. Additional studies are required to definitively separate the direct (physical distension) from the indirect (CO2 alteration of smooth muscle tone or ion channel conductance) effects on SAR spike time consistency.

Methodological considerations. In this study, the K-means clustering algorithm was used to create a single model response that captures the temporal pattern of spikes produced by a given SAR to a repeated stimulus. This method has been used to sort spikes generated by multunit recordings (Zouridakis and Tam 2000) and to demonstrate multiple distinct response patterns from the same neuron to a given stimulus (Fellous et al. 2004). In this study, we used the positions of the model spikes as reference points around which SDs of actual spike times were calculated. Depending on how this method is implemented, bias in SD measures may be produced. In the present study, steps were taken to avoid these problems. For example, responses were included in the analysis as long as they had ≥K spikes. Responses containing <K spikes were not used so that the same number of observations included in the SD calculation for each cluster within a given model was the same and therefore could be directly compared over the course of the response (e.g., Fig. 4). The most important aspect of our analysis is the use of SD as a measure of spike time consistency, the same measure used by many other investigators (e.g., Billimoria 2006; Marsálek et al. 1977; Tateno and Jimbo 1999).

In accord with our findings, studies of various sensory systems have demonstrated that spike time reliability increases with stimulus amplitude. For example, Rokem et al. (2006) found that locust auditory receptor spike time reliability increased with increased mean of a continuous Gaussian-distributed stimulus, with spike times locked to large individual fluctuations in the amplitude of the sound pressure level at the preferred frequency of each cell. Likewise, Mainen and Sejnowski (1995) found that stimuli containing rapid fluctuations dramatically increased spike time reliability compared with constant stimuli. Reich et al. (1997) found that increases in the contrast of a sinusoidal grating produced reduction in spike time variability in cat lateral geniculate and retinal ganglion neurons. In the primate retina, Uzzell and Chichilnisky (2004) reported that spike time and count variability decreased systematically with stimulus strength. Of these, our results are most related to those of the Reich et al. (1997) study, because SAR spikes were not produced in response to rapid fluctuations in lung distension over the course of an inflation. Lung inflation changed smoothly and slowly compared with the typical SAR ISI, making the increased precision in SAR firing even more impressive (e.g., Fig. 3).

Physiological implications. A recent study by Yu and Zhang (2004) found that SARs possess distinct, physically separable functional endings. Indeed, they found that the same SAR may have low-threshold-type (near tonic) activity supplied by one “receptive field,” whereas another receptive field provides high-threshold-type output. Their finding provides an interesting insight into the potential encoding mechanisms of SARs and may relate to our findings in various ways. For example, if multiple receptive fields (which we assume arise from branching of sensory endings within the pulmonary tree) contribute to SAR activity, then it remains an open question whether larger lung inflations would increase, or decrease, the spike time consistency. On one hand, larger inflations may promote more

Fig. 8. Effects of bronchial constriction on TP waveform and spike response. A: typical response (spike times, vertical lines) of a high-threshold SAR to a 9-ml lung inflation and its associated TP waveform (solid line) during normal airway smooth muscle tone. B: same data as in A, for the same SAR, during bronchial constriction. C: superimposed TP traces for both conditions. Dashed and solid TP traces are bronchial constriction and normal conditions, respectively. Dashed (bronchial constriction) and solid (control) horizontal bars above TP traces show the periods of action potential discharge for the respective traces.

from 15- to 12-ml or from 12- to 9-ml inflations are known to cause bronchodilation, including in anesthetized humans (D’Angelo et al. 2001). Thus, the decrease in CO2 levels associated with increases in lung inflation volumes in this study may have augmented the decrease in spike time variability via increased mechanical coupling of SAR endings to the airway via smooth muscle contraction. We have no way of knowing if, or to what degree, this occurred because of other experimental factors, such as urethane anesthesia, known to diminish the effects of pharmacological activation of smooth muscle (Dringenberg et al. 1995). Nevertheless, our results support the conclusion that increased mechanical coupling (or dynamics therein) between SAR endings and pulmonary tissue is the dominant factor in determining spike time consistency, regardless of whether it is produced via (dynamics in) mechanical deformation caused by increased inflation volume or via smooth muscle contraction caused by either CO2 or direct M3.
rapid depolarization of the receptor endings, and this increased rate might result in more consistent discharge patterns at the spike initiation zone. On the other hand, if various endings have different mechanical thresholds, then they may be recruited in unpredictable ways that might result in higher variability in output at the spike initiation zone of the peripheral axon. We do not know that any of the SARs we analyzed had multiple receptive fields, so it is unclear to what degree these considerations are applicable to the interpretation of our results. Additional studies are needed to determine how multiple receptive fields affect SAR spike time consistency.

Our finding that SAR spike time variability decreased with increases in lung inflation volumes (Fig. 3), or even as lung inflation increased within a breath (Fig. 4), has profound implications for spatiotemporal integration of their synaptic inputs at postsynaptic target neurons. Action potentials in slowly adapting pulmonary stretch receptors cause brief excitatory postsynaptic potentials in target neurons, particularly in pump cells (0.65- to 1.25-ms half-widths; Berger and Dick 1987). Decreases in synaptic jitter associated with larger lung inflations may therefore diminish second-order neuronal responses to convergent input from multiple SARs and as a result extend the dynamic range of their responses. Also, for near-synchronous convergent inputs, increases in spike time accuracy may result in more consistent patterned responses in second-order neurons, enabling another potential coding mechanism within central respiratory control circuits.

We found that the effect of dTP/dt on spike time reliability was largest during smaller inflation volumes (Fig. 7), during which the slower rates of change (dTP/dt) produced a more pronounced effect on absolute SD measures than during larger inflation volumes with higher dTP/dt values. In all inflation volumes, the minimum SDs during the stimulus occurred near the peak of the TP trace (dTP/dt −0), implying that high rates of change cause higher variability in spike time, although this effect was negligible at high inflation volumes (12 and 15 ml),

Fig. 9. Effects of bronchial constriction on SD. Top: SD vs. TP and dTP/dt for a typical high-threshold SAR in response to 9-ml lung inflation in conditions of normal airway resistance (control; red) or bronchial constriction (blue). Middle: SD vs. TP and dTP/dt for the same SAR as at top, under the same conditions, but in response to 12-ml lung inflation. Bottom: summary data, normalized and averaged over all nine SARs. Bar colors designate the same conditions as in scatter plots.
probably because SDs were already diminished in response to these stimuli. Since we did not independently vary dTP/dt and TP (i.e., high positive and negative dTP/dt values always occurred near the beginning and end of an inflation), it is not possible to definitively determine the effect of dTP/dt on SD from our data. However, our data show that at the same dTP/dt, higher TPs produced more reliable spike times (e.g., Fig. 7, A–D). This suggests that spike time reliability would improve with markedly increased inspiratory effort (hyperpnea), whereas more relaxed breathing (eupnea) would produce more variable SAR spike times. Arguments for spatiotemporal integration under these two conditions can be made similarly to those for low vs. high inflation volumes (see above).

Finally, we observed an increase in SAR spike time reliability during bronchial constriction compared with resting levels of airway resistance. Presumably, this was due to increased physical coupling between their sensory endings and the airway wall. Methylcholine exerts its effects through contraction of airway smooth muscle and not by a direct stimulation of SAR terminal endings (Sano et al. 1992; Schelegle and Green 2001). A previous study on the effects of vagal and acetylcholine-induced bronchial constriction demonstrated an increase in SAR activity with application of various M<sub>1</sub> receptor antagonists and agonists (Matsumoto 1997), and our results (Figs. 8 and 9) are in accord with this finding. Davenport et al. (1981) reported that the effects of bronchial constriction on SAR firing frequency depend on the location of their sensory endings relative to the carina, but these investigators did not evaluate the consistency of SAR spike times. Although the physiological implications remain to be determined, our results imply that under parasympathetic activation of pulmonary smooth muscle, the timing of SAR input to the brain stem is more consistent despite the fact that they are more difficult to activate (i.e., have increased mechanical thresholds).

Conclusion. In this study, the K-means clustering method was used to average hundreds of spike train responses into a single modeled spike train representing the response of an SAR to a given stimulus. The SD of the clustered spikes was then used as a measure of spike time consistency. An inverse relationship between TP (during the course of the lung inflation) and SD was observed. A positive relationship between dTP/dt and spike time SD was also demonstrated. SAR firing variability under bronchial constriction was smaller than that of its same-volume counterpart under normal airway resistance, during which the within-inflation relationship among SD, TP, and dTP/dt held. Thus we conclude that increased mechanical activation and coupling produce more consistent SAR firing patterns.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


