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Slowly adapting pulmonary stretch receptor spike patterns carry lung distension information

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ABSTRACT

Slowly adapting pulmonary stretch receptors (SARs) provide the respiratory and cardiovascular control systems with information regarding the rate and depth of breathing. Previous information theoretical analysis demonstrated that SAR spike count provides a reliable representation of lung distension. This study examines whether SAR spike patterns may also provide information about lung distension. To investigate this, artificial spike trains were generated with the same number of spikes (but randomized intervals) as those recorded from SARs in response to three different lung inflation volumes in urethane-anesthetized rabbits. Three different spike train classification methods were applied to estimate which stimulus evoked them, and the accuracy with which artificial spike trains were classified was compared to that of real SAR spike trains using the same methods. Because real SAR spike trains were classified with higher accuracies than artificial ones containing the same number of spikes, we conclude that SAR spike patterns, in addition to spike counts, contain information concerning the amplitude of lung distension.

Analysis of spike train responses in sensory systems has revealed that temporal patterns may convey information about a given stimulus [2,3,7,8,11]. Slowly adapting pulmonary stretch receptors (SARs) are visceral mechanoreceptors that provide information about the rate and depth of lung inflation to the cardiovascular and respiratory control systems. Previous studies have shown that the encoding and decoding functions of SARs are very reliable, with each SAR carrying up to 30% of the theoretical maximum mutual information regarding lung distension transmitted by the spike count (i.e., firing frequency) code [13]. Pump cells, SAR second-order neurons in the nucleus tractus solitarius, have been shown to convey approximately the same quantity of lung distention information in their spike count responses as SARs [9]. However, no studies have determined whether lung distension information may also be carried by the temporal pattern of spikes in an SAR response, in addition to (or as opposed to) their number.

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. The surgical preparation and recording techniques have been previously described in detail [4,5,13] and are therefore only summarized here. After deep anesthesia produced by intravenous administration of urethane (1.5 g/kg over ~40 min), the trachea was intubated and one femoral artery and vein were cannulated for measurement of blood pressure and administration of drugs, respectively. One cervical vagus nerve and nodose ganglion were dissected free from the surrounding tissue and immersed in the mineral oil pool in the neck via a lateral approach. Animals were then paralyzed (vecuronium bromide; 70 μg boluses, I.V. every 40–50 min) and artificially ventilated (40 breaths/min; Harvard Bioscience, Holliston, MA, USA).

During lung inflation, an SAR generates action potentials that are conducted in the vagus nerve from the spike initiating zone in the pulmonary tissue to the medulla oblongata, passively invading the stem axon and soma in the nodose ganglion en route. The electrical activity of individual SAR soma was recorded within the nodose ganglion using a tungsten microelectrode (Z ~1 MΩ at 1 kHz; FHC Inc., Bowdoinham, ME, USA), which was inserted into the nodose ganglion without removal of the connective tissue capsule. The lead wire was coiled into a spring in a "floating" electrode configuration [1,4,5,13]. The end of the lead wire was 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. SARs were identified by their faithful responses to manipulations in lung inflation (measured by tracheal pressure; TP) and by the unitary waveform in the spike-triggered average of the ipsilateral cervical vagus nerve [9,13]. SARs were further characterized as either high (silent periods between lung inflations) or low (tonic activity between lung inflations) threshold. TP was

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measured at the inspiratory sidearm of the Y-shaped trachea tube. SAR and cervical vagus nerve signals (300–3 kHz) were collected at 10,000 samples/s with 16-bit resolution (PowerLab 16 and Chart® version 5.3, ADInstruments, Colorado Springs, CO, USA).

Continuous recording of individual SARs was maintained for >1 h, during which the inflation volume was set to three different values: 9, 12, and 15 ml (each at 40 breaths/min). Each volume was presented for >20 min. Data collection commenced after a 5 min "buffer time" following a switch to a new volume, after which continuous recording was collected for approximately 15 min under that volume. Thus, spike train responses to approximately 600 lung inflations at each volume were collected.

Individual spikes were detected with the use of conventional software (Spike2 version 4.22, Cambridge Electronic Design Ltd., Cambridge, UK) using a simple threshold function, and responses were aligned via the TP waveform. Pattern analysis and classification computations were performed off-line by custom written M-files for MATLAB® (version 6.5, the MathWorks Inc., Natick, MA, USA), and some post-analysis was performed using Microsoft® Excel (version 10, the Microsoft Corp., Redmond, WA, USA).

From each set of SAR responses, 200 spike trains were selected randomly (from the 600 collected in response to each stimulus) in order to generate artificial spike trains and to classify as belonging to one of three models. The remaining 400 spike train responses to each stimulus (i.e., 9, 12, or 15 ml) were used to construct a model of the spike probability (JPBM) or of the instantaneous firing frequency (SPBM) for classification purposes (see below).

For each SAR, 200 artificial spike trains were generated based on real spike trains recorded in response to each lung inflation, using the following four constraints. First, each artificial spike train had the same number of spikes as its real counterpart. This guarantees the distribution of spike counts in a set of artificial spike trains was the same as that of the real data in response to a given stimulus, and avoids the possibility that differences in classification accuracy performance may be explained by differences in spike count. Second, the spike times in artificial spike trains were randomized, drawn from a uniform distribution. Third, all interspike intervals (ISIs) of an individual artificial spike train were greater than or equal to the minimum ISI of its corresponding real spike train, thereby avoiding the possible loss of information when bin widths larger than the minimum ISI are used (see below). Finally, artificial spike trains were set to possess the same mechanical threshold as their real counterparts for that SAR, which means their first and last spikes occur at the same TPs as their counterparts in the real data, thereby preventing the loss of spikes as response times vary. Thus, the only difference between a real and corresponding artificial spike train is the particular temporal pattern produced in response to a specific TP stimulus.

Artificial and real spike trains were classified using methods developed in our previous studies [4,5], all of which begin by discretizing time into equal bins. Three models (one for each lung inflation volume) were constructed for the joint probability-based classification method (JPBM) [5], each consisting of the probability of observing a spike in every time bin, based on 400 responses to each inflation volume. In the JPBM, each individual spike train to be classified is used to identify the appropriate spike/no-spike event probability in each time bin based on the model. The product of these probabilities is then calculated for each model, and the spike train is classified as belonging to the response model with which its events produce the highest joint probability (i.e., similarity over all bins). Our previous study showed that the JPBM outperforms the conventional Euclidean distance-based spike train classification method [5].

The sparse firing frequency-based spike train classification (SFBM [4]) is a simplified version of the commonly used PSTH-based classification method [6]. In this method, the instantaneous firing frequency of a bin where a spike occurs is calculated, and *only* bins containing spikes are used for the analysis. Those bins are compared with the same bins in the three models, each of which contains the average instantaneous firing frequency derived from 400 responses. The Euclidean distances between individual spike trains and each model are then calculated, and the spike train is classified as belonging to the model response (i.e., evoked by that particular stimulus) to which it is closest (see [4]). Our previous study demonstrated that the SFBM sacrifices very little classification accuracy compared to the "filled" firing frequency representation (FFBM; which uses *every* bin in the response) while saving considerable computational cost [4]. In this study, JPBM, SFBM, and FFBM were all used to classify both artificial and real spike trains.

As in our other studies, the only analysis parameter varied systematically was discretization bin width. Bin width was varied between 1 ms and just less than the minimum ISI recorded for a given SAR, in increments of 1 ms. The minimum ISI (i.e., highest instantaneous firing rate) was invariably recorded during 15 ml lung inflations. When displaying results from multiple SARs in one plot, all bin widths were normalized by their minimum ISIs for each individual SAR.

Classification accuracy, the performance metric, is defined as the fraction of test responses correctly classified within each lung distention stimulus category (at each bin width). In addition, overall classification accuracy (averaged from the accuracies to the three different stimuli) is also reported. Classification accuracy was assessed using 600 test responses (200 drawn from each of the three stimuli) to the stimuli that evoked them. Point-by-point differences in classification accuracy between real and artificial spike trains were also calculated for each method.

Nineteen neurons were recorded for long enough to perform the analysis described above, including ten high threshold and nine low threshold SARs. Fig. 1 displays the accuracy curves generated by the JPBM, SFBM and FFBM for the artificial spike trains. Some plots demonstrate increasing accuracy as bin width increases, while others show decreasing, unchanged or extremely low classification accuracy with increasing bin width (e.g., 12 and 15 ml plots in Fig. 1). The classification accuracies of most artificial spike trains increase with expanding bin widths, especially in the JPBM. When using the SFBM, most accuracy values markedly increase as bin widths exceed 40% of the minimum ISIs, though this phenomenon is not obvious when using the FFBM, confirming previous observations that the FFBM is less bin width-dependent than other methods [4]. In previous studies, real spike trains from low threshold SARs achieve very high (>0.9) classification accuracies [4,5]. By contrast, the present results indicate that some artificial spike trains of low threshold SARs demonstrate high, and others low, classification accuracy (Fig. 1).

The differences in overall classification accuracy between real spike trains and their artificial counterparts are illustrated in Fig. 2. The overall accuracy difference was used because it was averaged from all inflation conditions, and therefore allows for the most general evaluation of performance. As shown in Fig. 2, all differences were positive except for two low threshold SARs and one high threshold at 9 ml inflations (Fig. 2, *top*) and one low threshold SAR at 12 ml inflations (Fig. 2, *middle*), indicating that real spike trains are classified with greater accuracy than artificial ones.

Our main conclusion, based on the present findings, is that SAR spike *patterns* carry information regarding pulmonary distension. Even so, most artificial spike trains were classified at rates above chance (0.333...), implying that spike count must play a critical role in transmitting lung inflation information [13] since the effect of temporal patterns was excluded in the artificial spike trains. However, there were exceptions. Some artificial spike trains were