Nonspiking and Spiking Proprioceptors in the Crab: Nonlinear Analysis of Nonspiking TCMRO Afferents

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ABSTRACT

The proprioceptor that signals the position and movement of the first joint of crustacean legs provides an excellent system for investigating information processing and transmission in neurons that function in a graded (nonspiking) manner in the context of a simple motor system. The thoracic-coxal muscle receptor organ (TCMRO) spans the thoracic-coxal joint and transmits graded signals to the central nervous system via two large nonspiking axons.

The response characteristics and nonlinear models of the input-output relationship for the two nonspiking TCMRO afferents (S and T fibers) were determined using white noise analysis (Wiener kernel) methods. The best fitting linear responses of these neurons was similar, as were their second-order kernels. The gains of the afferents slowly increased with increasing frequency and reached a maximum at ~40-60Hz for the S fiber and 60-80Hz for the T fiber. Above this corner frequency, the gains of both afferents decreased at ~20dB/decade for the remainder of the 220Hz stimulus bandwidth. The shape of the first-order kernels, and hence the corresponding (linear) gain functions, of both afferents were similar when driven with different amplitudes of noise covering a 40-fold amplitude range. Predictions of the S fiber response based on the first two Wiener kernels were accurate, with the second order model producing a mean square error of 6-8%. Second-order Wiener models for the T fiber were less accurate with a mean square error of ~22-26%, but this accuracy improved to 10-16% with the incorporation of the third-order term in the Wiener expansion. The effect of cable properties on the transmission of the sensory potentials to the central nervous system was evaluated by determining the system
characteristics using membrane potentials 5-7mm distal to the transduction site. The major change after transmission along the axon was a low-pass filtering of the sensory signals and consequent reduction in signal bandwidth.
INTRODUCTION

Neurons usually transmit information as a sequence of action potentials due to the long distances these signals must travel compared with the comparatively short length constants ($\lambda$) of their axons. There are however, numerous instances of neurons that transmit information in a graded or nonspiking manner, either when the distances involved are short compared with the length constant or in cases where neuronal processes are specialized (large $\lambda$) for graded transmission. In many sensory systems, particularly the visual system, nonspiking neurons are ubiquitous elements in the initial levels of signal encoding and information processing and appear to be optimized for fast and accurate signaling over relatively short distances (Bush 1981; Roberts and Bush 1981; Pearson 1976; Egelhaaf and Warzecha 1999).

Nonspiking neurons have been described in various vertebrate and invertebrate nervous systems and these neurons play an important role in neuronal integration (Pearson, 1976; Roberts and Bush, 1981). In particular, nonspiking neurons have been found in sensorimotor and central pattern generator circuits and provide an important mechanism for the control of motor behavior (Angstadt and Calabrese 1991; Burrows et al. 1988; Burrows and Siegler, 1976, 1978; Büschges 1990, 1995; Büschges et al, 1994; DiCaprio and Fourtner, 1988; DiCaprio 1989, 1990, 1999; Graubard 1978; Nagayama and Hisada 1987; Paul and Mulloney 1985; Pearson and Fourtner 1975).
A substantial body of work on insect motor systems has examined sensorimotor pathways incorporating both spiking and nonspiking interneurons in reflex pathways. Nonspiking interneurons in the locust receive inputs from leg mechanoreceptors, and spiking and nonspiking local interneurons (Burrows and Laurent 1988; Laurent and Burrows 1988, 1989). The outputs of these interneurons are directed to motor neuron pools and can control the gain of reflex pathways. Nonspiking neurons are also involved in the control of reflex gain, time constant and sign in other arthropod nervous systems (Büschges, 1995; Büschges and Schmitz 1991; Nagayama and Hisada 1988; Nagayama et al. 1984).

The position, velocity, and acceleration of the first two joints of the crab leg are monitored by both nonspiking and spiking proprioceptors and therefore provide an ideal system for comparing spiking and nonspiking (graded) information transfer and processing in a simple sensory-motor system. The nonspiking thoracic-coxal muscle receptor organ (TCMRO) spans the TC joint, while the coxal-basal joint is monitored by the spiking CB chordotonal organ (CBCTO) and by nonspiking afferents arising from levator and depressor elastic strands. Feedback from these receptors and other limb proprioceptors is an important component of leg control systems during posture and locomotion. These inputs can directly modify the strength of motor neuron activity via reflex pathways, or act indirectly by providing input to central pattern generating networks (Marder and Bucher 2001; Pearson 2000, 1995).
The TCMRO is similar to other arthropod muscle receptor organs (Mill 1976) and consists of an elastic connective tissue sheath enclosing an intrinsic muscle that lies in parallel with the promotor muscle of the coxa spanning the thoracic-coxal (TC) leg joint (Alexandrowicz and Whitear 1957, 1967). This joint produces promotion (forward movement) and remotion (backward movement) of the more distal leg segments. The receptor is therefore stretched by leg remotion and relaxed by promotion. The TCMRO is the sole proprioceptor monitoring the TC-joint of the crab leg and is innervated by three afferent and two efferent neurons (Alexandrowicz and Whitear, 1957).

The three sensory neurons (T, S, and P fibers) that innervate the origin of the receptor have centrally located cell bodies in the thoracic ganglion. The S and T axons are large diameter (50-60 µm) that do not generate action potentials but instead conduct information to the central nervous system by graded transmission (Ripley et al., 1968; Bush and Roberts, 1971). Electrophysiological studies of the T and S fibers in Carcinus determined that these afferents have length constants in excess of 20mm (Mirolli 1981; Ripley et al. 1968). This is in striking contrast to the 1.6mm length constant of large (75µm diameter) crustacean motor neurons (Hodgkin and Rushton 1946). These studies also revealed a small (1-3 mV) voltage-dependent component of the T fiber response superimposed on the generator potential (Ripley et al. 1968) due to the presence of fast, TTX-sensitive sodium channels (Bush et al. 1980; Mirolli 1981). The P fiber is smaller than the S and T fibers (7-10 µm diameter) with an estimated length constant of 5mm in Carcinus (Wildman and Cannone 1996). This afferent produces a single action potential superimposed on a low amplitude graded receptor potential in response to stretch of the
receptor (Wildman and Cannone 1990). This mixed mode of signaling (spiking and nonspiking) has also been observed in afferent neurons of the oval organ in the crustacean ventilatory system (Pastor and Bush 1982).

We determined the transfer functions of the spiking and nonspiking receptors in order to compare their linear and nonlinear transfer characteristics and to provide a framework for functional comparisons of receptor properties, efferent control, synaptic transmission and information rates in these neurons. The best fitting linear estimates of S and T fiber response were very similar and characterized by broadly tuned gain functions with maximum gains occurring at 60-80Hz. The amplitude dependence of the linear response was also tested and found to be independent of movement amplitude. Nonlinear models of the S and T fiber responses based on the first and second-order Wiener kernels were able to accurately predict the response of the S fiber, but addition of the third-order Wiener term was necessary to produce similar accuracy for T fiber responses.
METHODS

An isolated ganglion-receptor preparation of male and female green shore crabs, *Carcinus maenas*, was used in all experiments. The walking legs and chelea were autotomized and the dorsal carapace, viscera and brain were removed. The sternal artery supplying the thoracic ganglion was immediately cannulated and the ganglion was perfused with chilled (16-17°C) oxygenated saline at a rate of 2-3 ml/min for 15-20 minutes before proceeding with further dissection. The saline composition was Na⁺, 500; K⁺, 12; Mg²⁺, 20; Ca²⁺, 12; Cl⁻, 576 (mM), buffered to pH 7.2 with 10 mM Tris maleate (Ripley et al. 1968).

The remotor, levator and depressor muscles were removed to expose the promotor muscle in the 5th leg segment. The promotor muscle tendon was then cut at the coxopodite and the main leg nerve cut. The skeletal plate containing the origin of the promotor muscle and the TCMRO was dissected free of surrounding structures along with the thoracic ganglion and leg nerves and transferred to a small volume (5ml) bath. The chamber was continuously superfused with chilled oxygenated saline for the duration of the experiment. The preparation was pinned to a Sylard base, and the TCMRO was exposed by removing the surrounding promotor muscle. A small piece of the promotor tendon was left attached to the distal end of the TCMRO and attached to the puller with a stainless steel pin. The nerve to the TCMRO was isolated from the leg nerve and supported with a small Sylgard block near the attachment of the TCMRO (Fig. 1).

The *in situ* length of the TCMRO was measured with a caliper when the TC-joint was held in the middle of the physiological range. The resting length of the isolated TCMRO was
set to this length by mechanically adjusting the position of the puller after the preparation was pinned to the substrate.

The electromechanical puller was constructed from a 5" diameter low-mid range speaker and the position of the speaker cone was monitored by an optical position sensor consisting of a light source, photodiode and an optical wedge (Hofmann and Koch 1985). The puller was controlled by a proportional-integro-differential (PID) controller operating in a length feedback mode. The frequency response of the puller is flat out to a cut-off frequency \( f_c \) of approximately 220Hz over a displacement range of ±1mm. All stimulus amplitudes (receptor length) in the text are given as the peak-to-peak values of the movement measured over at minimum 5s interval. For the size of animals used in these experiments (~4-5cm carapace width), a length change of 0.35mm corresponds to a total joint angle movement of ~15° where the maximum joint angle change is ~90 degrees (Clarac 1977). The TC joint is held in a relatively constant angle during lateral walking in the crab (Clarac 1977) but covers a 20-30° range during forward and backward walking in the lobster (Ayers and Davis 1977) and similar range in the fifth leg during swimming (Hartnoll 1971).

White noise was generated by a 31-bit pseudo-random number generator clocked at 10kHz resulting in a pseudorandom sequence length of >200,000s. The digital output of this generator was filtered to the desired bandwidth using a variable 8-pole low-pass filter (Wavetek 852), and DC-offset and amplified as required. Trapezoidal stimuli were generated by a custom-built waveform generator with variable rise/fall time, amplitude and duration.
Intracellular recordings from TCMRO afferents were made with microelectrodes filled with 2M KAc amplified with a bridge electrometer (NPI SEC 05L).

All signals were digitized on-line using a CED Power1401 laboratory interface (16-bit A/D converter, ±5 volt range, 0.4 µs conversion time) with intracellular and extracellular recordings sampled at 12.5 kHz while the position output of the feedback controller was sampled at 2.5 kHz.

**Systems Theory**

White noise (or Wiener kernel) analysis is a *nonparametric* approach to systems identification (Marmarelis and Marmarelis 1978) that has been used in the study of neurophysiological systems, primarily in visual (Marmarelis and Naka 1972), auditory (Eggermont, 1993) and mechanoreceptor systems (French and Wong 1977, Dickinson, 1990; Kondoh et al 1995). This approach to the system identification problem can be used to determine the transfer characteristic of a system without specifying (or assuming) an internal structure or specific mechanisms that may be present. The actual mechanisms are replaced with a filter with exactly the same transfer characteristics as the system under study. The mathematical basis for this analysis is comprehensively covered in Marmarelis and Marmarelis (1978) and in several more recent reviews (Westwick and Kearney 1998; French and Marmarelis 1999). A summary is given in the companion paper (Gamble and DiCaprio 200x, companion paper).

In classic linear systems theory the input-output relationship is described by the convolution integral:
\[ y(t) = \int_{0}^{\infty} h(\tau)x(t-\tau)d\tau \]

where \( h(\tau) \) is the impulse response of the system. This integral states that the output of the system, \( y(t) \) can be written as a **weighted sum** of the past inputs, \( x(t) \), where the weighting function at each time lag is \( h(\tau) \). If \( h(\tau) \) is known for a linear system then we may predict the system response to any input by application of the convolution integral. In practice however, most biological systems contain significant nonlinearities and nonlinear systems analysis techniques must be used.

The Wiener analysis is based on Volterra's (1959) approach to functional identification of a finite memory nonlinear system. Wiener showed that if the input to the system was Gaussian white noise, a series expansion could be constructed with mutually orthogonal terms and a convenient and computationally practical scheme for measuring the system kernels could be implemented. The input-output relationship for a system can then be written as:

\[ y(t) = \sum_{m=0}^{\infty} G_m[h_m(\tau_1,\ldots,\tau_m);x(t'):t' \leq t] \]

where \( G_m \) are orthogonal functionals if \( x(t) \) is a zero-mean Gaussian white noise signal and \( h_m \) is the \( m^{\text{th}} \) order Wiener kernel. The set of Wiener kernels \( h_m \) therefore characterizes the system and allows the prediction of the system response to any arbitrary input. The zero order kernel describes the DC response of the system, the first-order kernel is the best-fitting linear (impulse) response of the system, the second-order kernel describes the
nonlinear interaction of two inputs in the past on the output of the system in the present, and so on for higher-order kernels.

There are numerous methods for the calculation of the Wiener kernels operating directly in the time domain or after transformation of the input and output signals into the frequency domain (Marmarelis and Marmarelis 1978; French and Marmarelis 1999). In the work reported here, cross-correlation (Lee and Schetzen 1965) was used to calculate the first and second-order Wiener kernels, which is simple to implement and reasonably efficient given the computational power of contemporary laboratory computers. All system kernels were computed for a 30ms time range which was sufficient for the amplitude of all kernels to decay to near zero. All computations of first and second-order kernels were performed by programs written in the Spike2 script language (CED, Ver. 4.03).

The maximum noise bandwidth applied to the TCMRO was 220Hz, which was greater than the frequency of the maximum gain for the S and T afferents. This limit also served to minimize the error in kernel estimation due to excessive bandwidth, while reducing the error associated with the finite width of the autocorrelation function of bandlimited noise (Marmarelis and Marmarelis 1978). All system kernels were computed for a 30ms time range (75 time lags at a sample interval of 0.4ms) which was sufficiently long for all kernels to decay to zero. The number of data points (input-output pairs) used to estimate the kernels must be greater than the number of free parameters in the kernels. The use of 75 time lags to calculate the zero, first and second-order kernels results in 2926 free parameters. All calculations of first and second-order kernels were computed over a 25-
30s time interval, or 62,500-75,000 data points. First and second-order kernels were not smoothed and were plotted with Axum graphing software (Ver 5.0c, Mathsoft).

Where it was necessary to determine the third-order kernel for an afferent, software provided by Dr. A. S. French (KERNEL, Ver. 3.1.0.2) was used to calculate the system kernels. This software uses the parallel cascade method to determine the Volterra kernels of the system (Kornenberg 1991), which is more efficient when determining higher order kernels. This method is based on the fact that a wide range of nonlinear dynamic systems can be modeled by a parallel cascade of simple nonlinear systems, each consisting of a linear filter followed by a zero-memory nonlinearity. If a suitable set of cascades can be found, the Volterra kernels can be calculated from them. For a given input signal, x(t), the process starts with one cascade that gives an approximation of the output y(t), then the output of the cascade to x(t) is subtracted from the actual output, y(t), to leave a residual. A second cascade is then used to fit the residual, and so on, until the process reaches a set error level or maximum number of cascades. Although the input to the system can be any signal, it should be random and have a wide bandwidth, and if the input signal is Gaussian white noise, the kernels produced by this method are a good approximation of the Wiener kernels. The number of free parameters in a third-order kernel is \( \sim m^{3/3} \) (where m is the number of time lags). For the sampling interval of 0.4ms used in these experiments, 75 time lags (30ms total time) results in \( \sim 140,000 \) free parameters in the calculation of zero through third-order kernels. This calculation would therefore require at least 60s of data (150,000 data points), and 4-5 minutes of experimental data in practice, for the kernel estimation. In order to reduce the calculation time and the amount of experimental data
required, additional data channels were derived from the original records with a sample interval of 0.8ms. The number of time lags to span 30ms at this sample interval is reduced to 37, and the total number of free parameters in the computation of a third-order kernel is therefore reduced to \( \approx \frac{37^3}{3} = 16,884 \). This effective sample rate requires only \( \approx 65 \)s of data (81,250 data points) and 70-80 seconds of data were used when third-order kernels were determined.
RESULTS

Recordings were made from 10 TCMRO preparations that yielded 8 T fiber and 8 S fiber recordings. The resting membrane potential of these neurons was -65 to -70mV when the receptor was slack and depolarized to approximately -55mV when the receptor was held at a length equivalent to the mid-position of the TC-joint. Typical recordings from T and S afferents in response to a trapezoidal length change of the receptor are shown in Figure 2. The TCMRO afferents respond to trapezoidal stretch of the receptor with graded changes in membrane potential consisting of phasic and tonic components, that is, proportional to the rate of change of receptor length (velocity) and to its absolute length, although the ratio of tonic to phasic response amplitude is a distinctive characteristic of each afferent (Bush and Roberts 1971). At the beginning of a constant velocity stretch of the TCMRO, the T and S fibers respond with a very rapid large amplitude depolarization. This dynamic (velocity) component of the response, measured as the amplitude of the depolarization at the end of a ramp stretch, is approximately linear for the S fiber over a 100-fold range of velocity but is only approximately linear for the T fiber over a 10-fold velocity range (Bush and Roberts 1971). The amplitude of the T fiber velocity response remains relatively constant (55-60mV depolarization from rest level) for velocities greater than ~15mm/s (Bush and Roberts 1971). When the receptor is held at a constant length, the S and T afferents maintain a relatively constant level of depolarization that is roughly proportional to the receptor length (Bush and Roberts 1971).

The membrane potential recorded from T and S fibers in response to random movement of the TCMRO is shown in Fig. 3. These records were extracted from the middle of
a 60s period of stimulation. There was no noticeable transient response at the onset of stimulation (not shown), as the movement amplitude was increased from zero to the final desired amplitude over a 1-2s period in order to minimize response transients and possible damage to the receptor. The amplitude of the membrane potential fluctuation was essentially constant throughout the stimulation period with no significant adaptation. For example, the peak-to-peak amplitude of S and T fiber membrane potential measured over a 4s interval just after the start of stimulation, and for the same interval 50s later, was 33mV during both intervals for the S fiber and 51mV initially and 50mV after 50s for the T fiber. When the receptor length was changing rapidly over a large amplitude range, the response of the two afferents was relatively similar (Fig. 3, arrow 1) except for an approximately 2-fold membrane potential amplitude difference. During an interval when the movement amplitude was smaller, and hence lower velocity (Fig. 3, arrow 2), the membrane potential of the S fiber more closely followed the receptor length, while the T fiber still responded to a small increase in length with a large (maximal) depolarizing change in membrane potential (Fig. 3; * in boxed area).

**First-order linear response of TCMRO afferents**

The first-order kernel of an afferent is the best-fitting linear (impulse) response of the system and the units of this kernel are mV·mm⁻¹·s⁻¹. A positive kernel value therefore denotes an increase in membrane potential (depolarization) while a negative value indicates a decrease in membrane potential. All calculations of first and second-order kernels were computed over a 25-30s time interval with a time resolution (sample interval) of 0.4ms. Calculation of the system kernels over different overlapping or non-overlapping time intervals, or for larger periods, produced similar kernels in all experiments. The time axis for the all kernel plots are the time lag(s) and are shown with increasing lag(s) along the axis.
The first-order kernels and their associated gain functions for the S and T fibers are shown in Figure 4. The first-order kernels were calculated by cross-correlation of the input (receptor length) with the membrane potential of the afferent using a stimulus bandwidth of 200Hz. Gain functions were determined by calculating the Fast Fourier Transform (1024-point FFT) of the first-order kernel. The first-order kernels for the S and T fibers were very similar with a single positive peak followed by a much smaller and broader negative peak that decays to zero at a time lags greater than ~30ms. The major difference was that the width of the positive peak of the S fiber kernel was wider than the peak for the T fiber kernel, indicating a larger system time constant, and hence lower cutoff frequency, for the S fiber. This difference was also reflected in the gain curves for the T and S fibers. They are extremely similar, with a slowly increasing gain (slope ~10dB/decade) up to maximum gain at 30-40Hz followed by a decreasing gain of approximately -20dB/decade for the remainder of the stimulus frequency band. The difference in width of the first-order kernels results in a higher cutoff frequency of ~80Hz for the T fiber vs. ~40Hz for the S fiber. The first-order kernels and gain functions presented in Fig. 4 are typical of most of the data, although in some preparations (n=3), the width of the S fiber first-order kernel was shorter and therefore more similar to the T fiber kernel (for example, compare the S and T fiber kernels in Figure 5). The gain curve for the S fiber in these cases was similar to the one shown in Figure 4, but the cutoff frequency was higher (60-70Hz), although still slightly less than the cutoff frequency of the T fiber. The T fiber first-order kernels and gain functions were very similar in all 8 preparations. These gain functions are similar to the gain function for a first-order low-pass filter, although the gain for such a filter would be constant (flat) up to the cutoff frequency.

Stimulus amplitude, first-order kernels and gain

To determine if the first order response properties of S and T fibers depended on movement amplitude, random movements of several different amplitudes were applied to the
TCMRO. The peak-to-peak (p-p) membrane potential changes associated with these different amplitudes increased in proportion to the applied input amplitude for the T and S fibers at movement amplitudes greater than 0.05mm p-p. However, the peak T fiber depolarization was similar and close to maximum for all but the smallest movement amplitude, while the peak depolarization of the S fiber increased gradually as the movement amplitude increased (not shown). First-order kernels calculated for the T and S fibers with four amplitudes of stimulation (0.02, 0.05, 0.2 and 0.4 mm p-p), are shown in Fig. 5. The first-order kernels for each afferent are very similar for all amplitudes of movement, as are the corresponding gain functions (not shown). The best fitting linear response for the S and T afferents therefore appears to be independent of stimulus amplitude.

**Second order kernels**

Second-order kernels were calculated for S and T afferents by cross-correlation of the input (receptor length) with the membrane potential of the cell. In all cases, this kernel was non-zero, indicating that there was a nonlinear component to the overall response of the afferents (Fig. 6). The kernels are presented as 3D plots with the z-axis representing changes in membrane potential (units are mV·mm⁻²·s⁻²), while the x and y axes are the two time lags, \( \tau_1 \) and \( \tau_2 \). All kernels have their largest amplitudes on the diagonal (\( \tau_1=\tau_2 \)) with prominent but slightly smaller amplitude off-diagonal troughs. The T fiber second-order kernel has a large peak at small time lags followed by a smaller amplitude but broader ridge along the diagonal at longer time lags, along with two prominent off-diagonal troughs. The second-order kernel for the S fiber also has a peak at small time lags but this peak is broader and followed by a transition to a slightly smaller amplitude longer duration ridge extending along the diagonal for approximately 15ms. This broad "ridge" is flanked by two negative troughs running parallel to the diagonal. For time lags greater than 20ms, the value of both kernels is close to zero.
Model responses

In order to test the predictive ability of the Wiener kernels, the response of each neuron was modeled by convolving the first and second-order kernels with a 5s portion of the data set following the time interval over which the kernels were computed. The fit between the experimental data and the model prediction was assessed by computing the percentage mean square error (MSE) between the estimated output, $y_s$, vs. the real output $y$,

$$\text{MSE} = \frac{(y(n) - y_s(n))^2}{\bar{y^2}(n) - y^2(n)}$$

where the bar denotes the time average over all available samples $n$.

The model output for an S fiber based on the first-order prediction only (Fig. 7, $K_1$) is the filtered version of the input signal, as this model is produced by convolving the input with the first-order kernel and therefore gives the best fitting linear estimate of the system output. While the model output follows the experimental data, the MSE is high at 48%. Errors are especially evident when large changes in input amplitude are present and this first-order model consistently underestimates the amplitude of the S fiber response, especially the larger (peak) depolarizations. When the second-order model output is computed by convolving the second-order kernel with the input, and adding this result to the first order prediction, the resulting second-order model (Fig.7, $K_{1,2}$) has a MSE of only 6.5%. Similar levels of accuracy were obtained for all S fibers when second-order models were computed, with MSEs in the range of 6-9% ($n=4$).

The model output computed for a T fiber afferent is shown in Fig. 8. In this case, the prediction based on the first-order model alone had a high MSE of 90%, but the error
decreased markedly to 24% when the contribution of the second-order term in the Wiener expansion was added to produce a second-order model (Fig. 8, $K_{1,2}$). As the error of this second-order model was similar for all T fiber models ($n=4$) and higher than the second-order predictions for the S fibers, the third-order Wiener kernel was calculated for these afferents to produce a third-order model ($K_{1,2,3}$) of the T fiber response. The third-order kernels are four-dimensional (amplitude and 3 time lags) but can be visualized with one constant time lag as 3D "slices" of the kernel. The major feature of these slices was a large amplitude negative peak occurring at time lags less than 5ms with smaller amplitude positive peaks at slightly longer lags (~10ms) off the diagonal. Inclusion of the third-order term therefore corrects the overshoot and undershoot seen in the second-order model during large amplitude positive or negative length inputs. The MSE of the third-order model decreased to 11%, with the major deviations from the real output occurring on the slower hyperpolarizing portions of the response. These results and the overall level of accuracy of each (first, second or third-order) model were consistent for all neurons modeled, that is, S fiber second-order models had MSEs of 6-9% ($n=4$), while T fiber models only approached this accuracy with a third-order model (10-16% MSE, $n=4$).

**Cable properties**

In neurons that rely on the generation of action potentials to transmit information, recordings of neuronal activity can, in principle, be made at any point along the axon. However, in nonspiking neurons, the cable properties of the neuron may have a significant impact on the transfer characteristics of these cells and would at least be expected to low-pass filter the passively propagating signal (Jack et al. 1975). All of the results described above were made with recordings from the TCMRO afferents at a distance of approximately 0.5mm from the site of sensory transduction. Given the large length constants of these axons, this site
provides a reasonably accurate representation of the membrane potential in the transduction region.

In order to assess the role of cable properties in this system, and therefore the signals that would ultimately mediate graded synaptic transmission, T and S fiber membrane potentials were measured at the point where the axons entered the thoracic ganglion, with distances from the receptor origin of 5-7mm depending (roughly) on the size of the animal. Recordings made from two positions along the sensory axons are shown in Fig. 9. The length constants of these two axons were measured under steady-state conditions and were 1.85cm for the S fiber and 2.2cm for the T fiber. The amplitude of the membrane potential change in each axon is smaller, and the potentials are low-pass filtered when recorded distal to the receptor (gray traces) as evidenced by the decreased rise and fall times of the voltage compared to more proximal recording site (black traces). The first-order Wiener kernels and associated gain functions for S and T fibers calculated using data from the distal recording sites are shown in Fig. 10. As expected from the recordings shown in Fig. 9, the first-order kernels for the T and S fibers were similar to the kernels computed from recordings made near the transduction site, but had a longer duration when calculated with data taken from recordings made distal to the site of sensory transduction. The gain functions show a corresponding decrease in cutoff frequency for the best fitting linear estimate of the system response. Second-order kernels were also calculated (Fig. 10) and have a similar broadening of the peaks and troughs compared with the kernels determined close to the transduction region (compare with the second-order kernels in Fig. 6). The shift of the peaks of both kernels to larger time lags is due to the time delay in the signal propagation along the axon. When models were computed for the membrane potential of T and S fibers recorded at the more distal positions (not shown), similar results and levels of accuracy were obtained.
compared with the recordings made near the transduction site. As before, third-order models were required for the T fiber to produce a mean square error of ~10-16%.
DISCUSSION

The nonlinear transfer characteristics of the two nonspiking afferent neurons arising from the crab thoracic coxal muscle receptor organ were analyzed using white noise techniques. These neurons respond to movement of the TCMRO with graded changes in membrane potential that are passively propagated along the sensory axon to the central nervous system. The responses of the S and T afferents to deterministic stimulation of the TCMRO have been described in detail in previous studies (Ripley et al. 1970; Bush and Roberts 1971).

The first-order Wiener kernels provide the best fitting estimate of the linear portion of the system response and correspond to the impulse response of a purely linear system. In recordings made close to the transduction region of the afferents, the first-order Wiener kernels for the afferents were quite similar in overall shape and time course, although the peak of the S fiber kernel usually had a slight longer duration compared to the peak of the T fiber kernel. This indicates a slightly lower cutoff frequency for the S fiber as seen in the corresponding gain plots calculated by taking the Fourier transform of the first-order kernel. As noted, S fiber first-order kernels in three preparations were much closer in overall shape to the T fiber kernel, resulting in a similar frequency for the maximum gain.

The second-order Wiener kernels were also calculated for the S and T fibers and again were found to be roughly similar. These kernels describe the nonlinear interaction, and hence the deviation from a purely linear response due to inputs at different time lags or from amplitude dependent nonlinearities when the time lags are equal. The major difference in the two kernels was that the large positive ridge along the $\tau_1=\tau_2$ diagonal had a longer duration for
the S fiber, indicating a slightly longer system memory for this afferent. The amplitude of the second-order kernels for both afferents was close to zero after ~20ms.

Models of system response

Predictions of S and T fiber response were made by convolving the input signal (TCMRO length) with the first and second-order kernels, using experimental data that was not used for the calculation of the kernels. The response of the S fiber was well modeled by inclusion of the first and second-order terms in the Wiener expansion resulting in a MSE for the second-order model in the range of 6-8%. The second-order models for the T fiber response resulted in MSEs of only 25-35%. Prediction of the T fiber output with a similar degree of accuracy as the S fiber could only be made by including the third-order Wiener term. This third-order T fiber model improved the accuracy of the prediction and reduced the MSE to ~10%.

The need for a third-order model to improve the prediction of the T fiber response could be due to the presence of voltage dependent sodium channels in the T fiber and the inward currents that result from their activation. The S and T axons contain fast TTX-sensitive sodium channels (Bush et al. 1980; Mirolli 1981), although these channels may be restricted to the transduction region of the axon (Mirolli 1979; DiCaprio preliminary observations). These channels account for a small portion of the T fiber response (1-3 mV) that can be seen at the peak of the rising phase of the depolarization caused by stretching the receptor. This active component is not observed in the S fiber when the receptor is stretched and the sodium channels appears to be inactivated at the normal resting potential of the S fiber (Bush et al. 1980; Bush 1981). The small amplitude active component of the T fiber response is eliminated in TTX (Mirolli, 1981) and the remainder of the rising phase is otherwise unaltered (Bush
However, preliminary studies with TTX indicate that there is no effect of this treatment on the system kernels and the precise physiological role of this current, if any, is still unknown.

The need for the third-order term in the T fiber model may possibly be explained by the mechanics of transduction in the TCMRO. The TCMRO consists of elastic connective tissue sheath enclosing an intrinsic muscle (see Fig. 1B). The terminals of the S fiber axon lie in the connective tissue sheath near the origin of the receptor and are therefore mechanically in parallel with the receptor. The T fiber terminals are inserted in the base of the receptor muscle and tendon at the origin of the receptor and are therefore mechanically in series with the receptor muscle. The receptor muscle and tendon is therefore an additional mechanical element that determines the overall transduction for the T fiber receptor potential, but is not directly involved in the S fiber transduction. This difference in the receptor mechanics and transduction dynamics is demonstrated by experiments where the receptor efferent motor neurons are stimulated (Cannone and Bush 1981b). The resulting isometric contraction of the receptor muscle elicits a large T fiber depolarization but only a negligible S fiber depolarization, as the connective tissue sheath is not stretched by muscle activation. In addition, when the receptor muscle is severed close to the origin of the receptor, but leaving the connective tissue sheath intact, the steady state response of the T fiber to maintained receptor stretch is almost totally abolished, while the S fiber static response is essentially normal (Cannone and Bush 1981a). However, the dynamic response of the T fiber to trapezoidal stretch and to random stimulation is still present, although altered, when the receptor muscle is cut and the unstimulated receptor muscle appears to produce some resting tension (DiCaprio, preliminary observations). The mechanical properties of the receptor muscle and tendon therefore appear to govern mechanotransduction by the T fiber, while the mechanical properties of the connective tissue sheath may play the dominant role in sensory
transduction for the S fiber. Experiments are presently in progress to attempt to resolve and clarify this issue.

The effect of the cable properties of the afferents on overall system characteristics was evaluated by recording membrane potential of the S and T afferents 5-7mm distal to the site of transduction. As expected, given the electrotonic properties of a passive cable (Jack et al. 1975), the amplitude of the membrane potential change decreased and was low-pass filtered when recorded distal to the receptor. The first-order kernels for the T and S fibers were similar to the kernels computed from recordings made near the transduction site, but had a longer duration due to the filtering by the axonal cable. The gain functions for the best fitting linear estimate of the system response showed a corresponding decrease in cutoff frequency. Second-order kernels had a similar broadening of their peaks and troughs compared with the kernels determined close to the transduction site. When models were computed for the membrane potential of T and S fibers recorded at the more distal positions, similar results and levels of accuracy were obtained compared with the recordings made near the transduction site.

**Comparison with spiking CB chordotonal afferents**

The coxal-basal (CB) joint of the leg is the next most distal joint to the TC joint and the movement of the CB joint is monitored by a spiking proprioceptor, the CB chordotonal organ, and also by two nonspiking elastic receptor, the depressor and levator strands. The companion paper (Gamble and DiCaprio 200x) presents a white noise analysis of the spiking chordotonal organ afferents. This analysis confirmed earlier studies of chordotonal function in that the afferents could be classified into response categories that were sensitive
to position, mixed position-velocity, pure velocity and acceleration of the chordotonal organ. The response of these afferents was also nonlinear and second-order Wiener models were necessary to obtain reasonably accurate predictions of the afferent response. The major difference with respect to the first-order linear estimates of the receptor response is that S and T fibers have similar first-order kernels and the gain functions for the linear response were broadly tuned. Thus there is no subdivision of function with respect to the length of the receptor and derivatives of length (velocity and acceleration) as seen for the CBCTO afferents, and S and T afferents respond over a broad frequency range. However, the maximum frequency of the best-fitting linear response of the S and T afferents, although high, with a maximum cutoff frequency of 80Hz, is still less than the cutoff frequency of the CB chordotonal afferents, which is typically around 90-110Hz, and in some cases probably ranges up to 200Hz or higher (Gamble and DiCaprio 200x, companion paper).

A subset of the CBCTO afferents also respond to receptor acceleration and this type of response was not observed in the linear estimates of the S and T afferent response. However, as can be seen in the response to trapezoidal movements (Fig. 3), the rise time of the membrane potential is faster than the rise of the imposed length change, but any possible correlation between the transient acceleration of the receptor and this initial depolarizing response has not been investigated. Acceleration information for the TC-joint may also be provided by the P fiber response, as this afferent fires a single spike on initial movement of the TCMRO during a constant velocity stretch (Wildman and Cannone 1990, 1996), which is the expected response for a spiking acceleration sensitive afferent.
The chordotonal afferents and the TCMRO afferents are major sources of synaptic input to the reflex circuitry controlling their respective leg joints as well as to networks involved in locomotor control (Clarac et al. 2000). It would appear that there is a more explicit segregation of function in the CBCTO given the movement specific response classes of these afferents compared with the broad tuning of the TCMRO afferents. However, the demonstrated convergence of 2-5 CBCTO afferents on to leg motor neurons (El Manira et al. 1991) may result in functionally equivalent (broadly tuned) input from the CBCTO to postsynaptic neurons.

All of the experiments conducted in this study were done with the receptor efferent innervation cut, so that the TCMRO was operating in an open-loop configuration. The efferent input to the TCMRO is normally activated by stretch of the receptor, primarily via synaptic drive from the T fiber (Cannone and Bush 1981) and the activation of the efferent neurons produces a significant depolarization of the T fiber. The role of this positive feedback and its effect on the transfer characteristics of the S and T afferents is presently under investigation.

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FIGURE LEGENDS

Figure 1. A. Schematic diagram of the experimental preparation. The thoracic ganglion, the TCMRO and its sensory innervation were isolated and continuously superfused with oxygenated saline in a small volume chamber. The skeletal fragment containing the proximal attachment of the TCMRO was securely pinned to the Sylgard substrate of the chamber with 3-4 stainless steel pins while the distal end of the receptor is attached to the output shaft of an electromechanical puller by a pin inserted through a small piece of the receptor tendon. The electromechanical puller is constructed from a small loudspeaker and the position of the output shaft is monitored with a optical position sensor. The puller is in a length feedback control loop controlled by a proportional-integro-differential (PID) controller. Intracellular recordings of TCMRO afferent activity were made close to the point where the S and T fibers insert in the receptor. B. Anatomy of the TCMRO and innervation (modified from Roberts and Bush 1971). The view of the thoracic cavity (left) shows the position of the TCMRO in series with the promotor muscle of the TC joint. The S and T sensory fibers arise from the flanking connective tissue sheath of the TCMRO and at the base of the receptor muscle respectively, and are therefore in parallel (S fiber) or in series (T fiber) with the receptor (left). In all experiments the efferent nerve to the TCMRO was cut (x) and initial recordings made close to the origin of the receptor (right). Also shown above and to the right of the TCMRO is the elastic depressor receptor and its nonspiking sensory neuron (D fiber) that runs in the same nerve as the TCMRO afferents.

Figure 2. Response of T and S fibers to trapezoidal stretch of the TCMRO. The initial stretch of the TCMRO (len) produces a rapid depolarization of T and S fibers followed by a relatively steady depolarization at the new (longer) receptor length. The length change applied here is equivalent to ~20° of TC-joint movement.
Figure 3. Intracellular recording from T and S fibers during stimulation of the TCMRO with 220Hz bandwidth white noise. During the initial portion of this record, the movement of the receptor is relatively high amplitude and the responses of the S and T fibers are rather similar except for the marked difference in the amplitude of the membrane potential changes Arrow 1). When the movement is comparatively slower and slightly lower amplitude (Arrow 2), the response of the T fiber is still large amplitude in response to receptor stretch while the S fiber response appears to more closely track the changes in input length. Note that during this period, a small stretch of the receptor (* in boxed area) still produces a maximal depolarization of the T fiber.

Figure 4. First-order Wiener kernels and gain plots for T and S fibers. The first-order kernels (left) were computed by cross correlation of the input signal (receptor length) with the membrane potential of the sensory neuron. The first order kernels and associated gain functions are very similar for T and S fibers, although the width of the S fiber kernel is larger than the width of the T fiber kernel resulting in a higher cutoff frequency for the T fiber.

Figure 5. First-order kernels for S and T fibers calculated with 4 different amplitudes of stimulation covering a 20-fold range of amplitudes from 0.02 to 0.40mm peak-to-peak amplitude. The kernels for each afferent are essentially identical for each amplitude of stimulation, as are the associated gain functions (not shown).

Figure 6. Second-order kernels for T and S fibers shown as 3-D surface plots. The two time axis are the two time lags, $\tau_1$ and $\tau_2$, and the z-axis is the amplitude of the second-order kernel with units of mV·mm$^{-2}$·s$^{-2}$. The amplitude of the second-order kernels have been
normalized, but the maximum positive value for the T fiber kernel is approximately 10x larger than the positive peak of the S fiber kernel. The maximum values for the time axes are 30ms.

Figure 7. S fiber Wiener kernel models. The model output for an S fiber based on the first-order prediction only ($K_1$) is the filtered version of the input signal with a mean square error (mse) of 48%. When the second-order model output is computed by convolving the second order kernel with the input, and added to the first order prediction, the resulting second-order model ($K_{1,2}$) has a MSE of only 6.5%.

Figure 8. T fiber Wiener kernel models. The prediction of the T fiber based on the first-order model alone has a high mean square error (MSE) of 90%, but the error decreases markedly to 24% when the contribution of the second-order term in the Wiener expansion is added ($K_{1,2}$). The error was further reduced to 11% by addition of the third-order Wiener kernel term to produce a third-order model ($K_{1,2,3}$).

Figure 9. Cable properties of the T and S fibers. Simultaneous intracellular recordings from T and S fibers at two points along the sensory neuron. The proximal recordings (black records) were made approximately 0.5 mm from origin of the receptor and therefore within ~1mm of the sensory terminals. The distal recordings (gray records) were made close to the point of entry of the sensory nerve at the thoracic ganglion and were 7mm from the first electrode for the T fiber and 6mm for the S fiber.

Figure 10. First-order kernels, gain plots and second-order kernels calculated for the distal recording along the sensory cable for T and S fibers. The amplitude of the second-order kernels have been normalized, but the maximum positive value for the T fiber kernel is
approximately 10x larger than the positive peak of the S fiber kernel. The maximum values for the time axes are 30ms.
DiCaprio, Figure 1

A

input

position

TCMRO

thoracic ganglion

B

TC.joint

TCMRO

dep

S fiber

T fiber

D fiber

$V_m$
DiCaprio, Figure 3

T fiber
-58mV

S fiber
-56mV

len

50mV
20mV
0.4mm

40ms
DiCaprio, Figure 4
DiCaprio, Figure 5

![Graph showing T fiber and S fiber with time constant $\tau$ and mV/mm/s as the y-axis and 10ms as the x-axis.](image-url)
DiCaprio, Figure 6

T fiber

S fiber
DiCaprio, Figure 9

T fiber

\[ \lambda = 2.2\text{cm} \]

S fiber

\[ \lambda = 1.85\text{cm} \]

len

0.4 mm

40 ms

50 mV

20 mV
DiCaprio, Figure 10

T fiber

S fiber

10ms

Frequency (Hz)