Dynamics of Neurons Controlling Movements of a Locust Hind Leg
II. Flexor Tibiae Motor Neurons

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Newland, Philip L. and Yasuhiro Kondoh. Dynamics of neurons controlling movements of a locust hind leg. II. Flexor tibiae motor neurons. J. Neurophysiol. 77: 1731–1746, 1997. Imposed movements of a proprioceptor that monitors the relative position of the tibia about the femur, the femorotibial chordotonal organ (FeCO), evoke resistance reflexes in the motor neurons that control the movements of the tibia of the locust. The response dynamics of one pool of motor neurons, the flexor tibiae motor neurons, which are located in three groups (anterior, lateral, and posterior), have been analyzed by the Wiener kernel method. First- and second-order kernels that represent the linear and nonlinear responses, respectively, were computed by a cross-correlation between the intracellularly recorded synaptic responses in the motor neurons and the white noise stimulus applied to the FeCO, and were used to define the input-output characteristics of the motor neurons. The posterior fast, intermediate, and slow and the anterior fast and intermediate flexor tibiae motor neurons had biphasic first-order kernels with initial negative phases, indicating that they are velocity sensitive. The falling phases of the kernels had distinct shoulders, indicating that the responses of the motor neurons also had delayed low-pass components, i.e., position sensitivity. The anterior slow flexor motor neuron had a monophasic, low-passed, first-order kernel, indicating that it is position sensitive. The linear component of the motor neuron responses, predicted by convolving the first-order kernels with the stimulus signal, strongly resembled the actual response, whereas the second-order nonlinear component was small, particularly at >10 Hz. The power spectra of the fast motor neurons showed that they had the highest cutoff frequencies (at >8 Hz), whereas the slow flexor motor neurons had a gradual roll-off at 1 Hz. The intermediate flexor motor neuron had an intermediate cutoff frequency of ~2–3 Hz. The linear responses of the flexor motor neurons could be decomposed into low- and high-frequency components. The high-frequency components (>10 Hz) were velocity dependent and linear, whereas the low-frequency components (<10 Hz) were position dependent and nonlinear. The nonlinearity was a signal compression (or half-wave rectification). The results show that although the flexor motor neurons receive many common inputs during FeCO stimulation, each individual has specific dynamic response properties. The responses of the motor neurons are fractionated so that a given individual within the pool will respond best to position, whereas others will respond better to velocity. Likewise, some motor neurons respond best at low frequencies, whereas others respond best at higher frequencies of stimulation.

INTRODUCTION

Knowledge of the pathways and identities of the constituent neurons within a neuronal network is in itself not sufficient to understand the huge integrative tasks performed by the networks, because signals can be transformed at any stage. The properties of the individual neurons and their synapses, for example, can have profound influences on the way a signal is transmitted.

The neuronal pathways mediating resistance reflexes of a hind leg of the locust are known in detail (Burrows 1992). We have considerable knowledge of what sensory information is encoded about a mechanical stimulus to a leg (Hofmann and Koch 1985; Hofmann et al. 1985; Kondoh et al. 1995; Matheson 1990, 1992; Zill 1985), although we know little of how it is then transformed through the layers of spiking (Burrows 1987a) and nonspiking local interneurons (Burrows 1987a,b) comprising the network that produces a resistance reflex. Sensory neurons of a femoral chordotonal organ (FeCO) monitor movements of the tibia of a hind leg relative to the femur and encode different parameters of the stimulus, including position, velocity, and acceleration, in different combinations (Kondoh et al. 1995; Matheson 1990; Usherwood et al. 1968). Recently we showed that there is a systematic change in the coding properties across the entire population of FeCO sensory neurons, such that at one end of the range of response properties some sensory neurons encode only position, whereas at the other end some encode only acceleration. Between these extremes afferents will encode, to a greater or lesser extent, more than one movement parameter (Kondoh et al. 1995).

The sensory neurons from the FeCO make monosynaptic connections with motor neurons that innervate the muscles that move the tibia about the femorotibial joint (Burrows 1987b) in a resistance reflex. One of these muscles, the flexor tibia muscle, is innervated by nine excitatory motor neurons (Phillips 1981) divided into three groups, each of which contains a fast, intermediate, and slow motor neuron and each of which can be uniquely identified. For reflex adjustments to be appropriate for a particular behavioral context, it is essential that a neuronal network extract relevant features of a stimulus that are necessary to provide the appropriate adjustments of the limb. We know that during locomotion, the position and velocity of leg movement are controlled by the local circuits responsible for generating the walking pattern in both invertebrates (Cruse 1985a,b; Dean and Cruse 1986) and vertebrates (Desmedt and Godaux 1978; Stein 1982). Although we do know that locust flexor motor neurons receive velocity- and position-dependent inputs (Field and Burrows 1982), we do not have a detailed description of their dynamic response properties, nor do we know, in detail, how different members of a pool of synergistic
motor neurons differ in their responses to the same stimulus. The aim of this work was therefore to analyze the response properties of the flexor motor neurons and to compare them with the responses of the sensory neurons themselves to examine signal transformation in this neuronal network.

METHODS

Adult male and female desert locusts, *Schistocerca gregaria* (Forskål), taken from our crowded colony in Cambridge, UK, were used for all experiments. Locusts were immobilized ventral surface up in Plasticine, with a hind leg fixed anterior surface up at a femorotibial angle of 60° (zero mean position) (Fig. 1A). A small window was cut in the ventral thorax to expose the meso- and metathoracic ganglia, which were then stabilized on a wax-coated silver platform. The thorax was superfused with a constant flow of saline at room temperature (24°C). The posterior connectives of the metathoracic ganglion were cut, whereas the anterior connectives and lateral nerves remained intact. The sheath of the ganglion was treated directly with protease (Sigma type XIV) for 45 s before recording. Microelectrodes filled with potassium acetate and with DC resistances of 50–80 MΩ were driven through the sheath and into the somata or neuropilar processes of extensor and flexor tibiae motor neurons. Intracellular recordings were made with the use of an Axoclamp 2A amplifier (Axon Instruments). A pair of 50-µm copper wires, insulated except at the tips, was implanted in the extensor muscle of a hind leg and was used to stimulate the extensor muscle and evoke antidromic spikes in the fast extensor tibiae motor neuron (FETi).

Motor neurons innervating the flexor tibiae muscle of a hind leg occur in three groups in the metathoracic ganglion; anterior, lateral, and posterior, with each group containing a fast, an intermediate, and a slow flexor motor neuron. The anterior and posterior groups are readily accessible, and our results are confined to the motor neurons of these groups. Flexor motor neurons were identified with the use of a number of criteria: first, the relative positions of their small window was cut in the ventral thorax to expose the meso- and metathoracic ganglia, which were then stabilized on a wax-coated silver platform. The thorax was superfused with a constant flow of saline at room temperature (24°C). The posterior connectives of the metathoracic ganglion were cut, whereas the anterior connectives and lateral nerves remained intact. The sheath of the ganglion was treated directly with protease (Sigma type XIV) for 45 s before recording. Microelectrodes filled with potassium acetate and with DC resistances of 50–80 MΩ were driven through the somata in the metathoracic ganglion (Fig. 1B); second, the presence of short and constant-latency depolarizing potentials following antidromic spikes in FETi (Fig. 1C); third, the frequency of spikes evoked by injection of depolarizing current pulses through the recording electrode; and fourth, by the speed of tibial movement produced when depolarized. The results are based on 36 successful recordings from the posterior slow flexor tibiae (PSFlTi), 35 from the posterior intermediate flexor tibiae (PIFlTi), 35 from the poste-

![FIG. 1. A: drawing showing the location of the femoral chordotonal organ (FeCO) in the locust left hind leg (anterior surface). The FeCO apodeme was grasped, with the use of a pair of modified forceps attached to a vibrator, and cut distal to the forceps. B: location of the somata of the 3 groups of flexor tibiae motor neurons and the fast extensor tibiae motor neuron (FETi) in the metathoracic ganglion. Anterior is to the top. Lateral nerves 1–3, 5, and 6 are indicated. C: antidromic spike (†) evoked in FETi is followed at a constant latency by excitatory postsynaptic potentials in the somata of the posterior fast flexor tibiae (PFFlTi), posterior intermediate flexor tibiae (PIFlTi), and posterior slow flexor tibiae (PSFlTi) motor neurons recorded simultaneously. The excitatory postsynaptic potentials can lead to spikes that were usually of a small amplitude in the soma. D: Gaussian white noise (GWN) signals with cutoff frequencies (fc) of 27 or 58 Hz used as the stimuli, whose probability density functions are shown in E. The SD (σ) of the amplitude of the GWN with fc = 27 or 58 Hz was ~20 and 15°, respectively. F: power spectra of the white noise stimulus with fc = 27 and 58 Hz.](http://jn.physiology.org/)


rior fast flexor tibiae (PFFITi), 6 from the anterior slow flexor tibiae (ASFITi), 2 from the anterior intermediate flexor tibiae (AIFITi), and 6 from the anterior fast flexor tibiae (AFFITi) motor neurons from 56 locusts.

**Stimulation**

The apodeme of the metathoracic FeCO was exposed by cutting a small window of cuticle in the distal anterior femur, which was then grasped with fine forceps attached to a vibrator (Ling Altec, type 101) and cut distal to the forceps. A 500-μm displacement of the FeCO apodeme is equivalent to a 40° rotation of the tibia about the femorotibial joint (see Field and Burrows 1982). The forceps holding the apodeme were moved with conventional ramp wave stimuli or with Gaussian white noise (GWN) with cutoff frequencies (fc) of 27 or 58 Hz (Fig. 1, C–E). The white noise signal had a Gaussian probability density function (Fig. 1E) and a flat power spectrum in all frequency ranges examined (Fig. 1F).

The average intensity of the GWN stimulus, the middle of its operational range, was zero, i.e., the stationary state (a 60° femorotibial joint angle). The GWN was based on a random binary sequence generated with the use of a random binary generator (CG-742N, NF Circuit Design Block) band-limited from DC to 27 or 58 Hz by a low-pass filter (SR-4BL, NF Circuit Design Block) with a decay of 24 dB/octave. This band-limited white noise was used for both stimulation and cross-correlation. The peak-to-peak displacement of the white noise movement was measured with the use of two methods. First, the movements of the tips of the forceps were recorded on video and the displacement of the forceps was measured directly. Second, the linearity and peak displacement were confirmed with the use of a system of calibrated precision linear strain gauges (Measurement Group).

The SD, σ, was used to define the amplitude of the stimulus (in degrees of movement), and 99.7% of that deviation occurred within the range of −3σ to +3σ. The SD of the amplitude was 20° for GWN with fc = 27 Hz and 15° for GWN with fc = 58 Hz (Fig. 1D). Thus GWN with fc = 27 Hz covered the entire range of movement of the femorotibial joint from full flexion (0° joint angle) of the hind leg to full extension (120°), whereas GWN with fc = 58 Hz covered the range of 15°–105°.

**Data analysis**

Data were stored on magnetic tape with the use of a PCM-DAT data recorder (RD-101T, TEAC). The responses of the motor neurons were fed to a 16-bit personal computer (PC-9800VX, NEC) through a 12-bit analog-to-digital converter (ADX-98H, Canopus Electronics) at a sampling rate of 1–2 kHz for 20 s. Data were then transferred to a VAXstation 3100 or VAX 4000 computer (Digital Equipment) on which the software for white noise analysis, STAR, was run. To compute the first- and second-order Wiener kernels, data were digitally filtered at 0.5–200 Hz for both stimulus and synaptic response signals. First- and second-order Wiener kernels characterize the response dynamics of the extensor and flexor tibiae motor neurons. We used the cross-correlation method of Lee and Schetzen (1965) to compute kernels, and for estimation and convolution of kernels we used algorithms described by Sakuranaga and Naka (1985a,b). The first-order kernel is the first-order cross-correlation between the input (the white noise-modulated position of the tibia) and the output (the evoked synaptic response of a particular flexor motor neuron). The second-order kernel defines the multiplicative interaction between two parts of the input in the past, and is therefore a function of two time lags, t1 and t2. The magnitude of the nonlinear response is represented by contour lines on a two-dimensional plot. Solid lines indicate positive or depolarizing peaks and dashed lines indicate

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**FIG. 2.** Flexor tibiae motor neurons receive common synaptic inputs. A: simultaneous intracellular recordings from the somata of the 3 posterior flexor motor neurons show that they receive many common spontaneous synaptic inputs. B: simultaneous recordings from the same posterior flexor motor neurons while the FeCO apodeme was moved with a GWN signal with fc = 27 Hz. Although the 3 motor neurons do receive many common inputs evoked by FeCO stimulation, others are particular to only some members of the group.
negative or hyperpolarizing valleys. The unit for the first-order kernel is mV·deg⁻¹·s⁻¹, and that for the second-order kernel is mV·deg⁻²·s⁻² for synaptic responses. The detailed algorithms for computing the kernels, model responses, power spectra, and mean square errors (MSEs) have been described in Sakurana and Naka (1985a,b) and Sakai and Naka (1987).

Model responses of motor neurons were predicted by convolving the white noise input with the first- and second-order kernels. A quantitative measure of the agreement of the model with the experimental response is the MSE reduction. The MSE is given as a percentage by computing the ratio of the deviation between the response and the model, and indicates the accuracy of the model prediction (Marmarelis and Marmarelis 1978). For example, the MSE for the linear model represents the degree of linearity, i.e., the ratio of the linear component to the total response.

**RESULTS**

**Responses of the flexor tibiae motor neurons to FeCO stimulation**

**LINEAR RESPONSE PROPERTIES.** The flexor tibiae motor neurons share many common synaptic inputs. Simultaneous intracellular recordings from the PFFiTi, PIFiTi, and PSFiTi motor neurons show that all receive short-latency excitatory inputs following a spike in the FETi motor neuron (Burrows et al. 1989) (Fig. 1B). This centrally generated excitatory postsynaptic potential is mediated by monosynaptic connections between FETi and the flexor motor neurons. Moreover, all three posterior flexors receive many other common spontaneous synaptic inputs (Fig. 2A). During white noise movement of the apodeme of the chordotonal organ, however, it is clear that although much of the synaptic drive to the flexor motor neurons was very similar, some different synaptic inputs occur in particular neurons. For example, in the example in Fig. 2B the synaptic responses of PFFiTi and PIFiTi were similar, whereas PSFiTi received different inputs during some movements.

When stimulated by moving the FeCO apodeme with a white noise signal with fc = 27 Hz, the three posterior flexor motor neurons had differential, biphasic first-order kernels that had initial negative phases (Fig. 3, A–C). Because the first-order Wiener kernels are the best approximation of an impulse response, consisting of a rapid flexion of the tibia followed by a reextension, the initial negative phases showed that the flexor motor neurons received hyperpolarizing synaptic inputs during movements of the FeCO apodeme equivalent to flexions of the tibia. The subsequent positive peak showed that the motor neurons were depolarized during reextension, and the biphasic configuration of the kernels showed that all three flexors responded primarily to the rate of change of movement of the stimulus, i.e., velocity.

**FIG. 3.** First-order Wiener kernels from responses recorded in flexor motor neurons when the FeCO apodeme was moved with a GWN signal with fc = 27 Hz. A–C: 1st-order kernels of (A) the PFFiTi, (B) the PIFiTi, and (C) the PSFiTi flexor tibiae motor neurons computed from 26, 22, and 22 locusts, respectively. Kernels were normalized and superimposed. D–F: 1st-order kernels from individual recordings of (D) the anterior fast flexor tibiae (AFFiTi), (E) the anterior intermediate flexor tibiae (AIFiTi), and (F) the anterior slow flexor tibiae (ASFiTi) motor neurons. All kernels except that of ASFiTi were biphasic and had initial hyperpolarizations. This indicates that these motor neurons receive velocity-dependent inputs during FeCO stimulation, and that they are hyperpolarized during flexion movements and depolarized during extension movements. The 1st-order kernel of ASFiTi was more monophasic, indicating that it receives primarily position-dependent inputs.
The anterior group of flexors also contains three motor neurons, the AFFlTi, AIFlTi, and ASFlTi motor neurons. These also received short-latency depolarizing inputs following spikes in FETi, and other common synaptic inputs. In response to white noise stimulation at $f_c = 27$ Hz, AFFlTi and AIFlTi had differential, first-order kernels that had initial negative phases (Fig. 3, D and E). Thus these motor neurons had response properties similar to those of the posterior flexors, receiving depolarizing inputs during extension and hyperpolarizing inputs during flexion, and being sensitive to the velocity of movement of the apodeme of the chordotonal organ.

In contrast to these velocity sensitive flexor motor neurons, ASFlTi had a predominantly monophasic first-order kernel with an initial negative peak followed by a prolonged depolarization (Fig. 3F). This initial negative response corresponded to a negative valley on the diagonal in the second-order kernel (Fig. 5F), indicating that the ASFlTi motor neuron was position sensitive and received hyperpolarizing inputs during movements of the apodeme equivalent to flexions of the tibia and smaller depolarizations during extension.

The first-order kernels of all flexor motor neurons also had delayed positive monophasic components that formed distinct shoulders on the falling phase of their initial response, and because of their variability were best illustrated by averaging the first-order kernels (Fig. 4). This late component represents a delayed low-pass input (or position sensitivity). The anterior motor neurons also received similar delayed inputs (Fig. 3, D–F).

Nonlinear responses. The second-order kernels of the responses of the anterior and posterior flexors represent their nonlinear components. Those of the posterior flexors had an elongated depolarizing peak on the diagonal ($\tau_1 = \tau_2 = 25-75$ ms) with, or without, two small, off-diagonal valleys (Fig. 5, A–C). Both the hyperpolarizing valleys and the depolarizing peak were elongated parallel to the diagonal. A system whose second-order (or quadratic nonlinear) component is characterized by this type of diagonal peak can be modeled by a circuit containing a low-pass linear filter followed by a squarer (or a rectifier) and a second low-pass linear filter. Thus the second-order nonlinearity in the posterior group of flexor tibiae motor neurons is low-passed.

The second-order kernels of the anterior motor neurons were more differential (the peaks were elongated orthogonal to the diagonal) than those of the posterior group (Fig. 5, D–F).

An understanding of what the nonlinear components represent can be obtained by taking diagonal cuts through the second-order components where $\tau_1 = \tau_2$ (Fig. 6A) and superimposing these cuts on the first-order kernels (Fig. 6B). For ASFlTi, the second-order kernel had a similar configuration to the first-order kernel; a small on-diagonal valley (where $\tau_1 = \tau_2 = 10-30$ ms) and an elongated depolarizing peak on the diagonal (where $\tau_1 = \tau_2 = 60-100$ ms). Because the second-order term is a square function, it will not change depending on the polarity or direction of the stimulus. The configuration of the first-order kernel, however, is dependent on the stimulus direction, so that the kernels will sum during a flexion movement, but will cancel when the tibia is extended. This indicates that the nonlinearity is a half-wave rectification, which represents a directional coding property of the motor neurons. All other flexor motor neurons studied had a similar nonlinearity.

Our previous study on the response dynamics of FeCO sensory neurons showed that many sensory neurons encode more than one parameter of the stimulus (Kondoh et al., 1995b) and that these coding properties were revealed by changing the $f_c$ of the stimulus. Changing the $f_c$ of the stimulus did not, however, alter the configurations of the Wiener kernels of the flexor motor neurons. The first- and second-order kernels of PFFlTi obtained at $f_c = 27$ Hz (Fig. 7, Ai and Aii) were similar to those obtained at $f_c = 58$ Hz (Fig.
FIG. 5. Nonlinear response components of flexor tibiae motor neurons. A–C: contour plots of 2nd-order kernels from responses in (A) the PFFiTi (averaged from 26 locusts), (B) the PIFiTi (from 22 locusts), and (C) the PSFiTi (from 22 locusts), when the FeCO apodeme was stimulated with the use of GWN with \( f_c = 27 \text{ Hz} \). All kernels were normalized. Solid lines: positive (or depolarizing) peaks. Dashed lines: negative (or hyperpolarizing) valleys. The 2nd-order kernels all had an elongated depolarizing peak on the diagonal (for \( \tau_1 = \tau_2 = 25-75 \text{ ms} \) ) with 2 small hyperpolarizing valleys in off-diagonal areas. D–F: contour plots of the 2nd-order kernels of the responses of (D) the AFFiTi (from 5 locusts), (E) the AIFiTi (from 1 locust), and (F) the ASFiTi (from 4 locusts) flexor motor neurons.

7, Bi and Bii), being band-passed (biphasic), having delayed low-passed components in the form of a shoulder on the first-order kernel, and having monophasic, low-passed nonlinear components.

Model predictions and power spectra

Linear and nonlinear models of the responses of the motor neurons were predicted by convolving the first- and second-order kernels, respectively, with the stimulus input. For PFFiTi and PIFiTi motor neurons, the linear components (predicted from the 1st-order kernel) were large and similar to the actual response (Fig. 8, A and B), whereas the second-order nonlinear components (predicted from 2nd-order kernel) were much smaller, suggesting that these neurons are primarily linear. For example, the average MSE for the linear model (predicted by the 1st-order kernel) was 48.5% for PFFiTi, whereas that for the nonlinear model was 43.7%. The linear component, therefore, predicted 51.5% of the total response of the neuron, whereas the second-order nonlinear model only improved the prediction by an average 4.8% (Fig. 9A). An analysis of the responses in the frequency domain showed that the linear models of the PFFiTi and PIFiTi had powers of 5–20 dB larger than that of the second-order models in the frequency range of 0.5–10 Hz (Fig. 8, D and E). At higher frequencies, the power of the linear model was comparable with that of the actual response, suggesting that the response of the flexor motor neurons in this frequency range was linear.

The responses of the AFFiTi and AIFiTi were similar to those of their posterior counterparts, being mainly linear. For example, the MSEs of AFFiTi for the model predictions against the response suggest that the linear component comprised 48.5% of the total evoked response, whereas the second-order nonlinear component comprised only 12.1% (Fig. 9B). The linear model has a power comparable with that of the response in the frequency range of 5–20 Hz. The linear response in the high-frequency range was velocity dependent because of the differentiating first-order kernel (Fig. 3D). In a lower-frequency range of 0.5–5 Hz, the power of the linear and second-order models was similar, indicating that the response was nonlinear. The balance of the response not
falling off at 27 Hz, the upper \( fc \). The power of the linear model fell off at 1 Hz, then increased at 2 Hz, peaking at 3–5 Hz before falling off again. This double peak suggested that the synaptic response was likely to be composed of two different components whose optimal frequency ranges were different. In a high-frequency range (2–20 Hz), the power of the linear model was comparable with that of the response, indicating that the response was linearly related to the stimulus. In a low-frequency range (<2 Hz), the response was composed of the linear and second-order terms. This observation is explored in detail in a later section.

In common with the PSFlTi, the responses of ASFlTi were also low-passed. The MSE for the linear model was 73.8%, whereas that for the summation model of the linear and second-order models was 67.1%. Thus the linear component comprises 27.2% of the total response evoked, whereas the second-order term comprises only 6.7% (Fig. 9B). The power spectra of the response and the corresponding linear model in ASFlTi began to fall off at 2 Hz, indicating that the response in ASFlTi was low-passed as was expected from the monophasic first-order kernel with an initial hyperpolarization (see Fig. 3F).

Of all the posterior flexor motor neurons, the power of the response of PSFlTi was greatest at low stimulus frequencies (<1 Hz) (Fig. 8G). At higher stimulus frequencies, comparisons of power spectra when the apodeme was moved with white noise with \( fc = 27 \) Hz (Fig. 8, D–G) show that the response of PSFlTi declined at >1 Hz, whereas that for PIFlTi declined only slowly, at \( \sim 2-3 \) Hz, before falling off rapidly at the stimulus \( fc \). For PFFlTi, however, the power of the response declined only at >8 Hz, before declining rapidly at the \( fc \) of the stimulus. Thus the PFFlTi responds best during high frequencies of FeCO stimulation, and PSFlTi at low frequencies.

\section*{Gain and phase characteristics}

The gain and phase characteristics of the linear response were obtained by fast Fourier transformation of the first-order kernels, and are shown for the posterior group flexor tibiae motor neurons in Fig. 10. Phase shifts (i.e., delays) were negative in all frequency ranges examined (Fig. 10, Ai, Bi, and Ci) for these flexor motor neurons. Phase delay increased with increasing frequency, and reached \(-360^\circ\) at 20–30 Hz. No differences in phase between the three posterior motor neurons were found.

Gain in the PFFlTi and PIFlTi motor neurons was constant in the frequency range of 0.5–20 Hz when the FeCO apodeme was stimulated at \( fc = 27 \) Hz (Fig. 10, Aii and Bii). On the other hand, gain in PSFlTi was lower than that in PFFlTi and PIFlTi in the high-frequency range, decreasing at 8 Hz (Fig. 10Cii). Gain at \( fc = 58 \) Hz was similar to that at \( fc = 27 \) Hz. These curves suggested that the linear response in the posterior group of flexor tibiae motor neurons was constant gain and low-passed, although surprisingly the first-order kernels from which the gain curves were produced were all differential. This discrepancy suggests that the posterior flexor tibiae motor neurons receive information about the position of the tibia, in addition to the velocity-dependent inputs, and it is these velocity-dependent inputs that characterize the differenti-
Increasing the $f_c$ of the white noise stimulus had little influence on the responses of the motor neurons. $A_i$ and $A_{ii}$: 1st- and 2nd-order Wiener kernels, respectively, of the responses of the PFFITi obtained with a white noise stimulus with $f_c = 27$ Hz. $B_i$ and $B_{ii}$: 1st- and 2nd-order kernels, respectively, obtained with $f_c = 58$ Hz. Note the similarity of the kernel configurations at the different $f_c$s.

**Decomposition of the first-order Wiener kernels**

The coding properties of FeCO sensory neurons are dependent on the frequency of the stimulus input (Kondoh et al. 1995). Thus some sensory neurons code position at low stimulus frequencies ($f_c < 27$ Hz), but also velocity at higher stimulus frequencies ($f_c > 27$ Hz). The results shown in Fig. 7 indicate that if there is a transition then it must occur at frequencies lower than the upper $f_c$ of the white noise signal (i.e., $< 27$ Hz). To understand the contribution of the different components to the response of the flexor motor neurons at these lower frequencies, we analyzed the different components by decomposing the responses of the flexor motor neurons by passing their synaptic responses to white noise stimulation through a low-pass ($f_c < 10$ Hz) and a high-pass ($> 10$ Hz) filter, respectively (Fig. 12A). A decomposition of a white noise-evoked response in PFFITi into low- and high-frequency components produced first-order kernels of low-pass filtered and high-pass filtered responses (Fig. 12B) with power spectra that matched that of the response in low- and high-frequency ranges, respectively (Fig. 12C). The first-order kernel from the low-pass filtered response was monophasic with a time to peak of $\tau = 60–80$ ms, which corresponded to the time to...
FIG. 8. Model predictions of the responses of the posterior flexor motor neurons to FeCO stimulation. A–C: intracellular recordings of the responses in (A) the PFFITi, (B) the PIFITi, and (C) the PSFITi (top traces) to white noise stimulation at $f_c = 27$ Hz (bottom traces), and the corresponding linear and 2nd-order models. The responses are predominantly linear while the 2nd-order components are minor. D–F: power spectra of the responses of the same motor neurons shown in A–C, and those of the corresponding models and of the stimulus. In a high-frequency range (5–20 Hz), the linear models had powers comparable with those of the responses, indicating that the responses are linear in this range. Note that the power of the response of PFFITi falls off sharply at 27 Hz, whereas that of the PSFITi rolls off gradually at >1 Hz. G: power of the responses of the PFFITi, PIFITi, and PSFITi motor neurons plotted on the same graph for comparison.
The second-order nonlinearities were interpreted by taking cuts through the diagonals of the second-order kernels (Fig. 13, A and D). The diagonal cut of the second-order kernel of the low-pass filtered response in PSFITi was similar in shape to the first-order kernel, having an elongated depolarizing peak for \( t_1 = t_2 = 25–150 \) ms (Fig. 13B). Thus the linear and second-order terms of the response in the posterior group of flexor tibiae motor neurons were both low-passed in a low-frequency range (\(<10 \) Hz). Moreover, the amplitude of the cut was comparable with that of the first-order kernel, indicating that the second-order term contributes substantially to the low-pass filtered response. From the similarity of the two waveforms, it can be predicted that the depolarizing response in the linear term to the flexion response will be enhanced by the second-order term hyperpolarizing response during flexion (Fig. 13B), but canceled out during extension. This is because the second-order component is a square function. Thus the nonlinearity represented by the second-order kernel is a signal compression, such as a half-wave rectification, and again represents a directional coding property of the synaptic inputs. Figure 13C shows an example of the low-pass filtered response in PSFITi and the corresponding linear and second-order models. The MSE for the model predictions showed that the linear and second-order component comprised 31.0 and 23.9%, respectively, of the low-pass filtered response.

The second-order kernel from the high-frequency component had a hyperpolarizing valley (for \( t_1 = t_2 = 10–20 \) ms) and a depolarizing peak (for \( t_1 = t_2 = 50–60 \) ms) on the diagonal (Fig. 13D). The amplitude of the diagonal cut of the second-order kernel was much smaller than that of the first-order kernel, indicating that the second-order term was minor in the frequency range \( >10 \) Hz (Fig. 13, D and E). This was confirmed by the model predictions as shown in Fig. 13F. The MSE for the linear model against the response was 49.4%, whereas that for the summation of the linear and second-order models was 39.1%. Thus the linear component provides the major part (50.6%) of the response in the frequency range \( >10 \) Hz, whereas the second-order component was minor (\(<10\% \) of the total response). In the low-frequency range, therefore, the responses in the flexor tibiae motor neuron to movement about the femorotibial joint are position sensitive and half-wave rectified with a preferred direction of flexion, whereas they are velocity sensitive and linearly related to the stimulus in a higher-frequency range.

**DISCUSSION**

Although flexor motor neurons receive many common inputs during FeCO stimulation, each individual has specific dynamic response properties. Coding of tibial movement among the pool of motor neurons is fractionated, so that a given individual within the pool will respond best to position, whereas others will respond better to velocity. Likewise, some motor neurons respond best at low frequencies, whereas others will respond best at higher frequencies of stimulation.
Dynamic responses of the flexor motor neurons

In Wiener’s nonlinear theory, the first-order kernel is the best linear approximation of a system’s response to an impulse. For the leg movements we have analyzed, this would correspond to a brief flexion of the tibia. The initial negative valleys of the first-order kernels are generated by an imposed movement similar to a flexion of the tibia about the femorotibial joint, and the following positive peak is generated by an extension. Thus the first-order kernels predict that the flexor motor neurons receive inhibitory inputs during a flexion about the joint whereas they receive excitatory inputs during an extension. This is a resistance reflex about the femorotibial joint (Field and Burrows 1982) that counteracts deviations of one or more regulated variables from desired values. Cruse (1985a,b) and Dean and Cruse (1986) showed that two parameters of movement, position and velocity, are controlled variables during movements of the legs of stick insects. Positional information is used to monitor the anterior and posterior end points of the stance and swing phases during walking, and also to terminate these movement phases. Information about the velocity of a movement is needed to regulate force output by a leg during a stance phase of the walking cycle.

Close parallels exist between resistance reflexes of vertebrates and invertebrates. For example, in the stretch reflex of a vertebrate limb, muscle spindle afferents enhance the responsiveness of the feedback system by contributing a measure of the rate of change of the disturbance (i.e., velocity of the imposed movement) to the feedback loop. This makes the feedback signal more effective when the regulated variable changes rapidly. Moreover, like the chordotonal organ afferents of insects (Hofmann et al. 1985; Matheson 1990), group Ia afferents are not only sensitive to the rate of change of muscle length, but also to the length of the muscle (Matthews 1972). Again, as in insects, the neuronal networks involved in the reflex involve both mono- and polysynaptic pathways between the proprioceptive afferents and the motor neurons. Ia afferents in the medialis gastrocnemius motor neurons of the cat, for example, make direct excitatory connections with α-motor neurons innervating the same muscles from which they arise and with the motor neuron innervating synergistic muscles. They also inhibit motor neurons to antagonistic muscles through Ia inhibitory interneurons.

In the locust, the importance of controlling velocity is indicated by the dominance of velocity-dependent inputs in most of the flexor motor neurons. The primary response of all flexor motor neurons except ASFTi during an imposed repetitive movement is to the velocity of that movement. The ASFTi, however, responds best to positional information. Although most flexor motor neurons receive similar velocity-dependent inputs, each has a response property that is unique, but overlapping with those of other flexor motor neurons.
Bässler 1989; Field and Burrows 1982) respond best to slow movements of the apodeme of the FeCO, whereas fast and intermediate motor neurons respond best to faster stimuli. Thus there is a gradual drop-off of the power of the responses at high frequencies of stimulation in the order of the slow flexor first, followed at slightly higher frequencies by the intermediate flexor, and finally at higher frequencies still by the fast motor neuron.

On the other hand, the ASFITt motor neuron receives position-dependent inputs during FeCO stimulation, although its responses do have some dependence on velocity as indicated by the overshoot of the return phase following the initial response to extension in the first-order kernels. This difference in response properties to the other flexor motor neurons clearly indicates that there may be some degree of differential control of different flexor motor neurons.

Delayed excitatory inputs to the flexor motor neurons

All flexor tibiae motor neurons analyzed received position-dependent information during chordotonal organ stimulation, but with long delays. This is reflected in the long time to peak of the monophasic component in the first-order kernels. Because these positional components have such long delays, it appears certain that they are mediated through polysynaptic pathways, rather than by direct synaptic connection between the afferents and motor neurons. For example, we know that some of the sensory neurons from the FeCO make direct synaptic connections with the flexor motor neurons that generate the movements of the limbs (Burrows 1987a). These same sensory neurons make parallel connections with different classes of interneurons including spiking and nonspiking local interneurons (Burrows 1980, 1987a,b; Büschges and Wolf 1995), which in turn converge back onto the same motor neurons. Some of the spiking local interneurons respond to the position of the tibia (Burrows 1988), but we do not yet know in any detail the response properties of the nonspiking local interneurons.

What does this delayed component represent? The nonlinearity shown in the second-order kernels as an elongated diagonal peak defines an assistance reflex such that the flexor tibiae motor neurons (except for the ASFITt motor neuron) receive delayed excitatory inputs only when the tibia is flexed. The magnitude of the input is proportional to the position of the tibia, i.e., the smaller the femorotibial joint angle, the larger the inputs. Although these inputs do appear to oppose a resistance reflex, as has already been shown in locusts (Field and Burrows 1982), they are of a much lower gain than those that contribute to a resistance reflex (Bässler et al. 1986). These may simply reflect the long-lasting inputs that some local nonspiking interneurons receive from FeCO afferents (Burrows et al. 1988).

Comparisons with other studies

One of the best known examples we have of a joint control loop, at least at a linear level, comes from the many detailed studies on the stick insect (see reviews by Bässler 1983, 1993). Instead of using an escape behavior to avoid predators, stick insects exhibit a form of behavior...
termed catalepsy, in which they remain motionless (Godden 1974). Imposed movements of the limbs during this state produce responses that are too slow to be detected by a predator. Analyses of the control of joint movements during this behavior have revealed that catalepsy is critically dependent on two properties of a negative feedback loop. These are a marked dependence on the velocity of movement and a high gain of the feedback loop (Bäsßler 1983, 1993). These features ensure that even a small imposed movement is resisted with a large force (Bäsßler 1983). This is illustrated by intracellular recordings from the flexor tibia motor neurons, which show responses dependent on the velocity of imposed movement (Büsßges and Wolf 1995). Comparisons of the stick insect control loop with that of the locust, Locusta migratoria, have implied that the control loop of locusts is quite different from that of a stick insect because it fails to show either the high gain or velocity dependency (Ebner and Bäsßler 1983). Büsßges and Wolf (1995) suggest that the flexor motor neurons of the middle leg of L. migratoria exhibit responses that are not dependent on velocity. Our results on the hind leg of the locust, S. gregaria, contradict these findings. Five of the six flexor tibia motor neurons show a marked dependency on the velocity of FeCO apodeme movement. Time-derivative systems typically have gain curves with slopes of 20 dB/decade. Not only do chordotonal afferents have gain curves with slopes ≥20 dB/decade (Kondoh et al. 1995b), but so too do the flexor tibia motor neurons in the high-frequency range. This finding adds support to the original study of Field and
Burrows (1982), which showed that the response of a slow flexor motor neuron was dependent on the velocity of the stimulus. We show that this is only the case for the PSFITi motor neuron and not for ASFITi. In addition, the difference between the results presented here and those of Bütchges and Wolf (1995) implies that either the control loop is different between middle and hind legs of locusts, or that there is a species difference between S. gregaria and L. migratoria. Which of these possibilities is most likely remains to be examined in the future.
Signal transformation

Signals are transformed in a number of ways in neuronal networks controlling the movements of the limbs of locusts. Previous studies have shown that chordotonal afferents of insects can encode more than one parameter of the stimulus (Hofmann and Koch 1985; Hofmann et al. 1985; Kondoh et al. 1995b; Matheson 1990; Zill 1985), including different combinations of position, velocity, and acceleration. Kondoh et al. (1995) showed that the coding properties of FeCO sensory neurons were dynamic, and often dependent on the frequency of stimulation. Our results from the decomposition of the linear responses show that they also have frequency-dependent stimulus sensitivities, but with the transition frequency lower than that of the sensory neurons (at ~10 Hz compared with ~30 Hz for the FeCO sensory neurons), presumably due to low-pass filtering at the synapse and along the primary neurite of the motor neuron. With the exception of the ASFITI motor neuron, all other flexor motor neurons responded best to velocity at higher frequencies (>10 Hz) and to position at lower frequencies (<10 Hz).

In addition, all FeCO sensory neurons have nonlinear directional coding properties (Kondoh et al. 1995). The synaptic responses of the flexor motor neurons, however, are more linear and match well with the actual response over a wide range of frequencies. This is because the motor neurons are inhibited during flexion but excited during extension, with the inhibitory inputs making their responses linear (Kondoh et al. 1995a; Okuma and Kondoh 1996). The inhibitory input comes, most likely, from spiking local interneurons. For example, spiking local interneurons with somata at the ventral midline of the metathoracic ganglion receive direct excitatory inputs from FeCO afferents (Burrows 1987a). These interneurons reverse the sign of the input and make inhibitory output connections with leg motor neurons (Burrows and Siegler 1982).

The fcs of all the FeCO afferents are >80 Hz (Kondoh et al. 1995). Those recorded in the somata of the flexor motor neurons are much lower, at 1–10 Hz. This reduction must, to some extent, reflect the filter properties of the primary neurites of the motor neurons, because the recording sites are some distance away from the synaptic sites in the neuropil, but must also reflect a true loss of sensory information in the high-frequency range. Thus it is possible to represent the flexor arm of the feedback loop of this resistance reflex as a series of filters. First, the chordotonal afferents themselves act as a parallel array of low- and band-passed filters (Kondoh et al. 1995b). Second, the synapses betweenafferents and motor neurons can be represented by low-pass filters because of the reduction in the corner frequency of the power spectra. Finally, the flexor motor neurons themselves can be considered as band-passed filters. Clearly now we must also examine the dynamics of tibial movements and determine whether there is a further filtering of the signals due to the mechanical properties of the leg muscles and also whether the gain of movement of the hind leg is as low as has been suggested earlier.

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REFERENCES


BURROWS, M., WATSON, A. H. D., AND BRUNN, D. E. Physiological and ultra-
structural characterization of a central synaptic connection between identi-
BUSCHGES, A. AND WOLF, H. Nonspiking local interneurons in insect leg
motor control. I. Common layout and species-specific response properties
of femur-tibia joint control pathways in stick insect and locust. J. Neuro-
CrUSE, H. Which parameters control the leg movement of a walking insect.
I. Velocity control during the stance phase. J. Exp. Biol. 116: 343–355,
1985a.
CrUSE, H. Which parameters control the leg movement of a walking insect.
DIAN, J. AND CrUSE, H. Evidence for the control of velocity as well as
position in leg protraction and retraction by the stick insect. Exp. Brain
DEBRODT, B. AND BÄSSLER, U. Motor neurones of the flexor tibiae muscle
DESmedT, J. E. AND GODAUX, E. Ballistic skilled movements: load compen-
sation patterning of the motor commands. In: Cerebral Motor Control in
Man: Long Loop Mechanisms, edited by J. E. Desmedt. Basel: Karger,
EBNER, I. AND BÄSSLER, U. Zur Regelung der Stellung des Femur-Tibia-
Gelenkes im Mesothorax der Wanderheuschrecke Schistocerca gregaria
FIELDS, L. H. AND BURROWS, M. Reflex effects of the femoral chordotonal
organ upon leg motor neurones of the locust. J. Exp. Biol. 101: 265–
268, 1982.
GODDEN, D. H. The physiological mechanism of catalepsy in the stick insect
HOFMANN, T. AND KOCH, U. T. Acceleration receptors in the femoral
chordotonal organ in the stick insect Carcina impigra. J. Exp. Biol.
HOFMANN, T., KOCH, U. T., AND BÄSSLER, U. Physiology of the femoral
chordotonal organ in the stick insect Carcina impigra. J. Exp. Biol.
KONDOH, Y., HASIGAWA, Y., OKUMA, J., AND TAKAHASHI, F. Neural com-
putation of motion in the fly visual system: quadratic nonlinearity of
responses induced by picrotoxin in the HS and CH cells. J. Neurophysiol.
KONDOH, Y., OKUMA, J., AND NEWLAND, P. L. Dynamics of neurones con-
trolling movements of a locust hind leg: Wiener kernel analysis of the
responses of proprioceptive afferents. J. Neurophysiol. 73: 1829–1842,
1995b.
LEE, Y. W. AND SCHETZEN, M. Measurement of the Wiener kernels of a
MARMARELIS, P. Z. AND MARMARELIS, V. Z. Analysis of Physiological Sys-
MATHESON, T. Responses and locations of neurones in the locust metathor-
MATHESON, T. Range fractionation in the locust metathoracic femoral
MATTHEWS, P.B.C. Mammalian Muscle Receptors and Their Central Ac-
OKUMA, J. AND KONDOH, Y. Neural circuitry underlying linear representa-
sion of wind information in a nonspiking local interneuron of the cock-
1996.
PHILLIPS, C. E. An arthropod muscle innervated by nine excitatory motor
SACAL, H. N. AND NAKA, K.-I. Signal transmission in the catfish. V. Sensi-
Sakuranaga, M. AND NAKA, K.-I. Signal transmission in the catfish
389, 1985a.
Sakuranaga, M. AND NAKA, K.-I. Signal transmission in the catfish
Stein, R.B. What muscle variable(s) does the nervous system control in
USHERWOOD, P.N.R., RUNION, H. I., AND CAMPBELL, J. I. Structure and
physiology of a chordotonal organ in the locust leg. J. Exp. Biol. 116:
Zill, S. N. Plasticity and proprioception in the insects. I. Responses and
cellular properties of individual receptors of the locust metathoracic fem-