

THE DYNAMICS OF LOCUST NON-SPIKING LOCAL INTERNEURONS

Responses to Imposed Limb Movements

Oliver P. Dewhirst, Natalia Angarita-Jaimes, David M. Simpson, Robert Allen
Institute of Sound and Vibration Research, University of Southampton, Southampton, SO17 1BJ, U.K.

Philip L. Newland

School of Biological Sciences, Building 85, University of Southampton, Highfield Campus, Southampton, SO17 1BJ, U.K.

Keywords: Reflex Dynamics, Nonlinear System Identification, Wiener Laguerre.

Abstract: A key feature of the locusts hind leg control system is a reflex loop that uses a stretch sensor, the Femoral Chordotonal organ, to monitor the position and movements of the tibia relative to the femur. A population of non-spiking local interneurons in the metathoracic ganglia receive synaptic inputs from the sensory neurons in the chordotonal organ and indirect inputs from other interneurons. They function to integrate these signals and generate the motor pattern required for coordinated limb movement. Nonlinear Volterra models combined with Gaussian white noise stimulation have, for the first time, been used to characterise the dynamics of this population of interneurons. The results show that the interneurons can be clustered into three groups, those which are position, position/velocity and velocity sensitive.

1 INTRODUCTION

Reflexes are a critical part of vertebrate and arthropod motor control systems allowing posture and movement to be adapted to changes in the external environment. Greater understanding of the reflex control of limb movement should allow the features of such systems to be exploited to improve the design of engineering control systems (bio-inspired design) (Bar-Cohen, 2006). Arthropods provide an opportunity to develop new investigative techniques and gain insight into a relatively simple and accessible neuromuscular reflex control system. Three ganglia in the locusts thorax contain neurons responsible for controlling movements of the legs (Figure 1). A key feature of its hind limb reflex control system is a stretch sensor called the Femoro-tibial Chordotonal Organ (FeCO) (Burrows, 1996). This sensor monitors the movement of the tibia about the femoro-tibial joint (Figure 1). Movements of the tibia are converted into action potentials by sensory neurons located in the FeCO (~90 cells). These signals are processed by spiking local interneurons and then integrated with information from other sensors by the non-spiking local

interneurons. The non-spiking local interneurons transmit this information using graded potentials to the leg motor neurons which activate muscle contraction (Burrows, 1996). It is believed that the non-spiking interneurons have the ability to modulate the reflex response in one limb given information integrated from both local sensors and those on other limbs and hence play a crucial role in the production of coordinated limb movement (Burrows, 1996). Previous work has described the connections between the non-spiking local interneurons and the motor neurons in the hind leg of the locust (Laurent and Burrows, 1989). Little is known, however, of the range of inputs received by these interneurons. In this paper a nonlinear Volterra model combined with a Gaussian White Noise (GWN) stimulation signal has been used to characterise the input sensitivity of a population of non-spiking local interneurons to imposed movements of the locust hind leg femoro-tibial joint. A similar technique has been used to model a population of spiking local interneurons (Vidal-Gadea et al., 2009).

That study, however, used the Wiener series and a cross correlation parameter estimation method

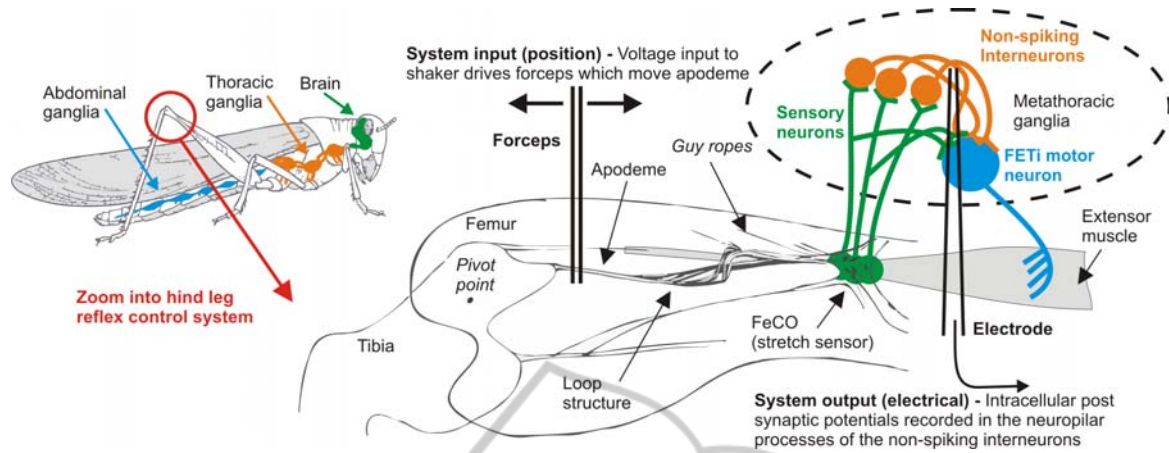


Figure 1: The locust hind leg control system and the non-spiking local interneurons modelled in this study.

(Schetzen, 1981). Whilst the cross correlation method is relatively computationally efficient, its accuracy relies on the properties of the input signal (Westwick et al., 1998). Parameter estimation accuracy is improved in the current study by estimating the parameters of a Volterra model using a Least Squares technique. Model complexity is significantly reduced using Laguerre basis functions (Marmarelis, 1993).

2 METHODS

2.1 Experimental Methods

Experiments were performed on 11 adult male and female desert locusts, *Schistocerca gregaria* (Forskål) at room temperature ($21.5 \pm 0.3^\circ\text{C}$, relative humidity $35.7 \pm 3.7\%$). Locusts were mounted ventral side uppermost in modelling clay. The apodeme of the FeCO (Figure 1) was exposed and attached by forceps to a shaker (Ling Altec 101). The FeCO was stimulated by applying a 27Hz low pass filtered GWN signal to the shaker (CG-742, NF Circuit Design Block). Intracellular recordings were made using a glass microelectrode which was inserted into the neuropillar processes of the interneurons in the metathoracic ganglia. The synaptic potentials were amplified using an Axoclamp 2A amplifier (Axon Instruments). Signals were stored on magnetic tape (digital format) using a PCM-DAT recorder (RD-101T, TEAC) operating at a sampling rate of 24KHz. The data were transferred to a computer using a PCMCIA interface card and QuikVu software (TEAC) and analysis was carried out using MATLAB (Mathworks, Cambridge UK).

2.2 Signal Processing: Theory

The second order Volterra series is written as

$$y(n) = h_0 + \sum_{\tau_1=0}^{L-1} h_1(\tau_1)u(n-\tau_1) + \sum_{\tau_1=0}^{L-1} \sum_{\tau_2=0}^{L-1} h_2(\tau_1, \tau_2)u(n-\tau_1)u(n-\tau_2) \quad (1)$$

where $u(n)$ is the input; h_0 , $h_1(\tau_1)$ and $h_2(\tau_1, \tau_2)$ are the zero, first and second order kernels and L is the number of lags. To facilitate parameter estimation, Wiener (1958) expressed the series in terms of a set of orthogonal functions:

$$y(n) = c_0 + \sum_{j_1=0}^{J-1} c_1(j_1)\mathcal{G}_{j_1}(n) + \sum_{j_1=0}^{J-1} \sum_{j_2=0}^{J-1} c_2(j_1, j_2)\mathcal{G}_{j_1}\mathcal{G}_{j_2} \quad (2)$$

where J is the number of functions in the decomposition and c are the coefficients of the Wiener kernels. The orthogonal basis functions $\mathcal{G}_j(n)$ are obtained using

$$\mathcal{G}_j(n) = \sum_{m=0}^L L_j(m)u(n-m) \quad (3)$$

$$L_j(n) = \alpha^{(n-j)^2/2} (1-\alpha)^{1/2} \sum_{k=0}^j (-1)^k \binom{n}{k} \binom{j}{k} \alpha^{j-k} (1-\alpha)^k \quad (4)$$

where L_j is the j^{th} order Laguerre function and α is the ‘‘decay parameter’’ controlling the damping of the Laguerre function. A lag of 100ms, $\alpha=0.5$ and J

= 6 were required to capture the dynamics of the interneurons. The outputs of the series can be calculated recursively using

$$\mathcal{G}_j(n) = \sqrt{\alpha}\mathcal{G}_j(n-1) + \sqrt{1-\alpha}\mathcal{G}_{j-1}(n) - \mathcal{G}_{j-1}(n-1) \quad (5)$$

with $\mathcal{G}_0(n)$ defined as:

$$\mathcal{G}_0(n) = \sqrt{\alpha}\mathcal{G}_0(n-1) + T\sqrt{1-\alpha}u(n) \quad (6)$$

where T is the sampling interval. The coefficients $c(j)$ (Equation 2) were calculated from the basis functions using the Least Squares method. The kernels were then obtained using

$$h_1(\tau_1) = \sum_{j=0}^{J-1} c_1(j) \cdot L_j(\tau_1) \quad (7)$$

$$h_2(\tau_1, \tau_2) = \sum_{j_1=0}^{J-1} \sum_{j_2=0}^{J-1} c_2(j_1, j_2) \cdot L_{j_1}(\tau_1) \cdot L_{j_2}(\tau_2)$$

2.3 Signal Processing: Application

Our analysis is based on that used in similar studies (Newland and Kondoh, 1997a and Vidal-Gadea et al., 2009) on different neurons. Models were fitted between the first 20 seconds of steady state adapted response (Figure 2B s3) and the corresponding samples of input signal. Validation was carried out using the last 4s of the recording by calculating the fitness function (Figure 2B s4).

$$fit = 100 \times (1 - \|y(t) - \hat{y}(t)\| / y(t)) \quad (8)$$

where $\|\bullet\|$ is the Euclidean norm (mean square value), $y(t)$ is the measured output and $\hat{y}(t)$ is the predicted output. In order to study patterns in the interneuron's responses kernels were clustered using the K-means algorithm (Hartigan and Wong, 1979). In order to focus on their sensitivity to position, velocity and acceleration (of primary interest to neurophysiologists), the gradient m of a linear function $y=mx+c$ fitted to the frequency response (magnitude only) calculated from the first order kernel between 2 and 15Hz was used as the feature for the k-means algorithm. The interpretation of the linear kernels is illustrated in Figure 3A and B. A position sensitive model has a kernel with a monophasic impulse response and flat frequency response. A velocity sensitive model has a biphasic kernel and a frequency response with a linear increase (20dB/decade, Figure 3B). A triphasic impulse response indicates an acceleration sensitive interneuron (40dB/decade, Figure 3B). Whilst the

first order kernel provides a means to describe the linear dynamic sensitivity of the interneurons, the majority of the interneurons have a nonlinear response, such as being primarily excitatory or inhibitory, or more sensitive to extension or flexion.

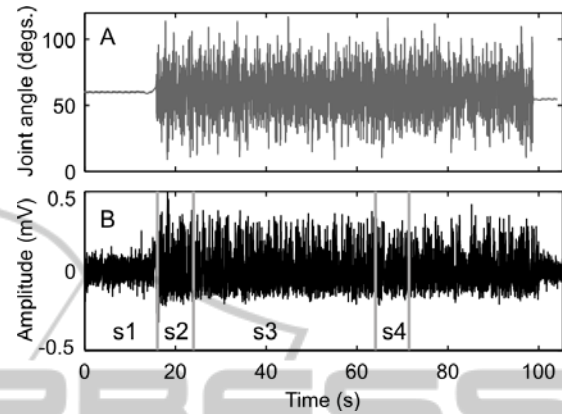


Figure 2: The band limited (0-27Hz) GWN input signal (A). The typical response of an interneuron (B) with spontaneous activity (s1), transient adapting (s2), steady state adapted response (s3) and validation section (s4).

This is illustrated in Figure 3C where the response of a model of an interneuron to sinusoidal movement of the tibia is shown. It should be noted that the linear model gives equal sensitivity to both flexion and extension, with inhibition during flexion and excitation during extension. The response of the nonlinear model, however, shows how this interneuron is only weakly inhibited during flexion of the leg but has a strong excitatory input when the leg is extended. The response to such a sinusoid will be used to illustrate the overall (linear and nonlinear) response of the neuron.

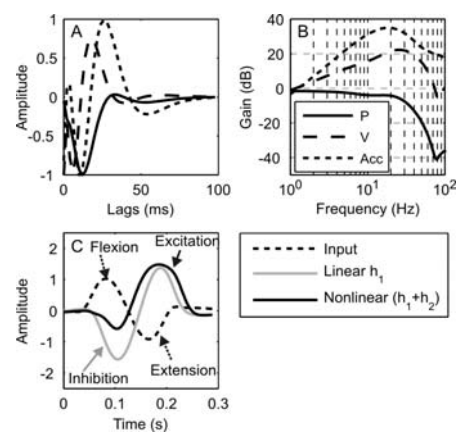


Figure 3: Illustrative 1st order kernels (A) and gain curves (B) showing position (P), velocity (V) and acceleration (Acc) sensitive models. Typical model response to a 6Hz sinusoidal input signal is shown in (C).

3 RESULTS

The linear dynamics of the 11 interneurons clustered into three groups according to the sensitivity of their responses to position, velocity or acceleration is shown in Figure 4. Clearly the idealized patterns in Figure 3B are not observed, but consistent patterns that indicate a range of sensitivities are evident in Figure 4D-F. The models of the interneurons in group 1 (Figure 4A, D) show a monophasic 1st order kernel. They have a flat frequency response in the range 0-20Hz and a decrease of -30dB/dec > 20 Hz indicating that interneurons in this group are primarily position sensitive. The models in groups 2 and 3 show a biphasic first-order kernel and a positive slope in their frequency response between 0-20Hz indicating that they mainly responded to the rate of change of movement of the stimulus. The two groups, however, differ in their response at lower frequencies. Whilst the models in group 2 show a constant slope in their frequency response (~20dB

/dec), in group 3 the responses are flatter at lower frequencies (0-3Hz) followed by a positive slope from ~3-20Hz (6dB/dec). No clearly acceleration sensitive interneuron models were found in this study. The second order kernels are shown in Figure 4G to I. The majority of interneurons in group 1 (Figure 4G) have a long positive excitatory peak along the diagonal line of their 2nd order kernel (Figure 4G) and a dominant negative peak in their first order kernel (Figure 4A).

Interneurons in the 2nd and 3rd groups have second-order kernels with a main inhibitory area (or excitatory depending on the direction of the dominant peak of the linear response) that are smaller compared to those in group 1 (Figure 4H, I). Also, these dominant areas peak closer to the origin reinforcing the hypothesis that these interneurons respond faster to stimulus changes.

An initial positive peak of the first order kernel

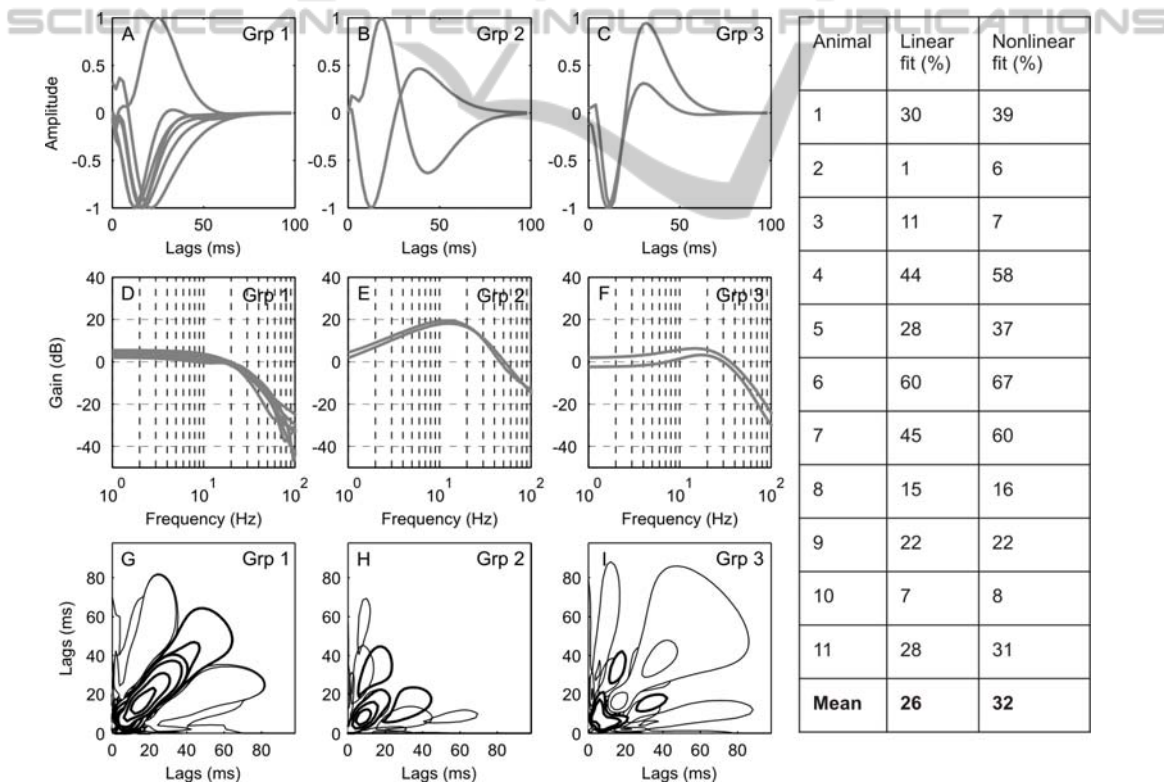


Figure 4: The impulse responses of the 1st order Volterra kernels of 11 interneurons separated into three groups using the K means clustering algorithm (A-C). Impulse responses have been normalised to unit peak value. The monophasic impulse response (A) and flat frequency response (D) indicate that group 1 is position sensitive. The biphasic impulse response (B and C) and positive slopes in the frequency response indicate that groups 2 and 3 are more velocity sensitive. The 2nd order Volterra kernels (G-I), positive values are represented by a thick line, negative values by a thin line. The kernels from group 1 are shown in G and have a dominant elongated peak along the diagonal. The kernels from groups 2 and 3 are shown in H and I respectively. They have dominant deflections closer to the origin than those in group 1 and are therefore faster to respond to stimulus changes. The predictive accuracy (fit) of the linear and nonlinear models is shown in the table.

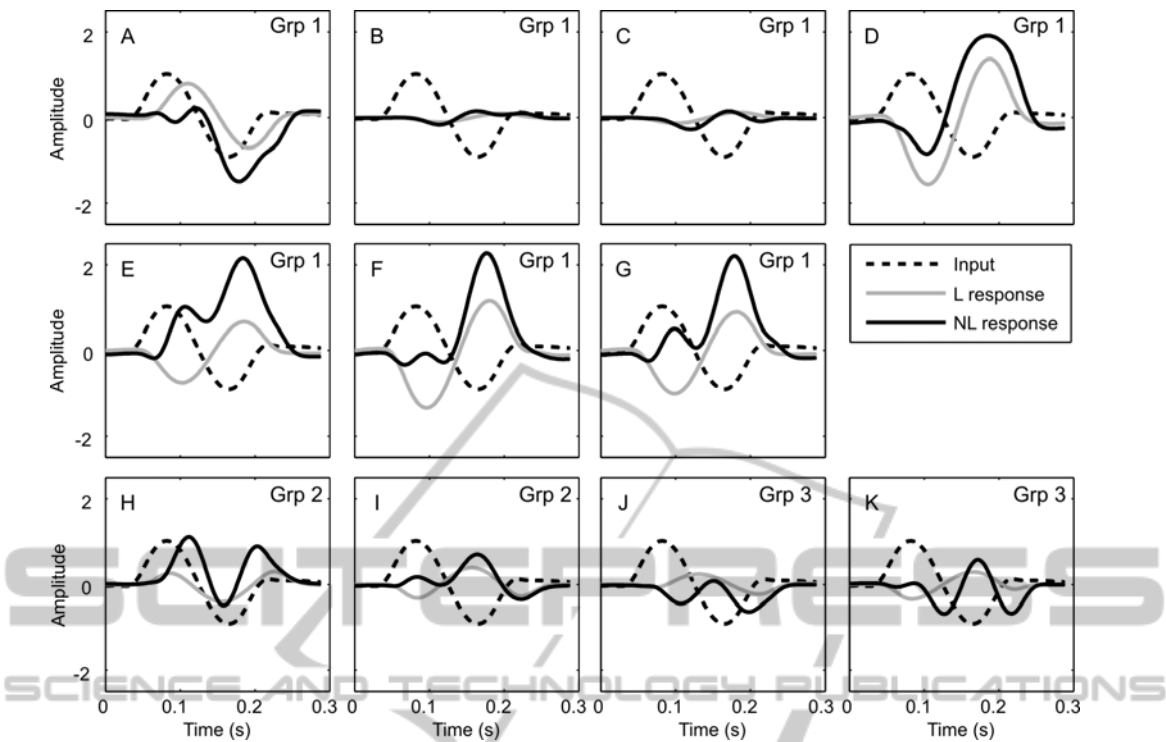


Figure 5: The response of the linear (L, first order kernel) of the nonlinear model and the nonlinear response of the model (NL, combined response of the first and second order kernels) to one period of a 6Hz sinusoidal input signal.

indicates that a neuron is excited during flexion and inhibited during extension. An initial negative phase would indicate excitation during extension and inhibition during flexion. In order to get the complete picture of the neurons characteristic, however, the response of the nonlinear model must be added. The effect that the first (linear) and the combined first and second order (nonlinear) kernels from group 1 have on a 6Hz sinusoidal input is shown in Figure 5A-G. The linear component gives equal sensitivity to both flexion and extension with inhibition during flexion and excitation during extension in 6 out of 7 cases (Figure 5A is the exception). When combined (linear + nonlinear) responses are taken, however, the interneurons in Figure 5E, F and G no longer respond with strong inhibition during flexion, and those in Figure 5D, E, F and G are strongly excitatory during extension. There would appear to be less consistency in the responses of the non-spiking local interneurons in groups 2 and 3.

The performance of the Volterra models was evaluated by comparing the predicted response given by the models and the response (synaptic potential) recorded from the non-spiking local interneurons. Model fit was calculated using validation data. The fit of the linear (first order) and

the nonlinear (first + second order) models for the 11 interneurons is shown in Figure 4. In some cases (especially for animals 2, 3 and 10) the fit is very poor, and here kernel estimates are probably not very reliable. It should be noted that these cases also show the lowest amplitude responses to sinusoidal input (Figure 5B,C and J). In the remaining cases, the NL model fit was better (or equal for animal 9) than for the linear model. The average model fit was 26% and 32% for linear and non-linear models, respectively, and thus rather poor. The recordings prior to the start of stimulation when the input is held constant (Figure 2B s1), however, show high levels of spontaneous activity.

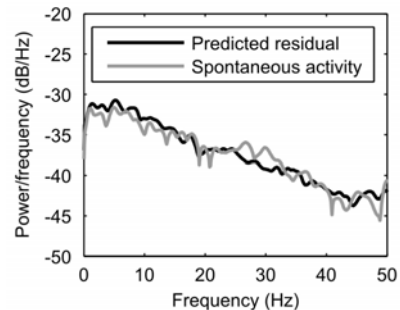


Figure 6: The power spectrum of the mean residual signal is compared with that of the spontaneous activity.

Analysis has shown that the spectrum of the residual signal (the difference between the model and the measured output signal) is very similar (Figure 6). This suggests that model fit is probably as good as might reasonably be expected, given that the model cannot predict spontaneous background activity (Marmarelis, 2004).

4 DISCUSSION

Previous work which characterised the dynamics of sensory, motor and spiking local interneurons in the locusts hind leg reflex control system has been extended to a group of non-spiking local interneurons. The models of the interneurons were classified into three groups using the k-means algorithm and the frequency response of the first order kernels. We found that 7 out of the 11 interneurons might be considered position sensitive; two were position/velocity sensitive and two were strongly velocity sensitive. The position sensitive interneurons were strongly sensitive to extension, with all but one having an excitatory input with extension. This was contrary to the results found by Vidal-Gadea et al. (2009) for the spiking local interneurons where extension caused inhibition. In general, the position/velocity and velocity sensitive interneurons received an excitatory input with movement of the tibia into extension. As was found by Vidal-Gadea et al. (2009) the non-spiking local interneurons were either sensitive to extension or to both extension and flexion. The current study found no evidence of a non-spiking local interneuron which responded solely to flexion.

While the members of the groups identified show common features, there are a range of responses included in each cluster. Further experimental work and analysis may identify additional clusters, or indicate that responses are graded rather than clustered, or can be separated into distinct clusters based on higher order features. The approach taken, using Gaussian white noise stimulation and system identification, has provided new insights into the operation of the neuronal network controlling reflex movements in the hind leg of the locust. In the continuation of this study we will probe the significance of these features during functional movements.

ACKNOWLEDGEMENTS

The authors would like to thank the BBSRC and the

EPSRC for their financial support.

REFERENCES

- Bar-Cohen, Y., 2006. Biomimetics-using nature to inspire human innovation. *Bioinsp. Biomim.* 1:1-12.
- Burrows, M., 1996. *The neurobiology of the insect brain.* Oxford University Press. Oxford, 1st edition.
- Hartigan, J. A., and Wong, M. A., 1979. A k-means clustering algorithm. *Appl Stats*, 28:100-108.
- Laurent, G. and Burrows, M., 1989. Intersegmental interneurons can control the gain of reflexes in adjacent segments of the locust by their action on non-spiking local interneurons. *J. Neuroscience*, 9(9):3030-3039.
- Marmarelis, V. Z., 2004. Nonlinear dynamic modelling of physiological systems. *Wiley IEEE press, first edition.*
- Marmarelis, V. Z., 1993. Identification of nonlinear biological systems using Laguerre expansions of kernels. *Ann Biomed Eng*, 21(6):573-80.
- Newland, P. L., and Kondoh, Y., 1997a. Dynamics of neurons controlling movements of a locust hind leg II. Flexor tibiae motor neurons. *J. Neurophysiol*, 77:1731-1746.
- Newland, P. L., and Kondoh, Y., 1997b. Dynamics of neurons controlling movements of a locust hind leg III. Extensor tibiae motor neurons. *J. Neurophysiol*, 77:3297-3310.
- Shetzen, M., 1981. Nonlinear system modelling based on the wiener theory. *Proc IEEE*, 69(12): 1557-1573.
- Vidal-Gadea, A .G., Jing, X. J., Simpson, D., Dewhirst, O. P., Kondoh, Y., Allen, R. and Newland, P. L., 2009. Coding characteristics of spiking local interneurons during imposed limb movements in the locust. *J. Neurophysiol*, 103:603-615.
- Wiener, N., 1958. Nonlinear problems in random theory. *The MIT press, first edition.*
- Westwick, D. T., Suki, B. and Lutchen, K. R., 1998. Sensitivity analysis of kernel estimates: implications in nonlinear physiological system identification. *Annals of Biomedical Engineering*, 26:488-501.