Wavelets of Excitability in Sensory Neurons

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Hasty, Jeff, J. J. Collins, Kurt Wiesenfeld, and Peter Grigg. Wavelets of excitability in sensory neurons. *J Neurophysiol* 86: 2097–2101, 2001. We have investigated variations in the excitability of mammalian cutaneous mechanoreceptor neurons. We focused on the phase dynamics of an action potential relative to a periodic stimulus, showing that the excitability of these sensory neurons has interesting nonstationary oscillations. Using a wavelet analysis, these oscillations were characterized through the depiction of their period as a function of time. It was determined that the induced oscillations are weakly dependent on the stimulus frequency, and that lower temperatures significantly reduce the frequency of the phase response. Our results reveal novel excitability properties in sensory neurons, and, more generally, could prove significant in the deduction of mechanistic attributes underlying the nonstationary excitability in neuronal systems. Since peripheral neurons feed information to the CNS, variable responses observed in higher regions may be generated in part at the site of sensory detection.

INTRODUCTION

When certain neurons are subjected to repeated presentations of an identical stimulus, the action potentials encoding the stimulus information have variable responses between presentations (Arieli et al. 1996; Britten et al. 1993; Dean 1981; de Ruyter van Steveninck et al. 1997; Hunter et al. 1998; Mainen and Sejnowski 1995; Rieke et al. 1997; Rose et al. 1969; Schiller et al. 1976; Shadlen and Newsome 1998; Snowden et al. 1992). While such variability is thought to be important in the processing of information by neurons in the CNS (de Ruyter van Steveninck et al. 1997; Mainen and Sejnowski 1995; Rieke et al. 1997; Shadlen and Newsome 1998), its potential role in peripheral neurons has not been appreciated (Koltzenburg et al. 1997; Merzenich and Harrington 1969). One central issue is whether such variability is utilized in the transfer of information (de Ruyter van Steveninck et al. 1997; Gerstner et al. 1996; Pei et al. 1996; Rieke et al. 1997) or whether it is merely a stochastic effect attributable to some underlying process. In other words, does a neuronal system reliably encode due to or in spite of such variability? A key to the resolution of this question is the determination of the source of the variability, as well as the deduction of an underlying mechanism for its generation. Here we report that when mechanoreceptors, recorded in isolated skin, are stimulated with mechanical sinusoids, the timing of responses in relation to the stimulus exhibits nonstationary, wavelike variability.

METHODS

Rapidly adapting (RA) mechanoreceptor neurons were recorded in a preparation of skin and nerve that was isolated from the hindlimb of adult rats, and studied in vitro. Single guard hair afferents were activated by moving hairs using periodic (sinusoidal) stimuli. We were interested in the phase relationship between the stimuli and the responses of individual neurons.

Preparation

Adult rats were anesthetized with pentobarbital sodium (Nembutal), administered intraperitoneally. The experimental preparation was an isolated sample of skin and nerve, taken from the inner thigh, and studied in vitro. The fur along the thigh was clipped to a length of approximately 2 mm. Then the skin sample, approximately 14 mm square, was excised along with its sensory innervation, a branch of the saphenous nerve. The sample was removed to an apparatus where it was maintained in a bath of artificial interstitial fluid kept at room temperature (20°C). The skin was supported from underneath by a platinum mesh. Thus the under side of the skin was maintained in the bath while the upper surface was dry. The cutaneous nerve was pulled into a small oil-filled plastic chamber for recording. The nerve was dissected into small fibers that were placed on a fine gold wire electrode for recording. The indifferent electrode was placed in the bath. Signals were amplified with a PARC 118 amplifier and filtered with a Riverbend Electronics Learning Filter. Guard hair afferents were sought by gently stroking the clipped hairs while recording from fibers. Recordings were often made from filaments containing several active neurons whose active hairs in the skin were far enough apart to allow them to be stimulated independently of each other.

Stimulation

When a suitable afferent was identified, the appropriate hair was actuated with a mechanical stimulator that consisted of a Cambridge Technology 300B lever system. This is a DC servomotor that rotates a shaft through controlled angular displacements. The motor actuated a 60-mm-long cantilever whose tip was brought into contact with the hair. The displacements that we used were small (<0.5 mm) so that the motion of the tip was essentially linear. The stimulator had a mechanical bandwidth of 0 to approximately 120 Hz. Stimuli were displacement-controlled sinusoids. Stimulus waveforms, along with a synchronization pulse, were generated with a Wavetek waveform generator. The stimulus amplitude was adjusted so as to elicit one spike per cycle and was kept constant throughout experimental runs. The phase of each action potential was measured in relation to the

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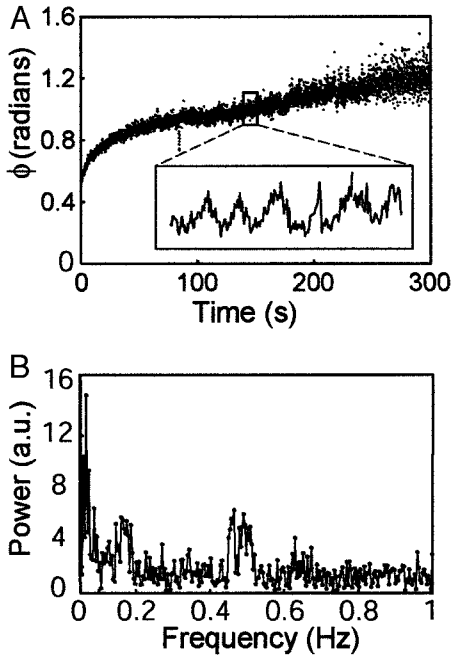

FIG. 1. Typical phase data from a mechanoreceptor. A trigger pulse is generated at the beginning of each actuation cycle, so that data collection periods contain the times of the trigger pulse and any subsequent action potentials. The phase, which represents the excitability of the mechanoreceptor response, is defined as $\phi_i = (s_i - z_i)2\pi f$, where s_i and z_i are the spike and trigger times ($s_i > z_i$), and f is the frequency of actuation. *A*: data for a 15-Hz stimulus frequency. During the entire run, the phase is bounded by $\pi/2$ as the mechanoreceptor is responding to the inward motion of the indenter. The phase slowly increases, indicating a slow decrease in excitability, which is likely due to adaptation. *Inset*: a section of the data is displayed at higher resolution, elucidating oscillations in the phase data. *B*: the power spectrum of the phase data in *A*. There are 2 distinct maxima at 0.18 and 0.50 Hz, along with a strong peak at very low frequency.

stimulus waveform (Del Prete and Grigg 1998; Hunter et al. 1998; Neiman et al. 1999; Read and Siegal 1996).

Data collection

Both mechanical and neuronal data were acquired using a Cambridge Electronic Design micro 1401 data acquisition system. We collected four analog signals. Two signals represented actuator position and the force applied by the actuator. In addition, we acquired the synchronization pulse that denoted the start of each stimulus cycle and the amplified neural recording. The data acquisition rate for the position and force signals was 500 Hz, and the acquisition rate for the synchronization pulse and neuronal spikes was 500 kHz.

Data analysis

The phase of the *i*th spike relative to the synchronization pulse is defined as

$$\phi_i = (s_i - z_i)2\pi f \tag{1}$$

where s_i and z_i are the spike and syncronization times ($s_i > z_i$), and f is the frequency of indentation. Defined in this way, the phase has units of radians and is a measure of the threshold or *excitability* of the mechanoreceptor system, i.e., it represents the magnitude of indentation required for action potential generation.

The continuous wavelet transform (CWT) of a time series $\phi(t)$ of length T is given by

$$\Phi(s,\tau) = \frac{1}{\sqrt{s}} \int_0^T \phi(t)\psi\left(\frac{t-\tau}{s}\right)dt \tag{2}$$

It is a measure of the degree to which the function $\phi(t)$ is periodic, with period proportional to the scale parameter s, and at a particular time denoted by τ. The function $\psi(t)$ is typically of Gaussian form (with compact support), and we utilized the function

$$\psi(t) = \frac{1}{\sqrt{2\pi\sigma^3}} e^{\frac{-t^2}{2\sigma^2}} \left(\frac{t^2}{\sigma^2} - 1\right) \tag{3}$$

The scale parameter s in *Eq. 2* is proportional to the width of the Gaussian and is, by analogy with the fast Fourier transform, a measure of the period of the signal. Operationally, the meaning of the wavelet transform can be illustrated as follows. Since the CWT is a function of both time and scale, consider fixing the width of the Gaussian in *Eq. 2* by letting $s = 1$ and superimposing it along with the time series at time $t = 0$. Then the CWT for $\tau = 0$ and $s = 1$ is obtained by integrating *Eq. 2* over time, and physically, this corresponds to the degree to which the Gaussian function and the time series overlay; i.e., the value of their convolution (the prefactor $1/\sqrt{s}$ is necessary for normalization purposes so that the transformed signal will have the same "energy" for each s). The Gaussian (at scale $s = 1$) is then translated in time to the location $t = \tau$, and the integral is recomputed to obtain the CWT at $t = \tau$ and $s = 1$ in the time-frequency plane. This procedure is repeated until the Gaussian has been translated to the end of the time series, resulting in the calculation of the CWT for fixed s, i.e., $\Phi(\tau, s = 1)$. Then, s is increased by a small value and the entire procedure is repeated. Note that this is a continuous transform, and therefore both τ and s must be incremented continuously. However, since the CWT is obtained numerically, both parameters are increased

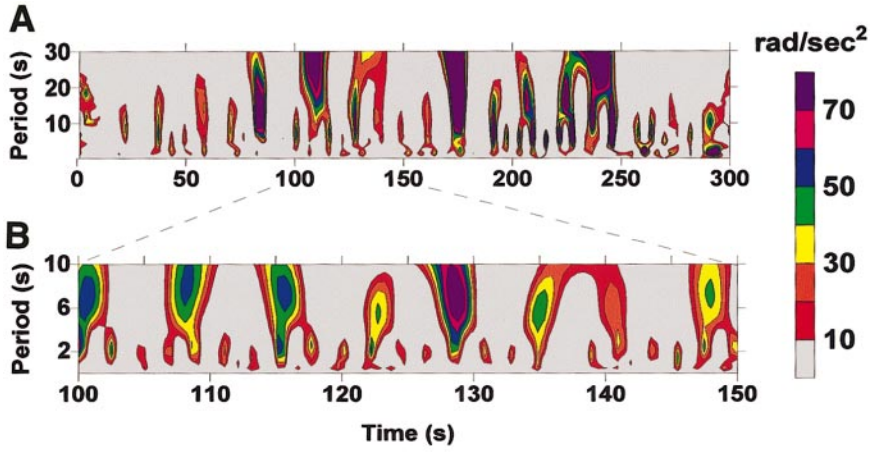

FIG. 2. Time-period diagrams obtained from a continuous wavelet analysis of the phase data in Fig. 1*A*. *A*: there are oscillations of several different periods, and, at times, there is significant frequency drift. The frequency drift is quite noticeable from 40 to 100 s and again from 150 to 200 s. The regions from 100 to 140 s and 200 to 250 s indicate coexisting waves of several different periods. *B*: a blowup of the time scale reveals more precisely dominant periods of approximately 2 and 6–8 s, respectively. These correspond to the peaks in the power spectrum of Fig. 1*B*.

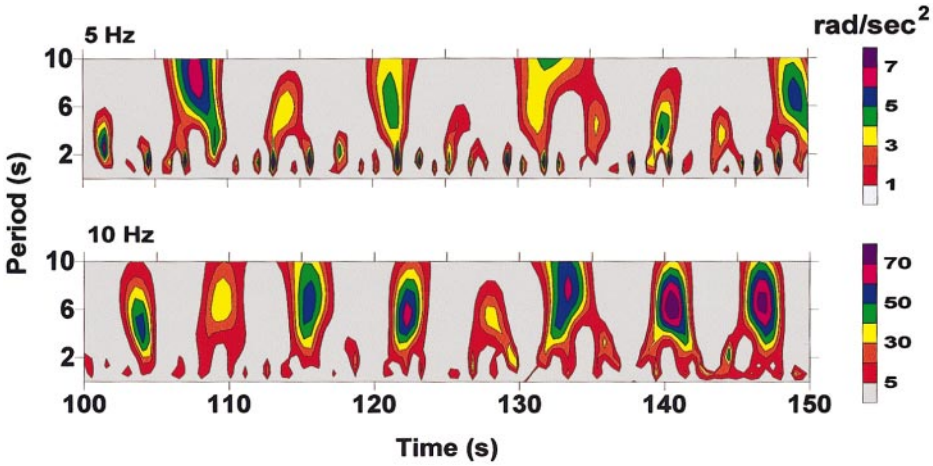

FIG. 3. Representative time-period diagrams for actuation frequencies of 5 and 10 Hz. The data sets were obtained under identical experimental conditions as the 15-Hz data used in Fig. 2, enabling comparison between Fig. 3 and Fig. 2*B*. In all 3 data sets, we observe periodicities of approximately 2 and 6 s, with the only major difference being the intermittency at 5 Hz. Compared with the 10- and 15-Hz data, the 5-Hz response shows significantly fewer periodic regions at low frequency.

by a sufficiently small step size, which corresponds to sampling the time-scale plane.

RESULTS

Data were collected from 14 neurons, and the results presented below are representative of the qualitative features observed in all of the neurons. Figure 1 shows the phase behavior of a typical neuron. The response shows a gradual, time-dependent increase in phase, a signature of the adaptation process. In addition, the variability of the response increases with time. We find that there is significant structure to the variability, which is seen to exhibit waves (Fig. 1, *inset*). To quantify the properties of the waves, Fourier analysis was utilized. The power spectrum of an entire data run of 300-s exhibits peaks at 0.18 and 0.50 Hz (Fig. 1*B*). Since the time series appears to be nonstationary, we investigated the temporal properties associated with the oscillations by partitioning the data into four sequential segments and analyzing each independently. We found significant qualitative differences in the power spectra generated from quarter segments in the data sets. For example, the power spectrum obtained using the first quarter of the time series in Fig. 1*A* does not clearly exhibit the 0.18- and 0.50-Hz maxima seen in Fig. 1*B*. However, analysis of the second and third quarter segments reveals significant power at these frequencies and, in addition, the birth of substantial peaks at low frequencies. Analysis of the fourth quarter segment exhibits maxima at 0.18 and 0.50 Hz, but relatively little low-frequency content.

Wavelet analysis is well-suited for describing a nonstationary time series such as the phase data in Fig. 1*A*. While the classical Fourier technique yields information only in the frequency domain, the wavelet approach quantifies periodicity in both the time and frequency domains and is thus capable of elucidating the nature of the time-dependent oscillatory regions noted above (see the caption of Fig. 2 for the definition of the continuous wavelet transform). Figure 2 shows the fluctuations in frequency and power during the run depicted in Fig. 1. We observe phase oscillations of several different frequencies and find that, at times, these waves exhibit a significant frequency drift. Such a variable response is of interest for two primary reasons. First, since the current opinion on population coding is that the function of a population of neurons is to produce responses in phase with a common stimulus, variability in the responses of single afferents should adversely affect this function. Further work is thus needed to determine what effect the waves have on the degree of synchronous behavior observed in groups of cells with a common stimulus. Second, the waves may provide insight into peripheral mechanisms involved in mechanoreceptor activation.

It is perhaps natural to conjecture that the adaptation process is somehow responsible for the oscillatory behavior. For example, in vertebrate auditory hair cells, the firing of an action potential induces a transport of calcium cations into the neuronal cell (Lumpkin and Hudspeth 1998; Lumpkin et al. 1997). These cations then act to increase the firing threshold through their interaction with the ion channel gating process. Thus for a periodic stimulus, each drive period could lead to a buildup of intracellular calcium, provided passive mechanisms responsible for export are unable to keep pace with the drive. Oscillations could then occur if an active export process, such as the transport of calcium out of the cell by protein pumps (Lumpkin and Hudspeth 1998), was triggered after a number of actuation cycles. If such an adaptation mechanism is indeed related to the phase periodicity, then the wavelet frequency response should depend on the actuation frequency. In Fig. 3, we plot representative time-period diagrams for actuation frequencies of 5 and 10 Hz. We observe variable periodicities of approximately 2 and 6 s for multiple stimulus frequencies. Since the stimulus represents the only periodic signal available to the neuron, it is of interest that the frequency of the phase response does not appear to be influenced by the drive frequency.

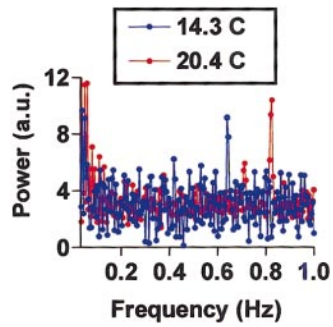

FIG. 4. The power spectrum of 2 sets of representative data obtained at 2 temperatures for an actuation frequency of 5 Hz. There is a temperature-induced shift from 0.81 Hz at 20.4°C to 0.62 Hz at 14.3°C. Additionally, at low frequency, there appears to be a 2nd shift downward, although the power spectrum in this regime renders it difficult to characterize this change quantitatively.

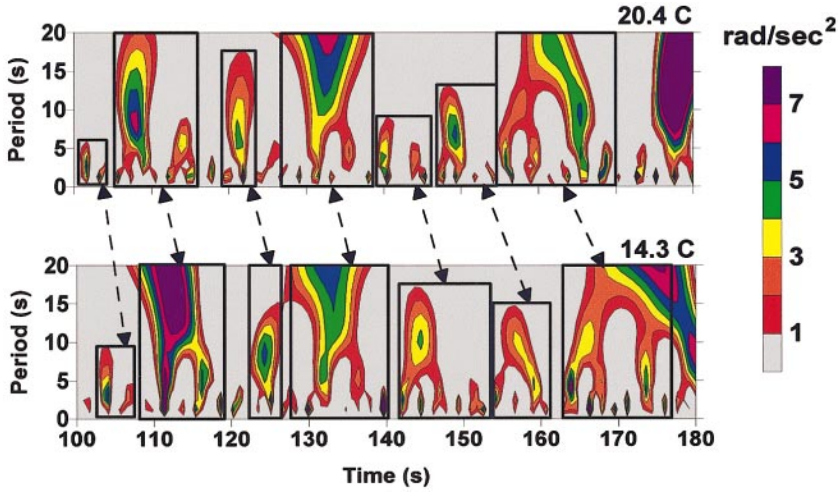

FIG. 5. Time-period diagram obtained from a wavelet analysis of the data used for Fig. 4. In both sets, there are significant oscillations at many different frequencies. Both sets have similar structure, and this has been highlighted with rectangles drawn around what appear to be complementary regions. By focusing on these regions, note that, relative to the data at 20.4° (*top*), the 14.3° (*bottom*) data have increased oscillatory periods at nearly all frequencies. The time delay in the occurrence of the regions in the *bottom diagram* is attributable to variations between data runs and is not a property of the temperature difference.

If the phase oscillations are linked to some metabolic process, then one might anticipate a decrease in the frequency of the waves as the sensory system is cooled. In Fig. 4, we plot the power spectrum of two sets of representative phase data obtained at two temperatures for an actuation frequency of 5 Hz. As the temperature is decreased, we observe a significant decrease of approximately 20% in the frequency of the phase response. In Fig. 5, the time-period diagram demonstrates that the oscillatory response is slowed throughout an entire experimental run.

DISCUSSION

The characterization of the waves of excitability reported here raises several significant issues. Of central importance is our finding that structured variations occur at the periphery of the sensory system. While variations in neuronal responses within the CNS have been the subject of much discussion (Arieli et al. 1996; Britten et al. 1993; Dean 1981; de Ruyter van Steveninck et al. 1997; Gerstner et al. 1996; Mainen and Sejnowski 1995; Pei et al. 1996; Rieke et al. 1997; Rose et al. 1969; Schiller et al. 1976; Shadlen and Newsome 1998; Snowden et al. 1992), it has been assumed that periphery responses that feed the central system are regular. Additionally, since the experiment entailed the stimulation of individual hairs and therefore single neurons, the oscillatory response cannot be attributed to a network of neurons. This suggests that the transduction mechanism in these sensory neurons is more complex than is typically appreciated. The fact that the waves appear to be independent of the stimulus frequency is remarkable, given that the only obvious time scales present are those set by the periodic drive and adaptation process. Perhaps the cells possess an underlying internal clock that operates independently of the drive. From an information-transfer point of view, the observed temperature effects could imply that the excitability waves are conveying useful information regarding the environment. It is also conceivable that the oscillations could lead to fractal statistics similar to those observed in the neuronal spike trains of other preparations (Ivanov et al. 1999; Teich 1989; Teich et al. 1997). Importantly, models incorporating correlations in the underlying ion channel kinetics have been shown to generate nonstationary statistics (Lowen et al. 1999), and such models might provide insight into the underlying mechanism for the excitability waves. Along these lines, further studies are needed to determine whether the phenomenon is a necessary component of the encoding apparatus or whether the system simply tolerates its existence.

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