Periodic Microstimulation of Single Mechanoreceptive Afferents Produces Frequency-Following Responses in Human EEG

Edward F. Kelly, Mats Trulsson, and Stephen E. Folger

Department of Diagnostic Sciences and Dental Research Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; Department of Physiology, Umeå University, S-901 87 Umeå, Sweden; and Department of Biomedical Engineering and Dental Research Center, The University of North Carolina, Chapel Hill, North Carolina 27599

Kelly, Edward F., Mats Trulsson, and Stephen E. Folger. Periodic microstimulation of single mechanoreceptive afferents produces frequency-following responses in human EEG. J. Neurophysiol. 77: 137–144, 1997. Dense multichannel recordings of scalp electroencephalogram were obtained in the vicinity of primary somatosensory cortex, time-locked to repetitive train microstimulation of single, physiologically characterized skin mechanoreceptive afferents in the median nerve of a single human subject. Frequency-domain analysis of cross-trial averages for fast-adapting or frequency-following responses (FFRs) may provide a type one and slowly adapting type one afferents revealed prominent, topographically organized “driving” responses in the electroencephalogram at the frequency of stimulation, which vanished under various statistical and experimental control conditions. The responses also exhibited systematic declines in amplitude both across and within trials, and orderly changes in scalp topography as a function of the location of afferents’ receptive fields on the hand. The observed response properties are tentatively explained in terms of characteristics of the pattern of afferent drive impressed on the cortex by microstimulation.

Introduction

Microneurography and microstimulation are well-established techniques with many useful applications in human sensory neurophysiology (Ochoa and Torebjörk 1983; Torebjörk et al. 1987; Trulsson and Johansson 1996; Vallbo and Hagbarth 1968; Vallbo and Johansson 1984; Vallbo et al. 1979, 1984). Among the many significant results produced to date, one of the most remarkable is the demonstration that a brief train of electrical stimuli activating a single skin mechanoreceptive afferent (MRA) can produce a distinct “bright” sensation that is referred to a skin region accurately reflecting the location, size, shape, and orientation of that afferent’s receptive field (RF) and that displays sensory qualities characteristic for afferents of that type. For example, with pulse trains on the order of 1 s in length and stimulus frequencies in the 20- to 40-Hz range, stimulation of fast-adapting type one (FA I) units typically yields sharply localized sensations of flutter, whereas stimulation of slowly adapting type one (SA I) units most commonly produces slightly more diffuse sensations of pressure or inward pulling; correspondingly, changes in stimulus frequency alter the pitch of flutter sensations and the intensity of pressure/pulling (Ochoa and Torebjörk 1983; Torebjörk et al. 1987; Vallbo et al. 1984).

The question arises as to what form(s) of cortical activity may accompany these sensory experiences. The neurophysiological analysis of flutter/vibration has traditionally coupled single-unit studies in animals with parallel psychophysical studies in monkeys and humans. It has been shown, for example, that mechanical flutter stimuli are capable of entraining the activity of single neurons at all levels up to and including primary somatosensory cortex (SI), and plausible hypotheses have been advanced as to the manner in which temporal and spatial properties of these cortical “driving” or frequency-following responses (FFRs) may provide a neurophysiological substrate for well-studied aspects of human vibrotactile psychophysics (Bolanowski et al. 1988; LaMotte and Mountcastle 1975; Mountcastle et al. 1990; Talbot et al. 1968).

Our laboratory is using high-resolution electroencephalographic (EEG) techniques (Wilkswo et al. 1993) to link the neurophysiological and psychophysical levels of observation in humans by combining vibrotactile stimulation with extraction of FFRs from scalp-recorded EEG (McLaughlin and Kelly 1993). These FFRs are small compared with the overall amplitude of background EEG, but they are much larger relative to background amplitudes at the driving frequencies and thus lend themselves to measurement with the use of frequency-domain techniques (Regan 1989). The experiments described below were undertaken to determine whether these techniques are capable of detecting EEG driving responses produced by train microstimulation of single MRAs in human skin.

Methods

Seven experimental sessions were conducted over a 5-mo period, all with a single volunteer subject, one of the authors (E. F. Kelly). All sessions consisted of repetitions of a basic sequence of three steps in which we attempted 1) to isolate and characterize functionally a single skin MRA; 2) to establish that we could selectively microstimulate that same afferent; and 3) to make high-density scalp recordings of the EEG activity associated with repeated delivery of the stimulus. Only for a small subset of the afferents we encountered could all three steps be successfully completed. All afferents successively isolated during experimental sessions lasting 3–4 h were studied in this manner.

Microneurography and microstimulation methods

The subject sat comfortably in a dental chair, the right forearm resting in a vacuum cast with the hand in a half-prone position. Impulses in single MRAs innervating the glabrous skin of the right
hand were recorded from the median nerve with coated tungsten needle electrodes inserted percutaneously 3–5 cm proximal to the wrist (Torebjörk et al. 1987; Vallbo and Hagbarth 1968). The electrodes had a shaft diameter of 0.2 mm, a tip diameter of ~5 μM, and an exposed tip length of 10–15 μM, with impedance 500–800 kΩ measured in situ at 1 kHz. When the nerve bundle had been impaled, the position of the electrode was carefully adjusted until single-afferent impulses from an MRA could be discriminated. The electrode was then left undisturbed in this recording position, supported only by the surrounding tissue. The single-unit recording was accepted if the spike amplitude was at least twice the background activity and the unit could be separated by an on-line triggering device (Edin et al. 1988) implemented in the sampling/analysis system (SC/ZOOM, Department of Physiology, Örebro University, Örebro, Sweden).

The extent of the RF was defined with von Frey hairs at 4 times the threshold of the unit and the RF boundary was marked on the skin with a fine-point ink pen (Johansson and Vallbo 1980). Units were classified as fast-adapting type one or two (FA I, FA II) or slowly adapting type one or two (SA I, SA II), in accordance with standard criteria described previously (Johansson and Vallbo 1979; Knibestol and Vallbo 1970; Vallbo and Johansson 1984). The electrode was then connected to a constant-current stimulator delivering positive square-wave electrical pulses 0.2 ms in duration. (The stimulation device and technique has been described in detail by Vallbo et al. 1984.) Pulse trains at 28 or 33 Hz lasting 1.0 s were triggered manually by the experimenter while stimulus amplitude was slowly increased from zero to the liminal point (threshold) for conscious detection. The subject reported the location of the sensation on the skin surface with the aid of a schematic drawing of the hand, where 21 separate skin regions had been labeled. After giving the code number for the appropriate region, the subject described the location in more detail. The final localization was achieved with a procedure that included alternating electrical stimulation and light mechanical indentation with a glass rod while the subject reported whether or not the two stimuli were felt at the same spot. Because the RF of the afferent had already been marked on the skin, it was immediately obvious to the experimenter whether the RF of the afferent and the perceptive field of the sensation were colocalized. If a matching sensation was reported the subject was asked to describe its shape, size, and quality. In early sessions this description was sometimes elicted with the subject blind as to the physiological classification of the unit. However, the sensory qualities associated with microstimulation of single units proved so clear and compelling as to preclude significant issues of subjective bias in the reports, and this experimental precaution was subsequently relaxed. If a matching sensation was not present the afferent was dropped.

During the subsequent EEG recording, the stimulus strength was initially set just above the threshold for a sensation (110–120% of the threshold value) to minimize the risk of exciting additional fibers. The stimulus intensity often had to be increased progressively during a run to continue to evoke the original sensation, probably because of slight movements or polarization of the electrode (Vallbo et al. 1984). However, the stimulus strength never exceeded 10 μA.

**EEG methods**

Combined microstimulation and EEG recording was carried out entirely under computer control (486 PC). One run was obtained for each afferent consisting of a sequence of 1-s microstimulus trains (trials). The pulse frequency within the trains was initially set at 28 Hz, but in later sessions this was changed to 33 Hz to escape residual background EEG activity in the 28- to 32-Hz range. EEG recordings were made concurrently with each microstimulus train and time-locked to its onset, including in later sessions a prestimulus period of 0.5 s. In all sessions after the first, we attempted to deliver 200 trials per run with a base interstimulus interval of 1.5 s and a superimposed random jitter of ±0.2 s to destroy synchronization with the ongoing EEG. Runs were terminated if the sensation disappeared, or if the attempt to restore it by increasing stimulus current resulted in recruitment of an additional afferent as indicated by the sudden appearance of a second distinct sensation localized elsewhere on the skin.

Scalp potential fields were sampled from a 5 × 5 array of custom-built high-impedance electrodes (2 cm center-to-center spacing) mounted in a rigid, snugly fitting acrylic helmet. This system minimizes coupling to external electromagnetic fields and provides for rapid and reproducible positioning of electrodes without the need for traditional skin preparation (see Dunseath and Kelly 1995 for details). The center electrode was located at a position known from previous cadaver studies to lie just anterior to the left SI hand area in most subjects (the C3 position of the 10/20 system) (Jasper 1958), and in a separate study this localization was specifically confirmed for the present subject by visualizing the cortex directly through his helmet with the use of magnetic resonance imaging techniques (Boyle and Kelly 1994). The right earlobe served as common reference for the recordings, with a forehead ground. All EEG channels and the stimulus waveform were sampled at 512 Hz and written to disk for off-line analysis.

For each run, the raw physiological records of all trials were visually examined, and trials containing gross artifacts on any channel (usually caused by large eyeblinks or head movements) were eliminated from the data sets. Attrition was typically 5–10%. Surviving trials were subjected to zero-phase digital filtering (Butterworth; passband 25–40 Hz for 28-Hz stim, 27–56 Hz for 33-Hz stim), downsampled to 128 Hz, and averaged in the time domain.

Driving responses were extracted from the averaged data in the frequency domain with the use of both conventional fast Fourier transform (FFT) and several “modern” or model-based autoregressive (AR) spectral analysis techniques. The AR approach first uses least-squares methods to fit a linear statistical model that best predicts the successive values of the time series from a specified small number (the model order) of immediately previous (and/or future) values. The spectrum implied by the fitted model is then normally calculated via a Fourier transform of the model coefficients. The return for the added computational burden associated with techniques of this type is that they can outperform the FFT in capacity to produce smooth, high-resolution spectra from short data records, provided the signal-to-noise ratio is adequate (Marple 1987). We used three variants on this basic theme: 1) the single-channel modified covariance AR algorithm of Marple (1987); 2) a related single-channel technique attributed to Franaszczuk and Blinowska (1985), which conceptualizes the observed time series as the summed oscillatory impulse responses of a bank of parallel filters, and directly calculates the frequency, amplitude, and damping parameters of the filters as functions of the roots of a polynomial formed from the AR model coefficients; and 3) a multichannel (matrix) extension of the basic AR model attributed to Franaszczuk et al. (1985), which predicts the observations in each channel from earlier values of all the channels simultaneously. By applying multiple spectral analysis techniques involving diverse computational formalisms, we intended to assure ourselves that observed driving responses were not computational artifacts associated with the properties of any single technique, in particular the more widely familiar FFT, which we emphasize in presenting our results.

**RESULTS**

Altogether, EEG recordings were attempted in conjunction with microstimulation of 14 afferents, and completed for half of these. Our presentation emphasizes results from...
TABLE 1. Data obtained from four typical mechanoreceptive afferents during microneurography and microstimulation

<table>
<thead>
<tr>
<th>Unit type</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Frey Threshold, mN</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Receptive field shape</td>
<td>Circular</td>
<td>Oval</td>
<td>Oval</td>
<td>Oval</td>
</tr>
<tr>
<td>Receptive field diameter, mm</td>
<td>4</td>
<td>3 × 4</td>
<td>4 × 5</td>
<td>3 × 5</td>
</tr>
<tr>
<td>Perceived field shape</td>
<td>Circular</td>
<td>Oval</td>
<td>Oval</td>
<td>Oval</td>
</tr>
<tr>
<td>Perceived field diameter, mm</td>
<td>4</td>
<td>3 × 4</td>
<td>5 × 6</td>
<td>3 × 6</td>
</tr>
<tr>
<td>Quality of sensation</td>
<td>Flutter</td>
<td>Pressure</td>
<td>Pressure</td>
<td>Flutter</td>
</tr>
<tr>
<td>Microstimulation threshold, μA</td>
<td>2.8</td>
<td>3.2</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Maximal stimulation, μA</td>
<td>6.5</td>
<td>9.5</td>
<td>4.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>

FA I, fast-adapting type 1; SA I, slowly adapting type 1.

Microneurography/microstimulation results

The 14 afferents selected for EEG recordings included 10 FA I, 3 SA I, and 1 SA II. The results obtained from microstimulation of these afferents were consistent with the findings of Ochoa and Torebjörk (1983) and Vallbo et al. (1984). That is, for FA I and SA I afferents, the evoked sensations matched in location, shape, and size the RFs of the afferents, with sensory qualities characteristic of the respective afferent type, whereas microstimulation of the SA II elicited no sensation whatsoever. Table 1 shows data from four typical MRAs (2 FA I and 2 SA I) that produced EEG effects when microstimulated. The location and extent of their RFs on the glabrous skin of the hand is illustrated in Fig. 1 together with examples of nerve recordings obtained during skin indentations.

EEG results

The basic form of the EEG effects observed, and statistical and experimental checks on their physiological reality, are summarized in Fig. 2 for an FA I with RF on the volar tip of digit 2 (unit 1) stimulated at 33 Hz. Time averages of the filtered data typically had RMS amplitudes in the range 0.1–0.4 μV and contained only the barest suggestion of a sustained response when visually inspected in the time domain, even though conventional somatosensory evoked potentials (SEPs) were present in averages of the raw data. By contrast, in the FFT spectra shown in Fig. 2A, a prominent peak at the frequency of stimulation is immediately evident in the posterior and lateral portions of the array. This driving response, the primary phenomenon of interest, appears superimposed on a stochastically varying and continuous EEG background. That it is not a computational artifact is indicated by Fig. 2B, in which the corresponding results are presented for AR spectra calculated with the use of the modified covariance method at four model orders in the neighborhood of the statistically optimum order (Marple 1987). As in this instance, results of the various AR analyses routinely confirmed (and sometimes extended) those obtained with conventional FFTs. The consistency of these outcomes, despite the computational diversity of the underlying spectral-analysis techniques, attests strongly to the reliability of the results. Figure 2C shows FFT spectra derived from ‘‘plus/minus’’ averages, in which the raw data are alternately added and subtracted on successive trials. This statistical control destroys any quasideterministic signal time-locked to stimulus onset, leaving behind an estimate of the noise in which it is embedded (Schimmel 1967), and it

![Image of receptive fields](http://jn.physiology.org/DownloadedFrom.jpg)
clearly destroys the driving response apparent in Fig. 2, A and B. Figure 2D shows FFT spectra derived from an experimental control run, carried out later in the same session, in which the microelectrode was withdrawn to a position outside the nerve but still in the tissue, and the entire protocol rerun at the final current intensity used to stimulate the afferent itself under the original experimental conditions. The subject experienced no sensation, and no trace of a driving response appears in the spectra. (Note also that the amplitude of the background EEG appears to be generally lower in this frequency range when no stimulus is present.) Four such control runs carried out in the course of the experiments produced no sign of a driving response.

Figure 3 uses another afferent, an SA I with its RF on the tip of digit 4 (unit 2), to illustrate additional systematic properties of the responses that provide further evidence of their physiological origin. The top row shows FFT spectra derived from separate averages of the trials in the first (Fig. 3A) vs. second (Fig. 3B) halves of the run. The driving response (33 Hz) remains highly consistent in topography, but it diminishes sharply in amplitude. The bottom row exploits the superior short-record properties of the modified covariance method (Marple 1987), and shows that a similar decline in the amplitude of the driving response occurs even between the first (Fig. 3C) and second (Fig. 3D) halves of the trial, i.e., the 1-s stimulation period itself. Both of these dynamic trends occurred for every afferent that produced a driving response.

Figure 4 illustrates dramatic changes in response topography observed as a function of RF location and unit type. The left column shows the afferents with RFs on digit tips (units 1 and 2); the right column shows the afferents with RFs near the digit bases (units 3 and 4). Similarly, the top row shows results for afferents with RFs on digit 4, and the bottom row shows results for afferents with RFs on digit 2. There is a sharp contrast between the topographies associated with tips and bases, with the former weighted toward the posterior half of the array and the latter concentrated toward the anterior half. One can also discern suggestions of the expected medial shifts between the centroids of the corresponding topographies for digits 2 and 4. These systematic changes provide further evidence of the physiological origin of the responses.

Finally, we recorded EEG activity associated with micro-
stimulation of the one SA II afferent, whose RF occupied the radial tip of digit 2. Stimulus intensity was set at 10 μA, higher than ever required for successful microstimulation of units of the other types, but in parallel with the absence of sensation no driving responses were observed in the EEG. These failures were not due to dislocation of the microelectrode, because we were still able to record activity from the afferent following the EEG run.

**DISCUSSION**

Our results fully establish both the existence of the basic driving phenomenon and the feasibility of combining microneurography/microstimulation and associated psychophysical procedures with EEG studies of cortical neuronal population responses in humans. Late in the course of our experiments we became aware of a paper by Kunesch et al. (1995) that also reports scalp-recorded potentials evoked by intraneural microstimulation. The stimulation procedures used in that study, however, employed constant-voltage stimuli applied to microelectrodes having larger exposed tips (100 μM) and lower impedances (100–200 kΩ), and, as the authors themselves acknowledge, assured only that ‘‘one or a few’’ afferents were being activated. Kunesch et al. also delivered these stimuli at a rate of only three per second to permit time-domain extraction of the conventional SEP, whereas we measured the evoked EEG activity in greater spatial detail and in terms of a specific response component closely tied to the neurophysiological substrate of vibrotactile psychophysics. Apart from these differences, however, our results are consistent and mutually supportive.

The orderly character of our results presents, even at this early stage, three primary challenges to interpretation. 1) Why are the responses so large? 2) What accounts for their across-trial and within-trial dynamic trends? 3) How can we explain the variability of their scalp topographies? We hypothesize that these response features all arise directly from the physiological properties of microstimulation.

We underscore first the surprising size of the EEG driving responses evoked by single-afferent stimulation. We ourselves were initially doubtful that any response other than the mass SEP would be detectable. However, FFRs were not only detectable but at least as large as those previously produced in the same subject by a 24-Hz, 50-μM vibrotactile stimulus delivered to the tip of digit 2 via an 8-mm contactor, i.e., by a tactile stimulus that, although roughly comparable in subjective magnitude activated ≥200 MRAs (Johansson and Vallbo 1979). Indeed, the size of the driving responses produced by periodic
microstimulation seems to provide an electrophysiological counterpart to the surprising clarity of the associated sensations. This paradox certainly cannot be resolved in terms of factors such as attentional effects or the numbers of trials available for averaging. Kunesch et al. (1995) also commented on the size of their responses relative to those produced by whole nerve stimulation, and suggested that this must somehow reflect abnormal “selectivity” of the input to SI. Microstimulation clearly provides a degree of selectivity unattainable with conventional mechanical or electrical whole nerve stimuli, approaching as closely as one ever can a limiting pattern of input drive consisting of synchronous activation of the middle-layer cortical terminations of some unknown but minimal number of thalamocortical afferents, i.e., a highly local patchwork of cortical columns (Jones 1991). Such a pattern could readily account for responses of the size we observed, because a momentary potential difference of 100 μV across even a single cortical column can contribute on the order of 1 μV to the EEG observed on overlying scalp, with a spread function extending over many centimeters because of the “smearing” effects of distance and the resistivity of the skull (Nunez 1981). Transcortical potentials of at least this magnitude have been observed at multiple locations in SI following median nerve stimulation (Allison et al. 1989).

What really needs to be explained, therefore, seems rather why the response associated with the tactile stimulus is not larger. We think this results from the fact that any vibrotactile stimulus (even if matched exactly to the RF of one particular MRA) necessarily activates additional afferents, but with phase dispersion arising from both the response times of the mechanoreceptors themselves and the varying conduction velocities of the corresponding afferents. These factors, in turn, can lead both to inhibitory physiological interactions at multiple levels of the somatosensory pathway (e.g., lateral inhibition, pericolumnar and corticocortical competitive interactions), and to field cancellation effects among local cortical FFRs bearing variable phase relationships to the driving stimulus (Pfurtscheller and Cooper 1975).

The systematic trends we observed, i.e., loss of driving response amplitude both across and within trials, strikingly resemble effects we have previously observed in our ongoing EEG studies of vibrotactile adaptation, and invite interpretation within that framework. That is, they may be electrophysiological signs of a pericolumnar competitive process that optimizes the capacity of the evolving cortical activity pattern to encode stimulus properties such as amplitude, frequency, and location (McLaughlin and Kelly 1993; Whitsel...
et al. 1991). The most striking difference is that with microstimulation the process seems to move faster, especially within trials. This makes sense, however, if one considers that the cortical activity pattern initially set up by microstimulation probably resembles—again because of its relatively high specificity—patterns that would appear later in the course of adaptation to a commensurate tactile stimulus. This interpretation of the response trends as central adaptation effects, although plausible, is not yet compelling, however. Even though the subject experienced the original sensation on almost every trial of every run we analyzed, we cannot at present rule out the possibility that the observed trends in microstimulation-induced FFR amplitudes may have been due in part to stimulus failure. Our vibrattractive studies control for this by the use of surface recordings of the first-order population response (Kelly et al. 1996), and for future work on microstimulation it would be desirable to implement an analogous control, with the use of low-impedance needle electrodes (Buchthal and Rosenfalck 1966) proximal to the site of stimulation both to verify single-fiber activation and to assess the consistency of the first-order response. Parenthetically, our hypotheses regarding the size and temporal dynamics of the cortical activation patterns set up by microstimulation of MRAs could be directly evaluated in animal subjects with the use of IR optical imaging techniques (Tommerdahl and Whitesell 1996) and compared with the patterns set up by vibrattractile stimuli matched to their RFs.

The early components of SEPs evoked by electrical median nerve stimulation have been studied extensively and are known to be dominated by sources in area 3b occupying the posterior wall of the central sulcus, which extend more variably into area 1 on the anterior crown of the postcentral gyrus (for review, see Allison et al. 1989, 1991; McLoughlin and Kelly 1993). The primary source, located in area 3b, is “tangential,” i.e., oriented roughly parallel to the skull, and expresses both poles of its activity simultaneously, reflected across the central sulcus. The scalp topography of these projected potential fields is spatially extensive and varies widely but systematically between subjects, reflecting small variations in the locus and orientation of the activated tissue within the representational field of the hand. We hypothesize that the large but orderly variations in response topography observed within our single subject similarly reflect relatively small spatial displacements of the much more focal source configurations set up by microstimulation. On the basis of the available data, moreover, these appear to be associated primarily if not exclusively with RF locus rather than unit type. The slight medial shift between the topographies for digits 2 and 4, for example, is consistent with the known somatotopic organization of the digit representations. Similarly, the dramatic posterior-to-anterior shift in response topography between afferents with RFs on the digit tips and digit bases could be explained by an orientation change of just a few degrees as the effective electrical source is displaced along the curvature of the postcentral gyrus. Our method thus also appears to provide significant new opportunities for application and validation of emerging electromagnetic source localization techniques that use subject-specific anatomic and functional imaging data to constrain the solutions (e.g., George et al. 1995; Wikswo et al. 1993).

We thank our colleagues W. Maixner, D. McLoughlin, and B. Whitesell for reviewing earlier versions of this paper.

This research was supported by National Institute of Dental Research Grant DE-07509 to E. Kelly and S. Folger and Grant 8667/11087 from the Swedish Medical Research Council to M. Trulsson.

Address for reprint requests: E. F. Kelly, 112 Dental Research Center, CB #7455, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599-7455.

Received 17 April 1996; accepted in final form 27 September 1996.

REFERENCES


date reference format


