Modulation and transmission of peripheral inputs in monkey cuneate and external cuneate nuclei

Claire L. Witham and Stuart N. Baker
Institute of Neuroscience, Medical School, Newcastle University, Newcastle upon Tyne, United Kingdom

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THE DORSAL COLUMN NUCLEI ARE the first stage of the ascending cutaneous and proprioceptive pathways to the cortex. These nuclei, together with the thalamus, are commonly known as relay nuclei (Gordon 1973), a misleading terminology that implies peripheral signals are simply passed to the cortex with little or no processing. A number of studies have shown that the ascending signals undergo substantial modulation in the cuneate, involving interactions both between ascending signals and with descending cortical inputs (Aguilar et al. 2003; Gordon 1973; Soto et al. 2004). The majority of research to date has focused on the cuneate nucleus of the cat.

Relatively little is known about processing in the cuneate and external cuneate of monkeys, which differ from cats in several key regards. In cats the external cuneate projects to the cerebellum, whereas in monkeys it projects also to the thalamus (Boivie and Boman 1981; Boivie et al. 1975). Cortical projections to the cuneate differ between the two species (Bentivoglio and Rustioni 1986). Behavioral differences between the two species may also have important functional consequences for somatosensory processing. Primates use their hands to explore and interact with the world around them (active touch), whereas in cats the paw shows a more limited behavioral repertoire of locomotion and reaching. A small number of studies have demonstrated widespread inhibition in the main cuneate nucleus of the primate, including descending inhibition from the sensorimotor cortex (Biedenbach et al. 1971; Harris et al. 1965). Several functions have been proposed for this inhibition including center-surround inhibition to sharpen receptive fields (Soto et al. 2004) and reduction of reafferent signals (Chapman et al. 1988).

Poisson train stimulation is a useful method for quantitative assessment of the relationship between neural inputs and outputs. This can yield both an estimate of the neural frequency filtering properties and also details on how different inputs might interact (Christakos et al. 1984). A Poisson stimulus train has several advantages over using stimulus trains of fixed frequency. It has a flat (white) power spectrum, allowing responses to be measured over a broad frequency spectrum all at once, and it is much quicker to apply than multiple stimulus trains at different frequencies (Windhorst et al. 1989; Windhorst et al. 1988). Coherence analysis can provide an estimate of the input-output relationship as a function of frequency. If multiple independent Poisson stimulus trains are applied, the conditioning effects of one input on another can be analyzed for many different inter-stimulus intervals (Rosenberg et al. 1989).

We used Poisson train stimulation of four peripheral nerves to investigate the ability of cuneate and external cuneate neurons to code afferent inputs. In particular, we assessed convergence from different peripheral nerves onto single cuneate neurons, interactions between different nerves, and the ability to process different input frequencies. We show that the cuneate carries out appreciable transformations of afferent input, which will influence upstream processing.

METHODS

Experimental Setup

General. All animal procedures were performed under UK Home Office regulations in accordance with the Animals (Scientific Procedures) Act, 1986, and were approved by the Local Research Ethics Committee of Newcastle University. Recordings were made from two adult female rhesus macaque monkeys (M. mulatta; monkeys E and M).

Address for reprint requests and other correspondence: S. N. Baker, Institute of Neuroscience, Henry Wellcome Bldg., for Neuroecology, Newcastle Univ., Framlington Place, Newcastle upon Tyne, NE2 4HH, UK (e-mail: stuart.baker@ncl.ac.uk).
Preparation surgery. In an initial surgery monkeys E and M were implanted with eight bipolar flexible nerve cuffs placed bilaterally on the median and ulnar nerves in the arm, and the deep radial (DR) and superficial radial (SR) nerves at the elbow. Wires from the nerve cuffs were tunnelled subcutaneously from an incision on the back to the implant sites. Spare wire was looped into a silicone pouch to prevent encapsulation with connective tissue and stored under the skin at the shoulder. Surgeries were carried out under aseptic conditions, and general anesthesia (3.0–5.0% sevoflurane inhalation in 100% O₂) supplemented with a continuous intravenous infusion of alfaxan, 15 μg·kg⁻¹·h⁻¹). A full program of postoperative analgesia (10 μg/kg buprenorphine; Vetergesc, Reckitt and Colman Products; 5 mg/kg carprofen, Rimadyl, Pfizer; 0.15 mg/kg meloxicam, Metacam, Boehringer Ingelheim Animal Health) and antibiotic care (8 mg/kg cefove- cin, Convenia, Pfizer and 15 mg/kg ibafloxacin, Ibaflin, Schering-Plough Animal Health or 15 mg/kg amoxicillin, Clamoxyl LA, Pfizer and 5 mg/kg enrofloxacin, Baytril, Bayer Animal Health) followed surgery.

Nonrecovery procedures. Deep anesthesia was induced with sevoflu- rane (3.0–5.0% sevoflurane inhalation in 100% O₂) supplemented with a continuous intravenous infusion of alfaxan (15 μg·kg⁻¹·h⁻¹). After tracheotomy, central venous and arterial lines were inserted via the major neck vessels. The silicone pouches containing wires from the nerve cuffs were exposed. The brain stem was exposed by dissecting the muscles from the back of the head, and removing a small window of occipital bone extending ~6 mm above the foramen magnum, and ~8 mm on either side of the midline. The anesthetic regimen was then switched to an intravenous infusion of propofol (15–50 mg·kg⁻¹·h⁻¹) and alfaxan (15–50 μg·kg⁻¹·h⁻¹). The monkey was transferred from the operating theater to the laboratory. The vertebral column was clamped at high thoracic and midlumbal levels. The head was fixed in a stereotaxic frame angled to produce a 71° angle between the transverse plane of the stereotaxic frame and the vertebral clamps. The dura at the occipital craniotomy was removed, and the underlying fourth ventricle was opened, to expose the brain stem at obex. Neuromuscular blockade was then commenced (atracurium, 0.7 μg·kg⁻¹·h⁻¹). Throughout experiments we monitored continually the animal’s central arterial pressure and heart rate, end-tidal CO₂, blood oxygen saturation, and core and skin temperature. Temperature was maintained via a blanket filled with warmed air and a thermostatic heating pad. Fluid balance was maintained by infusion of Hartman’s solution (to make up a total infusion rate of 5 ml·kg⁻¹·h⁻¹ including drug solutions). Brain oedema was reduced by giving methylprednisolone (initial loading dose 30 mg/kg iv, followed by infusion of 5.4 mg·kg⁻¹·h⁻¹ iv). Inspired gas had an oxygen concentration around 50% to avoid pulmonary oxygen toxicity, which can occur with high oxygen concentrations over long periods. Respiration rate and tidal volume were adjusted to maintain end-tidal CO₂ concentration around 5%. Infusion rates of the anesthetic agents were adjusted to maintain a stable plane of anesthesia, judged by heart rate and arterial blood pressure and the responses of these two measures to noxious stimulation. Including surgical preparation time, experiments lasted from 2 to 3 days.

Recordings and stimulation. Spike waveforms were recorded with a 16-channel Eckhorn microdrive (Thomas Recording, Giessen, Germany) using glass-insulated platinum electrodes. The guide tubes were positioned on the surface of the brain stem, and the electrodes were advanced into the brain. Penetrations were angled between 0° and 25° relative to the brain stem surface. The cuneate and external cuneate were located on the dorsal surface of the brain stem (see Fig. 1A) and identified by receptive field testing and short latency responses to peripheral nerve stimulation. After the brain stem had been extensively explored on one side, and responses to ipsilateral peripheral nerve stimulation were measured, we switched to the opposite side and gathered further data. Spike waveforms (300 Hz-10 kHz bandpass, gain 2–10 k) were sampled continuously at 25 kHz and discriminated offline using custom written software (Gatsby; SN

Baker). Each of the four nerve cuffs was stimulated using independent random Poisson train stimulation (constant current bipolar stimulus pulses, 0.2 ms per phase, proximal electrode negative on first phase; mean rate 10 Hz, minimum interval 9 ms monkey E and 3 ms monkey M; see Fig. 1B). The threshold stimulus intensity for motor activation was estimated for the three motor nerves (median, ulnar, and DR) just before neuromuscular blockade was commenced. During recordings, these nerves were stimulated at 1.5–2.0 times motor threshold. For the SR nerve, the stimulus intensity was chosen to produce clear cuneate field responses. Stimulus currents varied from 0.6 to 21 mA (median, 2.25 mA).

In some recordings focal mechanical stimuli were delivered to up to three skin locations containing the receptive field of recorded cells. The custom stimulators comprised a 4.7 mm diameter probe with a rounded tip (radius of curvature, 2 mm), which could be extended up to 2 mm from the center of a 22 mm diameter flat circle by the action of a solenoid (part number 533-6887; RS Components, Corby, UK). The circle was placed flat against the skin, with the probe centered over the receptive field. On each stimulus, the solenoids were activated for 2.5 ms. Mechanical stimuli were given as independent Poisson trains as described above, over the same period as the nerve stimuli.

Analysis

Classification. Standard raster plots and peristimulus time histograms (PSTHs) were produced to show the response of each cell to each nerve (PSTH bin width, 0.1 ms). The mean and SD of the PSTH baseline (taken over the 19.5 ms before the stimulus) were calculated. Responses were classified as facilitation if there was a rise in the PSTH greater than the baseline mean + 4 SD and suppression if the
PSTH fell below the baseline mean of 4 SD. In cells that showed both facilitation and suppression, the earliest response was used for classification. Inter-spike interval (ISI) histograms were produced using intervals of spikes occurring between 0 and 20 ms poststimulus. These were used to class facilitatory responses as bursting (defined as more than 20% of the intervals less than 2 ms in duration) or nonbursting.

To create average PSTHs, single-cell PSTHs were first normalized by dividing by the baseline counts (assessed in the 19.5 ms prestimulus period) and then smoothed by convolution with a Gaussian kernel of width 0.2 ms. These normalized and smoothed PSTHs were then averaged across all cells for a particular condition.

The response width for facilitations was measured across the region where PSTH bins exceeded the baseline counts + 4 SD. The response amplitude $s$ was assessed as:

$$s = \frac{1}{N} \sum_{i=1}^{N} X_i - X_{Baseline}$$  \hspace{1cm} \text{Eq. 1}

where $X_i$ are the counts in individual bins of the PSTH, the response runs from bins $i_1$ to $i_2$, $N$ is the number of stimuli, and $X_{Baseline}$ is the mean number of counts per bin in the PSTH over a prestimulus region. $s$ measures the mean number of extra spikes elicited by each stimulus. It is negative for suppressions; in plots here, we show $s$ values separately for suppressions and facilitations and plot the absolute value in each case.

**Coherence analysis.** The coherence between the stimulus and spike trains was calculated by transforming each point process into a time series by counting events in successive 1-ms bins. The time series were divided into nonoverlapping windows 1,024 bins long, and a discrete fast Fourier transform was applied, yielding a frequency resolution of 0.98 Hz. Coherence was calculated as follows:

$$Coh(f) = \frac{\left| \sum_{i=1}^{L} X_i(f)Y_i(f) \right|^2}{\sum_{i=1}^{L} X_i(f)X_i(f)\sum_{i=1}^{L} Y_i(f)Y_i(f)}$$  \hspace{1cm} \text{Eq. 2}

where $X_i$ and $Y_i$ are the Fourier transformed signals from the $i$th window, $f$ is the frequency, and $L$ is the total number of nonoverlapping sections (Baker et al. 2006; Evans and Baker 2003). Coherence was considered significant ($P < 0.05$) if it was greater than $Z$ where

$$Z = 1 - 0.05^{\sqrt{(L-1)}}$$  \hspace{1cm} \text{Eq. 3}

Coherence magnitude was averaged across all cells in a given category; significance limits were assigned to these average coherence spectra as described in Evans and Baker (2003).

**Conditioning effect.** The conditioning effect of stimulating one peripheral nerve [conditioning stimulus (CS); Fig. 2A] on the response in cuneate cells to a second peripheral nerve [test stimulus (TS)] was calculated by selecting all TS from the Poisson train, which were preceded by a CS at a fixed delay (± 1 ms precision). The CS-TS interval used was varied from 0 to 512 ms. For each interval the PSTH in response to the selected test stimuli was calculated and smoothed using a Gaussian kernel with a width of 2 ms (Fig. 2B, first row). Bin counts were normalized by the number of stimuli, yielding spiking probability per bin. This PSTH will contain features corresponding to the response to the CS and TS; at brief intervals, the CS response will overlap the TS response and make quantification of its amplitude difficult. Accordingly, the average PSTH response to the CS (Fig. 2B, second row) was subtracted from the combined PSTH (Fig. 2B, third row), yielding an estimate of the response to the TS alone. This procedure was repeated for each delay, and the results were plotted as a two-dimensional display using a false color scale (Fig. 2, C-E). On these plots, the abscissa represents time after the TS, the ordinate represents the CS-TS interval, and the color scale shows the probability of cell responses.

To summarize the effect of conditioning across cells, we first identified the region of the PSTH for each cell (compiled from all TS), which exceeded the baseline mean + 4 SD. For the conditioned PSTH at each CS-TS interval, we measured the response amplitude by summing the bin probabilities in this region and subtracting the baseline. Because all stimuli were given as simultaneous Poisson trains, there was no TS alone condition, which could serve as a control against which the conditioned responses could be compared. Accordingly, we measured the average response over CS-TS intervals between 400 and 512 ms, which is sufficiently long that the CS would be expected to have no effect, and used this as the control response against which comparisons were made. The conditioning effect $R$ at the CS-TS interval $\tau$ was thus measured as:

$$R(\tau) = s(\tau)/\bar{s} \times 100\%$$  \hspace{1cm} \text{Eq. 4}

where $s(\tau)$ is the response amplitude measured for the CS-TS interval $\tau$ and $\bar{s}$ is the average response amplitude measured for CS-TS intervals between 400 and 512 ms. The values of $R$ measured for individual cells were then averaged across all cells in a particular category, and the SE was calculated at each interval $\tau$.

**RESULTS**

We successfully recorded from both the cuneate and external cuneate nuclei; under the anesthetic regime described, cells showed good levels of spontaneous and stimulus-evoked activity over a long period of time (~48 h). A total of 317 cells

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were recorded in monkeys E and M (all cells were present for a minimum of 300 s of Poisson train stimulation). Of these, 183 cells had cutaneous and 63 cells had proprioceptive receptive fields. The remaining 71 cells either had no clear receptive fields or were lost before fields could be identified. Cells were classified based on their responses to stimulation of the peripheral nerves; examples of three cells are shown in Fig. 3 as rasters for each nerve separately (Fig. 3A) and overlain PSTHs (Fig. 3B). Cell 1 had a proprioceptive receptive field (wrist extension) and showed a narrow-peaked excitatory response to ulnar nerve stimulation. Cell 2 had a cutaneous receptive field on the dorsal forearm and responded to median, ulnar, and DR nerves with clear suppression of activity lasting ~25 ms; by contrast, following SR nerve stimulation there was a brief facilitation followed by suppression. Cell 3 had a cutaneous receptive field located on the dorsal surface of the most distal segment of the thumb. Activity was facilitated after stimulation of three of the nerves. Although the response to median nerve was a single spike, the cell responded to DR stimulation with a burst of two to three spikes at relatively fixed latency. The response to SR stimulation typically contained two spikes, but with a high latency jitter, leading to a broad PSTH peak. It is noteworthy that a brief stimulus could produce a longer-lasting modulation of neural discharge.

These three classes of responses were typical of most of the cells studied. Of the cells with identified receptive fields 38% showed facilitation (43% of cells with cutaneous receptive fields and 25% of cells with proprioceptive receptive fields) and 32% showed suppression (33% of cells with cutaneous receptive fields and 32% of cells with proprioceptive receptive fields). Some cells such as cell 3 in Fig. 3 showed a bursting response to single stimuli. Cell responses were classified as bursting if their ISI histogram (constructed from intervals occurring between 0 and 20 ms poststimulus) had more than 20% of the intervals below 2 ms. Overall 22% of facilitated responses were classified as bursting. Cells with cutaneous receptive fields were much more likely to have bursting responses (26%) compared with cells with proprioceptive fields (6%, χ²-test; P < 0.001).

The response for each cell-nerve combination was characterized by the response width and amplitude s (number of extra spikes per stimulus; see METHODS). The relationship between these measures is shown in Fig. 4 as scatter plots for three categories of response (A, facilitation with bursting; B, facilitation with no bursting; C, suppression). The results for all four nerves have been overlaid. There were no significant differences in s values between the peripheral nerves, for all three categories of response (P > 0.05, Kruskal-Wallis test). The combined distribution of s values across all nerves is indicated as a cumulative probability plot by the black line in each panel of Fig. 4. The width of facilitations produced by the different nerves was not significantly different, but for suppressions the response width for median nerve was significantly briefer than for the other three nerves (median response of 11.2 ± 1.4 ms for median nerve; 25.0 ± 3.6 ms for ulnar nerve; 28.6 ± 2.3 ms for DR nerve; 31.2 ± 3.2 ms for SR nerve; P < 0.001, Kruskal-Wallis test).

Convergence

Cuneate cells commonly showed significant responses to more than one peripheral nerve. In some cases all responses were facilitations (cell 3 in Fig. 3), and in others all responses were suppression; a mixture of facilitation and suppression responses could also occur (cell 2 in Fig. 3). We were interested to see whether the types of response followed recognizable patterns for particular types of stimulation. We first compared responses to the DR (a purely motor nerve) with those to SR (a purely cutaneous nerve), in the 183 cells that had a cutaneous receptive field. If convergence from proprioceptive and cutaneous afferents was organized in a reciprocal way, for example, we might expect that the discharge of these cutaneous responsive cells would be facilitated by SR, but suppressed by DR stimulation. A total of 120/183 cells showed significant responses to stimulation of both nerves. These cells were classified according to whether each response was facilitation or suppression; the averaged PSTH responses for each class, together with the number of cells in each class, are shown in

Fig. 3. Single cell examples of responses to peripheral nerve stimulation (Stim). A: raster plots for 3 cells in response to median (black), ulnar (green), DR (red), and SR (cyan) stimulation. Responses to only a small number of stimuli (500) have been plotted. Values in top right corner of box represent stimulus intensity. B: peristimulus time histograms (PSTHs) in response to the 4 peripheral stimuli (color scheme as above). Inset shows location of receptive field. Dotted line shows stimulus time.
proportional arm and 28/44 related to hand; significantly different, \( \chi^2 \)-test; \( P < 0.001 \).

An alternative principle of organization for convergence onto a given cell might be reciprocal responses to input from flexor versus extensor muscles. To test whether this was the case, we examined the responses of the 63 cells with proprioceptive receptive fields to stimulation of the median/ulnar nerves (flexor muscle related) and DR nerve (extensor muscle related). A total of 39 cells had significant responses to both median/ulnar and DR stimulation; of these three had inconsistent responses to median and ulnar stimulation (facilitation to one, suppression to the other). The remaining 36 cells were classified according to the type of response following each nerve; averaged responses for each class are displayed in Fig. 5B, together with the number of cells displaying each combi-

Fig. 5. Average PSTH plots for cells responding to more than 1 nerve. A: average PSTHs for cutaneous-related cells responding to DR and SR stimulation with facilitation and/or suppression (see METHODS for details of the normalization and averaging procedure). Rate is expressed as a fraction of the baseline (prestimulus) rate. PSTHs for DR and SR stimulation have been overlaid. B: average PSTHs for proprioceptive-related cells responding to flexor motor nerve (median/ulnar) and DR nerve stimulation with facilitation and/or suppression. PSTHs for flexor and extensor stimulation have been overlaid.

Fig. 4. Characteristics of single cell responses to peripheral nerve stimulation. A: scatter plot of response amplitude (s) versus response width for facilitated cells with bursting responses. Amplitude (s) represents the number of extra spikes above baseline elicited per stimulus (see METHODS for more details). Data for the separate nerves have been overlaid. Black line represents the cumulative probability histogram for s. B: as in A for facilitated cells with nonbursting responses. C: as in A for cells with suppression responses (absolute magnitude of s plotted, representing the reduction in number of spikes emitted per stimulus).

Fig. 5A (individual cell PSTHs have been normalized by their baseline rate and smoothed before averaging; see METHODS for further details). All four combinations of response occurred. There was a significant association between the response type following DR stimulation and that following SR, with cells tending to respond to both stimuli with facilitation or suppression (\( \chi^2 \)-test; \( P < 0.01 \)). The cells that showed suppression in response to both DR and SR stimulation tended to have more proximal receptive fields (17/30 cells related to elbow, upper arm, or shoulder and 10/30 related to hand) than the cells that responded to both stimuli with facilitation (7/44 related to proximal arm and 28/44 related to hand; significantly different, \( \chi^2 \)-test; \( P < 0.001 \)).

A: scatter plot of response amplitude (s) versus response width for facilitated cells with bursting responses. Amplitude (s) represents the number of extra spikes above baseline elicited per stimulus (see METHODS for more details). Data for the separate nerves have been overlaid. Black line represents the cumulative probability histogram for s. B: as in A for facilitated cells with nonbursting responses. C: as in A for cells with suppression responses (absolute magnitude of s plotted, representing the reduction in number of spikes emitted per stimulus).
 Relative to the incoming volley of 0.7 ms, 0.9 ms, and 1.6 ms connections. Vertical line represents 1 ms cut-off latency for monosynaptic response latencies (relative to arrival of afferent volley) to median nerve stimulation. For example, cell 3 in Fig. 3 had a response onset latency for DR nerve stimulation 1.1 ms after the afferent volley; this could be monosynaptic or disynaptic in origin. The distribution of response latencies relative to the arrival of the afferent volley for all facilitated cells is shown in Fig. 6. A total of 39 responses to median, 17 responses to ulnar, 12 responses to deep radial, and 10 to superficial radial nerve were classified as putative monosynaptic (<1 ms, vertical line). The cells with likely monosynaptic latencies had significantly larger responses than those with responses mediated by uncertain pathways (Fig. 6F; Wilcoxon rank-sum test; P < 0.001) for all four nerves.

**Response Latencies**

We measured the onset latencies of the facilitated responses relative to the first peak of the field response for each nerve, which correspond to the arrival of the primary afferent volley (see Fig. 6A). Cell responses having an onset within 1 ms of this field potential were classified as putative monosynaptic effects. For example, cell 3 in Fig. 3 had response latencies relative to the incoming volley of 0.7 ms, 0.9 ms, and 1.6 ms to median, DR, and SR nerve stimulation, respectively. The responses to median and deep radial nerves were thus likely to be monosynaptic; that to the SR nerve could be oligosynaptic, or it could be a monosynaptic response to more slowly conducting afferents. Cell 1 in Fig. 3 had a response onset latency after ulnar nerve stimulation 1.1 ms after the afferent volley; this could be monosynaptic or disynaptic in origin. The distribution of response latencies relative to the arrival of the afferent volley for all facilitated cells is shown in Fig. 6. A total of 39 responses to median, 17 responses to ulnar, 12 responses to deep radial, and 10 to superficial radial nerve were classified as putative monosynaptic (<1 ms, vertical line). The cells with likely monosynaptic latencies had significantly larger responses than those with responses mediated by uncertain pathways (Fig. 6F; Wilcoxon rank-sum test; P < 0.001) for all four nerves.

**Coherence**

To investigate how cuneate cells respond to different frequencies of peripheral inputs, we applied peripheral stimulation as a Poisson train, which has a white power spectrum containing a broad range of frequencies (see Fig. 1D). The coherence between the stimulus train and cell spiking was then calculated (see METHODS). Figure 7 shows the average PSTHs and coherence plots for four classes of cell responses (facilitation with bursting, facilitation with no bursting, suppression or no effect); results from the four nerves are overlaid in different colors.

Cells that responded to single stimuli with a burst of spikes (median, n = 24; ulnar, n = 21; DR, n = 23; SR, n = 28) showed the largest coherence with the stimulus trains (Fig. 7, A and B), with only a slight decline in coherence values at higher frequencies. For some cells, coherence remained at similar levels up to the maximum frequency measured of 330 Hz (fixed by the minimum stimulus interval used of 3 ms). Facilitated cells that did not exhibit bursting behavior (median, n = 118; ulnar, n = 62; DR, n = 83; SR, n = 85) also showed significant coherence values over a wide frequency range (Fig. 7, C and D). However, these spectra also showed a peak in coherence at ~10 Hz; this peak of elevated coherence appeared to end around 30 Hz, and coherence declined at a slower rate for higher frequencies.

Cells that responded with suppression in the PSTH showed a different pattern of coherence spectra (Fig. 7, E and F; median, n = 24; ulnar, n = 67; DR, n = 132; SR, n = 79). As well as having lower coherence values than for the facilitated cells, there was a greater dependence on frequency. For the median, ulnar, and DR nerves there was a peak at ~7 Hz and a sharp fall-off at 20 Hz. The SR nerve showed no peak, but rather a more gradual decay in coherence up to ~20 Hz.

For cells where there was no significant response to stimulation in individual PSTHs, the PSTH averaged across the population showed a very small suppression (Fig. 7G), indicating that some of these cells were affected by the stimuli, but so weakly that it needed the improvement of signal-to-noise ratio produced by population averaging to be detected. Coherence averaged across this population was significant at frequencies below 20 Hz, but of very low magnitude (note the greatly expanded scale on Fig. 7H).

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Fig. 6. Response latencies to peripheral nerve stimulation for facilitated cells, relative to arrival time of the afferent volley in the brain stem. A: field response to median (Med) nerve stimulation and PSTH response to median nerve for the neuron illustrated as cell 3 in Fig. 3. Arrow indicates stimulus onset. Dotted lines represent arrival of 1) afferent volley and 2) response onset. B: histogram of response latencies (relative to arrival of afferent volley) to median nerve stimulation. Vertical line represents 1 ms cut-off latency for monosynaptic connections. C: as in B for responses to ulnar (Uln) nerve stimulation. D: as in B for responses to DR nerve stimulation. E: as in B for responses to superficial radial nerve stimulation. F: comparison of response amplitudes (s) for identified monosynaptic responses and unidentified responses to each peripheral nerve.
histograms from Fig. 4 have been overlaid (thin lines) for those observed for responses to peripheral nerve stimulation. The coherence values were lower than for nerve stimuli (except for the bursting cells where they were similar), but there were clear peaks in the spectra for both facilitated and suppressed cells at ~10 Hz (Fig. 8, C, F, and I). Coherence remained well above significance up to high frequencies for facilitated cells but dropped close to the significance limit at around 50 Hz for suppressed cells.

Cuneate Versus External Cuneate

So far no distinction has been made between cells recorded from the main cuneate (cutaneous and proprioceptive) and external cuneate (proprioceptive) nuclei. We mapped the locations of cutaneous and proprioceptive cells recorded from monkey M onto the stereotaxic map of the brain stem produced by Smith et al. (1972). This is shown in Fig. 9A. For this monkey some recordings were clearly in either cuneate or external cuneate (marked by black or red ovals) with the rest of the recordings in the overlap between the two nuclei. Because proprioceptive cells might be recorded from either nucleus, only cells within the two ovals were used for the subsequent analysis of this figure. For monkey E (not shown) most of the proprioceptive cells were recorded from the border area between the two nuclei and therefore only cells from monkey M were used in this analysis. The responses to median and ulnar nerve stimuli (both mixed cutaneous and proprioceptive nerves) were divided up into facilitation (bursting and nonbursting responses were combined for this analysis) and suppression and the response characteristics, normalized PSTHs and coherence spectra calculated as in the previous figures (facilitations, Fig. 9, B, D, and F; suppressions, Fig. 9, C, E, and G). There were no significant differences between main and external cuneate cells in either response amplitude or width for either facilitated or suppressed cells (P > 0.05; Wilcoxon rank-sum test). The average size of peak facilitation (Fig. 9D) and suppression (Fig. 9E) were similar for main cuneate and external cuneate cells. Facilitated external cuneate cells showed smaller coherence than facilitated main cuneate cells (Fig. 9F), and suppressed external cuneate cells had a narrower coherence peak than suppressed main cuneate cells (Fig. 9G), although neither of these differences was significant (t test on individual cells’ coherence values at each frequency, P > 0.05 Bonferroni corrected for multiple comparisons of different frequency bins).

Conditioning Effects

All cells that were facilitated by stimulation of one or more nerves were analyzed to look at the conditioning effect of either the same nerve or another which produced either a significant facilitation of suppression of firing. The analysis followed the procedure described in Fig. 2. Figure 10 shows the conditioning effects on an example cell (cell 3 in Fig. 3). The cell was facilitated by stimulation of three nerves (median, DR, and SR); there was no response to ulnar nerve stimulation. Figure 10 shows the effect of all possible combinations of conditioning (columns) and test stimuli (rows); the plots on the leading diagonal show the results when the same nerve was used for the conditioning and test stimuli. DR nerve stimulation suppressed the short latency response to the median nerve and SR stimulation (center column) at short interstimulus

![Graphs and diagrams showing the relationship between firing rate and frequency](http://jn.physiology.org/).
intervals (less than 20 ms). Similar effects were seen for other combinations, although the effects on the DR nerve responses were less marked (middle row).

The average effects of conditioning across the recorded cell population are shown in Fig. 11A, in which responses to the median and ulnar nerves have been combined for ease of presentation. The response to the test stimulus was measured from the individual cells at different condition-test intervals and expressed as a percentage of that seen at long (400–512 ms) intervals, which was used as a control condition. Both the effects when the conditioning stimulus produced facilitation of firing (black traces) and the effects when the conditioning stimulus produced suppression of firing (blue traces) are shown. Depending on the combination of nerves used as condition and test stimulus, the response at short intervals was between 72.6% and 93.6% of the control level when the conditioning stimulus produced facilitation. The reduction in response magnitude lasted around 40 ms after the conditioning stimulus. The effects when the conditioning stimulus produced suppression were similar although the traces were noisier. Although the response to the test stimulus reached a consistent amplitude for condition-test intervals >40 ms, we cannot exclude the possibility of some long-lasting (>512 ms) conditioning effect. This could not be tested in our paradigm, since stimuli were given continually as simultaneous Poisson trains.

When stimulation of one nerve causes suppression of the response to stimulation of another nerve shortly afterward, this is clearly an interaction between different afferent pathways. However, interpreting the effects seen when the same nerve is both the conditioning and test stimulus is more complex. Suppression could then occur due to interactions between fibers with different receptive fields, which just happen to run within the same nerve. Alternatively, suppression could occur because the initial activation of fibers suppresses responses to those same fibers. This ambiguity cannot be resolved when examining the responses to electrical nerve stimulation, which activates a large number of afferent axons.

By contrast, mechanical stimulation over a small cutaneous receptive field excites a much smaller number of afferent inputs; in that case, any interactions are most likely to be produced by effects from the same afferents activated by both conditioning and test stimuli. Figure 11B shows the effect of conditioning a mechanical stimulus with the same mechanical stimulus. A similar suppression is seen as in Fig. 11A, implying that there is a homotopic response suppression.

**DISCUSSION**

This study provides a detailed analysis of the responses of primate cuneate cells to peripheral stimulation. Many features of our results appear broadly consistent with what is known of the organization of cuneate circuitry in the cat. We also provide a quantitative assessment of the ability of cuneate cells to encode temporal fluctuations in peripheral inputs, which may be especially important in permitting the fine tactile processing of the primate hand.

**Underlying Circuitry**

Cells responded with both facilitation and suppression of discharge (Fig. 3). Interactions between or within nerve stimuli showed suppression of facilitation responses over a time course of ∼40 ms (Fig. 11), which is similar to the average time course of the suppression responses (Fig. 7E). The cuneate in cat contains both GABAergic and glycinegic inhibitory interneurons (Galindo et al. 1967); suppression responses are likely to be mediated by GABAergic cells (Galindo et al. 1967). The majority of afferent inhibition in monkey results from presynaptic inhibition (Biedenbach et al. 1971), although significant postsynaptic inhibition occurs in cat (Andersen et al. 1964a).
Bursting responses to a single stimulus (e.g., Fig. 3, cell 3) are a common feature of cuneate neurons (Canedo et al. 1998; Galindo et al. 1968). Although bursting was more likely in cutaneous-related cells, in contrast with previous reports (Galindo et al. 1968), we also found bursting proprioceptive cells. Multiple mechanisms could underlie bursts, including excitation by other cells (projection neurons send collaterals to other projection neurons; Canedo et al. 2000), a prolonged excitatory postsynaptic potential (Andersen et al. 1964b) and an intrinsic tendency to rhythmic firing (Galindo et al. 1968). Soto et al. (2004) proposed a circuit whereby projection neuron collaterals excite glycinergic interneurons, which in turn inhibit the GABAergic neurons; the resulting disinhibition of the projection neurons produces bursts.

Convergence

One of the surprising findings of this study was the number of cuneate cells (both cutaneous and proprioceptive related) that responded to more than one peripheral nerve stimulation (with facilitation and/or suppression). This high proportion of cells (87% of all cells recorded responded to more than one input) is likely to be due to the relatively strong stimulation (1.5 to 2× motor threshold for the motor nerves), which would have activated many fast-conducting sensory afferents within the stimulated nerve. This high level of convergence seems incompatible with the general functions proposed for the cuneate such as receptive field processing or sensory gating. However, similar high levels of convergence have been reported for identified cuneocerebellar cells in the cuneate and external cuneate nuclei of the cat (Cooke et al. 1971). Convergence also occurs in cuneothalamic cells, although not to the same extent as in cuneocerebellar cells (Cooke et al. 1971). Convergence has also been reported at the level of the thalamus in awake monkeys (Butler et al. 1992).

In this study we did not identify cuneate cells as thalamic or cerebellar projection neurons. It is possible that the cells that showed the highest levels of convergence were cuneocerebellar cells. Another possibility is that they are the interneurons underlying the widespread afferent inhibition reported previously (Biedenbach et al. 1971). The function of this convergence is unclear. An arrangement of afferent input, which allows a single cell to be strongly activated by the widely disparate inputs coming from different nerves, would seem incapable of coding the location of a stimulus with any accuracy. However, some natural stimuli activate cutaneous receptive fields over a large area: an example would be the moment when the hand contacts an object, which can lead to Pacinian receptor (RAII) activation over much of the forelimb (Westling and Johansson 1987). In this case, the key feature to encode is not the stimulus location, but the precise time of occurrence. The highly converging input patterns, which we observed in some cells, may be well suited to subserve this function.

**Representation of Input Patterns in Cuneate Firing**

Many studies on neural responses to external inputs use single punctuate stimuli, with an interstimulus interval chosen to minimize interactions between successive events. This yields information on aspects of input convergence, and on response amplitude, but it cannot show how cells process temporal information contained in the input. In the somatosensory system, timing information is important for several functions; this is especially the case in the primate hand. As already noted above, during a reach and grasp movement the moment of hand contact with an object signals a key transition in the motor program. Exploration of an object with the hand involves scanning the skin over the object, effectively converting spatial features of the surface into spatiotemporal patterns of discharge in cutaneous afferents (Johnson and Lamb 1981). In agreement with previous studies in cat (Gynther et al. 1995; Vickery et al. 1994), we observed that cuneate neurons can...
preserve temporal features of afferent input to high fidelity (Fig. 7, B and D); this could allow subsequent thalamic and cortical processing to take advantage of temporally coded information.

Previous work studied the fidelity of transmission between discharge of a single peripheral afferent, and a cuneate neuron receiving input from that fiber (Gynther et al. 1995; Vickery et al. 1994) using trains of stimuli at different frequencies. The assumption in that approach was that 1:1 locking between afferent and cuneate firing was the ideal and that deviations from this reflected a failure of the cuneate to transmit information effectively. This differs from the present approach using coherence analysis, which is better suited to measure transmission of subtle modulations in afferent discharge. Naturally many afferents will be active simultaneously, and even with a small degree of convergence 1:1 transmission will be impossible, given the limitations in maximal sustained firing rate. In this situation, we believe that coherence analysis provides a better representation of how complex modulations in afferent population discharge will be sent to upstream centers. Results from the two approaches are not easily related to one another. A train of pulsatile stimuli at a given frequency will have Fourier components at multiple harmonics of the train frequency, so that multiple bins of the coherence spectrum will relate to the ability of a cell to represent a given stimulus train. Single unit coherence values are typically rather low (generally less than 0.1), for reasons we have explored previously (Baker et al. 2003), although coherence with a population of similar units is considerably higher (Baker et al. 2003). The magnitudes of coherence that we report here therefore reflect high fidelity transmission of afferent modulations by a small population of cuneate units.

One of the best understood aspects of primate cutaneous processing is roughness perception. Connor et al. (1990) investigated several possible neural codes to account for psychophysical estimates of roughness. One option was to measure spatial variations in receptor activation across the skin surface; another was to detect temporal variations in activity. Using the former model, a measure of spatial variability, which was maximally sensitive to differences between points separated by 2.2 mm, yielded the best match to psychophysical data in all conditions; this is approximately the spacing of SAI receptor endings in the skin of the human fingertip. By contrast, to give good fits to the psychophysical data, the temporal variability model required measurement of variation over different time intervals, depending on the fingertip velocity. The authors therefore concluded that roughness perception was most likely to be mediated by assessment of spatial variability, a conclusion backed up by several subsequent studies (Connor and Johnson 1992; Yoshioka et al. 2001). Nevertheless, because subjects typically adopt a fairly constant fingertip velocity around 20 mm/s when assessing a novel tactile target (Vega-Bermudez et al. 1991), it is still possible that assessment of temporal variation over a fixed timescale (around 130 ms; Connor et al. 1990) could contribute to roughness perception. In the present data, the conditioning analysis (Fig. 11) characterized experimentally over what timescale cuneate neurons can measure activity differences. We showed that a stimulus could suppress responses to a subsequent stimulus. However, this suppression was greatest at short intervals and declined to zero for interstimulus intervals larger than 40 ms. This is far from the selective suppression at an interval of 130 ms required for the temporal variation model of Connor et al. (1990) to match psychophysical data. However, our data do show that temporal fluctuations in afferent input are preserved in cuneate discharge (Figs. 7 and 8), such that measurement of temporal
variation to support roughness perception could be performed further upstream of the cuneate, in thalamic, or cortical circuits.

A further source of temporal fluctuations in afferent input could be oscillatory activity endogenously generated within the nervous system. We have shown previously that synchronized oscillations around 15–30 Hz in the primary motor cortex (the β-band) are propagated to muscle via the corticospinal tract (Baker et al. 2003) and then carried back to the central nervous system by afferents (Baker et al. 2006). This leads to a bidirectional coupling between cortex and muscle at these frequencies (Riddle and Baker 2005; Witham et al. 2011; Witham et al. 2010), which may have implications for the functional role of this activity (Baker 2007). Lower frequency oscillations, around 10 Hz, are responsible for overt physiological tremor. Motor cortical oscillations at this frequency can also synchronize with muscle; in this case, the dominant pathway appears to be sensory feedback (Williams et al. 2009). The present results show that many cuneate neurons are well able to encode oscillations in these two physiologically important ranges (Fig. 7, B and D). However, cells that responded to stimulation with a suppression showed a marked fall in coherence for frequencies around 20 Hz (Fig. 7G), as did cells with proprioceptive receptive fields located in the external cuneate nucleus (Fig. 7F, black line). Although these neurons will be able to encode afferent fluctuations around tremor (10 Hz) frequencies, and at the lower end of the β-band, they will markedly attenuate inputs in the upper β-range (20–30 Hz).

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: C.L.W. and S.N.B. conception and design of research; C.L.W. and S.N.B. performed experiments; C.L.W. and S.N.B. analyzed data; C.L.W. and S.N.B. interpreted results of experiments; C.L.W. prepared figures; C.L.W. and S.N.B. drafted manuscript; C.L.W. and S.N.B. edited and revised manuscript; C.L.W. and S.N.B. approved final version of manuscript.

Fig. 11. Average conditioning effects. A: average conditioning effects for interactions between peripheral nerves. Columns show conditioning stimulus, and rows show test stimulus. Data are shown as the percentage of the control response (response to test stimulus for condition-test intervals between 400 and 512 ms) for ISIs between 0 and 100 ms. Thick black line shows effect when the cell is facilitated by the conditioning stimulus. Blue line shows effect when cell is suppressed by conditioning stimulus. Shaded areas show mean response ± SE. Thin black line marks 100% (i.e., response equal to control). B: self-conditioning effects for mechanical stimulation over cutaneous receptive fields.
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