Coding of Tactile Response Properties in the Rat Deep Cerebellar Nuclei

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INTRODUCTION

The cerebellum receives a large number of somatosensory afferents both from ascending spinal and trigeminal pathways as well as from descending cortical pathways via the pontine nuclei. In anesthetized rats, tactile stimulation of different body parts leads to strong cerebellar cortical responses in the granule cell layer of the cerebellar hemispheres (Kassel et al. 1984; Morissette and Bower 1996; Shambes et al. 1978) and overlying Purkinje cells (Bower and Woolston 1983). Strong sensory responses to perioral stimulation in the granule cell layer of crus IIa are also maintained in the freely moving awake rat (Hartmann and Bower 2001). The receptive field structure of these responses has been described as a fractured somatotopy (Kassel et al. 1984; Shambes et al. 1978). That is, the receptive fields of adjacent cerebellar cortical patches do not represent adjacent skin areas, and multiple patches exist for the same skin area (Bower and Woolston 1983; Shambes et al. 1978). In the paramedian lobule, input from paws, hindlimb, trunk, and perioral structures can be adjacent to each other, whereas in Crus IIA, a finer grained map of predominantly perioral structures is found (Shambes et al. 1978). Climbing fiber input to the same cerebellar cortical areas also shows small cutaneous receptive fields broken up into parasagittal zones (Ekerot and Larson 1980; Garwicz et al. 1998) or patches (Brown and Bower 2001) that do not represent a contiguous body map.

Cerebellar cortical activity does not control cerebellar output directly, as an additional processing stage is inserted in the deep cerebellar nuclei (DCN), the sole target of Purkinje cell output except for a small projection to the vestibular nuclei. In the DCN, collaterals of ascending mossy fibers and climbing fibers have an excitatory action via AMPA and N-methyl-D-aspartate (NMDA) receptors (Anchisi et al. 2001). In contrast, Purkinje cell input to DCN neurons relays the processed output from the cerebellar cortex via GABA_A inhibitory synapses, specialized to follow high-frequency Purkinje cell firing (Telgkamp et al. 2004). Primarily, DCN activity has been studied with respect to eye and limb movement execution. In behaving animals, a clear modulation in activity is present in relation to eyeblink responses (Gruart and Delgado-García 1994; Gruart et al. 2000), smooth eye movement and saccades (Gardner and Fuchs 1975; Hepp et al. 1982; Ohtsuka and Noda 1991), or limb movement (MacKay 1988; Thach 1968; Van Kan et al. 1993). Limb-movement-related activity is enhanced when movements are executed with respect to sensory inputs (Gao et al. 1996; Gibson et al. 1996). Fewer studies have examined sensory responses of DCN neurons. Consistent sensory responses were found for electrical nerve stimulation (Armstrong and Rawson 1979b; Armstrong et al. 1975; Eccles et al. 1974a), and tactile (Armstrong et al. 1975; Cody et al. 1981; Eccles et al. 1974a) stimulation. These responses are generally composed of a brief early spike response followed by a strong inhibition, which then may be followed by a late phase of increased activity. During the execution of trained behaviors, DCN neurons in monkeys exhibit sensory responses to visual, auditory, and somesthetic cues (Chapman et al. 1986). Previous studies of cutaneous responses in the DCN did not address their receptive field structure or the coding properties of tactile responses with respect to stimulus parameters such as intensity and duration. The primary goal of our study therefore was to determine how DCN responses compare with cerebellar...
cortical responses in their receptive field structure when matching stimulus conditions in the anesthetized rat preparation are used. Furthermore, we were interested in determining whether tactile responses were sensitive to the amplitude and duration of the stimulus because this aspect has not been previously investigated. To address these questions, we recorded responses in all three deep cerebellar nuclei to focal cutaneous air-puff stimulation with varying stimulus positions on the face and the limbs and in separate experiments with varying stimulus intensity or duration.

**Methods**

**Surgery**

All procedures were approved by the Institutional Animal Care and Use Committee of Emory University and were in accordance with National Institutes of Health guidelines. Twenty-one male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) of age 50–100 days (300–600 g) were anesthetized with a mixture of ketamine (100 mg/kg), xylazine (5.2 mg/kg), and acepromazine (1 mg/kg) administered intraperitoneally. After an initial injection, the anesthetic mixture was continually infused by a syringe pump at a rate sufficient to suppress the foot-withdrawal reflex of the animal. The rate was increased as needed during the experiment when the foot-withdrawal reflex became clearly noticeable with a strong toe pinch. The rate was decreased when the rate of the heart beat, which was digitally displayed and made audible through a speaker, slowed significantly. The animal’s body temperature was monitored using a rectal temperature probe and maintained at 36°C by feedback through a heating blanket. The animal’s head position was secured using a custom-built stereotaxic frame that allowed access to the orofacial area of the animal for air-puff stimulation. The skull overlying the cerebellum was removed and the dura underneath resected. A ring of the animal for air-puff stimulation. The skull overlying the cerebellum was removed and the dura underneath resected. A ring of

**Recording**

Glass electrodes were pulled from 1.2 mm OD capillaries with filament (World Precision Instruments, Sarasota, FL, Model No. TW120F-3) to a tip diameter of 5–10 μm (mean impedance = 5 MΩ). The electrodes were filled with 3% Chicago Sky Blue (Sigma-Aldrich, St. Louis, MO, No. C-8679) in 0.5 M sodium acetate for extracellular recordings from the deep cerebellar nuclei (DCN). Electrodes were lowered between 3 and 4 mm below the cerebellar cortical surface to reach the DCN. DCN neurons were provisionally identified by recording depth and their acoustic signature on the sound monitor. The signal was filtered between 300 Hz and 5 kHz and amplified 10,000 times using a differential AC amplifier (A-M Systems). A chloridized silver wire implanted subcutaneously behind the skull was used as reference electrode. The amplifier output was visualized on an oscilloscope and discrete trials of 3-s duration were digitized at a sampling rate of 10 kHz and stored on a PC using custom-written data-acquisition software. Neurons were selected for recording based on the emergence of single, identifiable spikes from the noise level on oscilloscope traces and an apparent responsiveness to ipsilateral upper-lip air puff stimulation. Neurons that showed no sign of a response to the stimulus were abandoned. This was the case for ~25% of all recordings. Recordings were also aborted when clear shifts in baseline firing properties were observed.

After recording from a neuron, the recording site was marked by iontophoresically ejecting Chicago Sky Blue dye with the recording electrode as positive and a subcutaneous silver wire as negative. Continuous current (5 μA for 5 min) was used to emit enough Chicago Sky Blue dye to result in a clearly identifiable blue dot of ~50–100 μm diam in histological sections. Only neurons with a histologically verified location in the deep cerebellar nuclei (see following text) were included in the data set.

In a smaller number of animals (n = 5), recordings of local field potential activity in the granule cell layer of crus IIA were obtained during spontaneous activity and with the same orofacial air-puff stimuli as used for DCN recordings. The signal from 5-MΩ tungsten microelectrodes (A-M Systems, Model No. 575300) was band-pass filtered between 1 and 500 Hz, amplified 1,000 times, and digitized at 10 kHz. The recording electrode was placed 4 mm laterally from the midline and in the granule cell layer in the center of crus IIA of each hemisphere. Electroencephalographic (EEG) recordings were also obtained from five animals in conjunction with DCN single-neuron recording. A skull screw (Fine Science Tools, 19010-00) was attached to a wire lead and implanted over contralateral frontal cortex. The reference potential for the EEG recording from this skull screw was obtained from chloridized silver wire inserted below the skin of the neck. The signal was band-pass filtered between 1 and 500 Hz and digitized at 10 kHz.

**Histology**

At the end of each experiment, animals received an ip injection of 1 ml of Nembutal (50 mg/ml, Abbott Laboratories, North Chicago, IL). After 10 min, the absence of all deep reflexes was verified, and animals were perfused transcardially with a solution of 15% sucrose in 10% phosphate buffered formalin (No. SF100-4, Fisher Scientific, Pittsburgh, PA). The cerebellum was removed and placed in this same solution for 24 h and then transferred to a 30% sucrose +10% phosphate buffered formalin solution for an additional 24 h. The cerebellum was sectioned in 50-μm horizontal slices using a Reichert-Jung Kryostat (2800 Frigocut-E, Leica Microsystems, Bannockburn, IL) and sequentially mounted onto microscope slides. The boundaries of the nuclei were apparent in fresh tissue, as were the Chicago Sky blue marks. A mild cresyl violet counterstain was applied to enhance the visibility of distinct nuclei. Recording locations were identified as being in a particular nucleus based on the location of the blw marker with respect to cresyl violet stained nuclei. Those slices that contained blue markers inside the nuclei were digitally photographed and stored on a PC. All chemicals were obtained from Sigma Chemical unless otherwise noted.

**Stimulation protocols**

Air-puff stimulation was used throughout as a convenient means to cause precisely timed skin indentation of adjustable amplitude in different positions. This type of stimulation has been shown to be a reliable and convenient method to exert controlled pressure pulses on the skin (Hashimoto 1999). Air puffs were applied via Pasteur pipettes (tip diameter = 1 mm) with the tip placed 2–4 mm from the skin surface. A Picospritzer III (Parker Instrumentation, Fairfield, NJ) was used to regulate air-supply pressure and to control the air-puff timing via digital pulses to the external valve controller. This instrument allows the timing of valve control with a 1-ms time resolution. The time to full opening/closing of the valve is specified as 2.5 ms by the manufacturer. Air-supply pressure was adjusted between 10 and 50 psi. These supply pressures corresponded to the following gram weights of static air pressure when the tip of the stimulation pipette was directed onto an electronic balance at a distance of 3 mm: 10 psi: 0.29 g; 20 psi: 0.49 g; 30 psi: 0.95 g; 40 psi: 1.56 g; and 50 psi: 1.85 g. A delay between triggering the Picospritzer and the onset of the air puff was given by the movement of the air puff through the 10-in length of access tubing. This delay was measured as 5 ms by puffing onto the shaft of a microelectrode, which evoked an immediate microphonic response. An ultra-high-speed video (500 frames/s) of
the air-puff stimulation on the face showed a primary indentation of the skin subjacent to the stimulus pipette (see supplementary on-line material). A wider circle of skin of ~1–2 cm diameter showed a 10-ms lasting ripple due to the elastomeric properties of the stimulated skin, which induced a brief deflection of some macrovibrissae. This secondary response and whisker deflection was not visible by eye, however. A direct whisker deflection by air-movement was carefully avoided. A quick mechanical advance of the stimulation pipette to indent the lip showed a similar sequence of primary indentation and secondary brief spread of a ripple and whisker deflection when examined by high-speed video. We chose air puffs over the use of a mechanical tapper because the latter are typically bulky and relatively immobile, which makes them unfit for multiple relocations while holding a single cell recording. The Picospritzer valves also produced an audible click with each air puff. To determine whether a possible auditory component of observed neural responses was present, the air-puff pipette was turned away from the animal while the valve was kept in the same place. In all cases, this control stimulus led to a complete cessation of field-potential responses in the granule cell layer (n = 10) and single-neuron responses in the deep cerebellar nuclei (n = 4).

In a first set of experiments DCN neurons were recorded while the air-puff stimulus was varied among six different locations on the animal’s body: ipsi- and contralateral upper lip, lower lip, and forepaw. All paw stimulation was performed on the dorsal hairy surface of the paw. An air puff duration of 5 ms and an intensity of 30 psi supply air pressure were kept constant during these “location” experiments. For each location, a block of 50 trials of DCN activity was recorded, in which 0.5-s prestimulus and 2.5-s poststimulus activity were acquired. In a second set of experiments, the stimulus location was kept constant at the ipsilateral upper lip, while the intensity of 5-ms air-puffs was varied among 10, 20, 30, 40, and 50 psi. In a third set of experiments, the stimulus duration was switched between 5- and 500-ms duration while the stimulus location was held constant at the ipsilateral upper lip and the intensity at 30 psi air supply pressure. For each recording, the sequence of different stimuli used was randomized using a random number generator and blocks of 50 trials were obtained for each stimulus condition.

**Data analysis**

Spike times were extracted from analog data off-line using a software-based window discrimination procedure. The isolation of spikes in the raw data signal was generally excellent (see Fig. 2A for typical signal) due to the fine tip and high impedance of the electrodes used and manual optimization of electrode positioning for maximal signal-to-noise ratio. Spontaneous activity was analyzed from 10 trials of 10-s duration without air-puff stimulation. These data were used to construct inter-spike interval (ISI) and autocorrelation histograms and to analyze mean firing rates and spike irregularity measured by the coefficient of variation (CV) of ISIs. Peristimulus time (PST) histograms were constructed from recorded blocks of 50 trials for each stimulus condition for the analysis of sensory responses. To perform a statistical analysis of spike rate changes with respect to air-puff stimulation, an analog representation of each spike train was constructed using Gaussian local rate coding (Paulin 1995). In this method, each spike in a trial is first convolved with a Gaussian with an area of 1.0 and a specific width (time between peak of Gaussian and decay to 1/e of peak amplitude), and then the sum of all Gaussians is obtained to result in an analog trace representing instantaneous spike rate. To identify statistically significant responses to air-puff stimuli, we looked for poststimulus excursions of the mean instantaneous frequency that exceeded three times the value of the mean SDs of the prestimulus period. The use of this method allowed a statistical comparison of poststimulus response amplitudes and durations across cells and stimulus conditions. The choice of width of the Gaussian determines the time window over which each spike contributes to the analog representation of instantaneous spike rate. We used a width of 1, 5, and 20 ms to determine the occurrence of spike rate changes of short, intermediate or long durations, respectively (see RESULTS). Different Gaussian widths were required to determine the presence of responses of different durations because the choice of width is analogous to a filter that represents spike rate changes around a limited range of durations (Fig. 3B).

**RESULTS**

**Anatomical location of recorded neurons**

We recorded neurons from all three DCN to be able to compare response properties to tactile stimulation between different functional cerebellar circuits. For each of our sets of experiments, varying stimulus location, intensity, and duration, neurons were sampled from most areas of each nucleus (Fig. 1), with the exception of the medial part of the nucleus interpositus. A total of 59 neurons were recorded with full data sets consisting of 50 trials for each stimulus condition.
Spontaneous activity of DCN neurons recorded in ketamine-anesthetized rats

All neurons recorded showed a high level of baseline activity with an overall mean spike rate of 55 Hz. The spike patterns for individual neurons ranged from ongoing irregular (Fig. 2A, left) to strongly bursting (Fig. 2A, right). These patterns did not represent two distinct classes of neurons, however, as a continuous range of spontaneous firing rates and CV was present (Fig. 2E). Furthermore, there was no correlation between mean firing rate and bursting as measured by the CV (Fig. 2F). The CV was found to be a reliable indicator of burstiness in these neurons, as high CVs (>1) were caused by the large increase in SD of ISIs when pauses between bursts were present. The ISI distribution of all neurons showed a modal interval of <20 ms and a pronounced tail of longer intervals (Fig. 2B), regardless of the presence or absence of bursting. Autocorrelograms of spontaneous activity did not show prominent side-peaks (Fig. 2C), indicating the absence of regularly spaced spikes or bursts of spikes. To assess possible differences among the three nuclei in spontaneous activity patterns, we calculated a one-way ANOVA of mean spike rates and ISI CVs across neurons with nucleus as a factor (Fig. 2D). This analysis revealed that neurons in the medial nucleus showed a statistically significant slower mean spike rate than interpositus and lateral nuclei and a significantly lower CV than the lateral nucleus (P < 0.05). Nevertheless, the overall firing properties in all three nuclei were quite similar. The burst pattern observed appeared similar to recordings previously obtained in subthalamic nucleus (Magill et al. 2000) and globus pallidus (Goldberg et al. 2003) in ketamine-xylazine-anesthetized rats. This pattern is characterized by a wide-spread synchrony of unit activity with cortical slow-wave EEG oscillations that are globally synchronous in both hemispheres. To examine the relation between EEG and DCN activity, we obtained a sample of simultaneous EEG and DCN single-unit recordings (n = 14). We found that activity in the DCN showed a significant peak in the cross-correlation with EEG (Fig. 2G) and was thus strongly coupled to cerebral cortical activity. Specifically, DCN neurons showed pauses in firing when the EEG was depolarized; this coincides with cortical spiking activity (Contreras and Steriade 1995).

FIG. 2. The spontaneous activity of DCN neurons recorded in ketamine-anesthetized rats. A: extracellular recordings from 2 typical neurons from our sample are shown. Left: neuron fires continuously; right: neuron fires in clearly separated bursts. B: interspike interval (ISI) plots of spike activity for the neurons shown in A. The ISI plots were computed from 10 trials of 10 s each of spontaneous activity for each neuron before stimulation trials began. Bursty neurons showed a higher coefficient of variation (CV) than continuously firing ones due to the large SD of ISI duration caused by long pauses and fast intraburst firing. Note that the overall mean firing rate of the bursty and nonbursty neuron was almost identical. C: autocorrelograms of spontaneous activity for each of the neurons shown in A. (See results for description.) D: the population mean rate and CV of spontaneous spiking is shown separately for neurons from each nucleus. The mean frequency of each neuron was calculated by averaging its spike rate during 10 trials of 10 s each of spontaneous activity recorded before stimulation trials began. Statistical comparisons of the mean frequency and CV of neurons in the 3 nuclei were determined by 1-way ANOVA and Tukey-Kramer’s post hoc analysis. *, significance level of P < 0.05 was reached for the comparison indicated by the lines above. All other comparisons were not significant. E: the histograms of mean frequencies and mean ISI CVs across neurons show unimodal distributions, which passed the Kolmogorov-Smirnov test for normality (P > 0.05). This indicates that subpopulations of recorded neurons cannot be identified based on spontaneous activity patterns. F: a scatter plot of frequency vs. CV and a linear correlation analysis revealed no statistically significant correlation (P > 0.05, line indicates slope of linear correlation) between these variables, indicating that the degree of burstiness was not related to mean spike rate. G: electroencephalographic (EEG) recordings from contralateral frontal cortex were obtained simultaneously with DCN neuron recordings (see METHODS). A cross-correlation analysis was performed between a gaussian analog representation of DCN spike trains (10-ms Gaussian width, see METHODS and Fig. 3B) and the EEG recording. Ten simultaneous EEG and DCN unit traces of 10-s duration were used for computing the cross-correlation function. The trough at the center of this cross-correlogram denotes a decrease in DCN spike rate when the cortical EEG was depolarized. The gray bar indicates the 99% confidence interval of the cross-correlation. Cross-correlations and confidence limits were computed with the Matlab xcov function.
This result suggests that cortico-pontine mossy fiber input during cortical slow wave activity has a net inhibitory effect on DCN activity, presumably via driving inhibitory Purkinje cell input.

**Cells in all nuclei respond to cutaneous input with three distinct response components**

An early study of nucleus interpositus responses to cutaneous nerve stimulation in chloralose anesthetized cats showed the presence of three distinct response components that could occur independently from each other: an increase in spiking at a short latency of 5–35 ms, an intermediate inhibition, and a late increase in firing at 50–500 ms (Armstrong et al. 1975). These three distinct response phases were also seen in nucleus interpositus recordings from awake cats with the same electrical nerve stimulation (Armstrong and Rawson 1979b) as well as with mechanical tapping on the foot pads (Cody et al. 1981). We found that the responses to air-puff stimulation in our recordings from ketamine-xylazine-anesthetized rats generally consisted of the same three response components (Fig. 3). As observed in the earlier studies we found that short- and long-latency increases in spiking as well as an intermediate-latency inhibition could occur in varying combinations. Figure 3 shows examples of the inhibitory response in combination with or without early and late increases in spiking. In some cases, we observed a biphasic expression of the inhibitory period (Fig. 3A4), suggesting that it may be due to multiple mechanisms or that an intervening excitation may occur. Although the latency and duration windows of the three response components were distinct from each other, the exact onset timing and response duration of each component could also differ among neurons. The examples in Fig. 3 highlight the typical range in the duration of the inhibitory response (3A, 1 vs. 3) and the late increase in spiking (3A, 2 vs. 3). To visualize the relation between the phase in the burst period of bursty DCN neurons and the expression of tactile responses, we sorted the spike trains in Fig. 3 with respect to the number of spikes in the 100 ms preceding the tactile stimulus. This manipulation put spike trains that were between bursts at the time of stimulation at the top of the raster plots. A visual inspection of raster plots shows that the expression and timing of response components was generally not dependent on the level of spontaneous spiking at the time of stimulation (Fig. 3A, 1–6).

For statistical analysis of responses to air-puff stimulation, we first represented the spike raster histograms as analog traces by convolving each spike with a Gaussian (see METHODS for details). The resulting traces represent the average instantaneous spike rate across all trials without any binning artifacts. Convolving spikes with narrow Gaussians leads to an analog representation of spike rate that follows fast responses well but masks long-lasting shallow responses with fast frequency noise (Fig. 3B). In contrast, convolving with wider Gaussians leads to a significant smoothing of the frequency response, which leads to a better discrimination of longer responses while sacrificing the detection of short responses (Fig. 3B). Thus we used Gaussian filters of 1-, 5-, or 20-ms width to detect responses of increasing duration (see METHODS for details).

Previous studies on responses to cutaneous inputs from the limbs in the DCN exclusively used recordings from nucleus interpositus because this nucleus is most directly involved in the control of limb movement (Martin et al. 2000). In contrast, we compared responses between all three nuclei to examine the hypothesis that responses in different nuclei will show significant differences. Given the different functional circuits in which each nucleus is embedded (Buissere-Delmas and Aung 1993), we were surprised to find that a large proportion of neurons in all nuclei showed robust responses to air-puff stimulation consisting of the same three components described in the preceding text (Fig. 4). For all nuclei, the most common response component was the inhibition, which was observed in almost all neurons showing any response. The short-latency excitation was also expressed by a large majority of neurons in the medial nucleus and interposed nuclei, but was less frequent in the lateral nucleus (Fig. 4). Finally, the long-latency excitation was the least-common response component in all nuclei. It should be noted that neurons required a visible response on oscilloscope traces to be recorded with a full set of stimuli. This selection criteria may have created a bias in the final distribution of response components observed.

To determine whether individual response components differed among the three nuclei, we compared the time of onset and duration of each response component in each nucleus. Figure 5A shows bar graphs of the mean time of onset and mean duration of response for each response component in neurons of each nucleus. To avoid duplicate measures for each neuron, the response characteristics were evaluated only for one stimulus location and amplitude, which was applied to all neurons. This stimulus consisted of an air puff at the ipsilateral upper lip with an intensity of 30 psi supply air pressure and a duration of 5 ms. The response statistics shown in Fig. 5 thus encompass only a portion of the responses summarized in Fig. 4. The time of onset and duration of each response component were statistically compared across nuclei using one-way ANOVAs. No systematic differences in response components among the nuclei were observed, except for a statistically significant difference in the mean duration of the short-latency excitation between the interposed and lateral nuclei (P < 0.05).
It should be noted, however, that in all nuclei this response was extremely brief (<2 ms) and generally consisted of one or two additional spikes aligned with the stimulus trigger at a delay of 15 ms.

The relation between bursting properties in the spontaneous activity and tactile responses was analyzed by plotting the onset time and duration of responses against the CV of spontaneous spiking, which itself was a good measure of bursting (see preceding text). The results show no relationship between CV and tactile response properties for inhibitory responses (Fig. 5B) or excitatory responses (not shown). Therefore the timing of responses was not significantly influenced by the presence of more or less pronounced DCN bursting.

**In each nucleus, cells responded to stimulation of a large area of the body**

Cutaneous receptive fields in the cerebellar granule cell layer are small and distributed in discontinuous patches representing specific skin areas, such as a small area on the upper lip or forepaw (Shambes et al. 1978). Multiple patches of granule cells and overlying Purkinje cells may be activated by a single stimulus location (Bower and Woolston 1983; Shambes et al. 1978). Deep cerebellar nuclei neurons receive input from mossy fiber and climbing fiber collaterals and from Purkinje cells. In this study, we investigated the receptive field structure of DCN neurons to cutaneous stimulation to determine whether it is similar to cerebellar cortical receptive fields or suggestive of a major reorganization of the somatotopic representation compared with cerebellar cortical receptive fields. Furthermore...
we compared the receptive field structure across the three nuclei.

To address these questions, we recorded from individual DCN neurons during air-puff stimulation to the skin at six different locations on the face and limbs (see METHODS). We found that DCN neurons exhibited surprisingly large bilateral receptive fields spanning multiple areas of the body surface (Figs. 6 and 7). Examples of these types of consistent responses are shown in Fig. 6 to the four orofacial locations used (ipsi- and contralateral upper and lower lips) as well as to ipsi- and contralateral forepaw stimulation. Within these extended receptive fields, the response properties of each neuron remained remarkably constant. In particular, each neuron displayed its specific combination of one or more of the three major response components described above throughout its receptive field (Fig. 6). Comparing the receptive field distributions between nuclei for each response component indicated that all three nuclei were similar in their somatotopic representation (Fig. 7). In particular, in each nucleus all response components showed large receptive field areas encompassing ipsilateral and contralateral facial areas and forepaws.

**Representation of stimulus intensity and duration in DCN responses to air-puff stimulation on the ipsilateral upper lip**

Little is known about the extent to which the cerebellum relies on specific parameters of tactile input such as stimulus intensity or duration in the control of movement. To examine whether such stimulus parameters are reflected in the response properties of DCN neurons, we systematically varied the intensity of the air-puff stimulus between 10 and 50 psi air supply pressure (see METHODS), and in another set we switched air-puff duration between 5 and 500 ms. In general it was striking that similar response patterns were obtained for all stimulation amplitudes (Fig. 8A, neuron 1), though in some neurons the response was clearly diminished for the weakest stimulation (Fig. 8A, neuron 2). We quantified the amplitude of each response component (short- and long-latency increase in activity as well as inhibition) for each stimulus intensity by normalizing the response components of each neuron to the amplitude observed at the highest stimulation intensity ($n = 18$ neurons). The resulting normalized response amplitudes increased with stimulus amplitudes ($P < 0.05$, Fig. 8B), indicating that some information about stimulus intensity was retained in DCN responses. We also examined whether the duration of response components found by our algorithm was a function of stimulus intensity, but found no significant relation (Fig. 8C).

In additional experiments, we recorded field potential responses in the granule cell layer of crus IIa to determine whether response amplitude coding in the DCN is comparable to that in the input layer of cerebellar cortex. Similar to the DCN responses, we found a statistically significant change in amplitude ($P < 0.05$) in the averaged normalized responses for 10 versus 50 psi stimuli (Fig. 9B). However, the variability in single trial responses was far greater than the response differences for different stimulus intensities (not shown), which

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**FIG. 6.** Cells responded to stimulation of a large area of the body. PST histograms for 3 sample neurons, with cutaneous stimulation at our 6 stimulation sites are shown (50 trials for each condition). ↑, stimulation time. Each neuron displayed a specific combination of response components that remained stable for different stimulus locations, though the response could be weaker for the contralateral forepaw.

**FIG. 7.** Distribution of response components in each nucleus for different stimulation sites. For each nucleus, the number of neurons displaying a particular response component at each stimulation site is represented by a proportionally sized dot. The total number of neurons recorded in each nucleus for this stimulation set is denoted by the $n$’s in the header strings.

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FIG. 8. The representation of stimulus intensity in DCN responses to air-puff stimulation on the ipsilateral upper lip. A: PST histograms from 2 sample neurons responding to 5 stimulus intensities (50 trials for each condition). In neuron 2, responses appear weaker at the lowest stimulus intensity, but the time course of responses generally remained unchanged. B: For each stimulus intensity, response amplitudes were calculated the following way: first, neurons that did not show a significant response with the 50 psi stimulus were discarded. (The fraction of neurons with such significant responses at 50 psi is indicated on the right side). Second, the response period determined for the 50 psi stimulus was analyzed for spike rate changes compared with baseline at all lower stimulus intensities regardless of whether our algorithm had identified a significant response at these lower stimulus intensities or not. Third, spike rate changes compared with baseline (response amplitudes) were normalized to 1.0 for the 50 psi stimulus to examine relative differences in response amplitudes across stimulus intensities. The analysis was performed in this way to include data for all intensities for all responsive neurons in a uniform manner. One-way repeated-measures ANOVAs were performed on the response amplitudes prior to normalization to test for differences in the mean amplitude of each response component. This analysis showed significant increases in response amplitude with increasing stimulus intensity for all response components. The rows of markers above each bar are arranged to indicate the results of tests for statistically significant differences in pairwise comparisons. Asterisks are used to indicate significant differences ($P < 0.05$), and dashes are used to indicate differences that did not reach statistical significance ($P > 0.05$). The top row of markers indicates the results of pairwise comparisons between the corresponding stimulus intensity and the 50 psi stimulus condition. Lower rows of markers indicate the results of pairwise comparisons with progressively lower stimulus intensities. C: The response durations for each stimulus intensity were taken from our Gaussian rate coding algorithm (see METHODS). The plotted mean durations are normalized to 1.0 for the 50 psi stimulus. One-way repeated-measures ANOVAs performed on the data prior to normalization did not reveal a statistically significant influence of stimulus intensity on response duration. The fractions above each bar denote the number of neurons that showed a significant response at the stimulus intensity indicated. Only these responses are included in the analysis.
precluded a reliable coding of stimulus amplitude by single trial field potential responses.

Similar analyses as for stimulus intensity were performed to compare responses between stimuli of 5- and 500-ms duration (n = 17 neurons). In the majority of neurons (n = 14), the response was not significantly affected by stimulus duration, and a complex response pattern was evoked even by a 5-ms stimulus (Fig. 10A, neuron 1). In one neuron, we observed a prolonged duration of inhibition that matched the time course of the 500-ms stimulus (Fig. 10A, neuron 2), whereas another small subpopulation (n = 2) showed a distinct offset response (Fig. 10A, neuron 3). For the whole population of neurons, a small but significant increase in response amplitude was found for the inhibitory response and long latency increase in activity with the 500-ms stimuli (Fig. 10B). The response duration did not change significantly across the population for any response component. (Fig. 10B). Overall, these data indicate that stimulus duration is not coded reliably by response duration but that a longer stimulus could result in a stronger response. However, it should be noted that our 5-ms stimulus condition may also be diminished in intensity due to incomplete build-up of air pressure tubing leading to the rat during this short stimulation time.

**Distinct and unvarying temporal profile of DCN responses to tactile inputs**

The preceding analyses suggest that responses of DCN neurons to tactile stimulation have a relatively fixed temporal profile regardless of stimulus location, intensity or duration. If DCN neurons were involved in generating temporal signals for patterns of muscle activations, one would expect that different neurons would show distinct temporal response profiles that were independent of the stimulus condition. We analyzed the differences in temporal response profiles to the same stimulus between neurons and compared this between-neuron variability with the within-neuron variability in the response to different stimuli (Fig. 11). To perform this analysis, we again first obtained analog traces of the instantaneous spike rate by convolving spikes with gaussians. We then normalized the firing rate of each condition to 1.0 for the baseline period before stimulus onset. In Fig. 11A, we show that the spike rate profile of a single neuron to stimulation of different locations on the body is almost identical. We then constructed a SD trace for the response profiles to six stimulus locations for each of the recorded neurons (n = 24). The average of the SD traces (Fig. 11A, 2nd panel) calculated for all neurons shows that for the whole population very little variability was present in the response profiles of individual neurons during the response period. The same analysis was performed for neurons subjected to different stimulus intensities (3rd panel) or duration (4th panel), and again the average SD of the spike rate profile for the poststimulus time period for individual neurons shows little variability in responses (Fig. 11A). A small increase in variability at 1 s for the duration condition is related to the offset response induced at this time by the 500-ms stimulus in some neurons, which creates a difference in the spike rate profile compared with the 5-ms stimulus condition. Nevertheless, the unvarying temporal response profiles between stimulus conditions are in contrast to significant differences in response profiles between neurons for the same stimulus condition (Fig. 11B). These differences are visible in the spike-rate profiles for five different sample neurons to ipsilateral lower-lip stimulation (Fig. 11B, top) and are confirmed for the population by constructing an average of the SD trace of 24 neurons for different stimulus locations (Fig. 11B, 2nd panel). Similarly, different neurons recorded for stimulus intensity (n = 18, 3rd panel) or duration (n = 17, 4th panel) experiments show different response profiles. The SD traces for the populations in each case show an early peak reflecting variability in the early increase in spiking, as well as a prolonged deviation reflecting variability in inhibition and long latency increases in spiking. These data indicate that different response profiles are present between neurons throughout 500 ms after a brief 5-ms air puff.

**Discussion**

We examined tactile responses at the output level of the cerebellum using air-puff stimuli in the anesthetized rat. Because similar stimuli have been used extensively with cerebellar cortical recordings in the same preparation, this experimental design allowed us to compare the representation of sensory input...
between cerebellar cortex and the deep cerebellar nuclei and thus to address potential transformations of tactile information in the cerebellar circuitry. We found that tactile receptive fields in all three DCN were bilateral and generally covered both facial and forepaw areas. Responses to 5-ms brief stimulation had an extended temporal profile consisting of a combination of short- and long-latency increases of activity with an intervening period of inhibition. This temporal profile was nearly constant for individual neurons across different stimulus intensities and durations. Similar response properties were found in all three DCN. These tactile responses are indeed quite different from those observed in cerebellar cortex and suggest a fundamental recoding of tactile information at the output stage of the cerebellum.

Spontaneous activity in the DCN

One concern in the use of anesthetized preparations is that the spontaneous activity and responsiveness of neurons may be highly dependent on the anesthesia used. Fortunately, recordings in the DCN have been previously obtained for different anesthetics and in awake animals, and the results give benchmarks to compare our recorded activity against. In general, spontaneous activity rates of DCN neurons have been reported to be high. In awake rats, a mean spike rate of 29 Hz was found at an age of 12–19 days, which increased to 43 Hz at an age of 20–26 days (LeDoux et al. 1998). In awake cats, mean spike rates of 34 (Armstrong and Rawson 1979a) or 41 Hz (Cody et al. 1981) have been observed. Chloralose or pentothal anesthesia in cats is associated with a substantial slowing of spontaneous activity to 10 Hz (Eccles et al. 1974a). Our result with ketamine-xylazine anesthesia in rats showed a mean rate of 55 Hz at an age of 50–100 days; this is consistent with the expected rate in awake rats at this age. The activity pattern of many neurons in our data showed pauses and bursts, which has not been described for the awake condition. These pauses and bursts were highly correlated to the prominent slow-wave cortical EEG activity, indicating that they are part of the global
synchronous activity patterns found in ketamine-xylazine anesthesia (Goldberg et al. 2003; Magill et al. 2000). Robust sensory responses were evoked at all phases of this bursty activity, however, and no dependence of response properties on this activity pattern was found. The DCN are known to contain at least three different populations of neurons: Large glutamatergic projection neurons constitute the largest population of neurons and connect to multiple targets including the red nucleus and thalamus; medium-sized GABAergic projection neurons form a second large population of neurons and connect to the inferior olive; and finally a much smaller population of neurons consists of small local inhibitory interneurons that are mostly glycinergic (Batini et al. 1992; Fredette and Mugnaini 1991; Palkovits et al. 1977; Sultan et al. 2002; Teune et al. 1995). We did not find clusters of neurons with distinct properties in spike shape, spontaneous activity, or stimulation responses. It is quite possible that we never recorded from interneurons due to sampling bias against small and rare neurons. It seems unlikely that we would not have data from both types of projection neurons in our sample, unless one type was generally quiescent. To our knowledge, none of the existing in vivo electrophysiological studies have found distinctions between two distinct populations of neurons. Thus the general firing and response properties of excitatory and inhibitory projection neurons may be similar, although only a combined recording and juxta- positional staining study could resolve this issue with certainty.

**FIG. 11.** Each neuron had a distinct and unvarying temporal profile to cutaneous inputs. The variability in response patterns of single neurons to different stimulus conditions was observed to be lower than the variability in response patterns of different neurons to a single stimulus. A, top: responses to our 6 different stimulation sites of a single neuron are superimposed. The traces represent instantaneous spike frequency obtained with our local Gaussian rate coding algorithm after normalization to 1.0 during the control period. A Gaussian width of 5 ms was used throughout. SD traces were computed from the 6 normalized traces of different stimulus sites for 24 neurons and averaged (2nd panel). Similarly, averaged STD traces across stimulus conditions varying with respect to stimulus intensity or duration are plotted in the 2 bottom panels. B: the same analyses as in A were performed across neurons. Responses to ipsilateral lower lip stimulation are shown for 5 sample neurons in the top panel. A SD trace was computed for each stimulus condition across neurons, and the average of the STD traces is shown. The variability of responses across neurons shown is much larger than the variability across stimulus conditions shown in A.
Three distinct response components in air-puff responses

Previous examinations of tactile responses in DCN neurons were restricted to stimulation of a single nerve or small cutaneous areas while recording from a single nucleus. In general, these studies revealed the same pattern of three major response components that we found, namely a short-latency increase in activity followed by inhibition and, in some neurons, by an increase of activity at a long latency. A set of early studies focused on the short latency activity increase and inhibition in the decerebrate cat preparation and delineated possible ascending pathways mediating these responses in the fastigial nucleus by analyzing delays and using direct electrical brain stem stimulation in distinct structures (Eccles et al. 1974a,b). The authors concluded that short-latency increases in activity in fastigial neurons after sensory stimulation are due to two components mediated by excitatory input from the lateral reticular nucleus and the inferior olive. They ascribe inhibitory responses of fastigial neurons both to mossy and climbing fiber input to the cerebellar cortex, which in turn leads to Purkinje cell excitation and DCN inhibition. The long-latency increases of firing after stimulation were examined in some detail in a later study (Armstrong et al. 1975), which revealed that such increases of firing at latencies from 50 to 500 ms could occur independently of previous short-term latency responses. These response properties were mimicked by direct electrical stimulation in the inferior olive, and they concluded that delayed activity in the DCN was most likely due to disinhibition caused by pauses in Purkinje cell activity following late olivary inputs. Our study confirms the presence of these three distinct response components in response to tactile inputs in all three DCN. The similarity in response properties with earlier studies using anesthetized and awake cats indicates that the responses we observed were not specific to ketamine-xylazine-anesthetized rats.

Receptive fields in cerebellar cortex and the DCN

Our study was designed so that receptive fields of DCN neurons could be compared with the existing body of data analyzing receptive fields in cerebellar cortex established through cutaneous stimulation in anesthetized rats. The representation of body surface in the granule cell layer in anesthetized rats has been described as a “fractured somatotopy.” Individual patches in the granule cell layer have a small receptive field representing for instance a small area of forepaw or the ipsilateral upper lip. Adjacent patches can represent disjoint areas on the body surface, and multiple patches exist for each body area (Shambes et al. 1978). Similar results were found in anesthetized cats (Kassel et al. 1984). Excitatory responses of Purkinje cells were found to have a receptive field generally matching that of the underlying granule cell layer (Bower and Woolston 1983). Using the same stimulation design in crus IIa of anesthetized rats, it was found that the most sensitive receptive field of climbing fiber responses matches that of field potential responses in the underlying granule cell layer (Brown and Bower 2001). However, secondary weak contralateral and larger climbing fiber receptive fields were also present. In a different set of studies, climbing fiber input to cerebellar cortex was found to be organized in parasagittal zones and microzones in both cat (Garwicz et al. 1998) and rat (Jörntell et al. 2000) based on zonal anatomical projection patterns from the inferior olive (Buïsseret-Delmas and Angaut 1993). More recently it was found that Purkinje cell receptive fields are plastic based on climbing fiber input patterns (Jörntell and Ekerot 2002) and that excitatory and inhibitory receptive fields have a different structure that both relate to the local climbing fiber receptive field (Ekerot and Jörntell 2001). Although the exact alignment of Purkinje cell receptive fields and their zonal or patchy organization remains somewhat controversial, the literature is in agreement that receptive fields in cerebellar cortex are small, and that multiple patches or microzones represent the same body surface. This organization is in stark contrast to our findings in the DCN, where single neurons in all areas respond to a large portion of the body surface. Our finding is somewhat surprising given a reported lack of divergence in the cortico-nuclear projection (Garwicz et al. 1996). Due to the fractured somatotopy in cerebellar cortex, however, input from a relatively small area of cortex would be sufficient to lead to the large receptive fields seen in the nucleus. Convergence of input from multiple cerebellar cortical areas to single areas in the DCN has been demonstrated in an anterograde corticoinuclear labeling study (Pantò et al. 2001). Another significant origin of large receptive fields could be given by direct climbing fiber input and mossy fiber input to the DCN, which show considerable divergence and bilateral projections patterns in the nuclei (Parenti et al. 2002; Shinoda et al. 2000; Sugihara et al. 1999; Wu et al. 1999). It should also be noted that with the exception of the short-latency excitation, our observed responses to air-puff stimulation had a late time of onset that make direct ascending sensory pathways to the cerebellum an unlikely candidate as causing their presence. Rather, such late responses could be expected to be the result of multiple stages of processing, which might well include cerebral cortex because it constitutes a major source of cerebellar input via pontine mossy fibers and can also influence olivary input to the cerebellum (Brown and Bower 2002). Our study did not address the pathways that cause DCN activation in response to tactile stimulation but overall indicate that the tactile representation in the DCN is fundamentally different in comparison to cerebellar cortex in that each neuron in the DCN can react to input from large parts of the body surface. This makes sense if DCN activity is organized along motor control coordinates of some fashion, such that movements of different muscle groups or with different functions (e.g., posture vs. grasping) can all be influenced by and coordinated with cutaneous sensory feedback. Behaviorally, it is readily apparent that a stimulus at a single site (for example an air-puff in the face) can lead to the interruption of and reorganization of the entire body’s movement (Cooke and Graziano 2003).

Temporal profiles of DCN responses and possible relation to motor timing

In examining the responses displayed by different DCN neurons, we found that they generally showed a temporal profile extending far beyond the offset of stimulation. Furthermore, we found that the temporal response properties of individual neurons remained relatively stable in the face of changing location, intensity, or duration of sensory stimuli. These findings are consistent with the often expressed hypoth-
assumes that the cerebellum is involved in the adaptive control of precise timing of motor output and the timing of perceptive and cognitive processes as well. This hypothesis is based on the considerable amount of evidence showing that cerebellar lesions produce disruptions in the timing of specific behaviors (Ivry et al. 2002) and that cerebello-olivary interactions allow the generation of precisely timed output patterns (Yarom and Cohen 2002). Examples of behaviors disrupted by cerebellar lesions include both motor and predictive behaviors, such as finger tapping (Ivry and Keele 1989) and eye-blink conditioning (Koekkoek et al. 2003; Perrett et al. 1993), as well as cognitive behaviors, such as speech perception (Ackermann et al. 1997; Mathiak et al. 2002) and time discrimination (Breukeelaar and Dalrymple-Alford 1999; Mangels et al. 1998; Nichelli et al. 1996). Our findings are consistent with the idea that DCN neurons may constitute temporal pattern generators that can contribute to the precise temporal control of motor or cognitive events. The idea of cerebellar output as a temporal pattern generator has been employed with variations in several influential theories of cerebellar function (Barto et al. 1999; Houk 1987; Houk et al. 1996; Medina and Mausk 2000; Medina et al. 2000; Ulloa et al. 2003). Although our data from anesthetized animals cannot address mechanisms of motor or cognitive timing directly, the extended temporal response patterns to tactile input that are invariant as to stimulus condition support the notion that the cerebellar output consists of a temporal pattern generator.

**Sensory coding in the DCN**

Besides the tactile input examined in the present study, the cerebellum receives a large amount of different sensory signals. Proprioceptive input to the cerebellum is likely important for encoding limb position (Casabona et al. 2004; Giaquinta et al. 2000). While most of the studies examining cerebellar proprioceptive input have focused on cerebellar cortex, a direct influence of ascending proprioceptive input to the DCN has been identified in a study employing perturbations of the locomotor cycle in the decerebrate cat (Schwartz et al. 1987). Visual and auditory stimuli can trigger responses in dentate nuclei in the behaving primate that precede the onset of movement (Chapman et al. 1986). In this study, a significant number of neurons showed responses to auditory and visual stimuli, supporting the notion of multimodal sensory receptive fields in the DCN. Responses to a somesththetic stimulus, however, were found in a largely nonoverlapping population of dentate neurons. Overall, these studies support the general concept that sensory inputs generate responses in the DCN that are linked to the execution of well timed movements. No systematic investigations of changing the sensory parameters of stimulation were undertaken, however. Thus the sensory coding capacity of DCN responses in the behaving animal has not been assessed to date. Our study in anesthetized rats suggests that DCN responses are primarily not linked to sensory properties of the stimulus but may reflect the temporal control of motor patterns. Given the scarcity of studies assessing sensory responses in the DCN, however, it is too early to tell whether some sensory inputs may be more quantitatively mapped. The proprioceptive system in particular may well serve a specialized function in accurately representing limb position information in the control of movement. Tactile input to facial and forepaw surfaces of the rat, in contrast, is more likely involved in controlling movement with respect to sensory information during exploratory behavior. Sensory responses associated with such explorative behavior have recently been identified in the cerebellar granule cell layer of the awake behaving rat (Hartmann and Bower 1995). Finally, a distinction between sensory responses associated with well-trained sensorimotor tasks and sensory responses to novel stimuli is likely to be important. Identified mechanisms of plasticity in the DCN include synaptically driven changes in excitability (Aizenman and Linden 2000), and LTP/LTD of the Purkinje cell-DCN synapses (Aizenman et al. 1998; Guardaouz and Sastry 2000). Such plasticity in conjunction with cerebellar cortical LTD/LTP is likely to lead to an experience-based shaping of sensory responses in the DCN.

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