Exp Brain Res (1996) 109:240–250

RESEARCH ARTICLE

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Contribution of somatosensory cortex to responses in the rat cerebellar granule cell layer following peripheral tactile stimulation

Received: 14 October 1994 / Accepted: 20 November 1995

Abstract The spatial coincidence of somatosensory cerebral cortex (SI) and trigeminal projections to the cerebellar hemisphere has been previously demonstrated. In this paper we describe the temporal relationship between tactilely-evoked responses in SI and in the granule cell layer of the cerebellar hemisphere, in anesthetized rats. We simultaneously recorded field potentials in areas of common receptive fields of SI and of the cerebellar folium crus IIa after peripheral tactile stimulation of the corresponding facial area. Response of the cerebellar granule cell layer to a brief tactile stimulation consisted of two components at different latencies. We found a strong correlation between the latency of the SI response and that of the second (long-latency) cerebellar component following facial stimulation. No such relationship was found between the latency of the SI response and that of the first (short-latency) cerebellar component, originating from a direct trigeminocerebellar pathway. In addition, lidocaine pressure injection in SI, cortical ablation, and decerebration all significantly affected the second cerebellar peak but not the first. Further, when tactile stimuli were presented 75 ms apart, the response in SI failed, as did the second cerebellar peak, while the short-latency cerebellar response still occurred. We found a wide spatial distribution of the upper lip response beyond the upper lip area in crus IIa for the long-latency component of the cerebellar response. Our results demonstrate that SI is the primary contributor to the cerebellar long-latency response to peripheral tactile stimulation. These results are discussed in the context of Purkinje cell responses to tactile input.

Key words Field potential · Timing · Lidocaine · Somatosensory cerebral cortex · Crus IIa · Mossy fiber · Cerebellum · Rat

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Introduction

For several years we have been studying the influence of peripheral tactile stimulation on the granule cell layer of the rat cerebellar hemispheres (Bower and Kassel 1990; González et al. 1993). Tactile projections to this cellular layer are organized in a fine-grained, fractured somatotopic pattern (Shambes et al. 1978a, b; Welker 1987) that is remarkably similar between different individuals (Bower and Kassel 1990). Electrophysiological experiments have demonstrated that the fine structure of these projection patterns appears to reflect the complex, but apparently precise, branching pattern of mossy fiber afferents (Woolston et al. 1981; Welker 1987). Neurons in the trigeminal complex with upper lip receptive fields project exclusively to regions of the granule cell layer that also respond to upper lip stimulation (Woolston et al. 1981). These direct trigeminal projections also appear to be responsible for the very short latency of granule cell layer responses to peripheral stimulation (Woolston et al. 1981).

In addition to the organized pattern of direct trigeminal projections to the granule cell layer, projections from somatosensory cerebral cortex (SI) through the pontine nuclei also follow a precise pattern (Bower et al. 1981). These projections are organized such that regions of SI responding to a particular location on the skin influence regions in the granule cell layer receiving information from the same location on the skin directly from the trigeminal nuclei. In other words, tactile information reaching the cerebellum indirectly through SI is in register with information received directly from the trigeminal nucleus.

In the current paper we examine in detail the relative timing of these two different influences in the cerebellar granule cell layer by recording simultaneously from SI and the cerebellum. Kennedy et al. (1966) have shown in cats that cerebral cortex has a large influence in tactilely responsive cerebellar regions and that this influence occurs at a later latency than the direct projection. We have confirmed that this is the case for rats and have extended

these previous results to carefully quantify the spatial extent of the influence of SI in the cerebellar regions. We have also documented differences in the variability in the timing of the short- and long-latency cerebellar responses to peripheral stimulation with respect to the SI response. We believe that these different temporal and spatial patterns of the direct and indirect tactile projections to cerebellum (Fig. 1A) are likely to have important implications for cerebellar cortical processing. An abstract describing these results has been previously published (Morissette et al. 1991).

Materials and methods

Animal preparation

Eight adult Sprague-Dawley albino rats (one male, seven females) were anesthetized with sodium pentobarbital (Nembutal 12 mg/kg body weight) and ketamine hydrochloride (50 mg/kg) injected intraperitoneally. Supplemental doses of ketamine (15 mg/kg) were given as needed to suppress reflexive activity. The right cerebral cortex (SI) and the left cerebellar cortex (crus IIa) were surgically exposed and covered with mineral oil, using standard procedures (Bower et al. 1981). The trachea was cannulated to facilitate respiration. Rectal temperature was maintained at 36–37°C and heart rate was monitored (280–410/min). Further details on surgical procedures can be found in previous publications (Bower and Kassel 1990; González et al. 1993). The experiments conformed to the *Principles of laboratory animal care* (NIH publication 85–23, revised 1985).

Electrophysiological procedures

Multiunit activity and/or field potentials were recorded in the granule cell layer (400–700 μm deep) of crus IIa of the cerebellum and in layer IV (600–1000 μm deep) of SI. Glass microelectrodes filled with 2 M NaCl, with tip diameter of 5–10 μm and impedance of 1–3 MΩ, were used. Neural signals were amplified, selectively filtered (multiunit recording: 300 to 3000 Hz bandpass; field potential: 1 to 1000 Hz bandpass), displayed on an oscilloscope, and made audible via speakers using standard procedures.

As has been previously noted (Bower and Kassel 1990), multiunit and field potential recordings in cerebellum do not distinguish between electrical activity originating from mossy fibers and that originating from granule cells. We are therefore not claiming that our recordings represent exclusively one or the other source. Instead, in these experiments, we have only attempted to assess the patterns of activity induced by peripheral stimuli at particular locations in the granule cell layer. Previous studies have made it clear that tactile maps obtained using the same procedures as ours actually represent the spatial pattern of mossy fiber inputs to these regions of the cerebellum (Woolston et al. 1981). It has also been shown that the granular layer responses are well correlated spatially and temporally with overlying Purkinje cell responses, using both extracellular (Bower and Woolston 1983) and intracellular (Jaeger and Bower 1994) recordings in vivo. Thus, these physiological studies indicate that activity recorded in the granule cell layer is also correlated with the activation of the synapses of the ascending granule cell axon on Purkinje cells.

Tactile stimulation

In each experiment, controlled tactile stimulation of the facial surface was obtained using a custom-built tactile stimulator based on a Ling vibrator from Gearing and Watson Electronics (Chubbuck 1966). Direct positive feedback control of the stimulator was achieved by directly sensing displacement of the probe position. In the current experiments, a square wave (5, 10, or 50 ms width) with a total probe excursion of 0.5 mm was used. The tip of the stimulation probe was less than 1 mm in diameter. Stimulus trials consisted of 5–50 sequential stimuli presented with an interstimulus interval of 2 s. Timing of stimulus trials was controlled using custom software running on an IBM personal computer. More information on electrophysiological procedures can be found in González et al. (1993).

Experimental design

For six of the animals, standard receptive field mapping techniques (see Welker 1971, 1973; Shambes et al. 1978a, b; Bower et al. 1981) were first used to locate regions of SI and the cerebellar granule cell layer with common receptive fields. As mentioned in the introduction, previous mapping experiments have demonstrated that an SI locus influences cerebellar regions with which it shares overlapping peripheral receptive fields (Bower et al. 1981). In several cases, the influence of these SI locations on the locus of cerebellar recording was directly reconfirmed by electrically stimulating SI and observing the responses in the granule cell layer (not shown, but see Bower et al. 1981).

Once corresponding regions of SI and the cerebellar granule cell layer had been established, two general strategies were used to determine the specific contribution of SI to peripherally evoked cerebellar responses. In the first, peripheral tactile stimuli were presented, at least 2 s apart, while recordings were made simultaneously in SI and crus IIa. The latencies and amplitudes of responses in both locations were then compared: (1) under normal conditions, (2) following a 0.15 cc peritoneal injection of sodium pentobarbital, and (3) as the time delay *within pairs* of tactile stimuli was shortened (the time delay *between pairs* of stimuli was at least 2 s).

In the second approach, cerebellar responses were recorded after several different methods were used to interfere with the physiological integrity of SI. In these experiments, cerebellar responses were recorded before and after: (1) local SI pressure injections of 2% lidocaine hydrochloride (approximately 30 μl) in layer V-VI of SI (1500–2500 μm), (2) local ablations of SI cortex (aspirations with a glass pipette), and (3) complete midcollicular decerebrations (knife cut at the brachium of the superior colliculus). In order to minimize the number of animals used in these experiments, several of these procedures were performed sequentially in each animal. For example, in one animal, recordings were made following a series of ever larger local ablations of SI and then following a complete midcollicular decerebration.

For the remaining two animals (one shown in Fig. 6), only the cerebellar cortex was surgically exposed. The tactile stimulator was then positioned at the precise location on the face that elicited the strongest response (see bottom inset of Fig. 6). Fifty peripheral tactile stimuli were given, with 2 s delay between each stimulation, and the field potential responses were recorded. Crus IIa was then mapped by making 40–50 electrode penetrations spaced 100–200 μm apart in three mediolateral columns. At each electrode penetration, the location on the face that elicited the strongest response when stimulated (i.e., the receptive field) was noted, but the tactile stimulator stayed in the same location (circle with "1" inside, bottom inset, Fig. 6) for the entire experiment. As the electrode was moved away from the center of the upper lip patch, the waveforms sometimes became more complex, occasionally exhibiting a third and/or fourth distinguishable peak within 50 ms after stimulation onset. The first and second cerebellar peaks described in this paper were identified by their latencies, and the peak amplitudes of both the short- and the long-latency cerebellar responses to 50 tactile stimulations (2 s between stimuli) were measured and averaged. The diameter of each filled circle in Fig. 6 represents the mean value at each electrode penetration.

Map construction

As in previous experiments (Welker 1987; Bower and Kassel 1990; González et al. 1993), cerebellar tactile maps were con-

structed by drawing enclosing boundaries around adjacent electrode penetration locations whose receptive fields were from the same body structures. In cases where stimulation of more than one peripheral location could induce a response in a particular penetration, the region eliciting the strongest response was recorded. When responses were of equal strengths, the boundary line was drawn through the site of the electrode penetration.

Data analysis

In order to quantify the effects of the experimental manipulations on cerebellar field potentials, analog responses were digitized and stored on a MassComp 5700 laboratory computer. Off-line analysis was done on a Sun SPARCstation 2. The digitized responses were read by a custom-developed C program that measured the latencies and amplitudes of single responses. The latencies were measured as the time elapsed from the onset of stimulator movement to the time at the peak of the evoked response. The amplitudes were measured as the difference between the mean of the potential for 100 ms before the onset of the peripheral stimulus and the peak value of the field potential. Values obtained from identical experimental conditions were averaged, in some cases, for comparison to other responses. Comparisons between control and drug applications (sodium pentobarbital and lidocaine) were done using a paired, nonparametric Mann-Whitney *U*-test. All measures of variability described here are standard errors (SE). *V* is the coefficient of variation, the standard deviation expressed as a percentage of the mean; and *r* is the coefficient of correlation.

Results

Cerebellar granule cell layer and SI cortex responses to peripheral stimulation

Punctate tactile stimulation to the face elicited a burst of activity in layer IV of SI and a double burst in the granule cell layer of crus IIa in the rat (Fig. 1). A brief (10 ms) stimulation of the upper lip led to characteristic bursts of multiunit activity in an upper lip region of both SI and crus IIa (top traces, Fig. 1B and C, respectively). Each burst of population spiking was associated with a negative peak in the local field potential (bottom traces, Fig. 1B and C). A typical cerebellar response to a tactile stimulus consisted of an initial short-latency response (range 8–10 ms) followed by a second component peaking in amplitude between 16 and 22 ms. Different animals showed slight differences in the range of latencies (less than 2 ms) for both cerebellar peaks. For a sample of 71 cerebellar and cortical responses to stimulation in one animal, we found that the latency of the first cerebellar waveform, or short-latency response, stayed fairly constant, 8.94±0.05 ms, V=4.6%; as did its amplitude, 1.10±0.01 mV, V=8.4% (Fig. 2A, latency only). In contrast, the second cerebellar waveform, or long-latency response, was more variable in latency, 19.19±0.17 ms, V=7.4%, and amplitude, 2.06±0.03 mV, V=11.8% (Fig. 2C, amplitude not shown). The cerebral cortex (SI) response to the same stimulus consisted of a single waveform with latencies ranging from 12 to 20 ms. As shown in Fig. 2B (latency only), the SI response was highly variable in latency and amplitude: 15.76±0.21 ms, V=11.3%; 2.77±0.10 mV, V=31.0%.

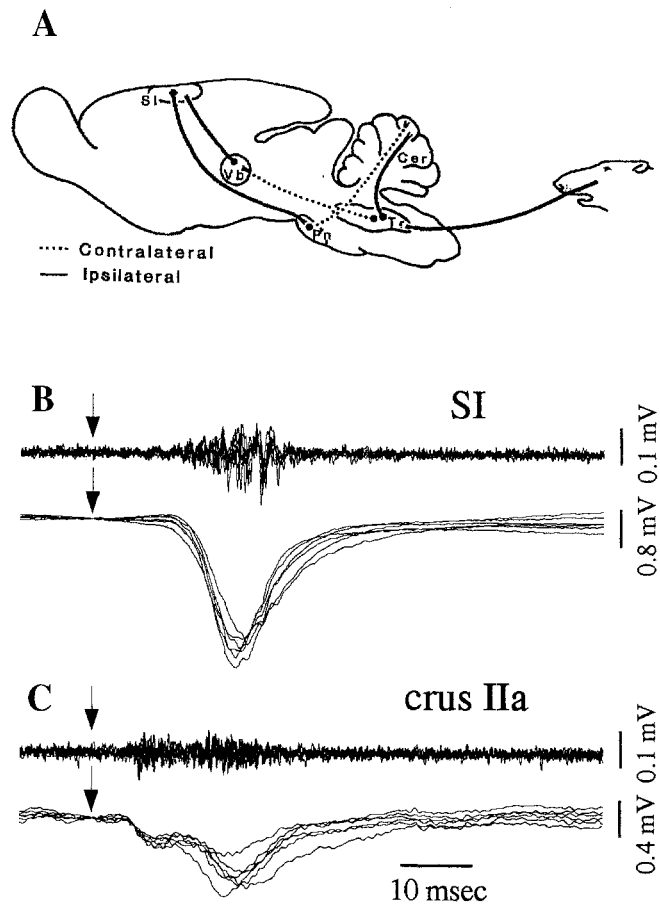


Fig. 1 **A** Simplified circuit diagram of tactile mossy fiber inputs to the crus IIa folium in the cerebellar hemispheres. The major pathways: a direct path from the ipsilateral trigeminal complex and an indirect path from the contralateral SI via various pontine nuclei. Several other areas not shown, including the superior colliculus, also project to crus IIa (see Kassel 1980; Brodal 1981; Huerta et al. 1983; Marfurt and Rajchert 1991). (*Cer* cerebellum, *Tr* trigeminal complex, *Vb* ventrobasal complex of the thalamus, *SI* somatosensory cortex, *Pn* pons; from González et al. 1993, with permission). **B, C** Cerebral (**B**) and cerebellar (**C**) cortical responses to a brief (10 ms) peripheral tactile stimulus of the upper lip. Traces show typical recordings of field potentials in layer IV of the SI (**B**) and in the granule cell layer of crus IIa (**C**). Recording electrodes were in an upper lip region in both SI and crus IIa. Responses to six consecutive stimuli, delivered 2 s apart, are superimposed. *Arrows* indicate the onset of the stimuli. *Top traces* Multiunit activity, bandpass-filtered digitally from 300 to 3000 Hz. *Bottom traces* Field potentials, same recordings as in top traces were here bandpass-filtered digitally from 1 to 1000 Hz. Positivity upwards in this and all subsequent figures

Correlation between the latency of the SI response and that of the second component of the cerebellar response

The latency of the second peak in crus IIa was highly variable, as was that of the SI response, but their latencies varied together. This is illustrated in Fig. 2D, which shows two consecutive pairs of simultaneously recorded cerebellar and cerebral responses to tactile peripheral stimulation of the upper lip. The two cerebellar peaks evoked by peripheral stimulation are denoted as "1" and "2" (Fig. 2D). We will henceforth refer to the first cere-

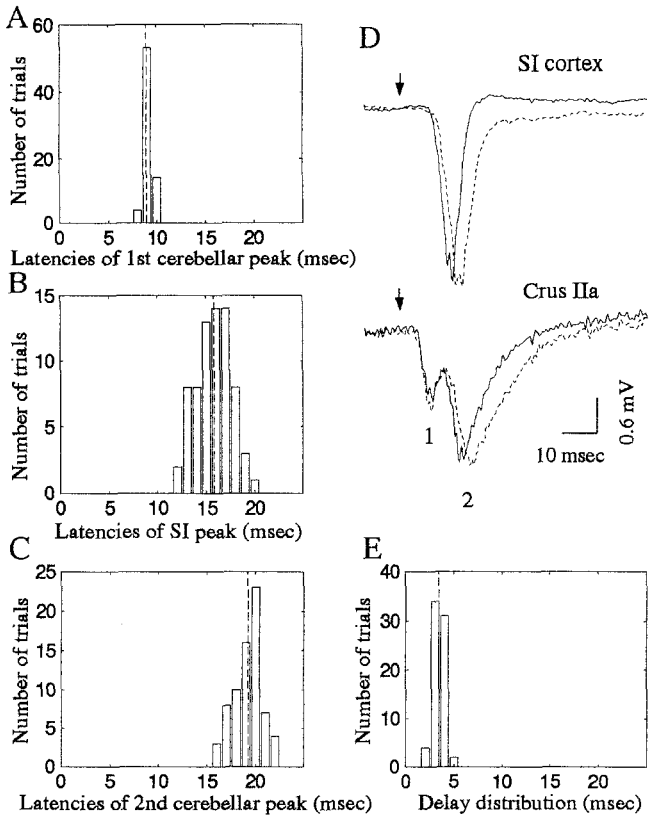


Fig. 2A–E Histograms of latencies of cerebral and cerebellar responses to tactile stimulation of the upper lip. Latencies were measured as the time elapsed from the onset of the stimulus to the time at the peak amplitude of the response. Histograms were constructed from the responses to 71 consecutive peripheral stimulations, at least 2 s apart, in one animal. All histogram bins are 1 ms. The *dotted lines* denote the mean latency (**A, B, C**) or mean of latency difference (**E**). **A** Histogram of the latencies of the short-latency component of the cerebellar field potentials (shown as *1* in **D**). **B** Histogram of the latencies of the SI responses to stimulation of the upper lip. **C** Histogram of the latencies of the long-latency component of the cerebellar granule cell layer field potentials (shown as *2* in **D**). Note how the cerebellar first peak latencies (**A**) are more tightly grouped together than are the SI (**B**) and second cerebellar (**C**) latencies. **D** Simultaneous cortical (*top traces*) and cerebellar (*bottom traces*) field potential responses elicited by peripheral tactile stimulation of the upper lip. Two consecutive responses are superimposed. Peripherally evoked cerebellar field potentials consisted of two components: one with short-latency (peak shown as *1*) and one with long-latency (peak shown as *2*). Response to first stimulus (*solid line*): cerebellar response peaked at 8.6 and 17.8 ms, cerebral cortex response peaked at 14.6 ms. Response to second stimulus (*dashed line*): cerebellar response peaked at 8.6 and 20.4 ms, cerebral cortex response peaked at 17.0 ms. *Arrows* denote the time of stimulation. **E** Histogram of the difference, for each trial, between the latency of the second cerebellar peak and that of the SI response following a peripheral stimulation

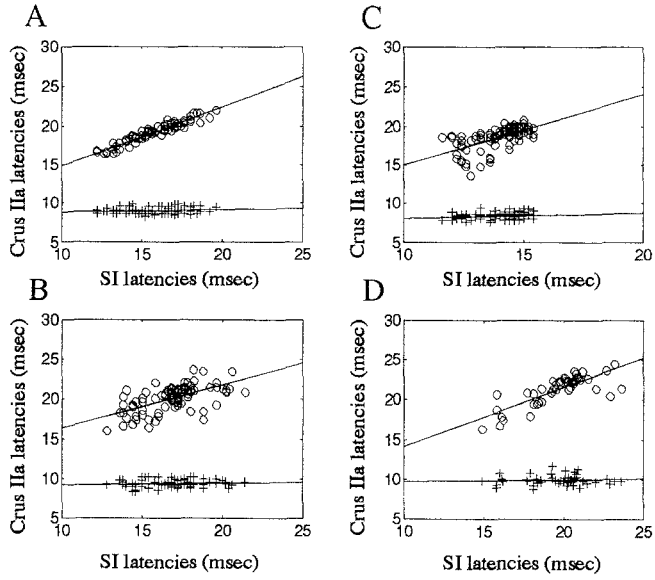


Fig. 3A–D Relationship between the latencies of the two peaks of the cerebellar field potential and that of the peak of the cerebral cortical field potential. The cerebellar first peak latencies are indicated by *crosses*, the cerebellar second peak latencies are shown by *open circles*. For four different animals (**A–D**), waveforms were recorded simultaneously in crus IIa and SI following tactile stimulation of the upper lip. The latencies of the second peak of the cerebellar waveforms varied considerably, as did the latencies of the peak of the SI cortical waveforms. The latencies of these two responses were highly correlated. In contrast, the latencies of the first peak of the cerebellar field potentials were more constant and not correlated with the SI response. Linear regression lines are shown for both cerebellar peaks for each animal; see text for *r* and *P* values

bellar peak ("1" in Fig. 2D) as the first, or short-latency, response and to the second cerebellar peak ("2" in Fig. 2D) as the second, or long-latency, response. The short-latency responses both peaked 8.6 ms after the onset of the stimuli. The long-latency responses peaked at 17.8 and 20.4 ms after the tactile stimuli, 3.2 and 3.4 ms, respectively, after the cerebral cortex response (Fig. 2D, top traces). The peak of the long-latency response in crus IIa occurred on average 3.44±0.07 ms after the peak of the SI response (V=17.8%, n=71 in one animal; Fig. 2E). While varying over a much smaller range than the SI or the second cerebellar responses (compare width of Fig. 2E with that of B and C), this delay between the response in SI and the long-latency component of the cerebellar response was not constant. When the latency of the tactilely-evoked SI response was in the later part of the normal range, the delay tended to be shorter than when the SI response was in the early part of the normal range (not shown).

Figure 3 compares the latencies of cerebellar and SI responses for four different animals. The latency of the second cerebellar peak and that of the cortical response to stimulation were highly correlated in each case: r=0.95, r=0.61, r=0.61, and r=0.80 (Fig. 3A-D, respectively, circles); and regression lines fit to the data were significant (P=0.0001). The slopes of the regression lines are m=0.7, m=0.5, m=0.7, and m=0.7 (Fig. 3A-D, respectively, circles). Note that these slope values are consistent with the delay between the SI response and the second cerebellar component response, being shorter when the SI response occurred later, as mentioned in the previous paragraph. In contrast, the latency of the first cerebellar peak was less variable and was not correlated with the cerebral peak latency (for all four animals shown in

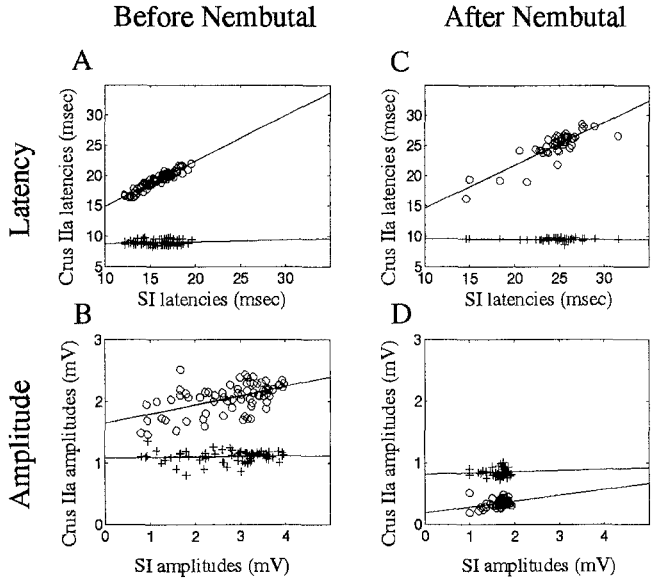
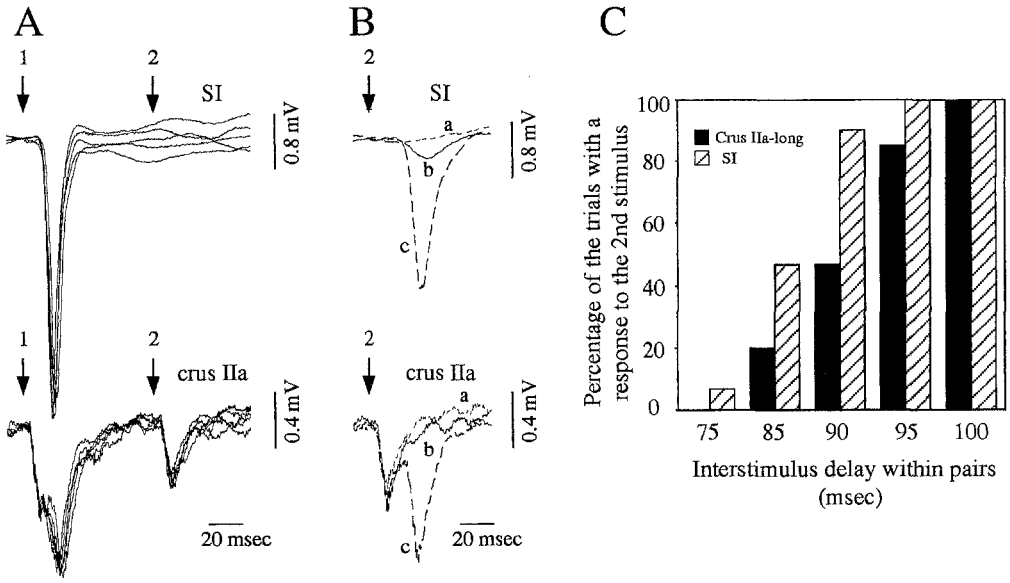


Fig. 4A–D Effect of a 0.15 cc intraperitoneal injection of sodium pentobarbital (Nembutal). Results are shown before (**A, B**) and 10 min after (**C, D**) the injection, in one animal. Measurements of the cerebellar first peak are indicated by *crosses*, while those of the cerebellar second peak are shown by *open circles*. Latencies (**A, C**) and amplitudes (**B, D**) of the two components of the cerebellar response are plotted as a function of those of the SI response to tactile peripheral stimulation of the upper lip. **C** Latencies of both cerebellar peaks as well as that of the SI peak increased significantly 10 min after a sodium pentobarbital injection. The latency of the second cerebellar peak was still highly correlated to the latency of the cortical peak, while the latency of the first cerebellar peak was not. **D** Amplitudes of the cerebellar and SI responses decreased significantly after the injection. The significance was judged by a Mann-Whitney *U*-test Fig. 3; crosses: $r<0.2$, regression analysis was not significant, $P>0.1$).

Effects of sodium pentobarbital on SI and cerebellar responses

A 0.15 cc intraperitoneal injection of the barbiturate sodium pentobarbital caused a significant (as judged by a Mann-Whitney *U*-test, $P=0.0001$) amplitude decrease in the cerebral peak as well as in both peaks of the cerebellar field potential (Fig. 4B, D). The injection also caused a significant (as judged by a Mann-Whitney *U*-test, $P=0.0001$) increase in the latencies of both the cerebral and cerebellar responses (Fig. 4A, C). However, the strong correlation between the long-latency cerebellar and SI responses persisted after the sodium pentobarbital injection: before, $r=0.95$; after, $r=0.86$; the slope of the regression line stayed constant before and after at 0.7 and was significant, $P=0.0001$ (Fig. 4A, C, circles).

Fig. 5 **A** Five superimposed field potentials of the responses in SI (*top traces*) and crus IIa (*bottom traces*) to a pair of tactile stimuli 75 ms apart. The responses to the first stimulus are typical (compare with Fig. 1B, C and 2D). When the second stimulus of the pair occurred, 75 ms later, the cerebellar recordings showed only the short-latency response, and the cortical traces showed very little or no response. The *arrows* denote the onset of the two stimulations. **B** Responses in SI (*top traces*) and crus IIa (*bottom traces*) to the second of a pair of tactile stimuli, 85 ms after the first one. The response to the first stimulus (not shown) was typical; see **A**. Three different field potential responses are superimposed. The onset of the second stimulus is denoted by an *arrow*. **C** Percentage of the trials in which the second stimulus elicited a response as a function of the delay between the two stimuli of paired stimulation. The delay between each pair of stimuli was at least 2 s. Percentages are shown for the long-latency cerebellar (*black bar*) and cortical responses (*hatched bar*). The short-latency cerebellar response occurred in all but one of the trials (in 164 out of 165 trials) for all interstimulus intervals shown. Each bar represents the percentage of trials with a response to the second stimulus for 30–40 paired stimuli



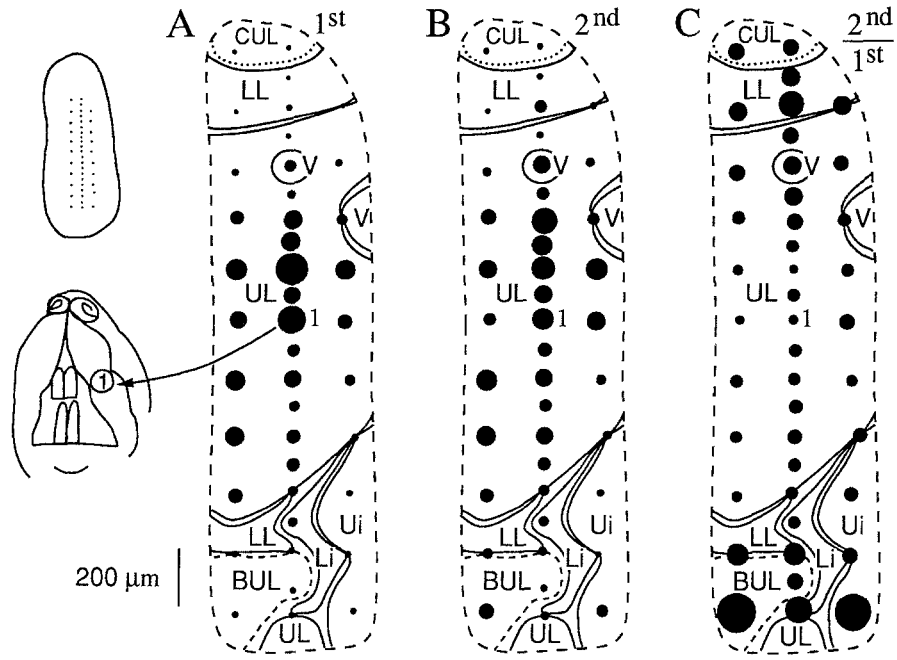


Fig. 6A–C Distribution of activity in cerebellar crus IIa following tactile stimulation of the upper lip. Each of the three maps (**A–C**) shows a different aspect of the same field potential data obtained in one animal. Each *circle* represents an electrode penetration and the diameter of each circle is proportional to: **A** the amplitude of the first, or short-latency, component of the response to peripheral stimulation; **B** the amplitude of the second, or long-latency, component of the response to peripheral stimulation; and **C** the ratio of the amplitude of the long-latency response to the amplitude of the short-latency response. The diameter of each *circle* was calculated by averaging the peak amplitude (or ratio of amplitudes) of the responses to 50 stimulations of the upper lip. *Top inset* Position of the electrode penetrations on the surface of the crus IIa folium. *Bottom inset Circle* with *1* inside indicates the area of tactile stimulation; it was stimulated at each electrode penetration, independent of what the receptive field was at that penetration. This upper lip area was the receptive field at the first electrode penetration shown as *1* on each map. *Top* is medial, *left* is rostral; *dotted lines*, contralateral structures; *dashed lines*, bilateral structures; *solid lines*, ipsilateral structures (*UL* ipsilateral upper lip, *BUL* bilateral upper lip, *CUL* contralateral upper lip, *V* ipsilateral vibrissae, *LL* lower lip, *Li* lower incisors, *Ui* upper incisors)

Effects of increased frequency of tactile stimulation

In this study, the standard time between each tactile stimulation was at least 2 s; however, in some experiments, paired pulses with variable interstimulus intervals (of less than 2 s) were given. As shown in Fig. 5, this had a significant, albeit different, effect on the SI and cerebellar responses. At a 75 ms interstimulus interval, the second stimulus elicited only the short-latency cerebellar response and not the long-latency cerebellar response. At these short interstimulus intervals, cortical traces following the second stimulus also showed very little or no response (Fig. 5A). Figure 5B shows three examples of responses obtained when the interstimulus interval was increased to 85 ms. Following the second stimulation of the pair, in some cases, neither cerebral nor cerebellar long-latency response occurred (Fig. 5B, dotted line, a). At other times (Fig. 5B, solid line, b), a small amplitude cerebral response was observed (7% of the amplitude of the response to the first stimulation) but no cerebellar long-latency response occurred; and finally some traces following the second stimulation showed a larger amplitude SI response (71% of the amplitude of the response to the first stimulation) as well as a cerebellar long-latency response (Fig. 5B, dashed line, c). In all cases, the cerebellar short-latency response occurred in response to both stimuli. When the interstimulus interval was decreased from 100 to 75 ms, there was a decrease in the number of long-latency cerebellar and SI responses to the second pulse of the pair (Fig. 5C). All cerebellar long-latency responses to the second stimulus occurred in trials where an SI response to the second stimulus was also present. At short interstimulus delay, there was a larger percentage of SI responses than long-latency cerebellar responses to the second stimulus. For paired stimuli with 75 to 100 ms interstimulus delay, when the second stimulus elicited a long-latency cerebellar peak in a trial, the amplitude of the SI response (1.56±0.07 mV, 62% of the amplitude of the SI response to the first stimulus; n=88) was on average twice as large as when there was no second cerebellar peak (0.79±0.07 mV, 32% of the amplitude of the SI response to the first stimulus; n=29).

Spatial distribution of the short- and long-latency cerebellar responses

In two animals, we attempted to quantify the spread of activity of both latency responses in crus IIa. In the animal shown in Fig. 6, we mapped the receptive field at 42 locations in the folium. Recordings were made in each location while stimulating a single location on the rat's upper lip (shown as the circle with "1" inside, bottom inset, Fig. 6). The largest responses were recorded in loca-

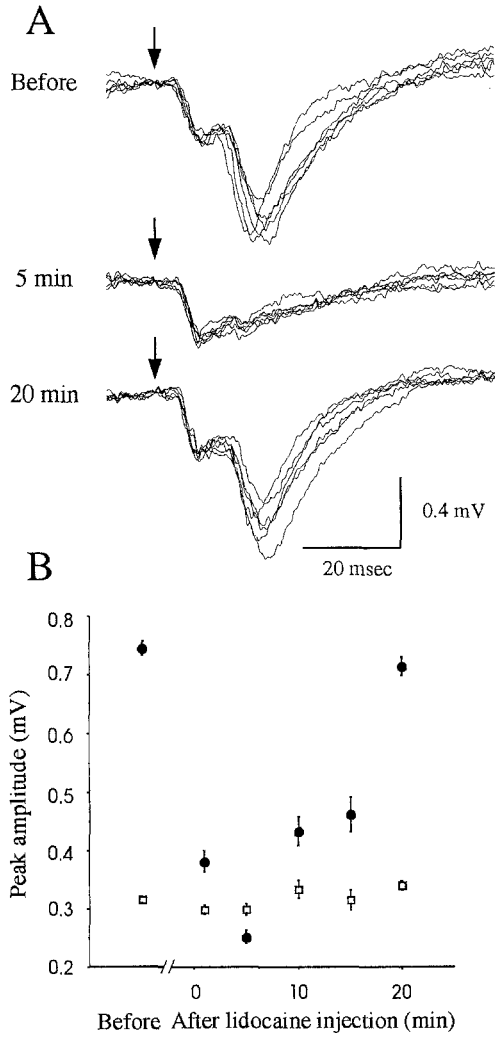


Fig. 7A, B Effect of lidocaine injection in SI on the cerebellar response to tactile stimulation of the upper lip. A pressure injection of approximately 30 µl of 2% lidocaine was applied to the corresponding upper lip region in layer V-VI of the somatosensory cortex. **A** Six superimposed consecutive cerebellar field potentials are shown before, 5, and 20 min after an injection. *Arrows* denote the onset of the tactile stimulus. **B** Amplitude of the two cerebellar peaks as a function of time after the lidocaine injection. Each point represents the mean amplitude ±SE of 50 traces like those shown in **A**. The cerebellar first, or short-latency, peak is denoted by an *open square* and the cerebellar second, or long-latency, peak is denoted by a *filled circle*. Lidocaine injection significantly decreased the amplitude of the cerebellar long-latency responses, but had little effect on the amplitude of the cerebellar short-latency responses. Effects were reversed in 20–30 min. The significance was judged by a Mann-Whitney *U*-test

tions where the receptive field matched the stimulated upper lip area. However, smaller responses were recorded throughout crus IIa. Comparing the mean amplitude of the two components of the response (Fig. 6A, B), it can be seen that the short-latency component decreased most rapidly away from the receptive field center, while the amplitude of the long-latency component decreased less, suggesting a somewhat larger projection region. The ratios of the amplitudes of the second and first cerebellar peaks, as seen in Fig. 6C, indicate that the second

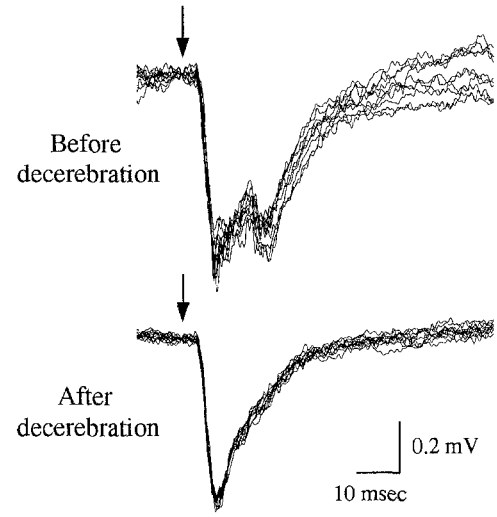


Fig. 8 Complete midcollicular decerebration abolished virtually all of the cerebellar long-latency response to tactile stimulation. Ten consecutive cerebellar responses to peripheral stimulation of the upper lip are superimposed before and approximately 40 min after decerebration. *Arrows* indicate the time of the stimulus

cerebellar peak becomes more significant further away from the receptive field relative to the first peak.

Disruption of SI selectively interferes with the long-latency cerebellar response

Several methods were used to interfere with the physiological integrity of SI to further confirm the influence of SI responses on the cerebellum. Figure 7 shows the effects of applying a local pressure injection of 2% lidocaine (approximately 30 µl) in the upper lip region in layer V-VI of SI while recording in a corresponding upper lip patch in the granule cell layer of crus IIa. After the injection, the amplitude of the second peak of the cerebellar response to tactile stimulation of the upper lips was significantly reduced (as judged by a Mann-Whitney *U*-test, P=0.0001). The maximal effect, with the cerebellar long-latency response almost completely vanishing, occurred 5 min after the injection. In contrast, the short-latency cerebellar response showed no significant changes (Fig. 7B). The effect of lidocaine on the second cerebellar peak was repeatable and reversed completely after about 20 min (Fig. 7).

We also examined the effects on cerebellar responses of local ablations of SI (not shown). When SI was partially ablated, the amplitude of the cerebellar long-latency component fell 48%. A more complete ablation in the same animal resulted in a greater decline in amplitude, 56%; while the amplitude of the short-latency component was not significantly affected.

The last and most extreme procedure involved a decerebration at the midcollicular level. It resulted in a virtually complete elimination of the long-latency component of the cerebellar response to peripheral stimulation of the upper lip (Fig. 8). Once more, this procedure af-

fected the second cerebellar peak quite selectively; the first cerebellar peak showed little change (before: 8.51±0.51 ms, 0.64±0.07 mV, n=97; after: 8.12±0.24 ms, 0.59±0.02 mV, n=100).

Discussion

These experiments carefully investigated the temporal relationship between tactilely-evoked responses in SI and the cerebellar granule cell layer. Our results were consistent with those obtained in the cat by Kennedy et al. (1966) in showing that SI is the primary contributor to the long-latency (second peak) cerebellar granule cell layer response elicited by tactile stimulation. By recording from SI and the cerebellum simultaneously, we have demonstrated a strong correlation between the latency of the SI response and that of the second cerebellar peak. Further, as described below, the onset of the SI-related response in the cerebellum appears to correspond with several features of Purkinje cell responses to peripheral tactile stimulation.

Origins of cerebellar granule cell layer responses to tactile stimulation

From 1940 to the late 1960s, a large number of studies of cerebellar afferent systems were conducted using evoked potential techniques (for reviews see: Bloedel 1973; Allen and Tsukahara 1974). These studies used large surface recording electrodes and electrical stimulation of peripheral nerves. These techniques have lower spatial resolution than the procedures used in this paper, which include recording the afferent potential in the granule cell layer (depth of 400–700 μm) and a more natural tactile stimulation of the periphery (review, Welker 1987). As a result of the lower spatial resolution in the earlier studies, the fine detail of tactile projections to cerebellum was obscured. For example, previous evoked potential experiments were interpreted as suggesting an organized somatotopic projection to the cerebellum (Snider and Stowell 1944; Provini et al. 1968), while Welker and his collaborators, using techniques like those used here, found the projection to be fractured (Welker 1987). Many previous studies also averaged large numbers of individual traces, which would have obscured the kinds of timing relationships reported here.

Short-latency component

Our results demonstrated that the first, short-latency, component of the cerebellar response to tactile stimulation was very regular in latency and amplitude. This short-latency response was almost certainly a result of a direct trigeminal projection. A direct projection from the trigeminal nuclei to the lateral hemispheres of the cerebellum has been demonstrated anatomically using horseradish peroxidase (HRP; Watson and Switzer 1978). Further, physiological experiments using antidromic collision techniques have demonstrated direct trigeminal projections to the same regions of the granule cell layer investigated in this study (Woolston et al. 1981). It has also been reported that the cerebellar hemispheres may receive projections directly from primary sensory nerves, at least in the case of the teeth (Elias et al. 1987). Whether direct or relayed through the trigeminal nucleus, the short-latency projections clearly provide a large, very fast, and temporally stable cerebellar input.

Contribution of circuits involving SI cortex to the long-latency component

The long-latency response to tactile stimulation was demonstrated to be more temporally variable and distinct from the short-latency response. We have provided clear evidence that these responses substantially involve the SI. Previous studies (Bower et al. 1981) have shown that direct electrical stimulation of SI induces responses in crus IIa. Numerous studies have described the anatomical properties of projections from cerebral to cerebellar cortex (for review see Allen and Tsukahara 1974; Angaut and Sotelo 1975). With respect to SI cortex and the lateral hemispheres of the cerebellum in particular, a large body of anatomical data shows that the pons receives input from layer V-VI cortical (SI) neurons and sends most of its afferents to the contralateral lateral hemisphere of the cerebellum, including crus IIa (Wise and Jones 1977; Brodal 1979, 1982; Mihailoff 1983; review, Brodal and Bjaalie 1992). There is also evidence of a secondary projection through the brainstem lateral reticular nucleus (Allen and Tsukahara 1974; Allen et al. 1979), but it projects primarily to the vermis, not the hemispheres (Clendenin et al. 1974; Newman and Ginsberg 1992). Thus, it is most likely that the effects described here are relayed through the pontine nuclei.

Contributions of other mid- and forebrain structures to the long-latency cerebellar response

While SI cerebral cortex clearly has a major influence on tactile regions of the lateral hemispheres, other structures, such as the motor cortex (Sharp and Evans 1982; Mihailoff et al. 1985) and the superior colliculus (Kassel 1980), have also been demonstrated to influence these regions of the cerebellum, presumably also through the pons (motor cortex, Mihailoff et al. 1985; superior colliculus, Dean et al. 1988). In the current study, we have shown that lidocaine injected into SI as well as direct SI cortical ablations substantially reduced, but did not eliminate, the long-latency component of the cerebellar response to peripheral stimulation. Complete midcollicular transection was required to completely abolish the response. This latter procedure should interrupt projections from all forebrain structures and all structures projecting

through the pons. Following decerebration, the first cerebellar peak was not significantly affected, whereas the second cerebellar peak disappeared. These findings are consistent with those of Kennedy et al. (1966) in the cat. These authors also showed a third peak occurring approximately 30–40 ms after the onset of the stimulus that reappeared 30–45 min after decerebration. We did not see any such late latency peak reappear after comparable time in our preparation. However, in normal rats, we found a number of responses with a third distinguishable peak when the peripheral area stimulated did not correspond to the primary receptive field of the location recorded from in the granule cell layer, such as when we stimulated the upper lip while recording from a lower lip patch (experiment described in Fig. 6). Since Kennedy et al. (1966) did not take into consideration the detail of the fine grain input map to the cerebellum, our data suggests that the "third peak" responses they describe may have been recorded from regions outside but near the forepaw receptive field patch. Further experiments will be necessary to determine the origin of that component of the cerebellar response to peripheral stimulation.

Temporal properties of cerebellar and cerebral cortical responses

Timing relationships between responses in SI cortex and the cerebellar long-latency response

Our data demonstrate that the latency of activity in SI cortex and the long-latency cerebellar response are quite variable but that they are highly correlated temporally. This is true both in the regular preparation as well as after the administration of barbiturates (Fig. 4). While barbiturates result in the lengthening of latencies in all responses measured, probably through a brain-wide increase in the $GABA_A$-induced Cl^- current, and thus inhibition of synaptic transmission (Snyder 1984), the latencies of the SI, and long-latency cerebellar responses still remain highly correlated. We have also demonstrated that there is relatively little variability in the delay between activity in SI and the long-latency response in the cerebellum on individual trials (Fig. 2E). Finally, we have shown that the latency of the short-latency cerebellar response, originating in the trigeminal nucleus, was much less variable and was not correlated with the timing of the SI response (Fig. 3).

Significance of the response timing in cerebellum

After a response has been induced in the cerebral cortex, the information is apparently relayed to the cerebellum rapidly and with essentially fixed latency. This was the case for tactile stimulation given every 2 s. Our data showed that when two stimuli were separated by less than 100 ms, the failure rate for SI and SI-related cerebellar responses increased. Previous studies showed similar results at the level of Purkinje cells, which were not capable of following stimuli applied at frequencies of 10 Hz (Bantli and Bloedel 1977).

We have also shown that the forebrain influence arrived in the cerebellum with no consistent temporal relationship to the initial burst of granule cell layer activity. For this reason, we speculate that the detailed timing of information originating in the forebrain and projecting to the cerebellum reflects the temporal requirements of forebrain, not cerebellar, processing. When considering these timing relationships, it is important to keep in mind that stimuli in these experiments were given irrespective of any intrinsic rhythms of the thalamus or cortex. Under more natural conditions, it is entirely conceivable that behaving animals coordinate the acquisition of afferent information so that it is in register with the intrinsic rhythms of their neural circuits. We have previously proposed that the primary role of the cerebellum may be in the general coordination of sensory data acquisition (Bower and Kassel 1990; Bower 1993). Presumably this coordination would involve not only the spatial but also the temporal use of sensory receptors. Accordingly, it is interesting to consider the timing of this long-latency input with respect to other cerebellar events induced by peripheral stimuli.

Comparison of our granule cell layer responses with extracellular and intracellular recordings from the Purkinje cells obtained in our laboratory (Jaeger and Bower 1994) shows that the SI influence on the granule cell layer coincides with two specific transitions in Purkinje cell responses to peripheral stimulation. First, the long-latency granule cell response coincides closely with the termination of the short-latency inhibition of Purkinje cells that often results from short duration peripheral tactile stimuli (Bower and Woolston 1983). Second, the long-latency response coincides with the beginning of a prolonged (50–200 ms) increase in simple spike firing frequency that can result from tactile stimulation. Intracellular work has demonstrated that this prolonged response is due to a prolonged, plateau-like Purkinje cell dendritic depolarization that probably also results from the initial activation of the granule cell layer, although it is masked by inhibition (Jaeger and Bower 1994). Intracellular recordings have shown that the long-latency granule cell layer response contributes a large part of the intracellular increase in potential in absence of inhibition (Jaeger and Bower 1994). These results suggest a different functional role for the direct and indirect cerebellar projecting tactile pathways, despite their spatial coherence. It has been suggested previously that peripheral and cortical afferents do not converge on common granule cells (Allen et al. 1974). For example, the pontine mossy fibers could activate different mossy fibers-parallel fibers mechanisms from mossy fibers arriving directly from the trigeminal nucleus. Our findings (Fig. 6) suggest that the SI-related component of the cerebellar responses to tactile stimulation is spatially more distributed than the trigeminal-related component and thus could influence different granule cells.

Proposed role of SI in cerebellar function

It has been known for many years that the cerebral cortex and the cerebellum are very strongly interrelated; the growth of cerebral cortex in mammals is paralleled by the growth of cerebellum (Stephan et al. 1981). In the current experiments, we have again demonstrated that SI provides a substantial input to the lateral hemispheres of the cerebellum. Our results, for brief tactile stimulation in anesthetized rats, showed that the SI response followed the direct sensory response from the trigeminal nucleus. In our data, the first component of the cerebellar response occurs at a latency of 8–10 ms, which is before SI is even activated. As it is also known that the initial cerebellar influence on motor output is very fast (Orlovsky 1972), our data suggest that an initial sensorimotor loop through the cerebellum may very well be completed by the time the response to the tactile stimulus arrives in SI and is relayed back to the cerebellum (Shambes et al. 1978b; Bower and Kassel 1990).

Most theories of cerebrocerebellar interaction focus on the putative role of cerebellum in motor control and therefore focus on the influence of motor pathways (Marr 1969; Albus 1971; Houk 1988; Thach et al. 1992). For the last several years we have been proposing an alternative hypothesis: that cerebellar circuits may be involved in monitoring and controlling the active acquisition of sensory information on which the performance of the rest of the nervous system is based (Bower and Kassel 1990; Bower 1993; González et al. 1993). Specifically, we have proposed that the cerebellum receives primary tactile sensory information from particular sets of sensory surfaces involved in active exploration, and then, through the motor system, adjusts the position of these tactile sensory surfaces with respect to each other. In this way we propose that the cerebellum uses the motor system to coordinate the acquisition of sensory data. Such a function for the lateral hemispheres is analogous to the known influence of the flocculus in the vestibulo-ocular reflex (Paulin et al. 1989). Our recent work using functional magnetic resonance imaging in humans (Gao et al. 1995, 1996) supports this proposal, demonstrating strong activation of the lateral regions of the cerebellum in tactile discrimination tasks irrespective of the occurrence of overt finger movements.

In the context of this hypothesis, we propose that the long-latency, forebrain-related response in the cerebellum could provide the cerebellum with information on the overall state of cortical networks, including information about the appropriate timing of data acquisition. Such information could, in principle, serve to modify Purkinje cell responses to the short-latency, raw afferent. In this way, ongoing control of sensory acquisition would be dependent both on the raw sensory information and the response of the cerebral cortex to that information. The timing of the SI influence during the plateau phase of the Purkinje cell response set up by the initial direct response to the stimulus is consistent with this idea.

Ultimately, a full understanding of the role of cerebral cortical circuits in cerebellar function, or of the cerebellum itself, will require a close examination of neural activity in behaving animals. For example, under natural behavioral conditions, tactile stimuli are likely to be of a longer duration, with much more complex timing relationships than those shown here for a single punctate stimulus. However, we would still expect any new stimulus to activate cerebellum first and SI second. Preliminary results from awake behaving animals in our laboratory show cerebellar double peak responses similar to the ones reported here (Hartmann and Bower 1993, 1995). These behavioral experiments and modeling efforts currently under way in our laboratory are intended to further shape and test these ideas.

Acknowledgements The authors wish to express thanks to Caroly Shumway, Leila González, and John Thompson for help with some of the techniques used, Dieter Jaeger and Wyeth Bair for valuable discussions, as well as Mitra Hartmann and the anonymous reviewers for helpful criticism of the manuscript. This project was supported by NIH grant NS22205.

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