GABAergic and Glutamatergic Modulation of Spontaneous and Motor-Cortex-Evoked Complex Spike Activity

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Lang, Eric J. GABAergic and glutamatergic modulation of spontaneous and motor-cortex-evoked complex spike activity. J Neurophysiol 87: 1993–2008, 2002; 10.1152/jn.00477.2001. Olivocerebellar activity is organized such that synchronous complex spikes occur primarily among Purkinje cells located within the same parasagitally oriented strip of cortex. Previous findings have shown that this synchrony distribution is modulated by the release of GABA and glutamate within the inferior olive, which probably act by controlling the efficacy of the electrotonic coupling between olivary neurons. The relative strengths of these two neurotransmitters in modulating the patterns of synchrony were compared by obtaining multiple electrode recordings of spontaneous crus 2a complex spike activity during intraolivary injection of solutions containing a GABA_A (picrotoxin) and/or AMPA [1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[g]quinoxaline-7-sulfonamide disodium (NBQX)] receptor antagonist. Injection of either antagonist led to increased synchrony between cells located within the same parasagitally oriented ~250-μm-wide cortical strip. Picrotoxin also increased complex spike synchrony among cells located in different cortical strips, leading to a less prominent banding pattern, whereas injections of NBQX tended to decrease complex spike synchrony among such cells, enhancing the banding pattern. The relative strength of these two classes of olivary afferents was assessed by first injecting one of the antagonists alone and then in combination with the other. The enhanced banding pattern of complex spike synchrony following injection of NBQX alone remained during the subsequent combined injection of both antagonists. Furthermore, the widespread synchronization of complex spike activity following injection of picrotoxin alone was partially or completely reversed by combined injection of picrotoxin and NBQX. Changes in the climbing fiber reflex induced by the intraolivary injections paralleled the changes observed for spontaneous complex spike activity, indicating that the effects of picrotoxin and NBQX on the synchrony distribution reflect changes in the pattern of effective coupling of inferior olivary neurons and demonstrating that synchronous complex spike activity does not require simultaneous excitatory input to olivary cells. Finally the pattern of synchrony during motor cortical stimulation was examined. It was found that the patterns of synchrony for motor-cortex-evoked complex spike activity were similar to those of spontaneous activity, indicating an important role for electrotonic coupling in determining the response of the olivocerebellar system to afferent input. Moreover, intraolivary injections of picrotoxin increased the spatial distribution of the evoked response. In sum, the results provide evidence for the hypothesis that electrotonic coupling of inferior olivary neurons via gap junctions is the mechanism underlying complex spike synchrony and that this coupling plays an important role in determining the responses of the olivocerebellar system to synaptic input.

INTRODUCTION

It is now well established that the olivocerebellar system generates synchronous complex spike (CS) activity among Purkinje cells of the cerebellar cortex (Lang et al. 1999; Llinás and Sasaki 1989; Sasaki et al. 1989; Sugihara et al. 1993; Yamamoto et al. 2001). Synchronous CS activity most often occurs among Purkinje cells located within the same parasagitally oriented strip of cortex; however, it can occur between Purkinje cells in different cortical strips and even between cells located on the vermis and hemisphere (De Zeeuw et al. 1996). Moreover, the patterns of synchronous CS activity are dynamic and can change significantly in relation to movements elicited by conditioned stimuli (Welsh et al. 1995). These changes stand in contrast to the relatively small modulations in single-cell CS firing rates during movements that are generally reported and suggest that changes in spatial patterns of activity rather than temporal variations in single-cell firing rates are central to the functioning of the olivocerebellar system. Thus it becomes critical to understand the mechanisms by which synchronous CS activity is generated.

Synchronization of olivocerebellar activity was proposed to result from the electrotonic coupling of inferior olivary (IO) neurons by gap junctions (Llinás 1974; Llinás et al. 1974; Sotelo et al. 1974). Consistent with this idea, electrotonic coupling between IO neurons has been directly demonstrated (Llinás and Yarom 1981a; Manor et al. 2000), and the average level of synchrony in different regions of the cerebellar cortex is correlated with the density of gap junctions in the IO subnuclei that project to these regions (De Zeeuw et al. 1997). Nevertheless, the extent to which spontaneous CS activity is synchronized as a result of such coupling as opposed to other mechanisms, such as simultaneous excitatory input to the IO, is unclear. This issue was addressed in a recent study in which glutamatergic input to the IO was blocked (Lang 2001). Injection of glutamate antagonists into the IO did not abolish CS synchrony but did alter its spatial distribution such that the parasagittal banding organization became more pronounced. That is, relative to control levels, CS activity among Purkinje cells within the same parasagitally oriented cortical strip be-
came more synchronized while the CS activity of cells in different strips became less synchronized following block of glutamate receptors. These results provided evidence that CS synchrony among parasagitally aligned Purkinje cells does not require excitatory synaptic input to the IO and by this exclusion support gap junction coupling as the underlying mechanism. However, whether synchronous CS activity between cells in different parasagittal strips is dependent on specific triggering afferent activity to the IO remains uncertain. One goal of present experiments is to address this issue.

The dynamic nature of the spatial distribution of CS synchrony suggests that modulation of the efficacy of electrotonic coupling of IO neurons is a major function of olivary afferents. Indeed, most gap junctions within the IO occur between dendritic spines that are also contacted by presynaptic terminals to form structures called glomeruli (King 1976; Sotelo et al. 1974). While synapses contacting any part of an IO neuron could affect its coupling to other cells, the strategic location of the intraglomerular synapses suggests that they are the major control mechanism. Many of these intraglomerular terminals are GABAergic (De Zeeuw et al. 1989; Sotelo et al. 1986), and generally attention has centered on the role of these inhibitory synapses in controlling the patterns of coupling among IO neurons and thereby synchronous CS activity. For example, previous multiple-electrode studies have shown that blocking γ-aminobutyric acid-A (GABA_A) receptors within the IO or destroying the GABAergic input to this nucleus produces a more widespread distribution of synchronized CS activity (Lang et al. 1996).

Despite their importance in regulating CS synchrony, immunohistochemical studies have shown that GABAergic terminals represent only about half of the intraglomerular terminals (De Zeeuw et al. 1989). Although the transmitters for most of the non-GABAergic terminals have not been identified, it is likely that the large majority of them utilize glutamate because the projection cells of the nuclei that give rise to these terminals are excitatory and appear to utilize excitatory amino acids as neurotransmitters (Onodera and Hicks 1995) and because presynaptic terminals containing other neurotransmitters that have been identified within the IO appear to avoid the glomeruli (King et al. 1984; Toonen et al. 1998; Wiklund et al. 1981).

Thus if the hypothesis of the glomerular origin of olivocerebellar synchrony is correct, these non-GABAergic, presumably glutamatergic, synapses should also play an important role in controlling the pattern of CS synchrony. Indeed, as mentioned in the preceding text, blocking intra-IO glutamate receptors leads to an enhancement of the banding pattern of CS synchrony (Lang 2001). Thus from the perspective of the banding organization of CS synchrony, glutamatergic and GABAergic inputs to the IO act in a complementary fashion. A natural issue then arises as to how these two neurotransmitter systems interact in generating the patterns of CS synchrony. Therefore a second goal of the present study is to compare the relative strength of these systems in determining the characteristics of spontaneous olivocerebellar activity and to establish the baseline distribution of CS synchrony in the absence of the two primary neurotransmitters that serve to modulate it.

The third goal of this study is to investigate the importance of electrotonic coupling in determining the response patterns of synaptically driven activity. Although much of the spontaneous activity of the olivocerebellar system may be generated intrinsically, it is clear that olivocerebellar activity can be triggered by excitatory input as well. To address this issue, motor cortical stimulation was used to evoke CS activity, and the patterns of synchrony of this evoked activity were compared with the synchrony patterns of spontaneous CS activity.

METHODS

Surgery

Extracellular recordings of CS activity were obtained from female Sprague-Dawley rats (250–300 g). Rats were initially anesthetized with ketamine (100 mg/kg ip) and xylazine (8 mg/kg ip). Supplemental anesthesia was given via a femoral catheter starting ~2–3 h after the initial dose, either as 0.1-ml boluses of a 0.9% saline solution containing ketamine (~6 mg/kg iv) and xylazine (~0.4 mg/kg iv) once every half hour or as a continuous infusion (ketamine, ~6 μg/min; xylazine ~1 μg/min). The exact rates of supplemental anesthesia were calibrated for each animal such that the anesthetic level was deep enough to prevent spontaneous vibrissal movements or reflex responses. This calibration took place during the implantation of the recording electrodes and once established was stable for the remainder of the experiment. The rectal temperature was maintained at 37°C by an electric heating pad. In some experiments, gallamine triethiodide (Flaxedil; ~20 mg/kg iv; Sigma) was given during the recording phase of the experiment.

The multiple-electrode technique used in the present experiments has been described in detail previously (Sasaki et al. 1989; Sugihara et al. 1993). In brief, following anesthetization, a femoral vein catheter for supplemental anesthesia delivery and tracheal tube for ventilation (when Flaxedil was administered) and delivery of supplemental oxygen were inserted. The animal was then placed in a stereotaxic apparatus, and the occipital bone and dura were removed to expose the dorsal surface of the cerebellum and medulla. A silicon-rubber platform was then cemented in place over crus 2a. Glass micro-electrodes (tip diameters of 2–5 μm, resistance ~5 MΩ, filled with a 1:1 solution of glycerol and 2 M saline) were individually inserted, using a piezoelectric micromanipulator (Burleigh, NY), through the platform into the molecular layer of the cerebellum until CS activity could be recorded, which was usually at depths of 70–100 μm below the cortical surface. At these depths CSs are observed, but simple spike activity is not (simple spike activity typically can be detected starting at depths of 120–150 μm), thereby allowing the isolated recording of CS activity. After isolation of CS activity an electrode was released from the manipulator and held in place by the platform. Successive electrodes were inserted in this manner until a rectangular array composed of 8–10 rostrocaudal columns and four to five mediolateral rows, with an inter-electrode distance of 250 μm, was completed. Following electrode implantation, the threshold for each recording channel was individually set to detect CS activity. To ensure that only CS activity was detected throughout the recording session, the activity of all electrodes was continuously monitored using a light-emitting diode (LED) panel and oscilloscope. The LED panel consisted of a rectangular array containing one LED for each electrode. Spike activity that crossed the voltage threshold for detection activated the LED corresponding to that electrode. Thus changes in the activity level of any cell that might occur, such as an increase in baseline noise levels in that channel or the appearance of simple spike activity if the electrode position changed, were immediately detectable as changes in the activity level of the corresponding LED and could then be investigated directly using the oscilloscope. Cells showing such noise or simple-spike contamination were eliminated from the data set. Only cells for which stable recordings were maintained through all experimental conditions were included in the analyses.

In one experiment, a new multichannel recording system (Multi-channel Systems, Germany) was employed. This system not only records the time stamps of the spikes, but the waveforms as well.
allowing for off-line confirmation of spike waveforms. The results obtained with this new system were identical to those obtained with the original recording system.

**Recording procedure**

Each experiment began by obtaining a control 20-min baseline recording of spontaneous CS activity. Next, CS responses evoked by motor cortex stimuli or via the climbing fiber reflex were measured. Following recording of the evoked responses, an intraventricular injection containing a single neurotransmitter antagonist [either picrotoxin or 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[1]quinoline-7-sulfonamide disodium (NBQX)] was initiated, and the spontaneous and evoked CS activity were recorded. A second injection containing both antagonists was then started, and the spontaneous and evoked CS activity during this condition were recorded. The lengths of the recording sessions during drug injection were identical to those of the control periods preceding the injections.

**Intra-IO injection procedure**

To position the injection pipette correctly within the IO, the location of the IO was first determined by lowering a microelectrode through the brain stem from the dorsal surface of the medulla while recording activity from the microelectrode. The correct localization of the pipette tip to the rostral IO was determined by the appearance of characteristic IO activity that was correlated with the CS activity recorded by the electrodes implanted into crus 2a. Following identification of IO activity, the stereotactic coordinates of the IO were noted, and the microelectrode was removed from the brain stem. An injection pipette was then lowered to the same coordinates. The location of the injection pipette was marked at the end of the experiment with an injection of alcian blue dye and con

**Motor cortex and cerebellar stimulation**

In experiments in which motor cortex stimulation was employed to measure the responses of the olivocerebellar system to synaptic input, the bone and dura overlying the motor cortex was removed, and a bipolar stimulation electrode was inserted into the vibrissal region of the motor cortex. The stimuli were usually (n = 6 animals) delivered as single pulses (100–200 μs, 500–1,000 μA) applied once every 2 s. In one animal, stimuli were delivered as 4-s duration stimulus trains in which pulses were delivered at higher frequencies (10–25 Hz). Between 294 and 2940 stimuli were applied in the different experiments.

Cerebellar stimulation was used to elicit the climbing fiber reflex (Eccles et al. 1966). The climbing-fiber reflex involves stimulation of the cerebellar white matter to elicit antidromic action potentials in olivocerebellar fibers. On reaching the IO, this antidromic volley spreads via gap junctions to excite electrotonically coupled IO neurons leading to an orthodromic CS response. The climbing-fiber reflex can thus be used to measure the pattern of electrotonic coupling among IO neurons. To elicit this reflex, a bipolar stimulation electrode was inserted into the white matter of crus 1b at a parasagittal plane that fell within the mediolateral limits of the recording array on crus 2a. Single shocks (60–500 μA, 100–200 μs) applied once every 2 s were used to evoke reflex responses.

**Multi-channel recording system**

The multi-channel recording system that was employed for most of the experiments has been described previously (Sugihara et al. 1993). Briefly, CS signals from all recording channels were converted to transistor-transistor-logic (TTL) pulses, stored on VCR tape, and captured onto a Pentium II personal computer (Dell) with a 1-ms inter-sampling period per channel using a digital I/O board (National Instruments).

A new multi-channel system (MultiChannel Systems, Germany) was employed for one experiment. This system consists of 128 channels (25 kHz per channel sampling; gain, 1,000 times; 0.2- to 8-kHz band-pass filters). Each channel has an independently adjustable single-level spike detector. This system records both the time stamps of the CSs and their waveforms.

**Histology**

On completion of the recording sessions, the solution in the injection pipette was switched to one containing alcian blue solution (10 mg/ml in saline), and a small amount (≤0.3 μl) of dye was injected to mark the tip position. The animal was then perfused intracardially with 0.9% saline followed by 10% formalin. The dissected brain was immersed in 10% formalin overnight followed by 30% sucrose formalin for ≥2 days. Coronal 60-μm-thick frozen sections of the brain stem were cut with a microtome and counterstained with cresyl violet. The histological sections were used to verify that the injection pipette was located in the rostral IO.

**Data analysis**

All data analyses were performed within IGOR (WaveMetrics, OR) using procedures written by the author for this programming environment. Statistical comparisons were made using two-sided Student’s paired t-tests. Mean values in the text are given with their associated SE unless otherwise stated.

Calculation of correlation coefficients was performed according to previously developed methods (Gerstein and Kiang 1960; Sasaki et al. 1989). The spike train of a cell is represented by the function \( X(t) \), where \( i \) represents the time step \( (i = 1, 2, \ldots, N) \), and \( X(t) = 1 \) if the onset of a CS occurred in the \( i \)th time step and otherwise \( X(t) = 0 \). \( Y(t) \) is defined the same as \( X(t) \), but represents the spike train of a second cell. The correlation coefficient, \( C(t) \), is then calculated using the standard formula for determining a correlation coefficient:

\[
C(t) = \frac{\sum_{i=1}^{N} V(i)W(i - t)}{\sqrt{\sum_{i=1}^{N} V(i)^2} \sum_{i=1}^{N} W(i)^2}
\]

where

\[
V(i) = X(i) - \frac{\sum_{i=1}^{N} X(i)N}{N}, \quad \text{and} \quad W(i) = Y(i) - \frac{\sum_{i=1}^{N} Y(i)N}{N}
\]

and \( t \) represents the time lag between compared times of the spike trains.

The degree of synchronous CS activity was measured by calculating the zero-time cross-correlation coefficient, \( C(0) \), using the equation for \( C(t) \) with \( t = 0 \). A time bin of either 1 or 5 ms was used for the different analyses. It should also be noted that while the cross-correlation coefficients of cell pairs displaying synchronized CS activity are small in absolute terms [with \( C(0) \) mostly ranging from 0.01 to 0.30], these values are statistically highly significant, as they are 1–2 orders of magnitude greater than expected by chance (Sugihara et al. 1993) and have a nonrandom spatial distribution. Further, the possibility of spurious correlations due to the rhythmic nature of CS activity and the finiteness of the data set is unlikely because calculation of \( C(0) \) values between cell pairs where the interspike intervals from one of the spike trains has been randomized yields correlations

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1–2 orders of magnitude smaller than those observed here (Lang et al. 1996).

RESULTS

In the present experiments, spontaneous or evoked CS activity was recorded under three successive experimental conditions: control, injection of a single receptor antagonist (either picrotoxin or NBQX), and simultaneous injection of two antagonists (picrotoxin and NBQX). To be included in the database, the average CS firing rate of a cell had to be ≥0.10 Hz in the control condition. Only cells that also displayed CS firing rates of ≥0.10 Hz in the last experimental condition were included in analyses that compared CS activity across conditions. These cells represented the large (≈90%) majority of cells in the database. The potential reasons for the disappearance of activity from the remaining 10% of cells are analyzed separately.

Modulation of average CS firing rates by excitatory and inhibitory IO afferents

Glutamate and GABA represent the major sources of excitation and inhibition to the IO. To assess the relative importance of these transmitters in modulating the level of olivocerebellar activity and to establish the baseline level of spontaneous olivocerebellar activity in the absence of such modulation, sequential injections of solutions containing NBQX alone and in combination with picrotoxin were made into the IO in three animals (n = 100 cells). Eighty-nine of the 100 cells remained throughout the recording and were used for the subsequent analyses. Intra-IO injection of NBQX decreased the average CS firing rate from 1.00 ± 0.06 Hz (mean ± SE; n = 89 cells) in control to 0.49 ± 0.04 Hz (n = 89; P < 0.001). Switch of the injection solution to one containing both NBQX and picrotoxin produced only a small, but statistically significant (P < 0.05) increase in average firing rate to 0.64 ± 0.05 Hz (n = 89). The firing rate changes induced by the drug injections in one typical experiment are shown by the rate meters in Fig. 1A. The rate meter shows the average CS firing rate for the 33 simultaneously recorded cells in the three experimental conditions. The average firing rates for the three conditions in this experiment were: control, 1.28 ± 0.09 Hz; NBQX, 0.56 ± 0.07 Hz; and picrotoxin + NBQX, 0.68 ± 0.07 Hz.

Because of the limited increase in firing rate with addition of picrotoxin to NBQX, several experiments (n = 8) were performed where picrotoxin was injected first to verify its effectiveness. Once an increase in spontaneous CS activity was observed, indicating the effectiveness of the picrotoxin injection, a combined injection of picrotoxin and NBQX was made. Of the 237 cells recorded in this set of experiments, stable recordings were maintained for 209 cells for all conditions, and these cells were used for the analyses. The average CS firing rate in control (1.54 ± 0.07 Hz) increased significantly during the picrotoxin injection to 2.15 ± 0.10 Hz (t = 5.24, P < 0.001). Switching to an injection solution that contained both picrotoxin and NBQX dropped the average CS firing rate below control levels to 1.01 ± 0.06 Hz (picrotoxin vs. picrotoxin + NBQX, P < 0.001; control vs. picrotoxin + NBQX, P < 0.001). An example of the effects of sequentially injecting picrotoxin alone and then in combination with NBQX is shown in the rate meter in Fig. 1B. In this experiment, the initial average CS firing rate was 0.84 ± 0.11 Hz (n = 21 cells). Intra-IO injection of picrotoxin increased this rate to 1.79 ± 0.26 Hz, whereas the firing rate during combined injection of picrotoxin and NBQX was 0.53 ± 0.08 Hz, significantly less than the control level.

Most, but not all, olivary neurons are spontaneously active

The preceding results suggest that the large majority of IO neurons generate spikes spontaneously in the absence of specific triggering input. However, in some cells, CS activity disappeared following the injections. Possible explanations for this disappearance include: IO cells were damaged by the injection, Purkinje cells were lost during the recording, and some IO neurons do not display spontaneous activity (i.e., they require excitatory synaptic input to trigger spikes). To attempt to determine the relative contributions of these factors, several categories were defined according to the conditions in which the spontaneous CS activity of a cell stopped and possibly returned. If the CS activity of a cell disappeared during a
period in which picrotoxin was added to the injection solution, the disappearance was classified as “damage-related” because picrotoxin acts to increase ring rates (i.e., 1st and 2nd possibilities). If the CS activity of a cell disappeared when NBQX was added to the injection solution, the cause was categorized as “uncertain” because it could reflect either a damage-related cause or a silencing of the activity by the loss of excitation with block of glutamate receptors. A third category of “silenceable” cells was defined as cells whose CS activity disappeared with injection of NBQX but returned on addition of picrotoxin to the injection solution.

In the experiments in which NBQX alone was injected initially, 88 of 100 cells displayed CS activity in all three recording periods (control, NBQX, NBQX + picrotoxin). There was one silenceable cell. Of the remaining 11 cells, 6 were lost during the NBQX injection (uncertain cause) and 5 during the subsequent combined injection (damage-related). Thus ≥94% (89 of 95) of the IO neurons did not require glutamatergic input to generate spontaneous action potentials. Similar results were obtained from the experiments in which picrotoxin was injected first. In these experiments, 209 of 237 cells displayed spontaneous CS activity in all three recording periods (control, picrotoxin, picrotoxin + NBQX), 5 cells were lost during the injection of picrotoxin (damage-related), and 23 cells were lost during the combined injection (uncertain cause). Thus in this experimental series ≥90% (209/232) of the cells displayed spontaneous CS activity in the absence of glutamatergic input to the IO.

Complementary actions of picrotoxin and NBQX on spontaneous CS banding patterns of synchrony

Loss of GABAergic activity (either by intra-IO injections of picrotoxin or by cerebellar nuclear lesions) leads to increased levels of CS synchrony and a more uniform spatial distribution, whereas block of glutamatergic activity within the IO leads to a sharper banding pattern (Lang 2001; Lang et al. 1996). Thus these two neurotransmitters act in a complementary fashion with respect to the banding organization of CS synchrony. In the present experiments, the relative strength of these two neurotransmitter afferent systems in determining the distribution of synchrony was compared.

Three experiments were performed in which an intra-IO injection of NBQX was followed by a combined injection of picrotoxin and NBQX. The bubble graphs of Fig. 2 show the distribution of CS synchrony with respect to two reference cells (A–C and D–F) for each of the experimental conditions from one experiment. The positions of the circles represent the relative locations of the recorded cells. The area of each circle is proportional to the degree of synchronous firing between the cell at that location and reference cell M. In control, the typical rostrocaudal banding organization of CS synchrony was observed with respect to both reference cells (Fig. 2, A and D). That is, the level of synchrony was high for cells located in the same parasagittal plane as the reference cell and fell rapidly for cells located at increasing distances from the reference cell in the medial (Fig. 2A) or lateral (Fig. 2D) direction. The injection of NBQX to the IO accentuated the banding patterns by increasing CS synchrony among cells located in the same parasagittal plane and decreasing CS synchrony among cells located in different parasagittal planes (Fig. 2, B and E). The combined injection of NBQX and picrotoxin further increased the CS synchrony among cells within the same parasagittal plane but only produced a small increase among cells located in different planes (Fig. 2, C and F); thus the bands of synchronous CS activity remained more sharply defined than they originally were in control (Fig. 2, compare A vs. C, and D vs. F).

Similar changes in the spatial distribution of CS synchrony were generally observed regardless of the choice of reference cell. This was demonstrated by calculating the zero-time cross-correlation coefficient, C(0), for all possible cell pairs and then plotting the average C(0) value as a function of the mediolateral separation between the cells. Such a plot for all cell pairs for the experiment shown in Fig. 2, A–F, is shown in Fig. 2G, where the enhancement of the banding pattern during the NBQX and NBQX + picrotoxin injections is demonstrated by the increase in average synchrony from control levels at 0 μm and the decreases at distances ≥500 μm. Similar results were found for all experiments as shown in Fig. 2H by the plot of average synchrony for all cell pairs from all experiments in which sequential injections of NBQX alone and then in combination with picrotoxin were made (n = 89 cells, n = 1,288 cell pairs).

Eight experiments (n = 209 cells) were performed in which picrotoxin alone was injected into the IO followed by a combined injection of picrotoxin and NBQX. The results from one typical experiment are shown in Fig. 3, A–D. In control, higher levels of CS synchrony are found primarily among cells located at small (≤250 μm) mediolateral distances from the reference cell M (Fig. 3, A and D). After injection of picrotoxin, the rostrocaudal banding pattern is almost absent because of high levels of synchronous CS activity among all cells of the recording array (Fig. 3, B and D). Switching to a solution containing both picrotoxin and NBQX rapidly led to the reappearance of the banding pattern (Fig. 3, C and D). Note that the addition of NBQX to the solution caused both a decrease in synchrony between the reference cell M and cells located ≥500 μm lateral to it, and a further increase from picrotoxin induced levels in synchrony between cell M and those located at mediolateral distances of ≤250 μm. Similar results were obtained in the other experiments as shown in Fig. 3E, where the average level of CS synchrony is plotted as a function of mediolateral distance between cells for all cell pairs (n = 2,806) in all experiments (n = 8) in which sequential injections of picrotoxin alone and then in combination with NBQX were made into the IO.

Modulation of climbing fiber reflex by intra-IO injection of picrotoxin and NBQX

One interpretation of the decreased spatial distribution of synchrony for spontaneous CS activity following injection of NBQX is that electrotonic coupling mediates synchrony among local clusters of IO neurons (those that project to the same parasagittally oriented strip of cortex), but that synchrony among Purkinje cells located in different cortical strips is driven by excitatory afferents that simultaneously activate multiple IO regions that project to the different cortical strips. To investigate this issue, the effect of intra-IO injections of picrotoxin and NBQX on the distribution of climbing fiber reflex evoked activity was examined. This reflex is mediated by the
gap junctions that electrotonically couple IO neurons and therefore does not involve synaptic transmission (Llinás et al. 1974). Thus if the spatially restricted distribution of synchrony that is observed for spontaneous CS activity following NBQX injections simply reflects the loss of synchronized excitatory afferent input to the IO, then the distribution of the climbing fiber reflex should be unaffected by such injections. Alternatively, if the reflex is affected, this would indicate that tonic glutamatergic activity acts to modulate the pattern of effective electrotonic coupling within the IO and suggest that electrotonic coupling of IO neurons also underlies synchronous CS activity between widely separated Purkinje cells.

The effect of intra-IO injections of picrotoxin followed by picrotoxin + NBQX on the climbing fiber reflex was tested in three experiments (n = 72 cells). In each case, picrotoxin injections increased the strength and spatial extent of the reflex response, whereas NBQX narrowed the response distribution. A typical example is shown in Fig. 4 from an experiment in which the climbing fiber reflex was evoked using a bipolar stimulation electrode inserted rostral to the recording array into the white matter of crus 1. The mediolateral position of the stimulating electrode relative to the recording array is indicated by “Stim” in Fig. 4, A–C. Single shocks (500 μA, 100 μs) evoked responses such as shown in Fig. 4D (top traces). The stimulus artifact (indicated by *) was followed by a short-latency response at 3.1 ms (arrowhead) due to intracerebellar branching of axons. The CS evoked by the climbing-fiber reflex occurred at a latency of 9.4 ms (arrow), approximately twice the conduction time of the olivocerebellar pathway in rats (Sugihara et al. 1993). The average latency of the reflex response ranged from 8.7 to 11.7 ms in the three experiments. The similarity of the reflex response to the spontaneous spikes shown in Fig. 4D, bottom traces, demonstrates the CS nature of the evoked response.

To measure the spatial distribution of the reflex response, 300 shocks were applied in each experimental condition, and the percent of stimuli evoking responses was calculated for each cell for each condition. In control, the stimuli primarily evoked reflex responses in cells located approximately in the same parasagittal plane as the stimulus electrode (Fig. 4A). During the injection of picrotoxin to the IO, identical stimuli were more likely to evoke a response and the reflex became

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Enhanced banding pattern of CS synchrony following injection of NBQX persists with injection of picrotoxin. A–F: bubble graphs showing the degree of CS synchrony between each cell in the recording array with the reference cell M. Locations of the circles represent the relative positions of the recording electrodes on crus 2a (see key on right of figure for general orientation of the recording array), and the area of each circle is proportional to the degree of synchrony. Scale on right of figure is for A–F. The pattern of CS synchrony is shown for the 3 experimental conditions (control, NBQX, NBQX + picrotoxin) for 2 different reference cells (A–C and D–F). G: average level of CS synchrony plotted as function mediolateral separation between cells for the experiment shown in A–F. Plots were generated by calculating synchrony values for all possible cell pairs and then sorting them according to the mediolateral separation of the cells. Inset: larger separation distances with an expanded y-axis scale. H: summary plot of CS synchrony as a function of mediolateral separation for three experiments (n = 89 cells, 1,288 cell pairs) in which sequential injections of NBQX alone and then in combination with picrotoxin were performed. Recording sessions were 20 min for each condition. Time bin for defining synchrony was 5 ms. Error bars are SE. Scale bars in A are for A–F and indicate distance on crus 2a.
more widespread than in control (Fig. 4, B and E). Instead of responses being confined to a 250-μm-wide strip of cortex, the area showing evoked responses extended for ≈ 1,750 μm in the mediolateral direction. Addition of NBQX to the injection resulted in a constriction of the area from which reflex responses were elicited back to roughly the control distribution (Fig. 4, C and E). However, within this narrow rostrocaudally oriented strip of cortex, the percent of stimuli evoking CSs remained high.

In two further experiments (n = 68 cells), the effect of NBQX injections on the climbing fiber reflex was tested. In both cases, the injection of NBQX primarily increased the reflex response percentages among cells that displayed responses in control but did not produce a widespread response distribution such as occurred with injection of picrotoxin alone. Subsequent injection of NBQX + picrotoxin led to a somewhat wider response distribution but not to the extent usually observed with injection of picrotoxin alone. The response distribution for each of the experimental conditions from one of these experiments is shown in Fig. 4F, which plots the average percent response of cells as a function of their mediolateral position relative to the lateral edge of the recording array (a higher distance indicates a more medial position). In the control condition of this experiment, responses were largely limited to cells located between 1,500 and 1,750 μm from the lateral edge of the recording array, consistent with the location of the stimulation electrode relative to the recording electrode. Injection of NBQX primarily increased the reflex responses of these cells, although some cells in the neighboring positions also showed a somewhat increased response rate. The width of this response distribution is similar to that of the synchrony bands observed for spontaneous CS activity after NBQX (Fig. 2, B and E). Addition of picrotoxin to the injection solution led to further increases in the response rates of cells already responding but generally did not produce responses in those cells that had not shown responses in control or during injection of NBQX.

**Comparison of synchrony patterns for spontaneous and synaptically evoked CS activity**

To investigate the importance of electrotonic coupling of IO neurons in determining the synaptic responses of the olivocerebellar system, motor cortex stimulation was employed to evoke CS activity, and the patterns of synchrony for spontaneous and synaptically evoked CS activity were compared (n = 7 experiments, 180 cells).

Motor cortical stimuli evoked CS responses at latencies of 17.00 ± 2.79 ms (n = 180) as calculated from the peak of peristimulus histograms with 2-ms bins (e.g., Fig. 8B). To assess the effectiveness of the stimulation, the percentage of stimuli evoking a CS response was calculated for each cell from its peristimulus histogram. First, the peak of the histogram was determined within a time window of 13- to 25-ms poststimulus. The percent response was then obtained by summing the height of the peak bin and all neighboring bins until the height of a bin fell below a threshold (defined as the baseline level of the histogram plus 20% of the peak height). The number of evoked responses was then expressed as a percent of the total stimuli applied. Overall the response rate was 17.9 ± 1.3% (n = 180), with the average single-cell response rates ranging from 2 to 54% for the individual experiments.

For each experiment, the pattern of synchrony for spontaneous CS activity was compared with the pattern during the 13-ms poststimulus time window in which the evoked CSs occurred. Results from two experiments that had clear rostrocaudal banding patterns for spontaneous CS activity but that...
had either weak (Fig. 5A) or strong (Fig. 5C) evoked responses are shown in Fig. 5. The spatial distributions of CS synchrony for these experiments are shown in Fig. 5, B and D, respectively. In control, the rostrocaudal banding pattern is demonstrated by the higher levels of synchrony for the 0-μm mediolateral separation as compared with the levels for cells that were separated in the mediolateral direction (Fig. 5, B and D, filled circles). During stimulation, the average level of synchrony for the evoked CSs was increased in both experiments, as indicated by the upward displacement of the evoked curves (open squares) from the spontaneous curves. However, despite this displacement, the evoked curves were similar in shape to those from spontaneous activity. This is demonstrated by scaling the spontaneous curves such that their average values are equal to the average values of the respective evoked curves (gray triangles). Thus the banding pattern of CS synchrony persists even during strong synaptic input.

To demonstrate further the relationship of synchrony patterns during synthetically evoked CS activity to those during spontaneous CS activity, the individual C(0) values for spontaneous and motor-cortex-evoked CS activity were calculated for the 2,442 cell pairs generated from the 180 cells of the seven experiments. A scatter plot of this data was generated such that for each cell pair the abscissa of its point was the C(0) value for the spontaneous activity of the cell pair and the ordinate was the C(0) value for its stimulus evoked activity (Fig. 5E). This plot shows the correlation between the synchrony levels during spontaneous activity and motor cortex stimulation (r = 0.70). Similar scatter plots generated for the individual experiments had correlations ranging from 0.55 to 0.80.

Although rostrocaudal banding is the predominant synchrony pattern for spontaneous CS activity, sometimes a more patch-like distribution is seen for individual cells, even when the majority of cells in an experiment show a band-like distribution. One such example is shown in Fig. 6A, where the highest correlations during spontaneous activity were found for cells located 250 and
Nevertheless, the pattern of synchrony for evoked CSs was highly correlated with the pattern displayed for spontaneous activity despite the fact that the stimuli evoked CSs throughout the array (the evoked response distribution is shown in Fig. 8A). That is, although the levels of CS synchrony during motor cortex stimulation were higher than during spontaneous activity, the same basic distribution of highly and weakly correlated cells was

**FIG. 5.** Similar rostrocaudal banding distributions of CS synchrony characterize spontaneous and motor cortex evoked CS activity. A: plot showing spatial distribution of CS responses to motor cortical stimuli for an experiment in which stimuli evoked responses in a limited distribution of cells. B: plot of average level of CS synchrony as a function of mediolateral separation between cells for the experiment in A. Curves show average CS synchrony for spontaneous activity (filled circles, solid line), activity during motor cortex stimulation (open squares, solid line), and for spontaneous activity after normalization to same average value as evoked activity (gray triangles, dash-dotted line). Error bars are SEM for the spontaneous and evoked activity. C and D: same as A and B for a 2nd experiment in which motor cortex stimulation was effective in evoking CS responses relatively uniformly throughout the recording array. E: scatterplot of C(0) values during spontaneous (x axis) and evoked (y axis) activity for all cell pairs (n = 2,442 cell pairs, 180 cells) in 7 experiments. Time bin for analysis was 1 ms. Scale at bottom right of figure is for A and C. Scale bars in A are also for B. Orientation key in B also for A.

**FIG. 6.** Similar spatial distributions of CS synchrony during spontaneous and motor-cortex-evoked CS activity for a cell with an atypical synchrony distribution. A and B: bubble graph showing distribution of CS synchrony with respect to cell M during a 20-min recording of spontaneous activity (A) and during a 20-min period in which stimuli were applied to the motor cortex (B). C: bubble graph showing the pattern of synchrony during spontaneous CS activity after normalizing the correlation values such that the average level of synchrony is the same as during motor cortex stimulation. D and E: plots of the average level of CS synchrony as a function of the mediolateral separation between cells for all cell pairs involving cell M (D) and for all cell pairs (E). In each plot, the curves for spontaneous activity (filled circles, solid lines), motor cortex evoked activity (open squares, solid lines), and normalized spontaneous activity (gray triangles, dashed lines) are shown. Time bin for synchrony calculation was 1 ms. Scale and orientation key for bubble graphs of A–C are given at bottom right of figure.
were applied at 2 intensities (500 and 860 \textmu A). The mean response rates and SDs were 20.4 ± 15.9\% for the lower intensity and 53.4 ± 18.8\% for the higher intensity. Comparison of the variability of the responses using the coefficient of variation (CV = SD/mean) confirms that there was a more uniform response to the higher intensity stimuli (low intensity, CV = 0.78; high-intensity, CV = 0.35). Given the different response distributions to the two stimulus intensities, the distribution of CS synchrony for the two conditions was compared. The average level of synchrony for spontaneous activity and for activity evoked during low- and high-intensity stimulation is plotted as a function of mediolateral separation between cells in Fig. 7C.

Stimulation produced higher levels of synchrony, and the amount of increase in synchrony varied with stimulus intensity. However, the basic banding pattern remained, even with the high-intensity stimulation, as demonstrated by the normalized synchrony curves (Fig. 7D). Stimulation does appear to flatten the synchrony curves to some extent, an effect that increases with stimulation intensity; however, the normalized curves all show higher levels of synchrony for 0–250 \textmu m distances than for larger separations, indicating that the banding structure of synchrony remains even when stimulation evokes responses throughout the recording array (Fig. 7D). Moreover, a high correlation was found between the individual cell pair C(0) values for spontaneous CS activity with the C(0) values for CSs evoked either by the low- or high-intensity stimulation (low intensity, r = 0.80; high-intensity, r = 0.72).

By varying the stimulus intensity, it was possible to evoke either a uniform or a nonuniform response to motor cortex stimulation across the recording array, as shown in Fig. 7. At the lower stimulus intensity, CSs were preferentially evoked in cells located on the medial three columns of the recording array, whereas at the higher stimulus intensity, responses to a large percentage of the stimuli were evoked throughout the array (Fig. 7, A and B). The mean response rates and SDs were 20.4 ± 15.9\% for the lower intensity and 53.4 ± 18.8\% for the higher intensity. Comparison of the variability of the responses using the coefficient of variation (CV = SD/mean) confirms that there was a more uniform response to the higher intensity stimuli (low intensity, CV = 0.78; high-intensity, CV = 0.35). Given the different response distributions to the two stimulus intensities, the distribution of CS synchrony for the two conditions was compared. The average level of synchrony for spontaneous activity and for activity evoked during low- and high-intensity stimulation is plotted as a function of mediolateral separation between cells in Fig. 7C.
1.1%). Similar results (i.e., no individual stimuli evoked CS responses throughout the array) were found in the other six experiments in which CSs were evoked with single shocks to the motor cortex.

With higher intensity stimuli or repetitive stimulation (which should phase-lock IO neurons to the stimuli), individual stimuli could evoke responses in all cells of the array (n = 2 experiments). Even in these two cases, which should provide the most favorable conditions for overriding the coupling pattern of IO neurons, it was still rare for a single stimulus to evoke responses throughout the array (≤1% of stimuli evoked responses in all cells), although individual stimuli often did evoke responses in the majority of cells (62 and 76% of stimuli evoked responses in ≥50% of the cells in the array). Yet even in these experiments the correlation structure remained similar to that in control (r = 0.72 and 0.77 for the relationship between individual C(0) values for spontaneous activity and during stimulation), which points to a second factor responsible for the maintenance of the synchrony patterns: the latencies of evoked CSs to an individual stimulus for cells that showed correlated spontaneous CS activity have less variability than the latencies of cells whose spontaneous CS activity was not correlated.

Intra-IO injection of picrotoxin alters CS response distribution to motor cortex stimulation

Picrotoxin affects the spatial distribution of spontaneous CS synchrony and climbing-fiber-reflex evoked CS responses, presumably by modifying the effective electrotoneic coupling of IO neurons. Thus if the pattern of electrotoneic coupling is shaping the spatial distribution of CS responses to motor cortical stimuli, intra-IO injections of picrotoxin should also change this distribution. The effect of intra-IO injections of picrotoxin on the distribution of motor cortex-evoked CSs was determined in three experiments (n = 91 cells). In each experiment, the average level of evoked responses during injection of picrotoxin increased significantly (P < 0.001) from control levels (experiment 1: control, 25.2 ± 2.6%, picrotoxin, 42.2 ± 3.6%, n = 26; experiment 2: control, 12.4 ± 1.3%, picrotoxin, 37.5 ± 3.3%, n = 30; experiment 3: control, 1.9 ± 0.4%, picrotoxin, 10.1 ± 1.6, n = 35). The changes in the distribution of motor cortex evoked activity for the second experiment are shown in Fig. 8. Not only does the response rate increase (Fig. 8, A and B, compare I and II), but the cell-to-cell variation in response decreases. Thus in control the coefficient of variation was 0.58, whereas during injection of picrotoxin, the coefficient decreased to 0.48. In the other two experiments the coefficient of variation in the picrotoxin condition also decreased from that in control (average difference = −0.18 ± 0.09, n = 3 experiments).

NBQX block of excitatory input to the IO

To confirm the efficacy of the NBQX injections in blocking excitatory input, the CS response to motor cortex stimulation (n = 4 experiments) or tactile stimulation (n = 2 experiments) was also measured during the injection of

**Fig. 8.** Intra-olivary injection of picrotoxin produces a more uniform response distribution to motor cortex stimulation. A: bubble graphs showing CS responses to motor cortex stimuli. Area of each circle is proportional to percent of stimuli to which that cell responded. Response distributions are shown for control (I) and during intra-IO injection of picrotoxin alone (II) and in combination with NBQX (III). Scale indicates percent of stimuli. B: peristimulus histograms showing the time course of the responses of 2 cells (indicated by numbers 1 and 2 in I) to motor cortex stimulation in the 3 conditions indicated in A.
NBQX. NBQX injections blocked evoked CS responses throughout the recording array. An example of this block is shown in Fig. 8, AII and BII. In this experiment, the prior injection of picrotoxin had led to high response rates throughout the array. Nevertheless, NBQX was effective in eliminating the evoked responses.

**Discussion**

In the present experiments, the effects on CS activity of blocking GABAergic and glutamatergic input to the IO were compared. The results demonstrate that the vast majority of IO neurons generate spontaneous spike activity in the absence of glutamatergic and GABAergic input, that these neurotransmitters have a largely complementary action on the spatial distribution of CS synchrony; and that the pattern of electrotetnic coupling within the IO plays an important role in shaping synaptically evoked responses of the olivocerebellar system.

**Origin and modulation of spontaneous olivocerebellar activity**

It has been hypothesized that the olivocerebellar system generates spontaneous oscillatory activity that can be employed as a timing signal for organizing motor commands (Llinás 1991). Indeed, investigations of IO neurons using in vitro techniques have revealed that the membrane conductances of these cells allow them to generate spontaneous oscillatory activity (Bal and McCormick 1997; Benardo and Foster 1986; Llinás and Mühlthaler 1988; Llinás and Yarom 1981a,b, 1986), and computer models also suggest that IO neurons can generate spontaneous activity, particularly when they are embedded within a network of electrotonically coupled cells (Manor et al. 1997). However, the cellular environment found under in vitro conditions may be quite different from that in vivo, and whether truly spontaneous olivocerebellar activity occurs in the intact animal needs to be demonstrated.

This issue was investigated in a previous study in which intra-IO injection of CNQX or NBQX was found to reduce, but not abolish, CS activity, suggesting that IO neurons are capable of spontaneous spike activity in the absence of fast excitatory synaptic transmission (Lang 2001). However, several questions concerning the origin of CS activity remained from this initial report. Is the activity that remains following block of excitatory input to the IO generated by the inhibitory feedback from the cerebellar nuclei via rebound low-threshold Ca spiking? If this is the case, then most or all olivocerebellar activity would still be driven by afferent activity. Second, does the NBQX-induced decrease in CS activity imply that some “spontaneous” CSs are directly triggered by excitatory inputs or is the decrease simply due to a negative biasing of the IO neurons as a result of the unopposed GABAergic pathways following glutamate blockade? In this case, virtually all spontaneous CS activity could be viewed as intrinsically generated, with tonic excitatory and inhibitory input simply acting in a modulatory role.

The present results provide evidence against either of these extreme possibilities and favor the interpretation of a dual origin for spontaneous CS activity, as previously suggested (Lang 2001). It was found that spontaneous CS firing rates were ~65% of control levels after the block of both glutamatergic and GABA<sub>A</sub> receptors, with 90% of cells continuing to display spontaneous activity. Of the remaining 10% of cells, the loss of CS activity in many cases can be explained by the damage caused by the injection procedure or loss of the Purkinje cell itself. This is consistent with the fact that injection of Ringer solution to the IO caused the disappearance of CS activity in a similarly small percentage of cells (Lang 2001). However, the numbers of cells whose activity disappeared with the addition of NBQX to the injection solution was higher than for Ringer injections or when picrotoxin was added. Thus there may be a small percentage of IO neurons whose spike activity is solely triggered by excitatory input. Regardless, the results indicate that for most IO neurons the majority of their spike activity is intrinsically generated and does not require specific triggering input.

Several caveats should be mentioned with respect to this conclusion. First, because NBQX does not block N-methyl-D-aspartate (NMDA)-type glutamate receptors, which are present in the IO (Petralia et al. 1994), it is possible that the remaining spontaneous CS activity may have been in response to NMDA-mediated excitation. However, this possibility is unlikely because of several reasons discussed previously (Lang 2001), including the voltage dependence of NMDA channels, the loss of glutamatergic-mediated, stimulus-evoked CSs with intra-IO injections of NBQX, and the fact that the anesthetic that was used, ketamine, is an NMDA channel blocker (Ebert et al. 1997) and was likely present in concentrations well above those needed to block NMDA receptors. Second, other transmitters found within the IO can influence the excitability of IO neurons and may be capable of triggering spike activity. While transmitters other than GABA and glutamate clearly influence IO neuronal excitability [e.g., serotonin has an excitatory action (Llinás and Yarom 1986; Placantonakis et al. 2000; Sugihara et al. 1995)] and the neuropeptides Leu-enkephalin and calcitonin gene-related peptide have inhibitory actions (Gregg and Bishop 1995; Schulman 1981), the fibers containing these other transmitters tend to terminate in a pattern indicative of a neuromodulatory/paracrine role and thus their activity is unlikely to be specifically tied to the timing of individual CSs (King et al. 1984; Toonen et al. 1998; Wiklund et al. 1981). Moreover, anatomical studies indicate that in comparison to GABAergic and presumed glutamatergic projections, afferents containing other neurotransmitters are quantitatively less important (De Zeeuw et al. 1989; Toonen et al. 1998).

Given that glutamate and GABA are the major mediators of fast excitatory and inhibitory synaptic transmission within the IO, it is interesting to compare their effectiveness in modulating IO excitability. The distribution of GABAergic terminals in the IO is unusual in that many of them terminate on dendritic spines (De Zeeuw et al. 1989). Nevertheless, they still form the majority of terminals contacting the soma and axon hillock (De Zeeuw et al. 1989, 1990), and therefore GABA should be the dominant transmitter for controlling the firing rate of IO neurons. Thus the present results are somewhat paradoxical in that the effect of NBQX on firing rate dominated that of picrotoxin and suggest that the activity of GABAergic IO afferents is relatively low.
under the experimental conditions. Indeed, although cerebellar nuclear cells have significant levels of spontaneous activity (e.g., Eccles et al. 1974a,b; McDevitt et al. 1987), large increases in these spontaneous rates can be induced (Andersson and Hesslow 1987), which in turn lead to a near complete suppression of CS activity, even with continued activation of excitatory IO afferents (Andersson et al. 1988; S. P. Marshall and E. J. Lang, unpublished observations). The presence of GABA\textsubscript{B} receptors in the IO (Margeta-Mitrovic et al. 1999) should also be noted as they are not blocked by picrotoxin and may contribute to GABAergic inhibition in the IO. However, their contribution is likely to be comparatively small because intra-IO injection of bicuculline, a GABA\textsubscript{A} blocker, eliminates GABA-mediated phenomena, such as postconditioning inhibition of IO activity (Andersson et al. 1988).

**Origin and spatial distribution of complex spike synchrony**

Multiple electrode studies have demonstrated that CS activity in different Purkinje cells is synchronized on a millisecond time scale (Lang et al. 1999; Llinás and Sasaki 1989; Sasaki et al. 1989; Sugihara et al. 1993). Based on morphological and electrophysiological data, it has been proposed that CS synchrony results from electrotonic coupling of IO neurons by gap junctions (Llinás 1974; Llinás et al. 1974; Sotelo et al. 1974). Furthermore, most IO neuronal gap junctions occur between dendritic spines within glomeruli that also contain presynaptic terminals, suggesting that the efficacy of the electrotonic coupling, and thereby the pattern of CS synchrony, is primarily modulated by the activity of these intraglomerular synapses (Llinás 1974; Llinás et al. 1974; Sotelo et al. 1974).

In support of these proposals, electrotonic coupling has been demonstrated directly with simultaneous recording of pairs of IO neurons in brain stem slices (Llinás and Yarom 1981a). Moreover, in a previous multiple electrode study, synchronous CS activity remained after block of glutamatergic input to the IO, indicating that excitatory input to the IO is not required for synchronization of CS activity (Lang 2001). However, in this same study, block of excitatory input by NBQX altered the spatial distribution of Purkinje cells displaying synchronized activity such that synchronous CS activity was almost exclusively limited to cells within the same ~250-µm-wide, rostrocaudally oriented strip of cortex. Thus while this study provided strong evidence that CS synchrony among Purkinje cells within the same parasagittally oriented cortical strip results from electrotonic coupling of IO neurons, it was less decisive on the question of what underlies synchronization of CSs between Purkinje cells in different cortical strips.

One possible explanation of the effects of NBQX is that while intra-band CS synchrony results from electrotonic coupling of IO neurons, synchronous CS activity among Purkinje cells in different parasagittal bands depends on specific triggering inputs to the groups of IO neurons that project to these different bands. In this case, either the activity of the different afferent axons would have to be precisely synchronized or the subthreshold oscillations could serve to synchronize asynchronous inputs (Lampl and Yarom 1993). Although straightforward, this explanation is not complete in that it does not account for the increased CS synchrony among cells of the same cortical band following NBQX injections. An alternative explanation is that rather than acting to trigger synchronous IO spikes directly, glutamate acts to modulate the effectiveness of the coupling between IO neurons (Lang 2001). Blocking glutamatergic activity should both increase the membrane resistance of IO neurons and hyperpolarize them. These effects should have opposing actions on the coupling of spike activity between IO neurons. Thus the action of the NBQX injections on the distribution of CS synchrony can be explained if the first effect (increased membrane resistance) is the dominant factor among neighboring IO neurons, which largely project to the same rostrocaudal strip of cortex (Sugihara et al. 2001), and the second effect (hyperpolarization) dominates the interaction of cells projecting to different cortical strips.

The climbing-fiber reflex was used to examine these alternative explanations for the role of glutamatergic activity in determining the distribution of CS synchrony. The climbing-fiber reflex measures the distribution of effective electrotonic coupling within the IO because this reflex is mediated by the gap junctions between IO neurons and does not involve synaptic transmission (Llinás et al. 1974). Thus if gap junctional coupling cannot mediate synchronization of CS activity among Purkinje cells in different parasagittally oriented cortical strips, it should not be possible to evoke a widespread reflex response. In fact, intra-IO injection of picrotoxin was found to result in just such widespread reflex responses here and previously (Lang et al. 1996), implying that electrotonic spread of spike activity can occur among IO neurons that project to different parasagittally oriented cortical strips. Furthermore, injection of NBQX resulted in a strong but narrowly distributed reflex response, which is virtually identical to its effect on the distribution of spontaneous CS activity. Because the timing of the cortical stimuli used to elicit the reflex response was independent of the timing of afferent activity, the effect of the NBQX injections on the reflex distribution most likely reflects a change in effective coupling of IO neurons caused by the loss of tonic glutamatergic input to the IO. Moreover, the near identical action of NBQX on spontaneous and evoked CS activity suggests that the mechanism of action is the same in both cases. Thus the present results support the idea that glutamate acts to modulate the effectiveness of the coupling between IO neurons and that synchronous CS activity is largely the result of spread of activity through the gap junctions. They do not, however, exclude the possibility of afferent activity directly triggering synchronous CS activity.

Anatomical results have shown that GABAergic synapses account for ~40–45% of terminals associated with gap junctions with nonGABAergic, presumably glutamatergic, terminals accounting for most of the remaining 55–60% (De Zeeuw et al. 1989). While the effect of GABA release from these terminals is almost certainly to decrease the effective coupling between IO neurons (by both hyperpolarization and increased membrane conductance), the action of glutamate is less straightforward. On the one hand, glutamate-mediated excitatory postsynaptic potentials (EPSPs) could directly trigger synchronous spike activity in coupled IO cells. Alternatively, the increased membrane conductance associated with the glutama-
tergic EPSP could reduce the current associated with spikes in one cell passing through the gap junction, leading to less synchronous activity between the coupled cells. While increased synchrony can occur with very strong synchronous activation of excitatory pathways (e.g., Fig. 7), the present results suggest that the second mechanism is the primary mode of operation because rather than falling, the level of synchronous CS activity among parasagittally aligned Purkinje cells increased following block of glutamate.

Beyond providing evidence concerning the underlying mechanism for generation of synchronous CS activity, the results characterize the baseline distribution of CS synchrony in the relative absence of modulation by afferent activity and thus reveal the anatomic structure on which physiological modulation of synchrony patterns occurs. When both glutamate and GABA were blocked, a pronounced banding pattern was observed in which synchronous CS activity occurred almost exclusively among cells within the same narrow 250–500 µm wide, parasagittally oriented cortical strip. This pattern is consistent with the overall topography of the olivocerebellar projection (Voogd and Bigaré 1981), and in particular reflects the fact that neighboring IO neurons primarily project to areas within the same 200–400 µm-wide narrow parasagittal plane (Sugihara et al. 2001). The relatively more abrupt drop in synchrony in the mediolateral direction observed following block of GABAergic and glutamatergic activity as compared with the control distribution suggests that the coupling of IO neurons is not homogeneous but rather that there are probably small groups of neighboring IO cells that are much more extensively coupled to each other than to adjacent groups of cells. This is consistent with anatomical evidence showing that IO neurons form clusters (Sotelo et al. 1974). Finally, despite the predominance of the banding pattern, there are occasional cells that have nonparasagittal banding patterns. The synchrony pattern for these cells probably reflects the fact that while olivocerebellar axons tend to branch within a single parasagittal plane, there can also be branching in the transverse axis (Ekerot and Larson 1982). Such transverse branching is relatively rare (Apps et al. 1991) and thus corresponds to the relative infrequency of observing synchrony patterns other than parasagittal banding under control conditions or following NBQX injections.

Despite the importance of the anatomical substrate, the results clearly indicate that both GABA and glutamate can significantly reorganize the patterns of CS synchrony. It was somewhat surprising to find that NBQX was at least as effective as picrotoxin in altering the distribution of CS synchrony. While this may indicate that glutamate has a similarly important role in controlling CS synchrony as GABA and is consistent with the similar proportions of GABAergic and non-GABAergic terminals found within glomeruli (De Zeeuw et al. 1989), other interpretations are possible. For example, the greater effect of NBQX may in part reflect a greater level of spontaneous glutamatergic activity under the experimental conditions. Nevertheless, the results indicate that these two afferent systems act in a complementary manner with respect to the banding structure of CS synchrony, and thus allow for flexible control of the patterns of synchrony in the olivocerebellar system.

Patterns of electrotonic coupling among IO neurons shape responses to synaptic inputs

Previous multiple electrode recording studies of CS activity have largely focused on the patterns of spontaneous CS activity and their modulation (De Zeeuw et al. 1996; Fukuda et al. 2001; Lang 2001; Lang et al. 1996, 1999; Lilnáš and Sasaki 1989; Sasaki et al. 1989; Sugihara et al. 1993; Yamamoto et al. 2001). Yet, results of the present and previous experiments (Lang 2001) indicate that some spontaneous CS activity is the result of excitatory inputs to the IO, and clearly, strong physiologic stimuli can evoke CS responses. Thus an interesting question arises as to the impact of electrotonic coupling of IO neurons on the olivocerebellar response pattern to afferent input.

In a prior multiple electrode study, the CS responses of crus 2a Purkinje cells to tactile stimulation of the peri-oral region were investigated (Lilnáš and Sasaki 1989). It was found that mechanical stimuli evoked CS responses in parasagittally oriented patches of cortex, similar to the typical banding patterns of spontaneous CS activity. Similar results were recently reported using single-unit recordings (Brown and Bower 2001). The present results show that in the rat, crus 2 also receives input from the face region of the motor cortex, suggesting a close correspondence between somatosensory and motor cortical inputs to this part of the olivocerebellar system, as has been demonstrated for the olivocerebellar projections to the anterior lobe of the cat cerebellum (Allen et al. 1974; Andersson and Nyquist 1983; Provini et al. 1968). The present results are also consistent with work done in monkey where it was shown that the lateral motor cortex (face and forelimb region) evoked CS responses in crus 2 (Sasaki et al. 1977).

However, the previous studies did not directly measure the patterns of synchronous activity in response to stimuli nor compare them to spontaneous patterns, and it could be argued that the response distributions simply reflect the topographic organization of the IO afferents and the olivocerebellar projection. The results of the present study support an important role for gap junction coupling in determining patterns of evoked activity. First, a close correspondence was found between the patterns of synchrony during evoked and spontaneous CS activity, strongly suggesting that gap junctional coupling is affecting the distribution of the synaptically evoked responses. Moreover, in the present experiments, relatively high stimulus intensities were used so that stimuli evoked CS responses throughout the recording array, removing topographic specificity as a major determinant of the response distribution, and yet the response patterns to individual stimuli still reflected the coupling patterns observed for spontaneous activity. The results of the picrotoxin injections further underscore the importance of electrotonic coupling in determining the response distribution to afferent input. Specifically, intra-IO injections of picrotoxin broadened the response distribution to motor cortical stimulation and made it more uniform, consistent with their action on the synchrony distribution of spontaneous CS activity. Finally, considering that the basic patterns of synchronous CS activity remain in the face of strong and widespread input to the IO, such as was generated by the electrical shocks employed in the present study, the response to more physiologic levels of activity should be determined to an even greater
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extent by the pattern of electrotonic coupling among IO neurons.

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REFERENCES


APPS R, TROTJR, and DIETRICH C. A study of projection in the branching from the inferior olive to the x and lateral cl zones of the cat cerebellum using a combined electrophysiological and retrograde fluorescent double-labeling technique. Exp Brain Res 87: 141–152, 1991.


