Association Between Dendritic Lamellar Bodies and Complex Spike Synchrony in the Olivocerebellar System

C. I. DE ZEEUW,[1,2] S.K.E. KOEKKOEK,[1,2] D.R.W. WYLIE,[2,3] AND J. I. SIMPSON[2]
[1]*Department of Anatomy, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands;* [2]*Department of Physiology and Neuroscience, New York University Medical Center, New York, New York 10016; and* [3]*Department of Psychology, University of Alberta, Edmonton, Alberta T6G 2EQ, Canada*

De Zeeuw, C. I., S.K.E. Koekkoek, D.R.W. Wylie, and J. I. Simpson. Association between dendritic lamellar bodies and complex spike synchrony in the olivocerebellar system. *J. Neurophysiol.* 77: 1747–1758, 1997. Dendritic lamellar bodies have been reported to be associated with dendrodendritic gap junctions. In the present study we investigated this association at both the morphological and electrophysiological level in the olivocerebellar system. Because cerebellar GABAergic terminals are apposed to olivary dendrites coupled by gap junctions, and because lesions of cerebellar nuclei influence the coupling between neurons in the inferior olive, we postulated that if lamellar bodies and gap junctions are related, then the densities of both structures will change together when the cerebellar input is removed. Lesions of the cerebellar nuclei in rats and rabbits resulted in a reduction of the density of lamellar bodies, the number of lamellae per lamellar body, and the density of gap junctions in the inferior olive, whereas the number of olivary neurons was not significantly reduced. The association between lamellar bodies and electrotonic coupling was evaluated electrophysiologically in alert rabbits by comparing the occurrence of complex spike synchrony in different Purkinje cell zones of the flocculus that receive their climbing fibers from olivary subnuclei with different densities of lamellar bodies. The complex spike synchrony of Purkinje cell pairs, that receive their climbing fibers from an olivary subnucleus with a high density of lamellar bodies, was significantly higher than that of Purkinje cells, that receive their climbing fibers from a subnucleus with a low density of lamellar bodies. To investigate whether the complex spike synchrony is related to a possible synchrony between simple spikes, we recorded simultaneously the complex spike and simple spike responses of Purkinje cell pairs during natural visual stimulation. Synchronous simple spike responses did occur, and this synchrony tended to increase as the synchrony between the complex spikes increased. This relation raises the possibility that synchronously activated climbing fibers evoke their effects in part via the simple spike response of Purkinje cells. The present results indicate that dendritic lamellar bodies and dendrodendritic gap junctions can be downregulated concomitantly, and that the density of lamellar bodies in different olivary subdivisions is correlated with the degree of synchrony of their climbing fiber activity. Therefore these data support the hypothesis that dendritic lamellar bodies can be associated with dendrodendritic gap junctions. Considering that the density of dedritic lamellar bodies in the inferior olive is higher than in any other area of the brain, this conclusion implies that electrotonic coupling is important for the function of the olivocerebellar system.

INTRODUCTION

The inferior olive (IO) is composed of subdivisions whose climbing fibers terminate in the cerebellar cortex in sagittally oriented zones (Groenewegen et al. 1979). The Purkinje cells of each zone project to a particular set of cerebellar and/or vestibular nuclei, which in turn provide a GABAergic feedback to the corresponding olivary subnucleus (De Zeeuw et al. 1989, 1994a; Fredette and Mugnaini 1991; Voogd and Bigaré 1980). The three-element anatomic loop comprising a particular zone with its climbing fibers and their collaterals, along with the corresponding Purkinje cells and the associated cerebellar and/or vestibular nucleus, has been called a module (Voogd and Bigaré 1980); this loop is considered to be the functional unit of the cerebellum (Andersson and Oscarsson 1978; De Zeeuw et al. 1994a; Oscarsson 1979). Although the different olivary subnuclei of different modules are generally considered as separate entities, recent studies in rat have shown at both the morphological and physiological level that neurons in different olivary subnuclei can, even when they are located on different sides of the brain, be electrotonically coupled by gap junctions (De Zeeuw et al. 1996b; Lang et al. 1996; Welsh et al. 1995).

Neuronal gap junctions can be demonstrated morphologically in the electron microscope (Angaut and Sotelo 1989; De Zeeuw et al. 1989; Rutherford and Gwyn 1977; Sotelo et al. 1974) and electrophysiologically with the use of multiple-unit recording (Bell and Grimm 1969; Bell and Kawasaki 1972; Lang et al. 1996; Llinás and Sasaki 1989; Llinás and Yarom 1981; Llinás et al. 1974; Sasaki et al. 1989). However, the ultrastructural characteristics of a gap junction, including its interneuronal gap of 2 nm, are technically difficult to demonstrate (Sotelo et al. 1974), and simultaneous impalement of two olivary neurons is at least as demanding (Llinás and Yarom 1981). The demonstration of neuronal gap junctions cannot be facilitated by any direct labeling method because the presently known antibodies and probes against the proteins and mRNA of gap junction channels do not detect specifically the presence of neuronal gap junctions (Dermietzel 1989; Matsumoto et al. 1991; Micevych and Abelson 1991; Nagy et al. 1988; Naus et al. 1990; Shiosaka et al. 1989; Yamamoto et al. 1989, 1990a,b). Therefore it is hard to obtain an adequate estimate of the density of neuronal gap junctions in the IO.

Recently, however, a new neuronal organelle, the dendritic lamellar body (DLB), has been discovered (Fig. 1) (De Zeeuw et al. 1995a). This organelle, which can be specifically labeled with an antiserum (α12B/18), is present

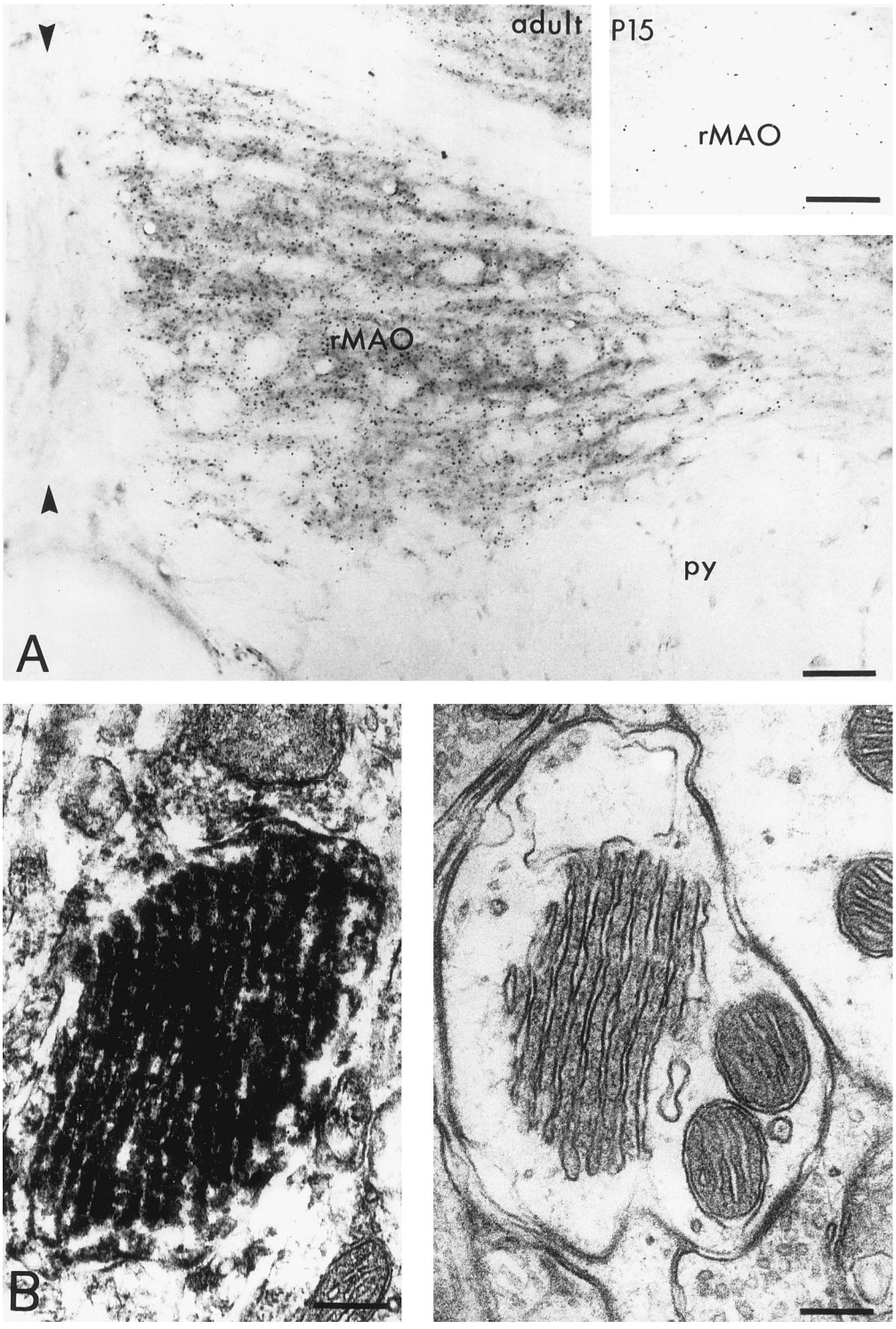

FIG. 1. Dendritic lamellar bodies (DLBs) in the rat inferior olive. *A*: light micrographs of the punctate labeling in the rostral medial accessory olive (rMAO) of an adult rat and of a 15-day-old rat obtained with the use of antiserum α12B/18. Each dot represents 1 DLB. Arrowheads: midline. py, Pyramidal tract. *B*: electron micrographs of an immunocytochemically labeled lamellar body (*left*) and a nonlabeled lamellar body (*right*) taken at the same magnification. Scale bars: *A*, 41 μm (adult) and 36 μm (postnatal day 15); *B*, 0.29 μm (modified from De Zeeuw et al. 1995a).

in all brain areas where dendrodendritic gap junctions are prominent. Most of the DLBs in the IO appear in development when gap junctions start to occur, i.e., between postnatal days 10 and 15 (Bourrat and Sotelo 1983). The density of DLBs in the IO is higher than in any other area of the brain. DLBs are present in all olivary subnuclei, but the density of DLBs varies among the different subnuclei. The density is highest in the rostral medial accessory olive (MAO) and lowest in the dorsal cap of Kooy (De Zeeuw et al. 1995a).

In the present study, two sets of experiments were performed to further investigate the association between DLBs and gap junctions. First, we investigated changes in the densities of DLBs and gap junctions in the rostral MAO following reduction of the GABAergic input that comes from the posterior interposed nucleus. Because a portion of these

GABAergic terminals is directly apposed to dendrites coupled by gap junctions (De Zeeuw et al. 1989), and because lesions of the cerebellar nuclei influence the strength of coupling between olivary neurons (Lang et al. 1996), we postulated that, if DLBs and gap junctions are related, then the density of both gap junctions and DLBs will change after reduction of the GABAergic terminals. Second, we evaluated the association between DLBs and electrotonic coupling at the electrophysiological level by comparing the densities of DLBs in different olivary subnuclei with the occurrence of complex spike (CS) synchrony in their respective climbing fiber zones. The flocculus of the cerebellum is useful for investigating the possible relation between DLBs and CS synchrony because its climbing fiber zones can be distinguished by different response patterns to optokinetic stimulation and because the olivary subnuclei that give rise to these different climbing fibers contain different densities of DLBs. The flocculus receives its climbing fibers from the dorsal cap, ventrolateral outgrowth, and rostral MAO (Tan et al. 1995). Both the dorsal cap and ventrolateral outgrowth contain significantly fewer DLBs than the rostral MAO (De Zeeuw et al. 1995a). Climbing fibers derived from the dorsal cap of Kooy and the ventrolateral outgrowth innervate Purkinje cells of zones 1–4 of the flocculus and carry direction-selective optokinetic signals (Graf et al. 1988; Leonard et al. 1988). The climbing fibers of zones 2 and 4 respond best to rotational optokinetic stimulation about the vertical axis, whereas those of zones 1 and 3 respond best to stimulation about a horizontal axis that is approximately perpendicular to the ipsilateral anterior canal (De Zeeuw et al. 1994b). The climbing fibers derived from the rostral tip of the MAO, which innervate Purkinje cells of zone C2 of the flocculus, do not carry optokinetic signals (De Zeeuw et al. 1994b); their activity may be related to head movement control (De Zeeuw et al. 1996a; De Zeeuw and Koekkoek 1997; Simpson et al. 1996).

METHODS

We chose to study the effects of cerebellar nuclear lesions on the expression of the DLBs in the rat, because in rats DLBs can be detected with the use of a specific antiserum (α12B/18) (De Zeeuw et al. 1995a); the major results were, however, confirmed in rabbits with the use of standard electron microscopy. The rabbit was chosen for the multiple unit recording experiments because *1*) in the rabbit the input and output relations between the olivary subdivisions and the Purkinje cell zones in the flocculus are known (Tan et al. 1995); *2*) the climbing fiber responses of Purkinje cells of different zones in the rabbit flocculus can be readily distinguished (De Zeeuw et al. 1994b); and *3*) the multiple-unit recording necessary to characterize climbing fiber synchrony can be readily performed in the vestibulocerebellum of the rabbit (Wylie et al. 1995).

Lesion-induced depletion

Ten male Wistar rats and two pigmented Dutch belted rabbits were used to study the effects of unilateral cerebellar nuclear lesions on the expression of DLBs and gap junctions in the IO. The animals were anesthetized with a mixture of ketamine (30 mg/kg), acepromazine (0.3 mg/kg), and xylazine (5 mg/kg). After the cisterna magna was exposed, the cerebellum was elevated and the right posterior interposed nucleus was mechanically or chemically lesioned. Mechanical lesions were made by suction; chemical lesions were made with the use of 1-μl injections of a mixture of *N*-methyl-D-aspartate (1 mM) and kainic acid (1 mM). Eight rats and the two rabbits were allowed to survive for 10 days, which is the optimal survival time for inducing depletion of γ-aminobutyric acid (GABA) in the IO after a lesion of the cerebellar nuclei (Fredette and Mugnaini 1991); the two remaining rats were allowed to survive for 5 days to measure intermediate effects.

After the survival time, the animals were anesthetized with Nembutal (100 mg/kg) and perfused transcardially with 200 ml saline followed by 1 l 4% formaldehyde and 0.9% NaCl in 0.1 M phosphate buffer (room temperature). The brain stems were removed 1 h after perfusion and cut coronally into 50-μm sections on a Vibratome. To stain the remaining GABAergic terminals and DLBs, the sections containing the IO were processed for immunocytochemistry with an antiserum against glutamic acid decarboxylase (GAD), the synthesizing enzyme of GABA (Fredette and Mugnaini 1991; Oertel et al. 1981), or with antiserum α12B/18 against DLBs (De Zeeuw et al. 1995a). The sections for GAD immunocytochemistry were blocked in rabbit serum, incubated in GAD antiserum (1:2,000), rabbit anti-sheep immunoglobulin G (1:50), and goat peroxidase antiperoxidase (PAP; 1:100), and stained in diaminobenzidine and H_2O_2. The rat sections to be processed for DLB immunocytochemistry were blocked in 5% normal donkey serum, incubated in DLB antiserum α12B/18 (1:1,000), goat anti-rabbit immunoglobulin G (1:50), and goat PAP (1:100), and stained in diaminobenzidine and H_2O_2.

All Vibratome sections, including those processed for immunocytochemistry, were analyzed at the light microscopic level and subsequently processed for electron microscopy. The Vibratome sections were osmicated in an 8% glucose solution (to allow visualization of the immunostaining after the osmication), block stained in uranyl acetate, directly dehydrated in dimethoxypropane, and embedded in Araldite (for details see De Zeeuw et al. 1988). Guided by observations made in the semithin sections, we prepared pyramids of the dorsal cap and rostral tip of the MAO. From these tissue blocks ultrathin sections were cut, mounted on Formvar-coated nickel grids, counterstained with uranyl acetate and lead (Hanaichi et al. 1986), and examined in the electron microscope. For both the ipsilateral and contralateral rostral MAO we quantified the number of GAD-positive terminals, the number of DLBs and lamellae per DLB, the number of gap junctions, and the number of perikarya. In the material from the rats, the numbers of GAD-positive terminals and DLBs were quantified both at the light microscopic and electron microscopic level; for the rabbits we quantified these structures only at the ultrastructural level. In the light microscopic analysis we determined the absolute number of immunoreactive boutons or puncta per surface area in one plane of focus with the condensor at a fixed level. The densities obtained in the different analyses were averaged and subsequently averaged across all animals.

Multiple unit recording in the vestibulocerebellum

Six pigmented Dutch belted rabbits were prepared for chronic recording with the use of sterile surgical techniques. General anesthesia was induced with a combination of ketamine (32 mg/kg im), acepromazine (0.32 mg/kg im), and xylazine (5.0 mg/kg im); supplemental doses (9 mg/kg ketamine, 0.09 mg/kg acepromazine, 2 mg/kg xylazine) were given every 30–45 min. An acrylic head fixation pedestal was formed and fixed to the skull by screws implanted in the calvarium. The pedestal was oriented so that the animal's nasal bone made an angle of 57° to the earth-horizontal plane. A craniotomy was made over the left paramedian lobule of the cerebellum, and a metal recording chamber, allowing introduction of microelectrodes into the flocculus, was fixed around the craniotomy by extending the acrylic head fixation pedestal.

This cylindrical chamber was oriented so that its axis was in a sagittal plane and made an angle of 27° to the vertical. The brain was covered by a Silastic sheet and the chamber was closed by a screw top. A search coil was implanted on the left eye to measure eye position (Robinson 1963). The coil, made of three turns of Teflon-coated stainless steel wire (Cooner No. AS632), was wound parallel to the limbus around the sclera under the superior and inferior recti and the inferior oblique muscles. The leads of the eye coil terminated in a plug fixed to the pedestal.

After a recovery period of 1 wk, Purkinje cell recordings and eye movement recordings were made. The animal was positioned with the implanted eye at the center of a magnetic field that alternated in spatial and temporal quadrature at 32 kHz. Calibration of the eye coil was achieved by rotating the field coils about the center of the eye while the animal maintained a stationary eye position. The activities of Purkinje cell pairs were recorded extracellularly with the use of glass microelectrodes (filled with 2 M NaCl; 2–4 MΩ) that were advanced parasagittally into the flocculus by two independent micropositioners. The neuronal activity was filtered with notch filters at the frequency of the magnetic fields used by the search coil system (32 kHz), amplified and filtered with a bandpass of 10 Hz to 10 kHz, discriminated with the use of window discriminators, and subsequently, together with the eye position, recorded on-line with the use of a CED 1401 signal capture device and the Spike2 (Cambridge Electronics Design) program on a personal computer. In addition, all data were stored on video tape.

The CS activity of Purkinje cells was identified by the characteristic waveform and low firing frequency (Eccles et al. 1966; Thach 1967). When possible, we also recorded the simple spike (SS) activity of the Purkinje cells. In these cases, the identification of a single Purkinje cell unit was confirmed by the presence of a pause in SS activity after the CS (De Zeeuw et al. 1995b; Simpson et al. 1996). The Purkinje cells were functionally categorized by determining the optimal axis for CS modulation in response to visual world rotation provided by a planetarium projector; the CS activity of vertical-axis Purkinje cells (VA cells) was modulated optimally when the planetarium rotated about the vertical axis, whereas the best response axis for horizontal-axis Purkinje cells (HA cells) was the horizontal axis oriented at 45° contralateral azimuth/135° ipsilateral azimuth (for details see De Zeeuw et al. 1994b; Graf et al. 1988; Leonard et al. 1988; Simpson et al. 1988; Soodak and Simpson 1988; Van Der Steen et al. 1994). The Purkinje cells from the C2 zone were characterized by an absence of a CS response to optokinetic stimulation; to avoid the possibility that cells outside the flocculus were identified as floccular C2 cells, only ''nonvisual'' cells that were located caudal to an optokinetically modulated cell were considered (for details see De Zeeuw et al. 1994b). During the search stimulus the planetarium rotated at a constant angular speed of 1°/s, which is close to the optimal speed for modulating the floccular visual climbing fibers (Barmack and Hess 1980; Kusunoki et al. 1990; Simpson and Alley 1974).

After the CSs were characterized, we recorded the activity of the pairs of Purkinje cells during spontaneous activity and during optokinetic stimulation about the axes of preference. During optokinetic stimulation the direction of the planetarium rotation usually reversed every 5 s, but occasionally we also used continuous stimulation in the excitatory direction. Each stimulus paradigm lasted from 5 to 30 min. Recording sessions generally ran 4 h, but were terminated if the animal showed signs of agitation. Between recording sessions the brain was covered by a Silastic sheet and the chamber was sealed.

To assess the temporal relationship of a Purkinje cell pair, cross correlograms were constructed with the use of existing routines in Spike2. Cross correlograms were constructed with the use of 1-, 2-, 5-, 10-, 20-, and 50-ms binwidths and always included 200 bins, 100 on either side of time zero. The cross correlograms contain two time zero bins (1 bin before and 1 bin after 0). To determine the number of times that two cells fired within 1 ms of each other, the two time zero bins of a cross correlogram were summed. The tendency of a neuron pair to fire within a given time period was determined as significant if one of the two time zero bins was denoted as a peak. A time zero bin was denoted as a peak if *1*) it had a value of ≥1 per 200 spikes combined from both cells of the pair, *2*) it was the highest bin, and *3*) it was ≥3 SD above the mean. The temporal relationship of the pair was designated as *1*) synchronous, when a time zero peak occurred in cross correlograms with 1- or 2-ms bins; *2*) perisynchronous, when a time zero peak occurred in cross correlograms with 5- or 10-ms bins; and *3*) contemporaneous, when a time zero peak occurred in cross correlograms with 20- or 50-ms bins. To further quantify the temporal relationship of a pair, the cross-correlation coefficient was used as a synchrony index (SI) (Gerstein and Kiang 1960; Sasaki et al. 1989; Wylie et al. 1995).

Calculation of SI was as follows

$$\mathrm{SI} = r = \frac{SS_{xy}}{\sqrt{SS_{xx}SS_{yy}}}$$

where

$$SS_{xx} = \sum X^2 - \frac{(\Sigma X)^2}{n}$$

$$SS_{yy} = \sum Y^2 - \frac{(\Sigma Y)^2}{n}$$

$$SS_{xy} = \sum XY - \frac{(\Sigma X)(\Sigma Y)}{n}$$

For an epoch time of duration T seconds, the spike trains of the two cells, $X(t)$ and $Y(t)$, are divided into n bins (T/binwidth) ($i = 1 \ldots n$). $X(t)$ and $Y(t)$ have the value of 1 (CS present) or 0 (CS not present) at time t. If cell X fires a times and cell Y fires b times during the epoch T, and on c occasions both cells fire within the same bin, then

$$\sum_{i=1}^{n} X_i = \sum_{i=1}^{n} X_i^2 = a$$

$$\sum_{i=1}^{n} Y_i = \sum_{i=1}^{n} Y_i^2 = b$$

$$\sum_{i=1}^{n} X_i Y_i = c$$

Consequently

$$SS_{xx} = a - \frac{a^2}{n}$$

$$SS_{yy} = b - \frac{b^2}{n}$$

$$SS_{xy} = c - \frac{ab}{n}$$

and

$$\mathrm{SI} = \frac{\left(c - \frac{ab}{n}\right)}{\sqrt{a\left(1 - \frac{a}{n}\right)b\left(1 - \frac{b}{n}\right)}}$$

RESULTS

Lesion-induced depletion

After a survival time of 10 days, lesions of the posterior interposed nucleus in the rat resulted in a substantial reduc-

tion of GAD-positive terminals in the contralateral rostral MAO (Fig. 2). Compared with the ipsilateral side, an average of 23 ± 1.7% (mean ± SE) of the terminals was left. The number of DLBs on the contralateral side decreased to 47 ± 3.4%, whereas the number of lamellae per DLB decreased from an average of 6.4 to 2.8 (44 ± 2.9%) (Fig. 3). The decrease in the number of lamellae per DLB was also reflected in the smaller size of the DLBs seen through the light microscope. In the ultrathin sections of the rostral MAO we found an average of 4.7 ± 0.52 and 1.7 ± 0.3 gap junctions per section on the ipsilateral and contralateral side, respectively. All differences between the ipsilateral and contralateral side mentioned above were significant (for GAD-positive terminals and DLBs, $P < 0.001$; for lamellae per DLB and gap junctions, $P < 0.005$; Student's *t*-test). There was no significant difference between the animals with mechanical or chemical lesions.

In the two rats killed after a period of 5 days, the changes were also detectable. The number of GAD-labeled terminals was reduced to an average of 38 ± 7.7%, whereas the number of DLBs and gap junctions was reduced to 57 ± 7.4% and 2.0 ± 0.4 gap junctions per section, respectively. The complexity of the glomeruli of these rats was also substantially reduced, but compared with the rats with a ten day survival time, relatively many degenerated terminals were visible inside the glomeruli. Therefore many degenerated terminals disappear from survival day 5 to 10. In the electron microscopic study of the rabbits we found that the posterior interposed nucleus lesion evoked the same effects on the ultrastructure of the rostral MAO as observed in the rats; the neuropil on the contralateral side was less complex than that on the ipsilateral side and contained significantly fewer GAD terminals (33 ± 15.7%), DLBs (49 ± 14%), and gap junctions (2.0 ± 0.5 vs. 4.4 ± 0.8).

To ascertain that the reductions in DLBs and gap junctions were not due to retrograde degeneration of the olivary neurons that provide axon collaterals to neurons in the contralateral interposed posterior nucleus, we counted in semithin sections the number of cell bodies on both sides of the rat IO. The number of olivary cell bodies with nucleoli on the side contralateral to the lesion was somewhat, but not significantly, smaller (86 ± 16%) than that on the ipsilateral side.

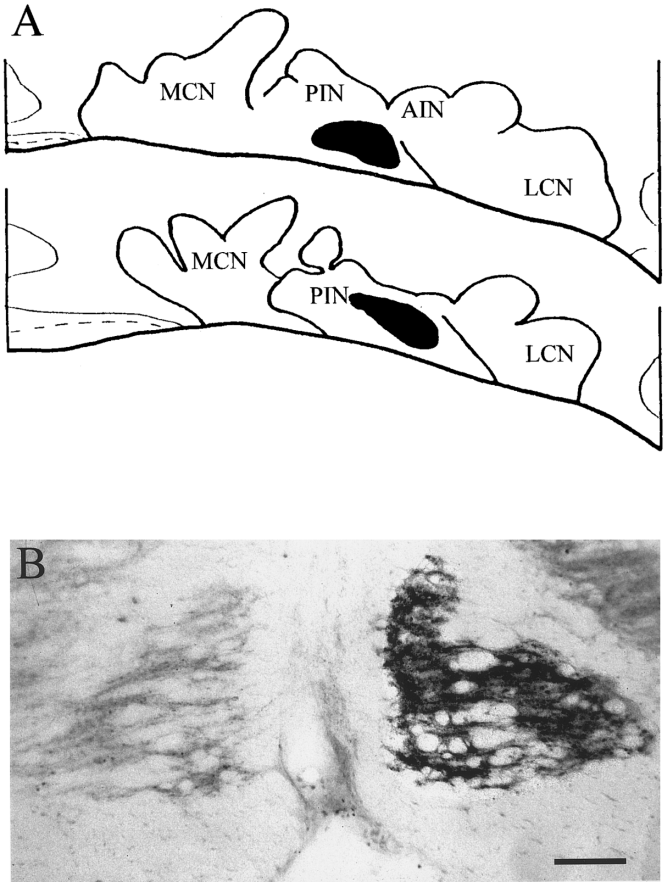

FIG. 2. Example of a physical lesion (center of lesion indicated in black) in the posterior interposed nucleus (PIN) of the rat cerebellum (*A*), and the resulting reduction of glutamic acid decarboxylase (GAD)-stained terminals in the contralateral rostral MAO (*B*). Transverse sections. MCN, medial cerebellar nucleus; AIN, anterior interposed nucleus; LCN, lateral cerebellar nucleus.

Multiple-unit recording in the vestibulocerebellum

The CS activity of 60 pairs of Purkinje cells was recorded in the flocculus of six alert rabbits. Of these pairs, 9 consisted of cells that did not respond to optokinetic stimulation (i.e., two C2-zone cells) (see also De Zeeuw et al. 1994b), 26 consisted of cells that had the same optokinetic response properties (i.e., 2 VA cells or 2 HA cells; referred to as visual ''like'' pairs), and 25 consisted of cells with different response properties (i.e., 1 C2-zone cell and 1 VA cell, 1 C2-zone cell and 1 HA cell, or 1 VA cell and 1 HA cell; referred to as ''unlike'' pairs). Cross correlograms were constructed for all pairs and the temporal relationship of each pair was categorized as described in METHODS. Examples of peristimulus time histograms and cross correlograms of two synchronous C2-zone cells and two synchronous HA cells are shown in Fig. 4, *B* and *C*, respectively. Tables 1 and 2 summarize the temporal relations of all cell pairs. Most of the pairs with the same response properties (78% of the C2-zone pairs and 70% of the visual like pairs) showed some sort of temporally related CS activity (either synchronous, perisynchronous, or contemporaneous), whereas only 36% of unlike pairs had temporally related CS activity. Of the nine C2-zone pairs, seven (78%) were synchronous and two were temporally unrelated; none was perisynchronous or contemporaneous. Of the 26 visual like pairs, 14 (54%) were synchronous, 2 (8%) were perisynchronous, and 2 (8%) were contemporaneous. Of the 25 unlike pairs, only 4 (16%) were synchronous, 2 (8%) were perisynchronous, and 3 (12%) were contemporaneous.

SI was calculated from the cross correlograms of each Purkinje cell pair at binwidths of 1, 2, 5, 10, 20, and 50 ms. Figure 5 shows the histograms of the mean SIs of each type of pair for 2-ms binwidths. All groups were compared pairwise with the use of the Mann-Whitney *U* test. The average SI of the C2 pairs was significantly higher ($P < 0.05$) than that of the visual like pairs, whereas the average SIs of both the C2 pairs and the visual like pairs were sig-

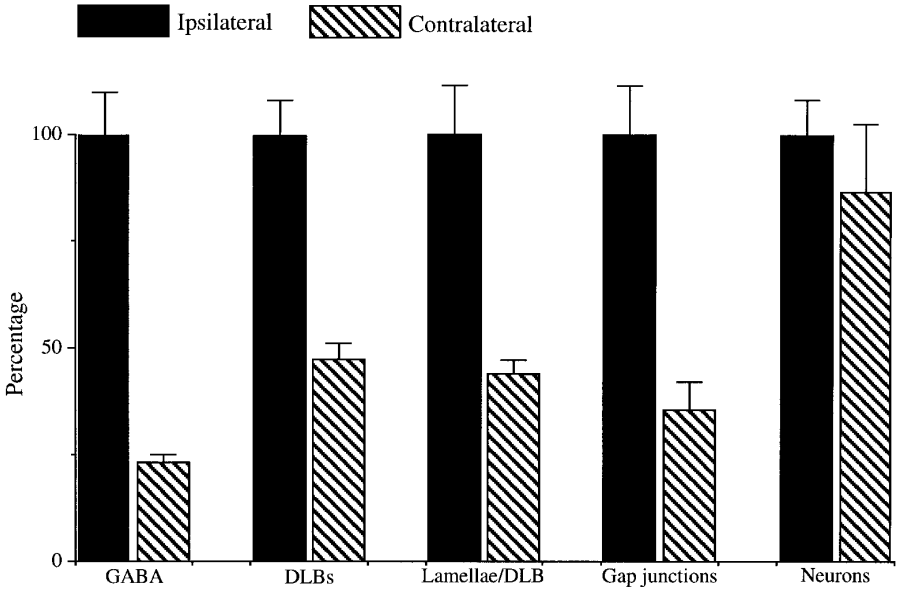

FIG. 3. Changes in the densities of DLBs and gap junctions in the rat rostral MAO after reduction of its GABAergic input from the contralateral posterior interposed nucleus of the cerebellum. Light microscopic and ultrastructural analyses demonstrate that lesions of the posterior interposed nucleus of the cerebellum resulted not only in a significant reduction of the number of GABAergic terminals [as revealed with the use of an antiserum against the γ-aminobutyric acid (GABA) synthesizing enzyme GAD], but also in a decrease in the number of DLBs, the number of lamellae per DLB, and the number of gap junctions. The number of olivary neurons did not change significantly after such lesions. For clarity of presentation, the values of the number of GABAergic terminals, DLBs, lamellae per DLB, gap junctions, and neurons in the ipsilateral rostral MAO are presented as control values and set at 100% (black columns). Bars atop columns: means ± SE. The total number of GABAergic terminals, DLBs, gap junctions, and neurons collected in the sections are 1,436, 476, 163, and 876, respectively.

nificantly higher than that of the unlike pairs (for difference between C2 pairs and unlike pairs, $P < 0.001$; for difference between visual like pairs and unlike pairs, $P < 0.005$).

Because the reversal of the direction of the constant-speed optokinetic stimulation produced substantially greater transients in the CS modulation in the alert rabbit than in the anesthetized rabbit (cf. Wylie et al. 1995), it was possible that the synchrony level in the alert rabbit was predominantly due to these transients (see also Fig. 4). Therefore we determined the synchrony level of all pairs for the excitation (ON) and inhibition (OFF) period of the CS modulation with and without transients (defined as the 0.5-s period after the turnarounds). SI ratios were calculated for each comparison [e.g., (ON − OFF)/(ON + OFF)], and a *t*-test was performed versus the null hypothesis that there was no difference [i.e., $H_o = (A - B)/(A + B) = 0$]. At the smaller binwidths (1–10 ms) the average SIs of the CSs accumulated during the ON periods and the OFF periods were not significantly different whether or not they included the transients (Fig. 6). At the larger binwidths the SIs of the transients became significantly higher, reflecting the high firing frequency during the transient periods. In addition, we compared the synchrony level of the CS activity when the direction of the rotation of the planetarium reversed every 5 s (i.e., with transients) with that when the optokinetic stimulus was continuously rotating in the excitatory direction (i.e., without transients). The synchrony level did not depend on the stimulus paradigm (Fig. 7). Finally, we investigated whether constant-velocity (with transients) or sinusoidal (without transients) optokinetic stimulation resulted in different SI levels. None of the stimulus paradigms we used evoked significant differences in synchrony. Taking these results together, we conclude that the occurrence of transients did not influence the percentage of pairs identified as synchronous and did not increase the average SI of the synchronous pairs.

To investigate SS synchrony and its relation to CS synchrony, we recorded simultaneously the CS and SS responses of 10 Purkinje cell pairs and calculated the SI of both the CS and SS responses for each pair (Fig. 8). The average SI of the SS activity of unlike pairs ($n = 6$) varied from 0.004 to 0.017 depending on the binwidth (1-ms binwidth: 0.004 ± 0.001, mean ± SE; 2-ms binwidth: 0.007 ± 0.004; 5-ms binwidth: 0.017 ± 0.01). For each binwidth the average SI of the SS activity of visual like pairs ($n = 4$) was significantly higher than that of the unlike pairs (Mann-Whitney *U* test, $P < 0.05$); they varied from 0.019 to 0.091 (1-ms binwidth: 0.019 ± 0.01; 2-ms binwidth: 0.026 ± 0.017; 5-ms binwidth: 0.091 ± 0.07). The average SIs for the SS activity were not significantly different from those for the CSs (for comparison with CS data see Table 2). Figure 9 illustrates the rank-ordered relation between the SIs of the CS and SS activity of the 10 pairs from which we were able to record simultaneously both types of spikes from both cells. The SI of the SS activity tended to increase as the SI of the CS activity increased.

DISCUSSION

The major results of the present paper are *1*) that the expression of DLBs and gap junctions can be downregulated concomitantly, *2*) that the density of DLBs in different subdivisions of the IO is correlated with the level of CS synchrony in the respective climbing fiber zones in the cerebellar cortex, and *3*) that the synchrony levels of CS activity and SS activity are related.

DLBs as a morphological correlate of electrotonic coupling

The vast majority of the olivary gap junctions are located in glomeruli, and the coupled dendrites in these glomeruli receive most of their GABAergic input from the cerebellar and vestibular nuclei and the nucleus prepositus hypoglossi (De Zeeuw et al. 1989, 1990, 1993, 1994a). Reduction of the GABAergic terminals in the rostral MAO that are derived from the contralateral posterior interposed nucleus of the

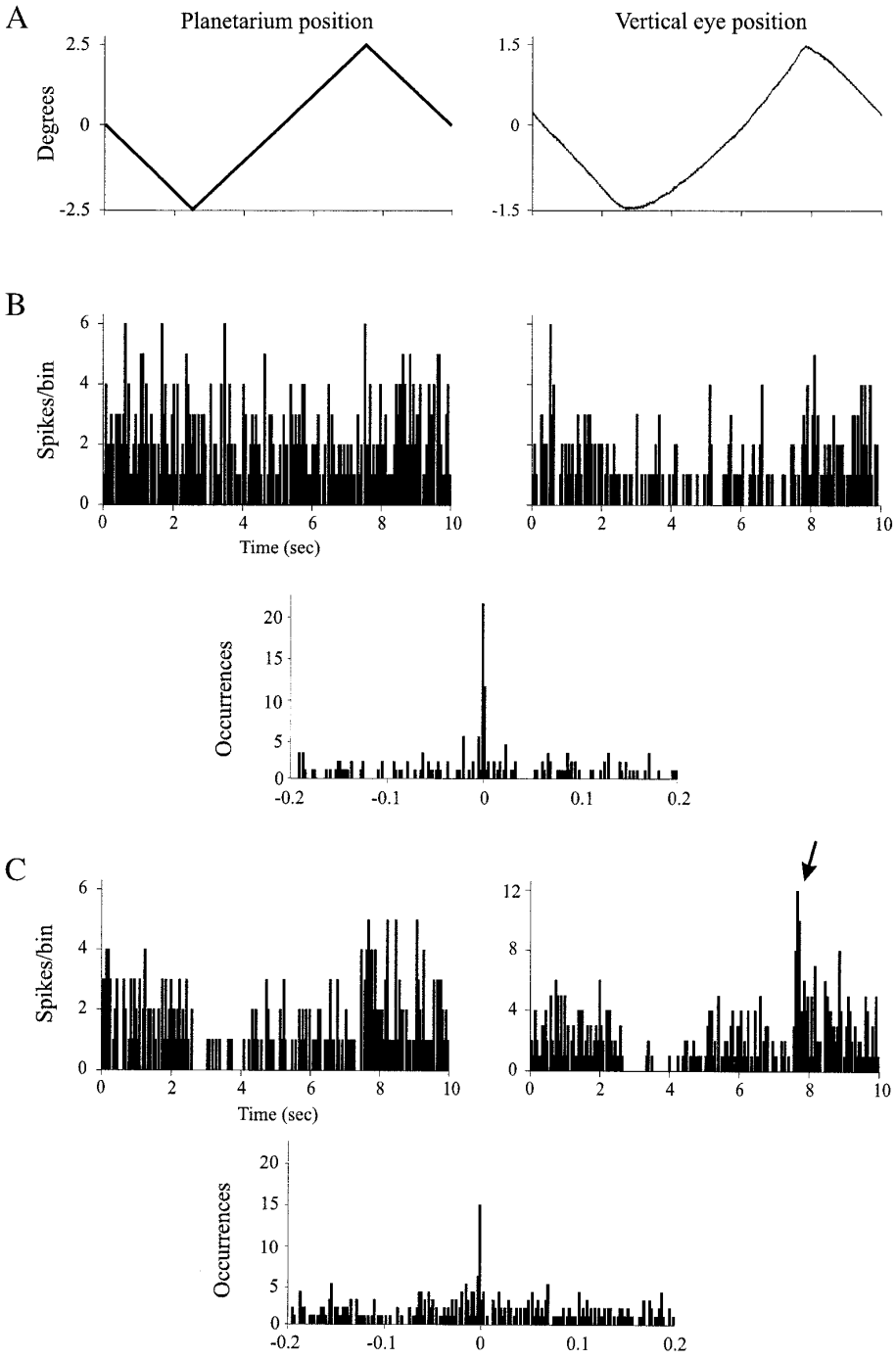

FIG. 4. Peristimulus time histograms and cross correlograms of the complex spike (CS) activity of 2 C2-zone cells (*B*) and 2 horizontal-axis Purkinje cells (HA cells) (*C*) in the flocculus of an alert rabbit during optokinetic stimulation with the planetarium projector oscillating at 0.1 Hz about the ipsilateral 135° axis in the horizontal plane (*A*). The peristimulus time histograms demonstrate that the HA cells, but not the C2-zone cells, are modulated. Because the total number of spikes analyzed for the C2 pair and the HA pair is the same (651 spikes), the relatively high time 0 bin in the cross correlogram of the C2 pair illustrates that the level of synchrony in this pair was higher than that of the HA pair; the synchrony indexes (SIs) of the C2 pair and HA pair were 0.097 and 0.025, respectively. Arrow in *C*: transient in the CS response. These transients are more pronounced in the CS responses of the alert rabbit than in those of the anesthetized rabbit. For the contribution of the transients to the synchrony level, see Figs. 6 and 7. Binwidths: 50 ms (peristimulus time histograms), 2 ms (cross correlograms). The recordings of both pairs were obtained from the same rabbit.

TABLE 1. *Classification of the temporal relationship of the CS activity of Purkinje cell pairs*

Pair Type	Total	Synchronous (1–2 ms)	Perisynchronous (5–10 ms)	Contemporaneous (20–50 ms)	Unrelated (>50 ms)
Non-visual like (C2-C2)	9	7 (78)	0	0	2 (22)
Visual like (HA-HA, VA-VA)	26	14 (54)	2 (8)	2 (8)	8 (30)
Unlike (C2-HA, C2-VA, HA-VA)	25	4 (16)	2 (8)	3 (12)	16 (64)

Values in parentheses are percentages. Pairs were only categorized as perisynchronous or contemporaneous when they did not belong to the synchronous category. CS, complex spike; HA, horizontal axis; VA, vertical axis.

TABLE 2. *SIs of Purkinje cell pairs calculated for different binwidths*

Pair Type	*n*	1 ms	2 ms	5 ms	10 ms	20 ms	50 ms
Non-visual like (C2-C2)	9	0.084 ± 0.051	0.115 ± 0.077	0.110 ± 0.078	0.115 ± 0.077	0.123 ± 0.075	0.142 ± 0.072
Visual like (HA-HA, VA-VA)	26	0.014 ± 0.005	0.018 ± 0.005	0.026 ± 0.006	0.031 ± 0.007	0.041 ± 0.007	0.067 ± 0.011
Unlike (C2-HA, C2-VA, HA-VA)	25	0.002 ± 0.001	0.003 ± 0.001	0.004 ± 0.001	0.007 ± 0.002	0.009 ± 0.002	0.017 ± 0.005

Values are means ± SE. For details on the calculation of synchrony index (SI) see Wylie et al. (1995). For abbreviations see Table 1.

cerebellum was followed by a significant reduction in the numbers of gap junctions, DLBs, and lamellae per DLB. This concomitant downregulation supports the hypothesis that DLBs are involved in the synthesis or turnover of gap junction channels. Because the numerical changes were only investigated at two times after the lesion (5 and 10 days), it is not possible to say whether the change in the number of DLBs occurred before or after the change in the number of gap junctions. One would expect that denervation of the coupled dendrites in the glomeruli first affects the gap junctions and then the DLBs, which are usually located just outside the glomeruli. This expectation is in line with the finding that the percent reduction in the number of gap junctions was somewhat greater than that of the DLBs. The reduction in gap junctions and DLBs was not due to retrograde degeneration of the olivary neurons, because the number of cell bodies was not significantly reduced.

The outcome of the multiple-unit recordings in the flocculus further strengthens the hypothesis that DLBs are associated with dendrodendritic gap junctions. The percentage of temporally related C2-zone pairs was significantly higher than that of visual like pairs and of unlike pairs. In addition, the average SI of the CS activity of C2-zone pairs was significantly greater than that of the visual like pairs, which is in line with the fact that the density of DLBs in the rostral MAO is significantly greater than that in the subnuclei that provide the climbing fibers to the visual floccular zones 1–4, i.e., the dorsal cap and ventrolateral outgrowth (De Zeeuw et al. 1995a). This outcome is consistent with the assumption that CS synchrony is caused predominantly by coupling via gap junctions between olivary neurons. Although we cannot rule out the possibility that the afferents also play a role in determining the synchrony level (see also De Zeeuw et al. 1996b), there is no reason to believe that the activity of excitatory afferents to the rostral MAO is better synchronized or more widely distributed than that to the dorsal cap and ventrolateral outgrowth. Furthermore, the density of GABAergic boutons in the rostral MAO is not smaller than that in the dorsal cap and ventrolateral outgrowth (Fredette and Mugnaini 1991; Nelson and Mugnaini 1988). These findings, in conjunction with the observation that the tran-

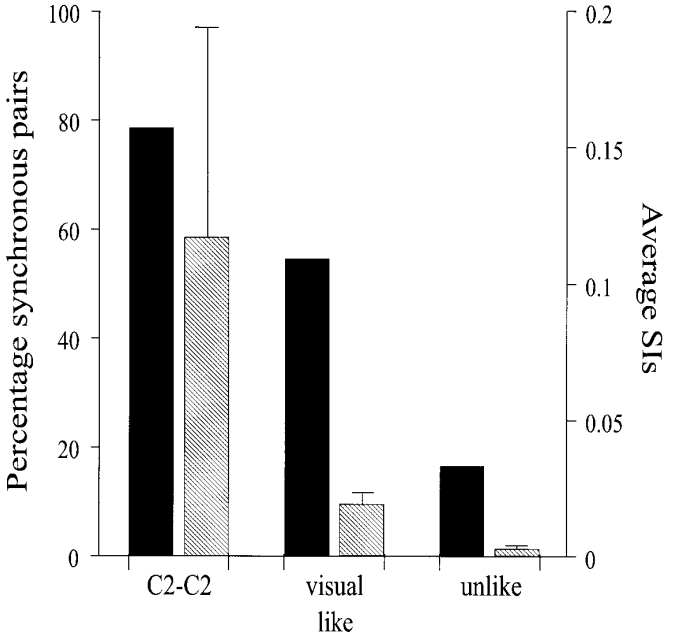

FIG. 5. Differences in synchrony between C2-C2 pairs, visual like pairs [HA-HA or vertical axis (VA)-VA], and unlike pairs (C2-HA, C2-VA, or HA-VA). Left column in each pair: percentage of Purkinje cell pairs that was synchronous. Right column in each pair: average SI for a binwidth of 2 ms. The synchrony between the C2 pairs was significantly higher than that of the visual like and unlike pairs, and the synchrony between the visual like pairs was significantly higher than that of the unlike pairs.

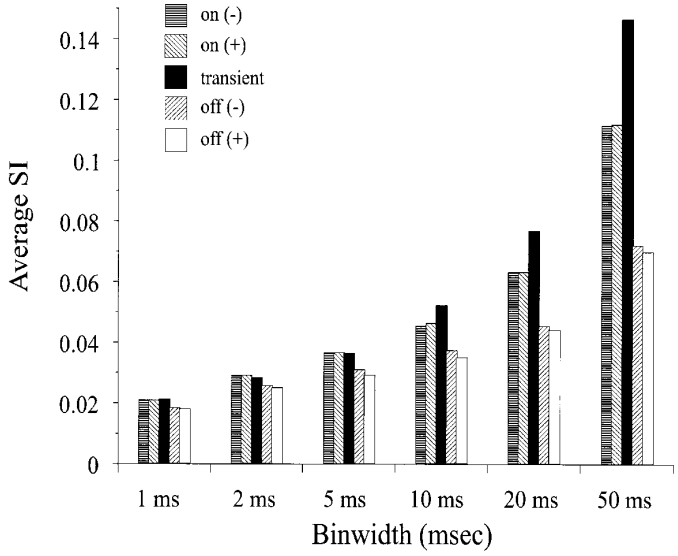

FIG. 6. SIs during different periods of the CS response. SIs were determined during the ON and the OFF period with and without the transients, and for the excitatory transients alone. Transients (see arrow in Fig. 4) were defined as the 500-ms period after the turnaround of the planetarium into the excitatory (ON) or inhibitory (OFF) direction. Plus and minus signs: values for the ON and OFF periods with (+) and without (−) the transients. At the small binwidths there were no significant differences in the SI values, indicating that the transients cannot be the sole factor responsible for synchrony.

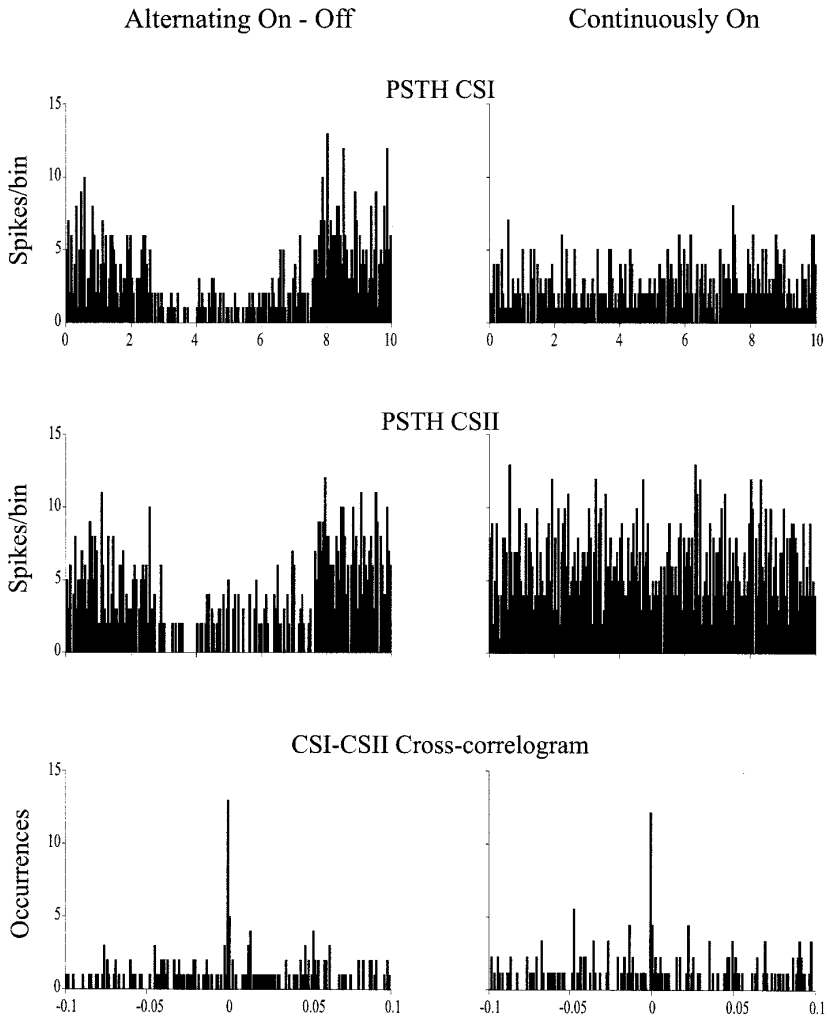

FIG. 7. Peristimulus time histograms and cross correlograms of the CS activity of 2 VA cells during alternating on-off optokinetic stimulation (*left*) and during continuous excitatory optokinetic stimulation (*right*). Both cross correlograms (binwidth 2 ms) were composed from the same number of spikes. The synchrony level during continuous excitatory stimulation was not significantly different from that during alternating stimulation. Binwidths: 50 ms (peristimulus time histograms); 1 ms (cross correlograms).

sients during CS excitation did not contribute more to the synchrony than CSs occurring in the rest of the response, suggest that CS synchrony is not predominantly caused by the afferents, but by another phenomenon, putatively the electrotonic coupling by dendrodendritic gap junctions in the IO.

At this point we can only speculate on the precise relation between DLBs and dendrodendritic gap junctions. Whether DLBs are involved in the formation or turnover of gap junction channels is not clear. Identification of the protein that is detected by antiserum α12B/18 and that is specifically located in DLBs may give a clue. Expression cDNA libraries of the IO and other brain areas where DLBs are prominent were recently produced and screened. The sequences of the positive clones that have been picked up in these libraries with antiserum α12B/18 suggest that the DLB protein is involved in the localization of cell-cell contacts and the transport of endocytotic vesicles to cytoplasmic organelles (De Zeeuw, unpublished data). The fact that the protein may be involved in endocytosis and the observation that DLBs are often surrounded by endocytotic vesicles (see also Fig. 1*B*) favor the possibility that DLBs are instrumental in the turnover of gap junction proteins, especially because gap junctions have a fast turnover of only a few hours (Hertzberg et al. 1989; Laird et al. 1991).

Relation between synchrony of CS activity and synchrony of SS activity

The present study is the first to demonstrate the occurrence of SS synchrony in the alert, behaving animal and to investigate its relation with CS synchrony. The average SIs of the SS activity were not significantly different from the SIs of the CS activity. Because the SI tends to decrease as the firing rate increases (Wylie et al. 1995), the similar magnitudes of the SS SIs and CS SIs indicate that the impact of the SS synchrony should not be underestimated. The synchrony of SSs of like pairs was significantly higher than that of unlike pairs (see Fig. 8), as also found by Wylie et al. (1995) for CS activity in the vestibulocerebellum of the aneasthetized rabbit and by us in the present study for CS activity in the alert rabbit. The relation found between the SIs of the CS and SS activity of individual pairs raises the possibility that SS synchrony and CS synchrony are not independent. Although not quantified, the same relation was also noted by Bell and Grimm (1969) and Bell and Kawasaki (1972),

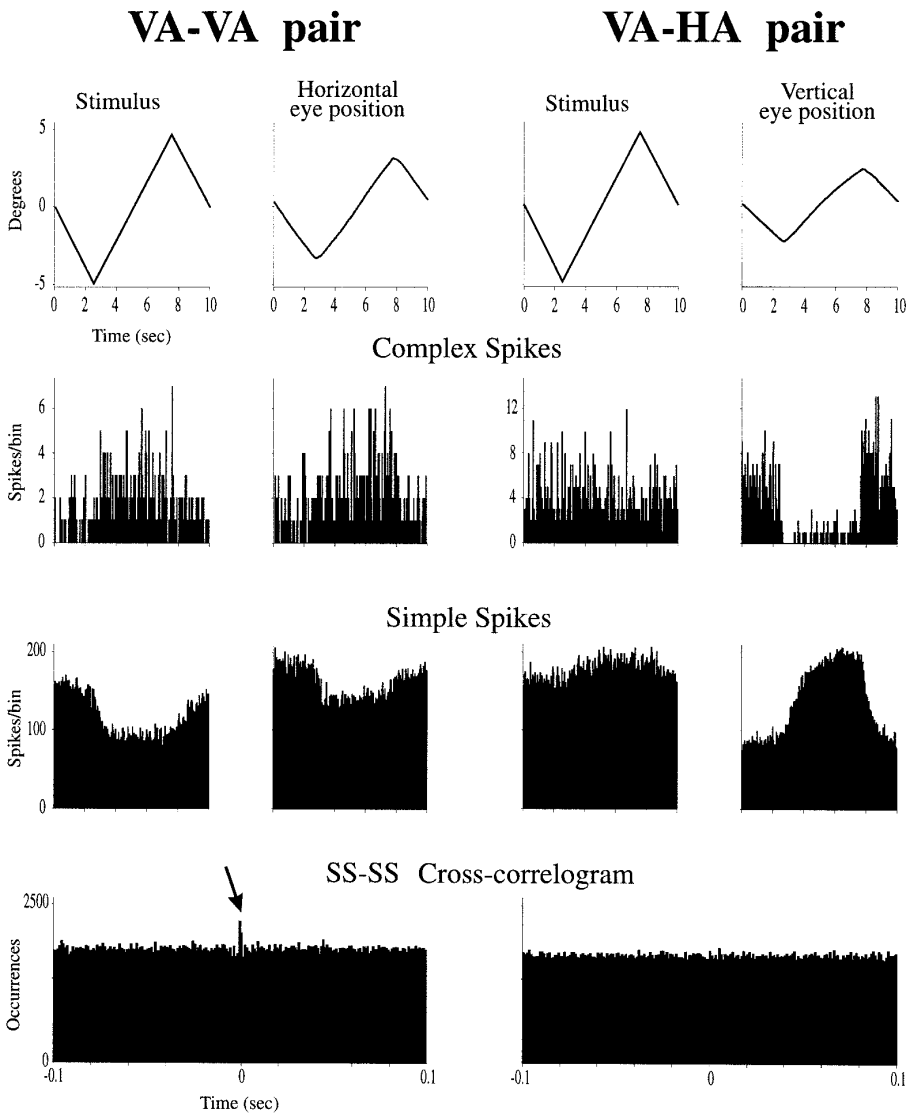

FIG. 8. Comparison of synchrony between the simple spike (SS) activity of a visual like (*left;* 2 VA cells) and unlike (*right;* HA cell and VA cell) pair. For both pairs, the peristimulus time histograms (binwidth 50 ms) of the CS and SS activity are presented at *top* and the cross correlogram (binwidth 1 ms) of the SS activity is at *bottom.* Note the relatively high peak (↘) in the cross correlogram of the SS activity of the visual like pair, and the absence of a peak in the cross correlogram of the unlike pair. The peristimulus time histograms and cross correlograms of both the visual like and visual unlike pair are composed of spikes collected over a total period of 490 s. The total numbers of CSs and SSs of the visual like pair are 704 and 60,266, respectively. The total numbers of CSs and SSs of the visual unlike pair are 1,322 and 59,228, respectively. The recordings of each pair were obtained from a different rabbit.

who recorded simultaneously both CS and SS activity of multiple Purkinje cells in the anesthetized cat and guinea pig. The finding that SS synchrony is restricted predominantly to Purkinje cells in the same climbing fiber zone is in line with that of Ebner and Bloedel (1981), who observed that short-duration positive SS correlations with short time lags of 1–2 ms occurred only between Purkinje cells separated by <100 μm, suggesting that the cells were in the same zone. Thus the present data in conjunction with earlier reports suggest that synchrony does not only occur between CSs but also between SSs, and that both CS synchrony and SS synchrony occur predominantly within single or functionally similar sagittal zones.

The findings presented above raise the questions of how the related CS and SS synchrony comes about and what function it may serve. With respect to the causal mechanisms of this relation, three factors may be involved, namely common afferents to the source of the parallel fibers and climbing fibers, directly induced SS synchrony by CS synchrony at the individual Purkinje cell level, and indirectly induced SS synchrony by CS synchrony via recurrent Purkinje cell collaterals (De Zeeuw et al. 1994b). Whether all three factors contribute simultaneously and equally to the related SS and CS synchrony cannot be determined at present, but considering the prominent density of DLBs and gap junctions in the IO it is parsimonious to ascribe an important role to the electrotonic coupling between olivary neurons, and thereby to an initial occurrence of CS synchrony. With respect to the teleological aspects of the CS and SS synchrony relation, it has already been demonstrated by several groups that the amplitude of the SS responses of Purkinje cells is correlated with the extent to which their climbing fiber inputs are synchronously activated (Lou and Bloedel 1992; Mano et al. 1986, 1989). Although the definition of synchrony in these latter experiments is less strict than that used by us or other colleagues (Lang et al. 1996; Llinás and Sasaki 1989; Welsh et al. 1995), their out-

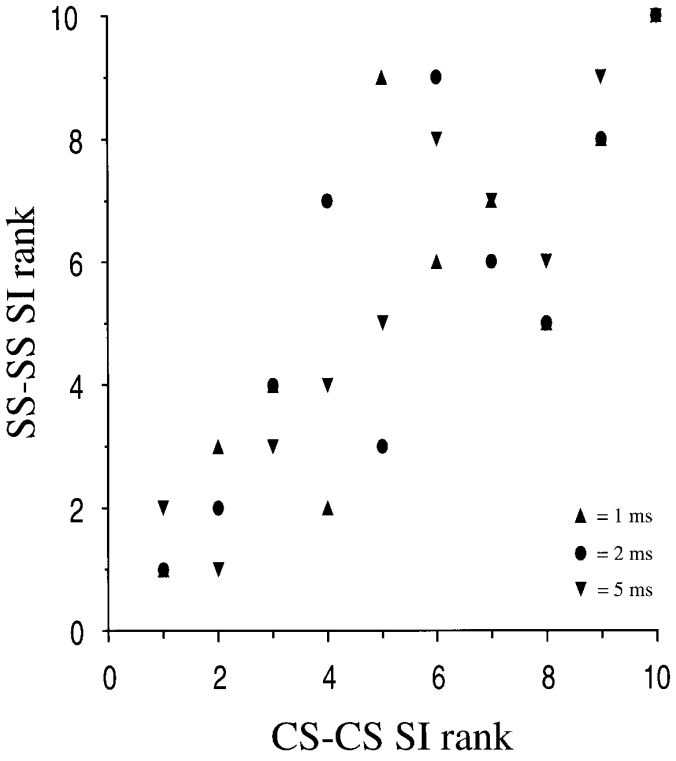

FIG. 9. Relation between the SIs of the CS and SS activity of 10 Purkinje cell pairs. The SIs of the CS activity and the SIs of the SS activity were ranked and then plotted according to the ranking for 3 different binwidths (1, 2, and 5 ms).

comes support the possibility that synchronous climbing fiber input may be responsible for mediating increased motor responsiveness.

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