

Temporal Relations of the Complex Spike Activity of Purkinje Cell Pairs in the Vestibulocerebellum of Rabbits

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Parasagittal zones in the vestibulocerebellum contain Purkinje cells whose complex spike (CS) activity is modulated in response to rotational optokinetic stimulation (OKS) about either the vertical axis (VA) or a horizontal axis (HA) that is approximately perpendicular to the ipsilateral anterior canal. In rabbits, there are two VA zones in both the ventral nodulus and flocculus, two HA zones in the flocculus, and one HA zone in the ventral nodulus. We investigated the temporal relationship of the CS activity of Purkinje cell pairs in the same or different zones of the vestibulocerebellum in ketamine-anesthetized pigmented rabbits. A synchronous temporal relationship was defined as the tendency of the CS of each Purkinje cell to fire within, at most, 2 msec of one another. Generally, neurons in the same zone showed a tendency to exhibit CS synchrony. Of 82 pairs consisting of two Purkinje cells in the same zone (e.g., two nodulus HA cells), 33 were synchronous. In contrast, none of 26 pairs consisting of two neurons in functionally different zones (e.g., a VA cell paired with an HA cell), showed CS synchrony. Pairs consisting of neurons in spatially separated VA zones in the ventral nodulus also showed a tendency to be synchronously related (6/16), as did pairs consisting of a nodulus VA cell and a flocculus VA cell (3/14). The CS synchrony was higher during OKS in the preferred direction than during spontaneous activity. This is the first demonstration that CS synchrony in the vestibulocerebellum can be manipulated with a natural sensory stimulus.

[Key words: synchrony, rhythmicity, optokinetic, inferior olive, flocculus, nodulus, correlograms, cerebellum]

The inferior olive (IO) projects as climbing fibers (CFs) to the cerebellar cortex where they synapse onto Purkinje cells and give rise to complex spikes (CSs) (Thach, 1967). Inferior olivary neurons and, consequently, CSs of Purkinje cells, have been observed to fire rhythmically at a characteristic frequency of about 10 Hz (Llinás and Yarom, 1981a,b, 1986; Llinás, 1986, 1991; Llinás and Sasaki, 1989; Sasaki et al., 1989). Gap junctions between neurons in the IO are the morphological correlates of

electrotonic coupling (Llinás et al., 1974; Sotelo et al., 1974), which is thought to be responsible for the phenomenon of CS synchrony. Synchrony is the tendency of CSs of Purkinje cells to fire within a very brief interval of one another, often within the same millisecond. This phenomenon was first reported by Bell and colleagues (Bell and Grimm, 1969; Bell and Kawasaki, 1972), who recorded from Purkinje cell pairs, and by Llinás and colleagues (Llinás and Sasaki, 1989; Sasaki et al., 1989; Sugihara et al., 1993), who recorded from arrays of up to 96 cells. The importance of electrotonic coupling was recently supported by the discovery of the dendritic lamellar body that is associated with dendrodendritic gap junctions (De Zeeuw et al., in press a). This organelle is present in all olivary subnuclei and its density is higher in the IO than in any other brain area.

Generally, the temporal relationship among CSs is strongest within a parasagittal plane (Bell and Kawasaki, 1972; Llinás and Sasaki, 1989; Sasaki et al., 1989; Sugihara et al., 1993), reflecting the sagittal zonal organization of the CF input to the cerebellum (Groenewegen et al., 1979). As shown in Figure 1, the ventral nodulus contains four sagittal zones and the flocculus contains five zones on each side of the brain. These zones can be identified on the basis of CS modulation in response to rotational optokinetic stimulation (OKS). Purkinje cells in zones VA1 and VA2 of the ventral nodulus and zones 2 and 4 of the flocculus receive their CF input from the caudal dorsal cap (cdc) of the IO and respond best to OKS about the vertical axis (Simpson et al., 1981; Balaban and Henry, 1988; Graf et al., 1988; Katayama and Nisimaru, 1988; Leonard et al., 1988; Kano et al., 1990a; Kusunoki et al., 1990; De Zeeuw et al., in press b; Tan et al., in press; Wylie et al., in press; see also Barmack and Shojaku, 1992). Zone HA of the ventral nodulus and zones 1 and 3 of the flocculus contain two Purkinje cell types whose CSs are modulated in response to OKS about a horizontal axis that is approximately perpendicular to the ipsilateral anterior semicircular canal (Fig. 1) (Simpson et al., 1981; Graf et al., 1988; Kano et al., 1990a,b; Kusunoki et al., 1990; De Zeeuw et al., in press b; Tan et al., in press; Wylie et al., in press). The two cell types differ with respect to the origin of their CF input and ocular dominance (see Fig. 4). Contra-45° (c45) cells receive their CF input from the rostral dorsal cap (rdc) and have a contralateral ocular dominance. Under monocular viewing conditions, c45 cells are maximally excited in response to clockwise (cw) rotation about an axis oriented at about 45° contralateral azimuth (Simpson et al., 1981; Graf et al., 1988; Leonard et al., 1988). Ipsi-135° (i135) cells receive their CF input largely from the ventrolateral outgrowth (vlo) and have an ipsilateral ocular dominance. Under monocular viewing conditions, i135 cells are maximally excited in response to counterclockwise (ccw) rota-

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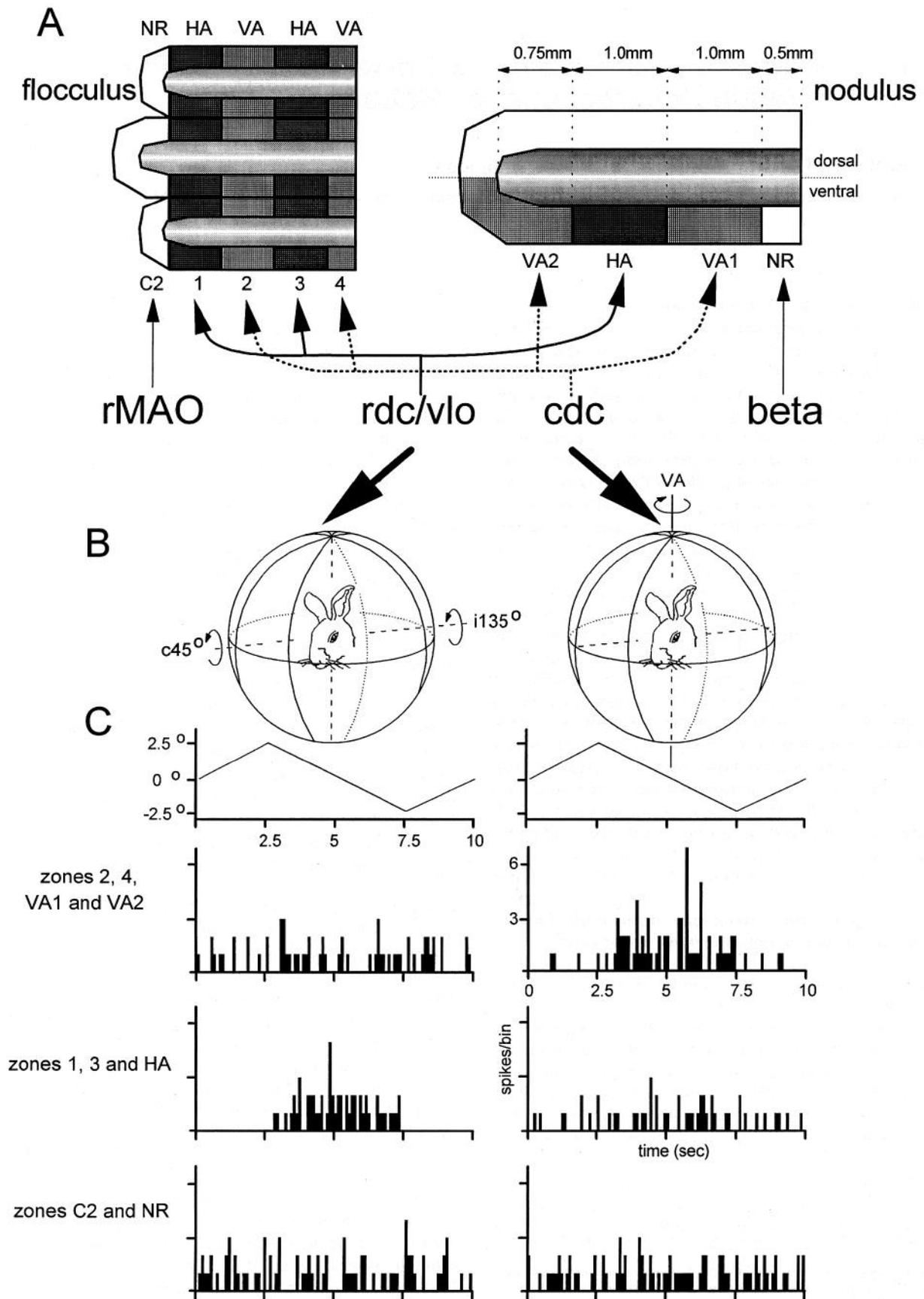


Figure 1. Zonal organization of the rabbit flocculus and ventral nodulus. The five zones of the flocculus, the four zones of the ventral nodulus, and the origin of their climbing fiber inputs are shown (A). C shows examples of the CS modulation of Purkinje cells in each of the zones of the left ventral nodulus and flocculus in response to optokinetic stimulation. The OKS was produced by a planetarium projector (Simpson et al., 1981;

Table 1. Classification of the complex spike temporal relationship of Purkinje cell pairs

	Total	Synchronous (1–2 msec)	Perisynchronous (5–10 msec)	Contemporaneous (20–50 msec)	Unrelated
Pairs consisting of two Purkinje cells in the same zone					
VA1-VA1	33	12 (36%)	4 (12%)	4 (12%)	13 (39%)
VA2-VA2	6	5 (83%)	1 (17%)	0	0
c45-c45	14	3 (21%)	4 (29%)	2 (14%)	5 (36%)
i135-i135	17	6 (35%)	4 (24%)	4 (24%)	3 (18%)
i135-c45	7	5 (71%)	0	1 (14%)	1 (14%)
NR-NR	5	2 (40%)	1 (20%)	0	2 (40%)
Total	82	33 (40%)	14 (17%)	11 (13%)	24 (29%)
Pairs consisting of two Purkinje cells in different zones					
"Like pairs"					
VA1-VA2	16	6 (38%)	3 (19%)	1 (6%)	6 (38%)
FVA-NVA	14	3 (21%)	5 (36%)	1 (7%)	5 (36%)
Total	30	9 (30%)	8 (27%)	2 (7%)	11 (37%)
"Unlike pairs"					
VA-HA	12	0	1 (8%)	0	11 (92%)
HA/VA-NR	14	0	0	0	14 (100%)
Total	26	0	1 (4%)	0	25 (96%)

tion about an axis oriented at about 135° ipsilateral azimuth (Simpson et al., 1981; Graf et al., 1988; Leonard et al., 1988). Since cw rotation about the axis at contra-45° azimuth is the same as ccw rotation about the axis at ipsi-135° azimuth (see Fig. 4), under binocular viewing conditions, the responses of ipsi-135° and contra-45° cells are indistinguishable. CS activity of Purkinje cells in the most medial zone of the ventral nodulus, zone NR, and zone C2 of the flocculus is not modulated by OKS (Kano et al., 1990a; De Zeeuw et al., in press b; Wylie et al., in press). Zone NR of the ventral nodulus receives CF input from the β -subnucleus (beta) of the inferior olive (Balaban and Henry, 1988; Katayama and Nisimaru, 1988), and its cells are responsive to vestibular stimulation originating in the otoliths and vertical semicircular canals (Barmack et al., 1989, 1993; Shojaku et al., 1991; Barmack and Shojaku, 1992). Zone C2 of the flocculus receives its CF input from the rostral tip of the medial accessory olive (rMAO) (Tan et al., in press). It is not known what stimulus modulates CS activity in zone C2 (De Zeeuw et al., in press,b).

The vestibulocerebellum is an ideal structure to study the phenomenon of CS synchrony because the olivocortical projections have been precisely delineated, the zones can be identified with physiological criteria (OKS), and the messages carried by the CFs to each of the optokinetic zones is known. By recording from Purkinje cell pairs in the rabbit's vestibulocerebellum, we addressed the following questions: (1) Do Purkinje cells in the same zone with the same CS response properties show CS synchrony? (2) Do Purkinje cells in different zones, but having the same CS response properties (e.g., zones VA1 and VA2 of the nodulus) exhibit CS synchrony? (3) Do neurons in the same zone but having different CS response properties (i.e., c45 cells

i135 cells) exhibit CS synchrony? (4) Is the CS activity of nodulus Purkinje cells temporally related to the CS activity of flocculus Purkinje cells receiving CF input from the same subnucleus of the IO? (5) What is the effect of modulating CS activity by OKS on the strength of the temporal relationship?

Materials and Methods

Surgery. Experiments were performed on 24 Dutch-belted pigmented rabbits. Animals were initially anesthetized with a ketamine (32 mg/kg)-xylazine (5 mg/kg)-acepromazine (0.32 mg/kg) cocktail (i.m.) and supplemental doses (9 mg/kg ketamine; 2 mg/kg xylazine; 0.09 mg/kg acepromazine) were administered every 30–45 min. Rectal temperature was routinely measured, and the animals were placed on a heating pad to maintain body temperature at 37–39°C. To penetrate the nodulus, a section of bone and underlying dura was removed from the back of the skull, exposing lobules VII, VIII, and IX of the cerebellar vermis. In some cases, the surface of the paramedian lobule was exposed to permit access to the flocculus.

Extracellular recording and optokinetic stimulation. Glass microelectrodes (2–3 M Ω) filled with 2 M NaCl were used to record extracellular potentials. Initially, the approximate boundaries of the zones of the ventral nodulus were mapped out by making successive penetrations 300 μ m apart in the mediolateral direction. The zones were identified on the basis of CS modulation of Purkinje cells in response to OKS (Fig. 1). Once the zonal boundaries were determined, penetrations were made with pairs of electrodes. The two electrodes were independently manipulable and could be placed within the same zone or different zones in the ventral nodulus. In some cases, one electrode penetrated zone 2 or zone 4 of the flocculus and another electrode penetrated a VA zone in the nodulus.

The extracellular potentials were amplified and band-pass filtered (1–3000 Hz; Tektronix AM 502). Purkinje cells were identified by the presence of the complex spike, which occurs spontaneously at about 1 spike/sec (Thach, 1967). Window discriminators (Frederick Haer & Co.) produced standardized square wave pulses, each representing a single complex spike. Raw signals and discriminated pulse were displayed on

Graf et al., 1988), which oscillated about either the vertical axis (*right*) or a horizontal axis oriented at 135° ipsilateral azimuth/45° contralateral azimuth (*left*), as indicated in *B*. The planetarium oscillated at 0.1 Hz with a constant speed of 1°/sec. The ordinate scale is the total number of spikes per 100 msec bin cumulated over 10 cycles. The stimulus position profile is also shown in *C*. Abbreviations, rostral medial accessory olive (rMAO), rostral dorsal cap (*rdc*), ventrolateral outgrowth (*vlo*), caudal dorsal cap (*cdc*), nonresponsive (*NR*), horizontal axis (*HA*), vertical axis (*VA*, *VA1*, *VA2*).

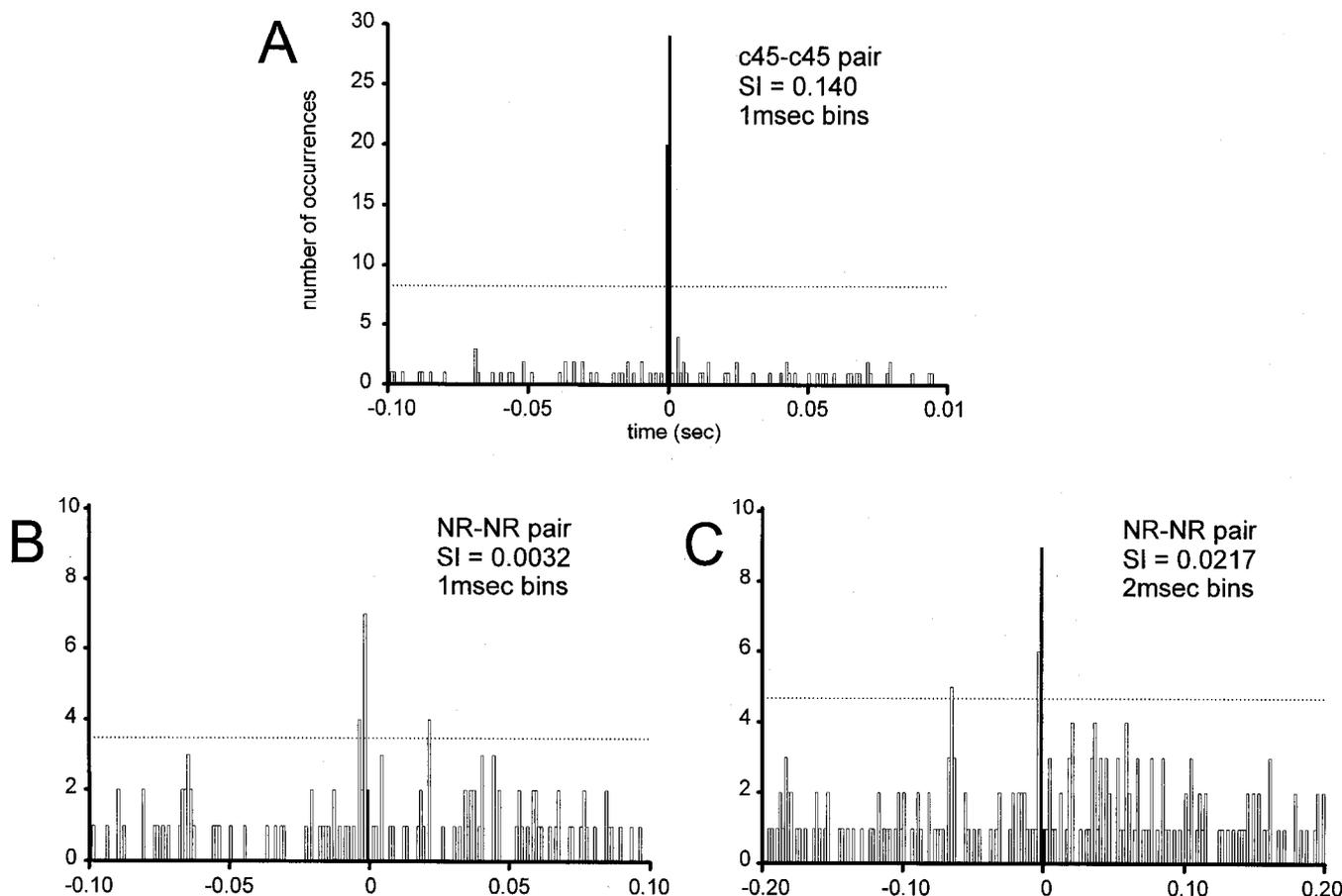


Figure 2. Synchronous CS activity of Purkinje cell pairs in the ventral nodulus. Cross-correlograms are shown for two Purkinje cell pairs that had a synchronous temporal relationship. Cross-correlograms were constructed and the temporal relationship was determined based on the three criteria described in the Materials and Methods section. The time-zero bins are shown in *solid black*, and the *dotted horizontal line* represents the mean + 3 SD. The synchrony index (*SI*) is also shown for each cell pair. In *A*, a time-zero bin met all three criteria. In *B*, a time-zero bin did not meet any of the criteria; however, when the same data was binned with 2 msec intervals (*C*), a time-zero bin met all three criteria. Thus, by our definition, these two cells have a synchronous temporal relationship, displaying a tendency to fire within the same 2 msec, although not within the same msec. In fact, in the cross-correlogram in *B*, one neuron tends to fire 2 msec before the other. The cell pair in *A* showed a remarkably strong correlation, although there were four other pairs that showed a stronger synchronous relationship. The highest synchrony index observed was 0.5. *A*: time = 500 sec; cell 1, 268 spikes; cell 2, 430 spikes. *B* and *C*: time = 400 sec; cell 1, 345 spikes; cell 2, 427 spikes.

a storage oscilloscope (Tektronix 5115) and fed to an audio monitor (Grass AM5).

A pair of cells was recorded for 5–20 min during spontaneous activity and/or during OKS about the preferred axis (for pairs consisting of two cells with the same axis preference). The OKS was produced by a planetarium projector, which consists of a light source positioned in the center of a sphere that was pierced by numerous small holes. The sphere was oscillated (0.025–0.1 Hz, 1°/sec constant speed) about an axis by a pen motor (MFE 54-150B), and the axis could be positioned at any orientation in space. The command signal for the pen motor was computer generated using ASYST software. The raw signals, discriminated pulses, and the command signal were stored on videotape with the aid of a multichannel Neuro-Corder (Neurodata DR-886). The pulses and command signal were also fed to a Cambridge Electronic Designs (CED) 1401 machine and peristimulus time histograms (PSTHs) were displayed on line with SPIKE2 software.

Off-line data analysis. To assess the temporal relationship of a cell pair, cross-correlograms were constructed using existing routines in SPIKE2. The cross-correlogram is a continuous histogram of the time intervals between the occurrence of the CSs of the two different cells. Cross-correlograms were constructed using 1, 2, 5, 10, 20, and 50 msec bin widths and always included 200 bins, 100 on either side of the zero-time. (e.g., cross-correlograms containing 1 msec bins were constructed from –100 to +100 msec). The cross-correlograms contain two “time-zero bins.” One time-zero bin indicates the number of times that CS1 fires *before* CS2 within the given bin width, and the second indicates

the number of times that CS1 fires *after* CS2 within the given bin width. Thus, to determine the number of times that two cells fire within 1 msec of each other, the two time-zero bins of a 1 msec bin width cross-correlogram are 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 at least 5, (2) it was the highest bin, and (3) it was at least 3 SD above the mean. The temporal relationship of the cell pair was designated according to the following definitions (see also Figs. 2, 3): (1) synchronous, time-zero peak in correlograms with 1 or 2 msec bins; (2) perisynchronous, time-zero peak in correlograms with 5 or 10 msec bins; (3) contemporaneous, time-zero peak in correlograms with 20 or 50 msec bins; (4) unrelated, no time-zero peak in any correlograms.

To further quantify the temporal relationship of a cell pair the cross-correlation coefficient was used as a synchrony index (*SI*) (Gerstein and Kiang, 1960; Sasaki et al., 1989; Sugihara et al., 1993; see Appendix). The *SI* was calculated for all bin widths except 50 msec. If available, cross-correlograms and *SI*s were obtained for spontaneous activity, OKS in the “ON” direction (i.e., the direction resulting in CS excitation; see Fig. 1), and OKS in the “OFF” direction (i.e., the direction resulting in CS inhibition; see Fig. 1). Unless otherwise stated, the *SI*s and cross-correlograms are based on the total (spontaneous + OKS) data available from the cell pair.

CS rhythmicity of individual Purkinje cells was examined by constructing autocorrelograms. Generally, 10 msec bin widths were used

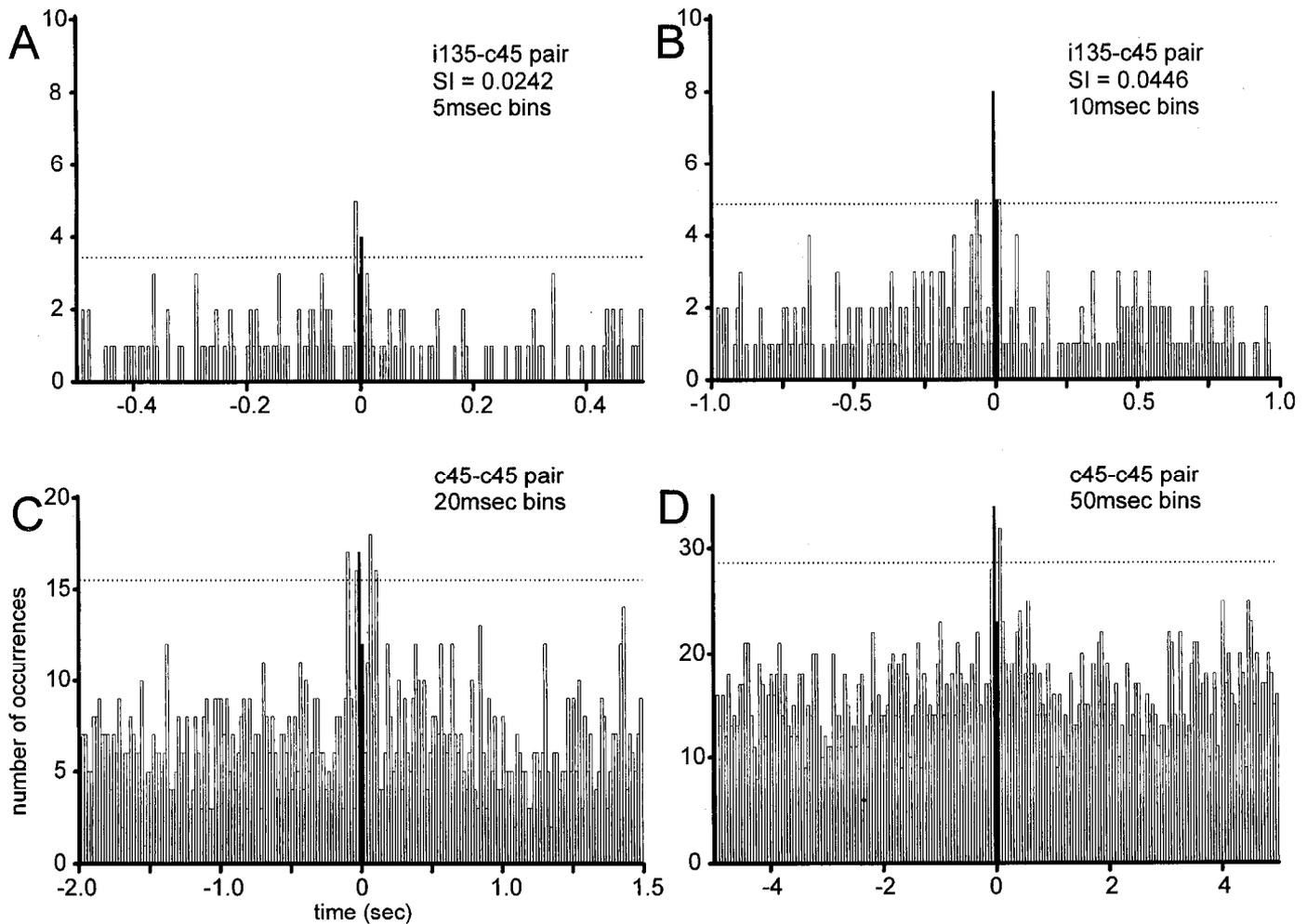


Figure 3. Perisynchronous and contemporaneous complex spike activity of Purkinje cell pairs. Cross-correlograms are shown for two Purkinje cell pairs that had perisynchronous (*A* and *B*) and contemporaneous (*C* and *D*) temporal relationships. In *A*, the cross-correlogram is binned with 5 msec intervals, but a time-zero bin is not the largest bin and does not contain at least five occurrences. When the same data is binned with 10 msec intervals, the criteria are met. Thus, this neuron pair showed a tendency to fire within 10 msec, but not within 5 msec. In *C*, a time-zero peak was greater than 5, and greater than the mean + 3 SD, but was not the largest bin. In *D*, the same data is binned with 50 msec intervals and a time-zero bin met all three criteria. Thus, this neuron pair showed a tendency to fire within 50 msec, but not within 20 msec. *A* and *B*: time = 600 sec; cell 1, 257 spikes; cell 2, 239 spikes. *C* and *D*: time = 1000 sec; cell 1, 454 spikes; cell 2, 691 spikes.

and 100 bins on each side of the time-zero bin were included. The frequency of rhythmic CS activity for a given neuron was determined from the time interval between peaks in the autocorrelogram and also by examining the power spectrum resulting from a fast Fourier transform (FFT) applied to the 'top' of the autocorrelogram. The FFT was only useful for those autocorrelograms that had two or more peaks on each side of time-zero (see Results). To analyze only the 'top' of the autocorrelogram, the period from 0 to 50 msec was removed.

Results

The CS activity of 138 pairs of Purkinje cells was recorded. Of these, 124 pairs consisted of two nodulus neurons, while the remaining 14 pairs consisted of one cell in flocculus zone 2 or zone 4 and one cell in nodulus zone VA1 or VA2 (NVA-FVA pairs; see Table 1). Cross-correlograms were constructed for all cell pairs, and the temporal relationship of the pair was categorized as outlined in the Materials and Methods section. Cross-correlograms of two synchronous cell pairs are shown in Figure 2, and cross-correlograms of a perisynchronous pair and a contemporaneous pair are shown in Figure 3. Table 1 summarizes the temporal relations of all cell pairs.

Pairs consisting of two neurons in the same zone

The CS activity of 75 pairs consisting of two neurons from the same zone of the ventral nodulus, and having the same OKS response properties, was recorded [33 VA1-VA1 pairs, 6 VA2-VA2 pairs, 14 c45-c45 pairs (from zone HA), 17 i135-i135 pairs (from zone HA), and 5 NR-NR pairs; see Table 1]. Most ($n = 52$; 69.3%) of these cell pairs showed some sort of temporally related CS activity. Twenty-eight (37.3%) were synchronous, 14 (18.7%) were perisynchronous, and 10 (13.3%) were contemporaneous. Figure 2 shows cross-correlograms of a c45-c45 pair and an NR-NR pair that both showed a synchronous temporal relationship (see also Fig. 3*C,D*).

The CS activity of seven pairs of neurons consisting of one c45 and one i135 neuron from the nodulus HA zone were also recorded (i135-c45 pairs). As previously mentioned, these Purkinje cell types respond best to OKS about a horizontal axis approximately perpendicular to the ipsilateral anterior canal, but they differ with respect to the olivary source of their CF input and their ocular dominance (see Fig. 4). Of seven i135-c45 pairs,

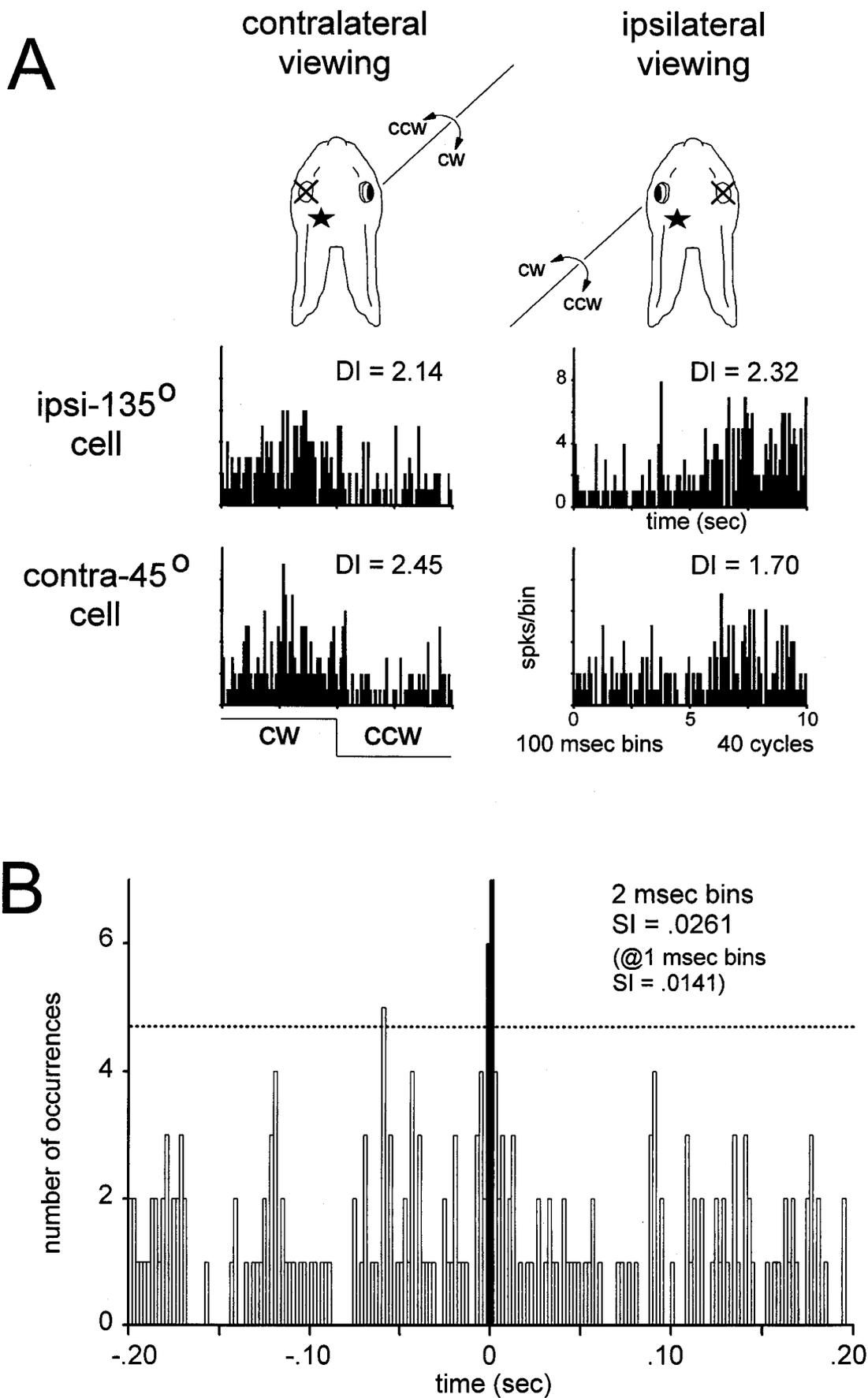


Figure 4. Synchronous CS activity of a contra-45° neuron and an ipsi-135° neuron in the HA zone of the ventral nodulus. A shows the CS modulation of these two cell in response to OKS about the horizontal axis oriented at 45° contralateral [ipsi (left) eye occluded] and to OKS about

five (71.4%) were synchronous, and one (14.3%) was contemporaneous. Figure 4 shows the cross-correlogram of a cell pair consisting of an i135 cell and a c45 cell that were synchronous (see also Fig. 3A,B).

Pairs consisting of neurons in different zones

Recordings were made from 56 Purkinje cell pairs consisting of two cells from different zones (see Table 1). Of these, 16 pairs consisted of a cell from each of the VA zones in the ventral nodulus (VA1-VA2 pairs) and 14 pairs consisted of one cell in zone 2 or zone 4 of the flocculus and one cell in either zone VA1 or VA2 from the nodulus (FVA-NVA pairs). The remaining 26 pairs were called "unlike pairs" because they consisted of two neurons that did not have the same best-response axis to OKS. Twelve pairs consisted of two visually responsive cells, one from the HA zone and the other from either the VA1 or VA2 zone (VA-HA pairs). The remaining 14 pairs consisted of one Purkinje cell from the NR zone and a visually responsive cell from the adjacent VA1 zone ($n = 11$) or the HA zone ($n = 3$) (NR-HA/VA pairs).

Similar to pairs consisting of two neurons from the same zone, the VA1-VA2 and NVA-FVA pairs showed a tendency to be temporally related. Of the 16 VA1-VA2 pairs, 6 were synchronous, 3 were perisynchronous, and 1 was contemporaneous. Of the 14 FVA-NVA pairs, 3 were synchronous, 5 were perisynchronous, and 1 was contemporaneous (see Fig. 6B-D).

In contrast, 25 of the 26 unlike pairs were temporally unrelated. The exception was a VA-HA pair that was perisynchronous. If one considers all but the unlike pairs, of 112 Purkinje cell pairs, 77 (68.8%) showed some sort of CS temporal relationship. Forty-two (37.5%) were synchronous, 22 (19.6%) were perisynchronous, and 13 (11.6%) were contemporaneous.

Groupwise comparison of synchrony indices

The SI was calculated from the cross-correlograms of each Purkinje cell pair at bin widths of 1, 2, 5, 10, and 20 msec. Figure 5 shows histograms of the mean SIs, grouped according to the type of cell pair, for 2 (A) and 10 (B) msec bin widths. All groups were compared pairwise using Mann-Whitney *U* tests. In A, with the exception of the c45-c45 and NR-NR groups, the SIs of all groups were significantly greater than the SIs of both groups of "unlike pairs" (one-tailed tests). In B, the SIs of all groups were significantly greater than the SIs of the VA/HA-NR group, and the SIs of all groups except the NR-NR group were significantly greater than the VA-HA pairs. At both bin widths, the SIs of the VA-HA pairs and VA/HA-NR pairs were not significantly different (two-tailed tests), and there were no significant differences between any other two groups (two-tailed tests).

Effect of optokinetic stimulation on the strength of the temporal relationship

Recordings were made from 88 neuron pairs during OKS about the preferred axis in the "ON" and "OFF" directions, and from 53 of these pairs recordings were also obtained during spontaneous activity. Cross-correlograms were constructed and SIs were calculated for each cell pair under each condition. The effect of OKS on the strength of the temporal relationship was determined, as outlined in Figure 6. 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., $H_0 = (A - B)/(A + B) = 0$]. Note that (1) the SIs during OKS in the "ON" direction were significantly greater than during spontaneous activity for all bin widths, (2) the SIs during the "OFF" direction were significantly greater than during spontaneous activity only for those calculated from the 10 msec bins, and (3) the SIs during OKS in the "ON" direction was significantly greater than the SIs during the "OFF" direction for those calculated from 1 msec bins. Figure 6B-E shows data from an NVA-FVA pair. Cross-correlograms (1 msec bins) are shown for OKS in the "ON" direction (B), "OFF" direction (C), and during spontaneous activity (D). Figure 6E shows the ratio comparisons. Typical of our sample, the temporal relationship of this cell pair was strongest during OKS in the "ON" direction, and weakest during spontaneous activity.

Rhythmicity of complex spike activity of individual Purkinje cells

The rhythmic firing of Purkinje cell CSs was examined by constructing autocorrelograms of 114 nodulus Purkinje cells. Of these, 36 (31.6%) showed a clear rhythmic firing pattern, as revealed by peaks in the autocorrelograms. The autocorrelograms of four cells are shown in Figure 7. For the 36 cells, one to six peaks on each side of the correlogram were apparent (mean = 2.4 peaks). While most (25) had two or more peaks (Fig. 7A-C), 11 had only one peak (Fig. 7D). (Cells showing a single peak in the autocorrelogram would accurately be described as producing pairs of action potentials separated by a characteristic time interval.) As shown in the right panels of Figure 7, fast Fourier transforms were applied to the tops of the autocorrelograms displaying more than one peak. The resultant power spectrum showed a peak corresponding to the characteristic frequency of the cell. The characteristic frequency of these 36 neurons averaged 8 Hz (range 2.5–12.5 Hz). Rhythmic activity of an individual neuron was independent of the temporal relationship of a recorded neuron pair. That is, for a pair showing a synchronous temporal relationship, one, both, or neither of the neurons may have been rhythmic.

Discussion

Purkinje cells in the same zone

We categorized the temporal relationship as either synchronous (within 1–2 msec), perisynchronous (5–10 msec), or contem-

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the horizontal axis oriented at 135° ipsilateral azimuth [contra (right) eye occluded]. The peristimulus time histograms show the modulation in response to constant speed oscillation about the indicated axes at a frequency of 0.1 Hz and amplitude of 2.5°. During the first 5 sec of each cycle the OKS is clockwise (CW) and during the remaining 5 sec the OKS is in counterclockwise (CCW). CW and CCW are with respect to the viewing eye. That is, CW about the axis at contralateral 45° azimuth with respect to the contralateral eye is the same direction in space as CCW about the axis at ipsilateral 135° azimuth with respect to the ipsilateral eye (see Graf et al., 1988). The direction index (DI) is the ratio of the response to OKS in the excitatory versus inhibitory direction. Note that the ipsi-135° cell has a slight ipsilateral dominance, whereas the contra-45° cell has a marked contralateral dominance. The star symbol indicates the recording side (left). In B, the cross-correlogram (2 msec bins) of this pair is shown. Time = 850 sec; cell 1, 635 spikes; cell 2, 335 spikes.

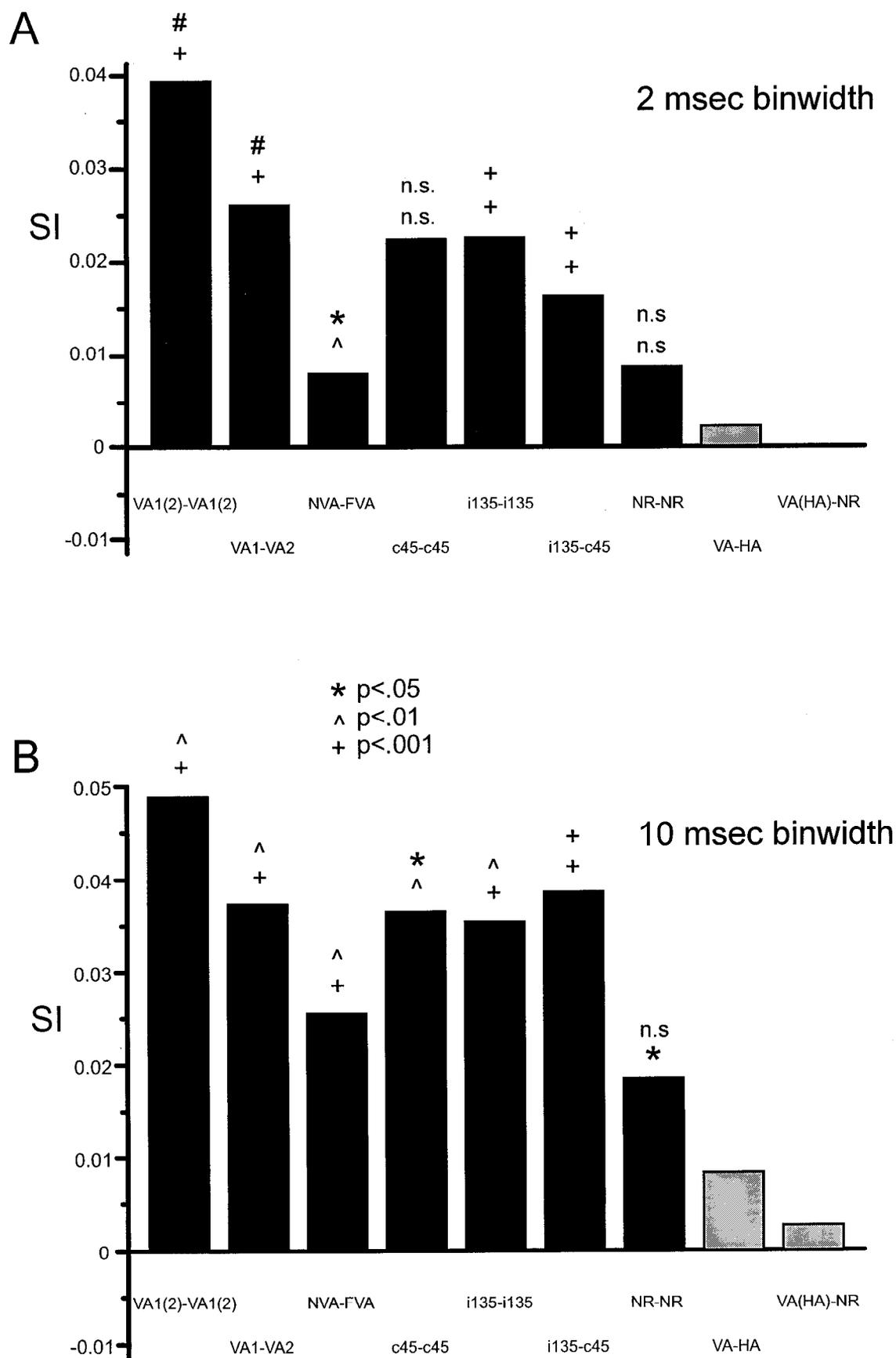


Figure 5. Average cross-correlation coefficients of the CS activity of Purkinje cell pairs in the vestibulocerebellum. The average synchrony indices (SIs) for each group were calculated from the cross-correlograms, and are shown in histogram form for those binned at 2 and 10 msec (A and B, respectively). Groups were compared using the Mann-Whitney U test. Above each solid bar, the upper and lower symbols indicate the significance

poraneous (20–50 msec), because previous studies describing “synchronous” CS activity were clearly describing different relationships. For example, Llinás and colleagues (Llinás and Sasaki, 1989; Sasaki et al., 1989; Sugihara et al., 1993) describe synchronous activity as occurring within a few milliseconds, whereas Lou and Bloedel (1986, 1992) describe “synchronous” activity in relation to a time window on the order of tens of milliseconds. It is noteworthy that, on some occasions, a neuron pair could show a ‘mixture’ of two relationships. For example, for the pair in Figure 6B, there are several occurrences of firing within 1 msec, but also a significant number of occurrences within 10 msec. Thus, there appears to be a “synchronous” tendency superimposed upon a broader “perisynchronous” relationship. In such cases, we maintained the three-point criterion and described this relationship as synchronous.

In the present study, we have shown that the complex spike activity of Purkinje cells in the same zone of the ventral nodulus is temporally related. Of 82 pairs, 40% were synchronous, 17% were perisynchronous, and 13% were contemporaneous. This confirms the observations of Bell and Kawasaki (1972) and Llinás and colleagues (Llinás and Sasaki, 1989; Sasaki et al., 1989; Sugihara et al., 1993) who found that the CS synchrony of Purkinje cells was strongest in the sagittal plane, but progressively decreased as the mediolateral distance between a pair increased. Whereas these researchers described this pattern of CS synchrony as a ‘spatial’ phenomenon, we describe CS synchrony with respect to functional properties of the zones. Purkinje cells in the same physiologically identified zone show CS synchrony.

The HA zones in the flocculus and ventral nodulus contain contra-45° and ipsi-135° Purkinje cells, which receive CF input from the rdc and vlo, respectively. Nevertheless, in the present study we showed that c45-i135 pairs were temporally related. Of seven such pairs, five were synchronous and one was perisynchronous. Since CS synchrony is most likely the result of gap junctions between IO neurons (Lang, 1989, 1990; Llinás and Sasaki, 1989; Sasaki et al., 1989), the present study suggests that different, although contiguous, olivary subnuclei (rdc and vlo) may be electrotonically coupled.

Vertical axis neurons in different zones

In the present study, we showed that Purkinje cells in the VA1 and VA2 zones were temporally related. Of 16 VA1-VA2 pairs, 6 were synchronous, 3 were perisynchronous, and 1 was contemporaneous. Moreover, we found that flocculus VA neurons were temporally related with VA neurons in the ventral nodulus. Of 14 NVA-FVA pairs, 3 were synchronous, 5 were perisynchronous, and 1 was contemporaneous. At first glance, this finding is not surprising given that these zones receive input from the same subnucleus of the IO, the dc (Balaban and Henry, 1988; Katayama and Nisimaru, 1988; Tan et al., in press). In fact, some CFs branch and innervate both the flocculus and the nodulus (Takeda and Maekawa, 1989a,b). However, synchronous CS activity between Purkinje cells in these spatially separated VA zones is remarkable when one considers that the CFs to different zones are likely of different lengths. Sugihara et al. (1993) have reported that CFs of different lengths in rats have uniform conduction times (but see Aggelopoulos et al., 1994).

They also showed that the longer branches of branching olivocerebellar fibers tended to have thicker diameters than the shorter branches.

Functionally, we are not sure what the different VA axis zones in the ventral nodulus and flocculus represent. One problem is that the ventral nodulus is rarely studied in isolation, and often the ventral and dorsal nodulus are grouped with the ventral uvula (Nagao, 1983; Waespe et al., 1985). In rabbits, flocculectomy results in a phase lag of the vestibulocular reflex (Ito et al., 1982; Nagao, 1983), whereas nodulectomy results in a phase lead (Nagao, 1983). Recent studies in primates have concluded that, in broad terms, the nodulus and ventral uvula are involved in the control of the velocity storage mechanism, whereas the flocculus is important in controlling the gain of the vestibulo-ocular and optokinetic reflexes (Waespe et al., 1983, 1985; Waespe and Hess, 1987; Cohen et al., 1992). In this regard, the VA zones in the nodulus and flocculus may be establishing timing information necessary for coordinating these different components of the compensatory response.

Effect of optokinetic stimulation on the strength of CS temporal relations

A characteristic feature of Purkinje cells in the VA and HA zones of the flocculus and nodulus is the CS modulation that occurs in response to rotational OKS about a particular axis (Simpson et al., 1981; Graf et al., 1988; Kano et al., 1990a,b; Kusonoki et al., 1990; De Zeeuw et al., in press b; Wylie et al., in press). In the present study, we have shown that temporally related CS activity is also a characteristic feature of Purkinje cells within the same zone. Moreover, we have shown a relationship between these two characteristics. We found that the cross-correlation coefficient was slightly (about 20%), but significantly higher during OKS in the “ON” direction versus spontaneous activity when calculated from the cross-correlograms constructed at all five bin widths. This is the first demonstration in the vestibulocerebellum that sensory stimulation can increase temporally related CS activity. Differences between “OFF” and spontaneous activity were less consistent and somewhat curious. The SI was higher for “OFF” than spontaneous, but this difference was only significant for those SIs calculated at the 10 msec bin width.

We believe that the effect of OKS on the temporal relationship of Purkinje cells will be larger and more consistent in an alert preparation for two reasons. First, in the anesthetized rabbit, eye movements do not occur, whereas the alert animal will make compensatory eye movements in response to visual and vestibular stimulation. Welsh et al. (1993; personal communication) have noted that CS synchrony in crus 2 increases during movement. Second, Lang et al. (1989, 1990) have shown that CS synchrony is affected by manipulation of the cerebellar nucleus neurons that provide the GABAergic innervation to the subnucleus of the IO giving rise to the climbing fiber innervation of the corresponding cerebellar zone (De Zeeuw et al., 1989). Destruction of the GABAergic input results in an increase in the CS synchrony. The GABAergic input to the dc and vlo is from the prepositus hypoglossi (De Zeeuw et al., 1993), the ventral dentate nucleus, and the dorsal group y (De Zeeuw et al., 1994).

←

level of the comparison versus the VA-HA group and the VA/HA-NR group, respectively (one-tailed tests). The two “unlike pair” groups were not significantly different, and there were no significant differences between any other two groups (two-tailed tests).

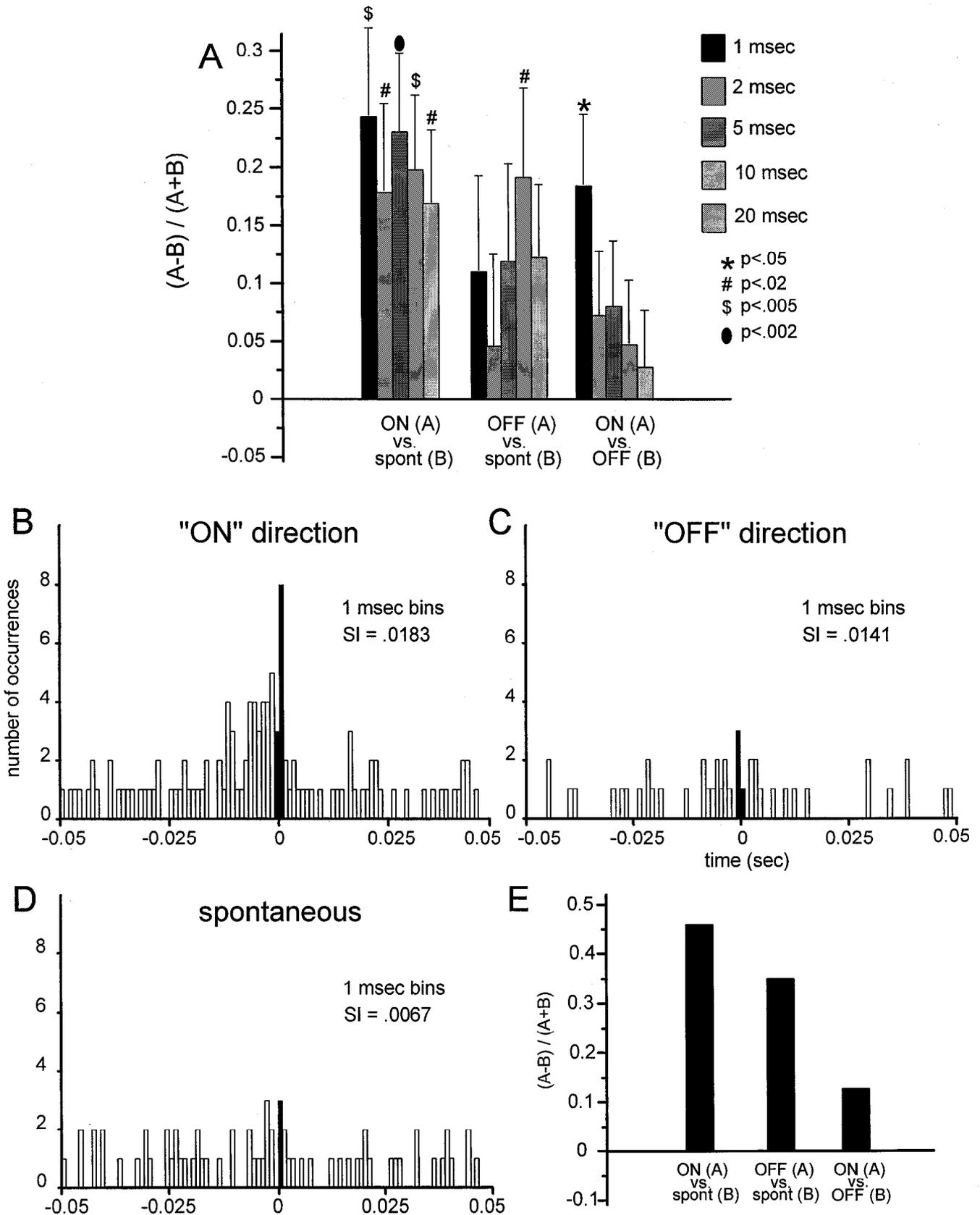


Figure 6. Effect of optokinetic stimulation on the temporal relationship of Purkinje cell pairs. **A**, The SI was calculated from the cross-correlograms of cell pairs that were recorded during optokinetic stimulation (excitatory and inhibitory) and spontaneous activity. To compare these three conditions, a ratio was calculated for each cell pair $[(A - B)/(A + B)]$, and a *t* test was performed versus the H_0 that there was no difference [i.e., $(A - B)/(A + B) = 0$]. This histogram shows the means of the ratio for "ON" versus spontaneous, "OFF" versus spontaneous and "ON" vs. "OFF" for five different bin widths. The error bars represent $+ 1$ SE. (Before transformation, any SI < 0.0001 was assigned a value of 0.0001.) **B–E** show a typical example of the effect of OKS on the strength of the temporal relationship of a Purkinje cell pair consisting of a nodulus VA1 cell and a

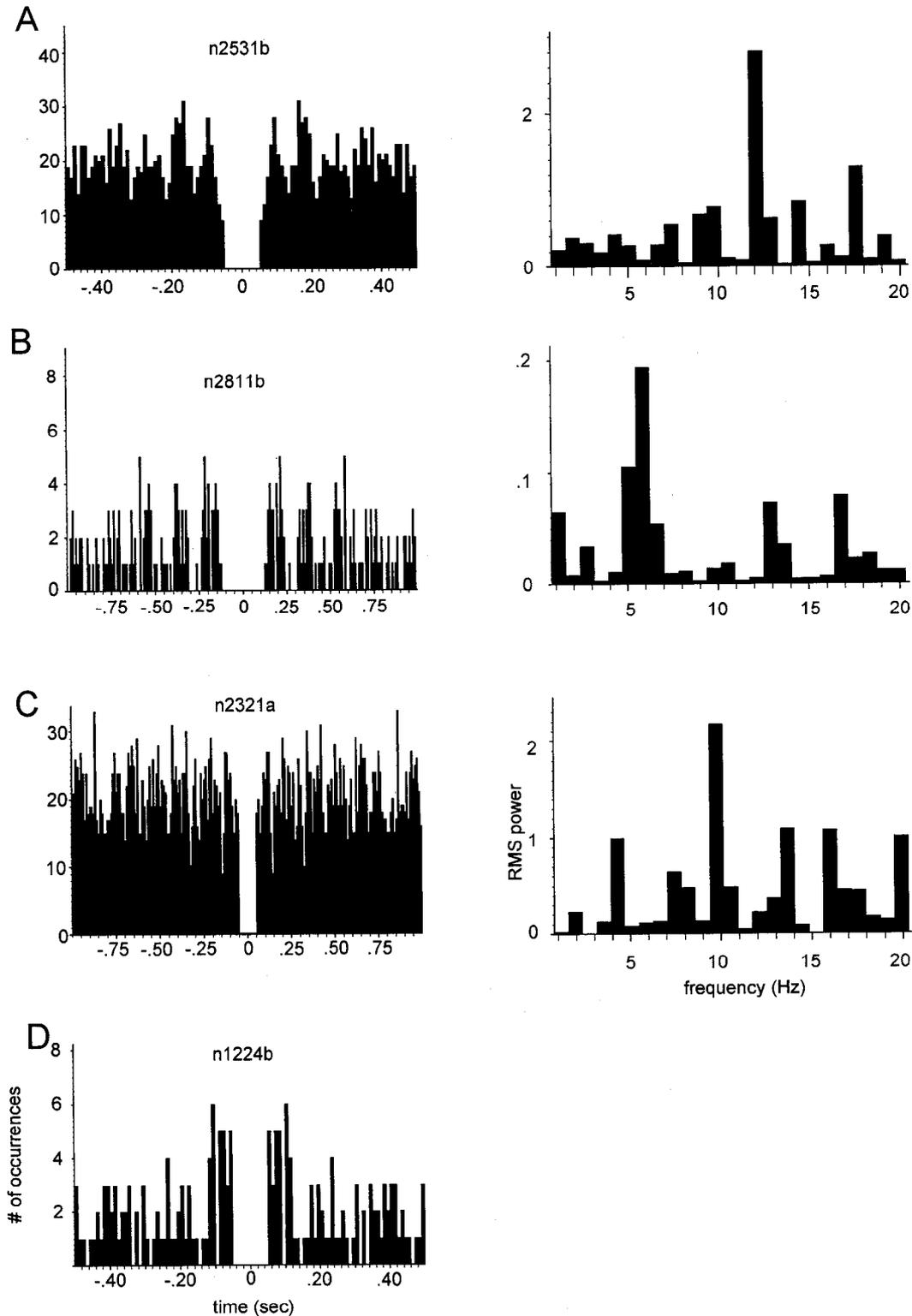


Figure 7. Complex spike rhythmicity of Purkinje cells. On the *left* the auto-correlograms of four Purkinje cells considered to be rhythmic are shown. The central peak (time-zero) was removed. On each side of the correlogram there are two to four peaks in *A*, three peaks in *B*, five to six peaks in *C*, and a single peak in *D*. The characteristic frequency of a cell was calculated by taking the inverse of the time between peaks. On the *right*, the fast Fourier transforms applied to the tops of the correlograms displaying more than one peak (*A*, *B*, and *C*) are shown. RMS (root mean squared) power is shown as a function of frequency. In these histograms, the *n*th bin is at the frequency $n/1.28$ Hz. That is, successive bins are incremented by 0.78 Hz. Note that peaks are seen at about 12, 5–6, and 10 Hz in *A*, *B*, and *C*, respectively.

flocculus VA cell that were synchronously related. Cross-correlograms (*1 msec bins*) constructed from recordings during OKS in the “ON” direction (*B*), OKS in the “OFF” direction (*C*), and spontaneous activity (*D*) are shown, and the SIs are indicated. *E* shows the ratios for this cell pair at this bin width. *B*: time = 500 sec; cell 1, 840 spikes; cell 2, 374 spikes. *C*: time = 500 sec; cell 1, 402 spikes; cell 2, 173 spikes. *D*: time = 500 sec; cell 1, 566 spikes; cell 2, 239 spikes.

Many of these GABAergic terminals synapse onto dendritic elements linked by gap junctions, which are thought to be responsible for the electrotonic coupling (Llinas et al., 1974; Sotello et al., 1974; De Zeeuw et al., 1989). Neurons in the prepositus and dorsal y are modulated during visual and vestibular stimulation in alert preparations (Lopez-Barneo et al., 1982; Chubb et al., 1984; Escudero et al., 1992; McFarland and Fuchs, 1992; Partalis et al., 1993) and this modulation may result in stronger CS synchrony.

Appendix

Calculation of synchrony index

The standard cross-correlation coefficient was used as a synchrony index (SI):

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

where

$$SS_{xx} = \sum X^2 - \frac{(\sum X)^2}{n}, \quad SS_{yy} = \sum Y^2 - \frac{(\sum Y)^2}{n},$$

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

For an epoch time of duration T sec, the spike trains of the two cells, $X(t)$ and $Y(t)$, are divided into n bins ($T/\text{bin width}$) ($i = 1 \dots n$). $X(t)$ 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

$$\sum_{i=1}^n X_i = \sum_{i=1}^n X_i^2 = a, \quad \sum_{i=1}^n Y_i = \sum_{i=1}^n Y_i^2 = b, \quad \sum_{i=1}^n X_i Y_i = c.$$

Consequently,

$$SS_{xx} = a - \frac{a^2}{n}, \quad SS_{yy} = b - \frac{b^2}{n}, \quad SS_{xy} = c - \frac{ab}{n},$$

and

$$SI = \frac{\left(c - \frac{ab}{n}\right)}{\sqrt{\left(a - \frac{a^2}{n}\right)\left(b - \frac{b^2}{n}\right)}}.$$

To illustrate the effect of absolute firing rate on the SI, suppose cell X fires 600 (a) times and cell Y fires 400 (b) times in 500 sec. Further suppose that for the 1 msec cross-correlogram the sum of the two time-zero bins is 50 (c). Because the two time-zero bins were summed, the bin width is 0.002 sec and therefore $n = 250000$:

$$SI = \frac{50 - \frac{(600)(400)}{250000}}{\sqrt{\left(600 - \frac{600^2}{250000}\right)\left(400 - \frac{400^2}{250000}\right)}}$$

$$SI = 0.1003.$$

Suppose that the two cells fire at half this frequency. If cell X fires 300 (a) times and cell Y fires 200 times (b) in 500 sec, and

for the 1 msec cross-correlogram the sum of the two time-zero bins is 25 (c), then

$$SI = \frac{25 - \frac{(300)(200)}{250000}}{\sqrt{\left(300 - \frac{300^2}{250000}\right)\left(200 - \frac{200^2}{250000}\right)}}$$

$$SI = 0.1011.$$

The SI is only slightly higher in the second scenario with the lower firing rates. This is because the number of bins in which both cells *do not* fire is higher, which contributes to the correlation.

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