Recoverable and Nonrecoverable Deficits in Conditioned Responses after Cerebellar Cortical Lesions

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This study reexamined the effects of unilateral damage to cerebellar hemispheral lobule VI on the rabbit's conditioned nictitating membrane (NM) response. Extensive unilateral removal of hemispheral lobule VI in 11 rabbits impaired ipsilateral conditioned responses as reflected by reductions of 52% in mean frequency and 53% in mean amplitude during test trials on the first postoperative session. The decreases in the amplitude and frequency of conditioned responses were highly correlated ($r = 0.82$). The frequency of conditioned responses recovered to control levels but their amplitudes remained reduced such that the correlation between these two measures of responding was no longer significant by the 12th postoperative conditioning session. The decrease in the amplitude of conditioned responses was not accompanied by changes in onset latency or rise time. There was no significant impairment of conditioned responses in surgical controls and animals with only partial damage to hemispheral lobule VI. It was concluded that hemispheral lobule VI plays an important role in the regulation of motor centers in the brainstem so as to facilitate the initiation and optimum execution of the conditioned NM reflex. This cortical regulation of the conditioned NM response may contain learned elements; however, these cannot be resolved with lesion methods, nor has their existence been proven in this or other lesion studies. Nevertheless, the results of this study do demonstrate that the cerebellar cortex cannot be considered as the single locus necessary for NM conditioning.

[Key words: cerebellar cortex, hemispheral lobule VI, rabbit, nictitating membrane, classical conditioning, motor function]

Aspiration lesions of hemispheral lobule VI (HVI) and ansiform lobule (ANS) were used by Yeo et al. (1985a) and subsequently by Lavond et al. (1987) to test the involvement of cerebellar cortex in classical conditioning of the nictitating membrane (NM)

response. These cortical areas were initially chosen by Yeo et al. (1985a) because the corneal air puff or periocular electrical pulse unconditioned stimulus (US) used in conditioning activates trigeminal afferents to areas in the inferior olive that project robustly to HVI and ANS (Miles and Wiesendanger, 1975a,b; Cody and Richardson, 1979; Gellman et al., 1983; Yeo et al., 1985b). These cortical areas also receive auditory and visual inputs (Snider and Stowell, 1944), which could lead to a convergence of CS and US and thus provide a locus for associative learning. Yeo et al. (1985a) and Lavond et al. (1987) observed a large postoperative decrement in conditioned responses (CRs) as measured by both their frequency and amplitude but no effect on the UR. Both Yeo et al. (1985a) and Lavond et al. (1987) interpreted the postoperative decreases in CR frequency to reflect a deficit in associative learning and not a performance deficit. However, there was disagreement as to the duration of this effect. Yeo et al. (1985a) failed to observe any recovery and concluded that the deficits were permanent, while Lavond et al. (1987) reported that animals demonstrated full recovery such that CR frequency and amplitude were no longer significantly different from control values. Subsequently, Lavond and Steinmetz (1989) examined initial acquisition of CRs following extensive removal of HVI and reported that these cortical lesions delayed the acquisition of CRs as measured by their frequency and amplitude but had no effect on the unconditioned response (UR). They concluded that although HVI was not essential for classical conditioning, it normally played an important role in learning through a modulatory influence on the interpositus nucleus, the latter being essential for learning. More recently, Yeo and Hardiman (1992) reported that cortical lesions enhanced the amplitude of URs, raising the possibility that performance variables might indeed be impairing CR production. In addition, Yeo and Hardiman (1992) found that there was a higher frequency of CRs on conditioned stimulus (CS)-alone test trials as compared with paired trials, an effect that could be due to the presence of a performance deficit that is, perhaps, related to an impairment in response initiation. However, Yeo and Hardiman (1992) observed that the depression of CR excitation characterized by impaired amplitudes, frequencies, and initiation did not relate in a simple way to the accompanying increase in excitability of the UR. A common performance deficit might not produce these differential excitabilities, and it is possible that CRs were depressed by a learning loss. The recovery of CRs with continued training, particularly by those subjects with subtotal lesions, could be consistent with a learning deficit embedded within general changes of excitability of the NM response.

We have reexamined the role of the cerebellar cortex in the regulation of CRs to determine the extent to which deficits in CR frequency and topography after cortical damage were recoverable, as suggested by the studies of Lavond et al. (1987) and Lavond and Steinmetz (1989), or were, at most, only partially recoverable, as suggested by Yeo et al. (1985a) and Yeo and Hardiman (1992). This was accomplished by examining the effects of extensive damage to HVI along with varying amounts of damage to the adjacent ANS on the rabbit's conditioned NM response. In addition, through the use of CS-alone trials, we also examined in greater detail how HVI lesions might affect the topography of CRs. Complete CR topographies, including the determination of peak CR amplitudes, can only be determined on test trials. For example, peak CR amplitudes are typically reached after the time of US onset and, thus, their measurement is obscured by the occurrence of the UR during paired CS-US trials. The accurate measurement of CR amplitudes during paired CS-US trials may be further compromised in animals with cortical lesions by the increase in UR amplitude reported by Yeo and Hardiman (1992). Finally, animals received 16 days of conditioning preoperatively so that lesion effects on CR production could be measured from stable, postasymptotic baselines.

Materials and Methods

Subjects. Rabbits of both sexes (New Zealand white albino), weighing 1.75–2.25 kg on arrival, were obtained from Hazelton Research Animals (Denver, PA). They were housed individually under a 12 hr/12 hr light/dark cycle, and maintained on rabbit chow and water.

Apparatus and general procedure. The apparatus, including the IBM PC-AT and ASYST software for stimulus control and data acquisition, have been described in detail (Romano et al., 1991). Briefly, animals were placed in Plexiglas restrainers and fitted with a headmount that supported a potentiometer and a 2-mm-diameter metal tube for delivery of the air puff US. The shaft of the low-torque, rotary potentiometer (Litton Potentiometer Division, PS091-023) was directly coupled to a suture placed in the NM. Movements of the NM were transduced to DC voltages and digitized every 5 msec with a resolution of 0.03 mm of NM movement per analog-to-digital (A/D) count.

Preoperative conditioning procedure. The general methods employed for conditioning of the rabbit's NM response followed those described by Welsh and Harvey (1989b) using the equipment described by Romano et al. (1991). Briefly, each animal was placed in a sound-attenuating chamber illuminated by two 24 V, 6 W lightbulbs positioned on either side of a 7.6 cm speaker (Boston Acoustics, model 703). Conditioning of the right nictitating membrane was accomplished by pairing a 250 msec, 84 dB, 1 kHz tone delivered through the speaker with a 100 msec air puff US delivered through the metal tube. The tube was positioned 6 ± 1 mm from the center of the right cornea, and air puff pressure measured at the end of the tube was 200 gm/cm². Each daily conditioning session consisted of 66 trials presented with a mean intertrial interval of 60 sec (range, 50–70 sec). The 66 trials were divided into six blocks of 11 trials. Within each block, the first 10 trials consisted of paired presentations of the tone CS and air puff US. The interval between CS and US onsets was 250 msec. The 11th trial in each block was a test trial during which the 250 msec tone CS was presented alone. Thirty-two animals received one 66 min adaptation session followed by 15 or 16 conditioning sessions preoperatively.

Surgery. Thirty-two rabbits underwent surgery 1 and 5 d after the last preoperative conditioning session using the general procedures described by Yeo et al. (1985a). All rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) supplemented with acepromazine maleate (1 mg/kg) and xylazine hydrochloride (5 mg/kg) followed by 50 ml of 20% (w/v) mannitol intravenously to dehydrate the brain. Animals were then placed in a stereotaxic headholder and maintained under halothane anesthesia administered through a 3.5 mm pediatric endotracheal tube. The skull overlying the right cerebellar cortex was removed and the underlying dura was cut and reflected to expose the superior surface of the cerebellar cortex. Seven animals received no further surgery and served as surgical controls.

Twenty-five other rabbits received aspiration lesions intended to remove HVI or HVI and the adjacent ANS. After surgery, the cortical surface was covered with sterile absorbable gelatin foam and the scalp was sutured.

Postoperative procedures. Beginning immediately after surgery, and during the next day, all animals received a 50 ml subcutaneous injection of a 5% dextrose, 0.9% sodium chloride solution to counteract dehydration produced by mannitol. Animals also received a 1.0 ml intramuscular injection of chloramphenicol sodium succinate (100 mg/ml) for 4 d. All animals appeared normal and active on the day following surgery. At 20 or 21 d following surgery, animals were exposed to 12 additional daily conditioning sessions that were identical to the preoperative procedures. At the end of the study, all animals, including the surgical controls, were injected with a lethal dose of sodium pentobarbital (195 mg, i.v.) and perfused through the heart with 0.9% saline followed by 10% formalin. The brains were removed, stored in 30% sucrose formalin for at least 2 weeks, and embedded in gelatin. Coronal sections, 60 μm thick, were obtained at 0.25 mm intervals throughout the cerebellum and inferior olive and stained with buffered cresyl violet. Based on microscopic examination of the histological material, the extent of each lesion was reconstructed onto a series of transverse plates of the rabbit cerebellum. Evaluation of these lesions was carried out independently by two of the authors (J.P.W. and C.H.Y), neither of whom were aware of the behavioral findings associated with each animal.

Response definition. The frequency and topography of responses were determined according to the procedures of Welsh and Harvey (1989b) and Romano et al. (1991). A response was defined as at least 0.5 mm (16 A/D counts) of membrane extension. On paired trials, responses were scored as CRs if they occurred during the 250 msec CS-US interval and as URs if they occurred within 800 msec after US onset. On CS-alone trials, responses were scored as CRs if they occurred within 800 msec of CS onset. Once a response was detected, its latency was determined by searching successively earlier A/D samples of the NM waveform to establish when the response first deviated from baseline by at least one A/D count (i.e., 0.03 mm). Measures obtained during paired CS-US trials included only the percentage occurrence of CRs and URs and their onset latencies. Analysis of the full topography of the CR could only be determined during the CS-alone test trials (see Welsh and Harvey, 1989b). CRs occurring during CS-alone trials were characterized in terms of their onset latencies, peak amplitude, latency to peak amplitude, and rise time.

Data analysis. A repeated-measures analysis of variance (ANOVA) using the SYSTAT statistical package (Wilkinson, 1988) was carried out on the various response measures. Follow-up tests of simple main effects were performed with the method of Dunnett to allow comparison of all experimental groups with the surgical controls (Winer, 1971). Significance for all statistical comparisons was set at $p \leq 0.05$.

Results

Locus of lesions

The lesions of cerebellar cortex could be divided into two groups. One group of animals (total HVI, $n = 11$; T1–T11) had either total or near total ablation of HVI characterized by a very slight sparing of Purkinje cells in the most ventral and medial part of the lobule at the depths of the primary fissure. In most cases there was accompanying extensive damage to the ANS involving Crus I and Crus II. None of the lesions extended into the deep cerebellar nuclei. These lesions were essentially identical with those reported by Yeo and Hardiman (1992). Figures 1 and 2 present photomicrographs taken through the major extent of these lesions for each of the animals (T1–T11). Serial reconstructions are presented for the overall largest lesion in the total HVI group (T1; Fig. 3), an intermediate lesion (T11; Fig. 4), and the smallest lesion (T8; Fig. 4). This overall smallest lesion, T8, was the most complete lesion of HVI and involved little other cortical tissue.

A second group of animals (partial HVI, $n = 14$; P1–P14) demonstrated less extensive damage to HVI with varying amounts of damage to the ANS. Figure 4 presents serial recon-
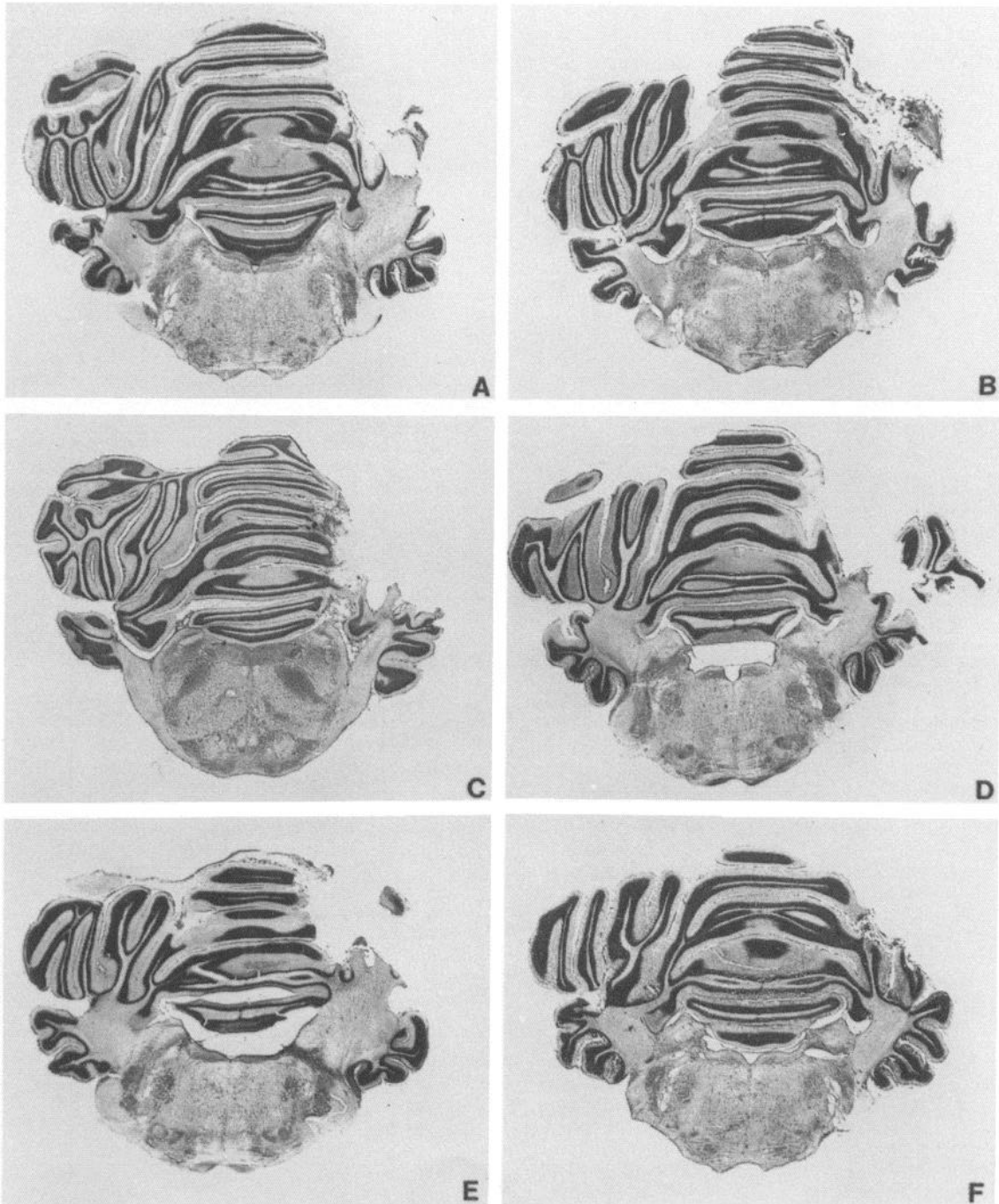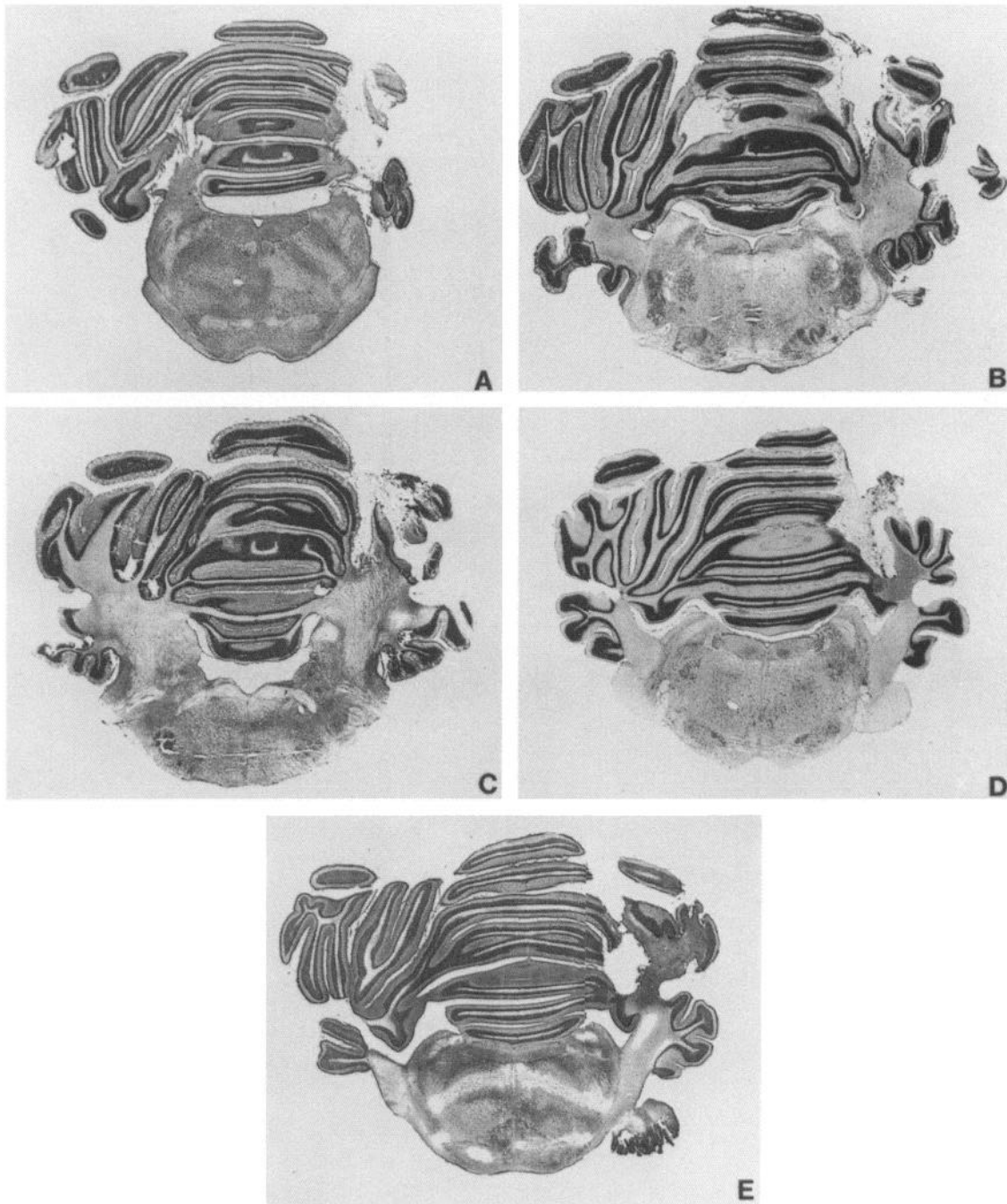
Figure 1. Photomicrographs (3× magnification) taken through the major extent of the aspiration lesions in animals of the total HVI group. *Letters* refer to animals shown in subsequent figures: *A,* T1; *B,* T2; *C,* T3; *D,* T4; *E,* T5; and *F,* T6.

structions of the largest lesion in the partial HVI group (P2) and a lesion of intermediate size (P4). A third group of animals consisted of surgical controls (*n* = 7; C1–C7). Subsequent analysis of the behavioral effects of cerebellar damage was based on a comparison of the three groups of animals described above: surgical controls (*n* = 7), partial HVI lesions (*n* = 14), and total HVI lesions (*n* = 11). Some animals in the total HVI group and in the partial HVI group exhibited gliotic reactions that extended ventrally from the sites of cortical damage through the white matter and into the deep cerebellar nuclei (dentate, interpositus, and/or fastigial). The increased gliosis occurred either in the

interpositus nucleus alone (T11), interpositus plus dentate (T3, T7, P11), interpositus plus fastigial (P10, P12), interpositus plus dentate and fastigial (P1, P2), or dentate alone (P7). The increased gliosis occurred in small portions of these nuclei from the level of plates −3.0 to −4.0 of Figure 3 with no evidence of neuronal degeneration (see Fig. 5).

Preoperative acquisition of CRs

All animals demonstrated robust acquisition of CRs across the preoperative conditioning sessions as measured by CR frequency (Fig. 6*A*), CR onset latency (Fig. 6*B*), or CR amplitude

Figure 2. Photomicrographs (3× magnification) taken through the major extent of lesions in animals of the total HVI group. *Letters* refer to animals shown in subsequent figures: *A,* T7; *B,* T8; *C,* T9; *D,* T10; and *E,* T11.

(Fig. 6*C*). All three measures had reached postasymptotic levels of performance by the last three preoperative sessions. In agreement with previous findings (Welsh and Harvey, 1989b), a significantly ($p < 0.01$) greater frequency of CRs could be detected on test trials as compared with paired trials during the first 3 d of conditioning (Fig. 6*A*). More importantly, there were no preoperative differences among the three experimental groups either in the rate of CR acquisition or in the final performance of CRs as measured by their frequency, amplitude, onset latency, latency of peak amplitude, and rise time (Table 1). These preoperative measures of the postasymptotic performance of CRs

were equivalent to those obtained in a previous study employing similar methodologies (Welsh and Harvey, 1989b).

Postoperative deficits in CR performance

Surgical controls and animals in the partial HVI group failed to demonstrate any significant decreases in mean CR frequency during postoperative testing (Fig. 6*D*). However, animals in the total HVI group demonstrated a significant ($p < 0.001$) reduction in the mean percentage of CRs on the first postoperative session whether measured during paired or test trials (Fig. 6*D*, Table 1). Thus, during test trials, animals in the total HVI group

T 1
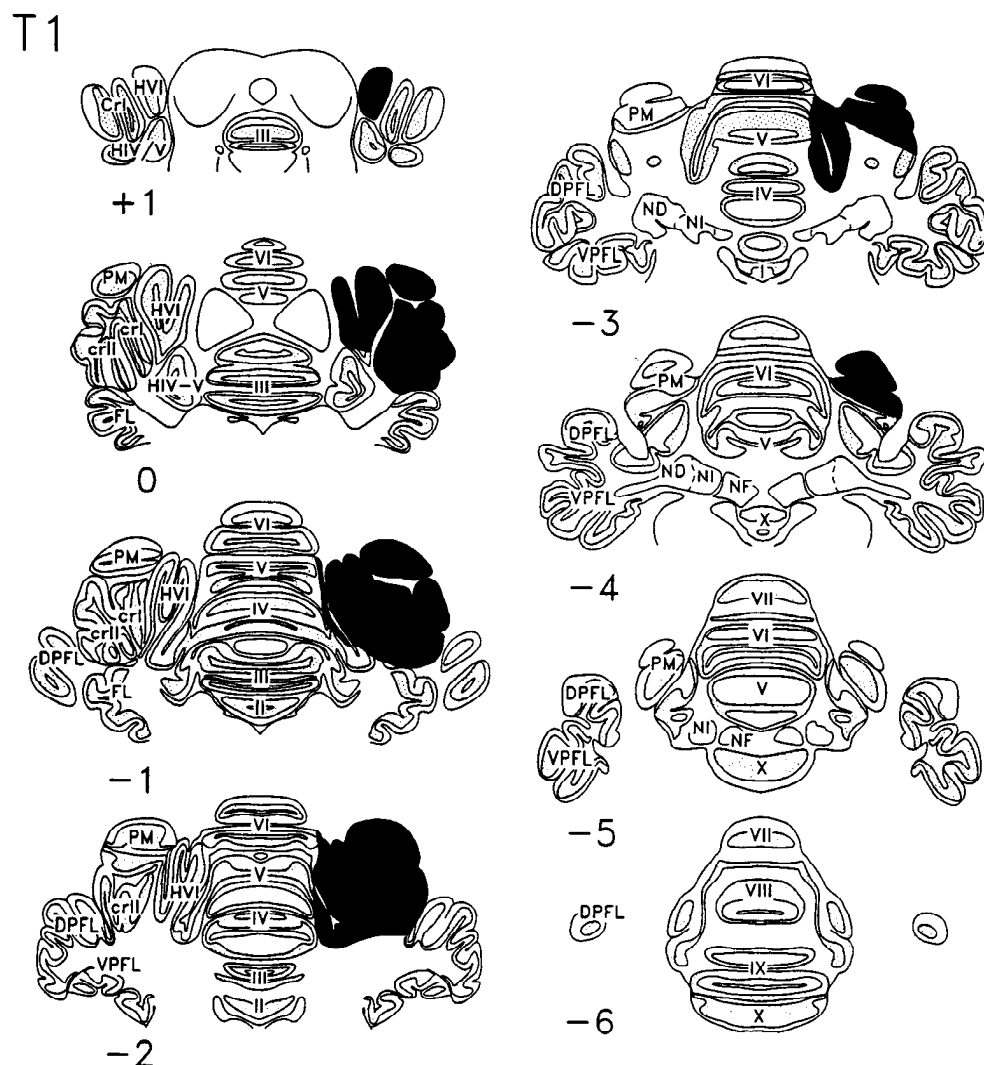
Figure 3. Serial reconstruction of the lesion sustained by animal T1 of the total HVI group using the transverse sections of Yeo and Hardiman (1992). The plates are numbered from 1 mm in front of lambda (+*1*) to 6 mm posterior to lambda (−*6*). This was the largest lesion in this study. Abbreviations on the plates relevant to this study are: *crI* and *crII, Crus I* and *Crus II* (ansiform lobe); *DPFL,* dorsaal paraflocculus; *FL,* flocculus; *ND,* dentate nucleus; *NF,* fastigial nucleus; *NI,* interpositus nucleus; *PM,* paramedian lobe; *HVI,* hemispheral lobule HVI; *II–VI,* vermian lobules II–VI.

responded to the CS on 45.9 ± 10.6% of trials during the first postoperative session as compared with 94.8 ± 3.4% for surgical controls and 86.4 ± 7.3% for the partial HVI group. It should be noted that all animals, including those exhibiting large decreases in CR frequency, always demonstrated a UR to the US on paired trials.

There was a gradual recovery of CRs over the 12 postoperative sessions with a significantly ($p < 0.05$) greater number of CRs being detected during test as compared with paired trials (Fig. 6*D*). The mean percentage of CRs of the total HVI group was no longer significantly different from that of the surgical controls by the last eight conditioning sessions when measured during test trials and by the last three sessions during paired trials. The effects of total HVI lesions on CR frequency were not associated with a significant effect on the onset latency of CRs (Fig. 6*E*), latency of peak CR amplitude, or CR rise time (Table 1). Nevertheless, it should be noted that the mean onset latencies of the total HVI group were consistently longer than those of animals in the control and partial HVI group (Fig. 6*E*), and these increases in onset latencies were consistent with the increased frequency of CRs during test as compared with paired trials (see Fig. 6*D*).

In contrast with the transient effects of cerebellar damage on

CR frequency, animals in the total HVI group demonstrated a large and persistent decrease in CR amplitude (Fig. 6*F*, Table 1). The mean CR amplitudes of the total HVI group were significantly ($p < 0.01$) reduced when compared with their preoperative values or with the mean of the control and partial HVI groups across all 12 postoperative sessions. In addition, there was no significant change in mean CR amplitudes of the total HVI group across sessions, indicating the absence of any recovery. The postoperative decrease in CR amplitudes was observed in each animal of the total HVI group. For example, the decreases ranged from 15% to 83% when expressed as a percentage change in the mean CR amplitude of each animal (calculated across all 12 postoperative sessions) from their preoperative baselines.

On the first postoperative session, animals in the total HVI group demonstrated equivalent decreases in both CR frequency (52%) and CR amplitude (53%) from their preoperative baselines. To examine further the relationship between these two measures of CRs, we obtained for each animal the percentage change in CR amplitude and CR frequency calculated separately for postoperative day 1 and day 12. We then determined the Pearson product–moment correlation coefficient between these two measures. There was a significant ($p < 0.01$) correlation of
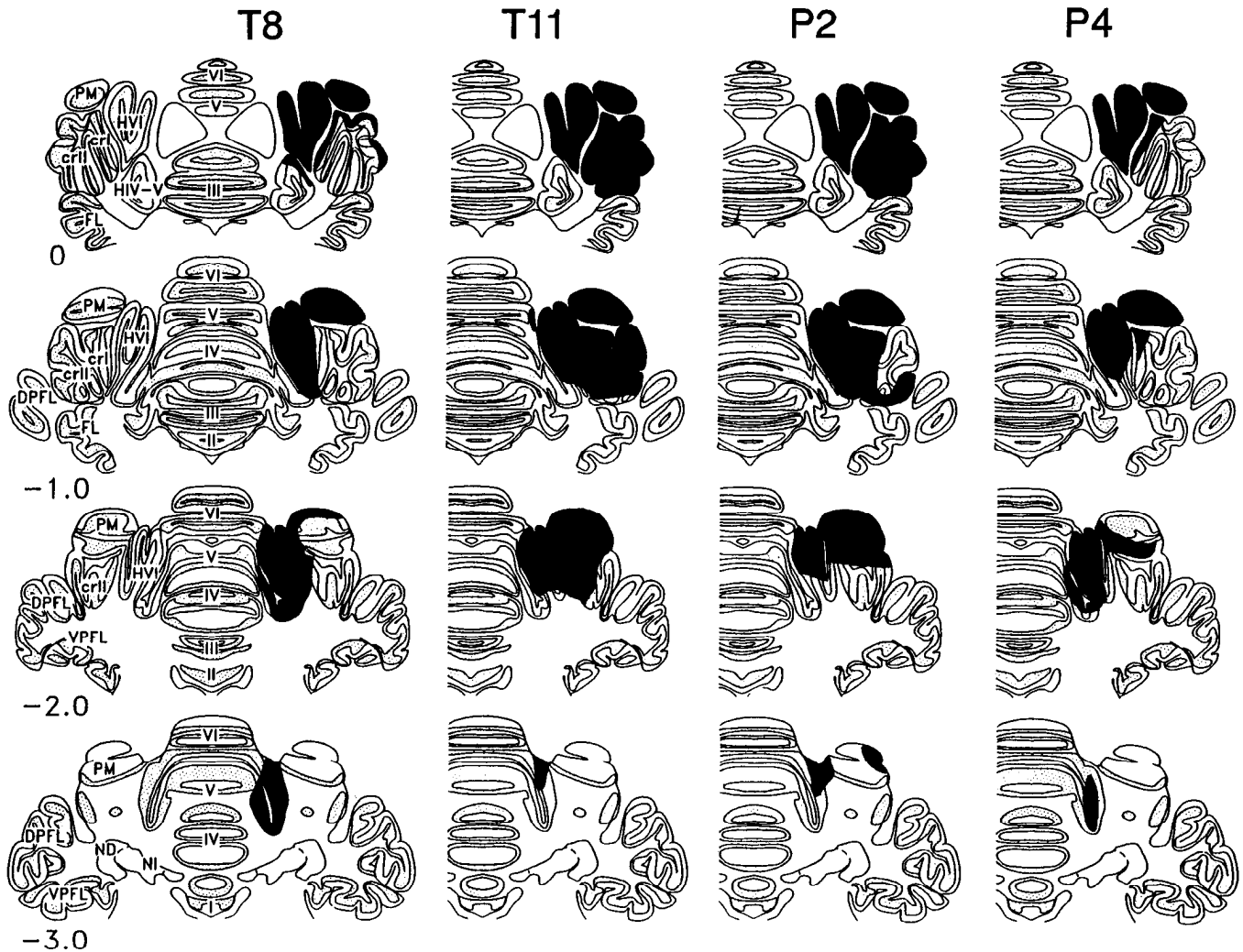
Figure 4. Serial reconstructions of the lesions sustained by animals T8 and T11 of the total HVI group and by animals P2 and P4 of the partial HVI group. Abbreviations are as in Figure 3.

0.82 between the percentage change in CR frequency and amplitude on day 1, but by day 12 the correlation had decreased to 0.36 and was no longer significant.

Relationship of cerebellar damage to CR deficits

Figure 7 presents the percentage of CRs on paired trials prior to surgery (point above P) and across the 12 postoperative sessions for each animal in this study. As can be seen, the seven surgical controls (C1–C7, top left panel) showed no deficits in CRs as compared with their preoperative scores. In the partial HVI group, 10 animals demonstrated greater than 80% frequency of CRs and four animals (P1, P4, P11, and P13) showed deficits in CR frequency followed by recovery (top right panel). Animals in the total HVI group demonstrated three behavioral effects. Two animals (T5 and T7, bottom left panel) showed little postoperative deficits in percentage of CRs. Two other animals (T3 and T11, bottom left panel) demonstrated a persistent impairment in their ability to perform CRs, which was also characterized by large day-to-day variability. Finally, seven animals (bottom right panel) showed large deficits in CRs on the first postoperative session followed by complete recovery.

The differences in the effects of HVI lesions on CR frequency

that were obtained in animals of the total HVI lesion group did not appear to be related to an extension of these lesions into more lateral areas of the cerebellum or into the deep cerebellar nuclei. For example, animal T1 had the greatest amount of damage to lateral cerebellar tissue (see Figs. 1A, 3). As can be seen in Figure 3, this lesion removed all but the most ventral part of HVI and also destroyed more than 90% of the ANS, with only the most lateral aspects of the posterior portion of the ANS (Crus II) being spared. In addition, a large extent of the paramedian lobule was removed. Although animal T1 did demonstrate a decrease in CRs postoperatively (Fig. 7, bottom right panel), the effects of this lesion were far less than those produced by the overall smallest lesion in this group (animal T8). In animal T8, there was a total ablation of HVI (greater than that of animal T1) but with little involvement of other cerebellar structures and no evidence of gliosis in any of the deep cerebellar nuclei (see Figs. 2B, 4). Animal T8 demonstrated a greater postoperative deficit in CRs and a longer recovery period than animal T1 (Fig. 7, bottom right panel). Finally, an animal that demonstrated a persistent impairment characterized by large day-to-day variability in percentage of CRs (T11, Fig. 7, bottom left panel) had an intermediate-sized lesion involving
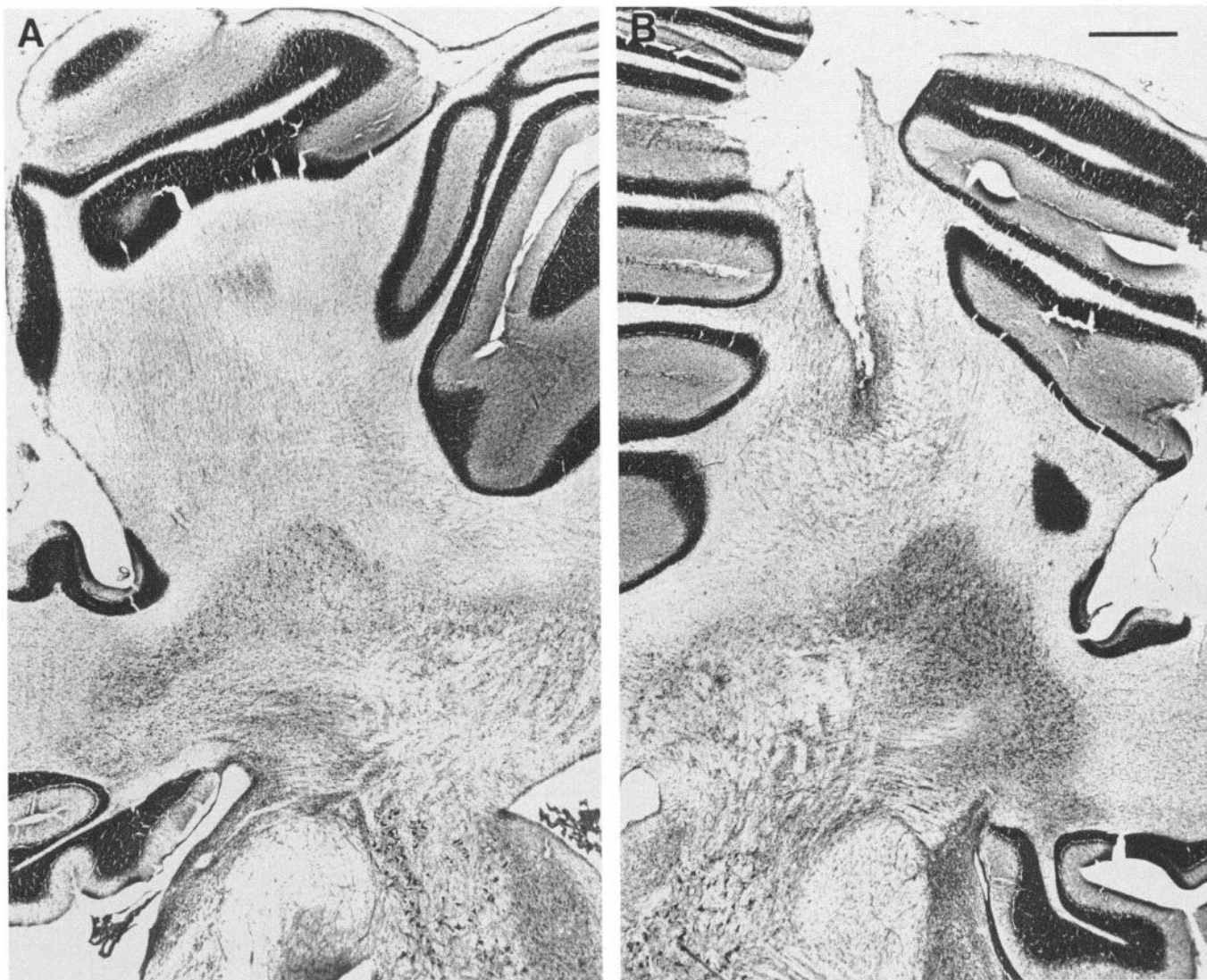
Figure 5. Photomicrographs of the left (*A*) and right (*B*) hemicerebellum of animal T7. Note the wedge-shaped lesion in *B*, which has selectively removed the most posterior HVI. Heavy gliosis can be seen in the right dorsolateral interpositus and dorsal dentate immediately ventral to the lesion in *B*. Neurons of normal size can be seen within the field of gliosis in the right deep cerebellar nuclei of *B*. Note also the normal appearance of neurons and the absence of gliosis in the contralateral, left, deep nuclei of *A*. Scale bar, 1 mm.

all of HVI but only the most medial portions of the ANS and paramedian lobules (Figs. 2*E*, 4).

The permanent deficit in mean CR amplitude demonstrated by all animals in the total HVI group also appeared to be due to damage to HVI and not due to additional damage to more lateral cerebellar cortex. For example, both animal T1 with the largest overall lesion and animal T8 with a lesion restricted predominantly to HVI demonstrated comparable decreases in CR amplitude of 40% and 60%, respectively.

None of the lesions extended ventrally into the deep cerebellar nuclei. As noted above, some animals demonstrated an increased number of glial cells in the ipsilateral dentate, interpositus, and/or fastigial nuclei with no evidence of any neuronal cell loss (see Fig. 5). However, there was no relationship between the presence, locus, or extent of such gliosis and deficits in CR performance in the total HVI or partial HVI groups. For example, there was increased gliosis in the interpositus nucleus of two animals (T3 and T11) that demonstrated persistent impairments in both the frequency and amplitude of CRs (Fig. 7),

but an equivalent amount of gliosis within the interpositus nucleus was also present in animals showing little or no CR deficits (T7, P1, P2, P10, P11, and P12).

Discussion

HVI modulates CR performance

The results of this study indicate that HVI is required for optimal performance of the conditioned NM reflex. The initial effect of HVI lesions was to produce an equivalent and highly correlated decrease in CR frequency and amplitude. These effects occurred in the absence of any significant changes in the temporal characteristics of the CR such as onset latency or rise time and in no case was there an abolition of CRs. The decrease in CR frequency was quite variable with two animals showing little change, seven animals showing CR deficits with subsequent recovery, and two animals showing deficits with no recovery. By contrast, HVI lesions produced a consistent and apparently nonrecoverable decrease in CR amplitude. The decrease in CR amplitude was due to the loss of HVI since it (1) occurred only
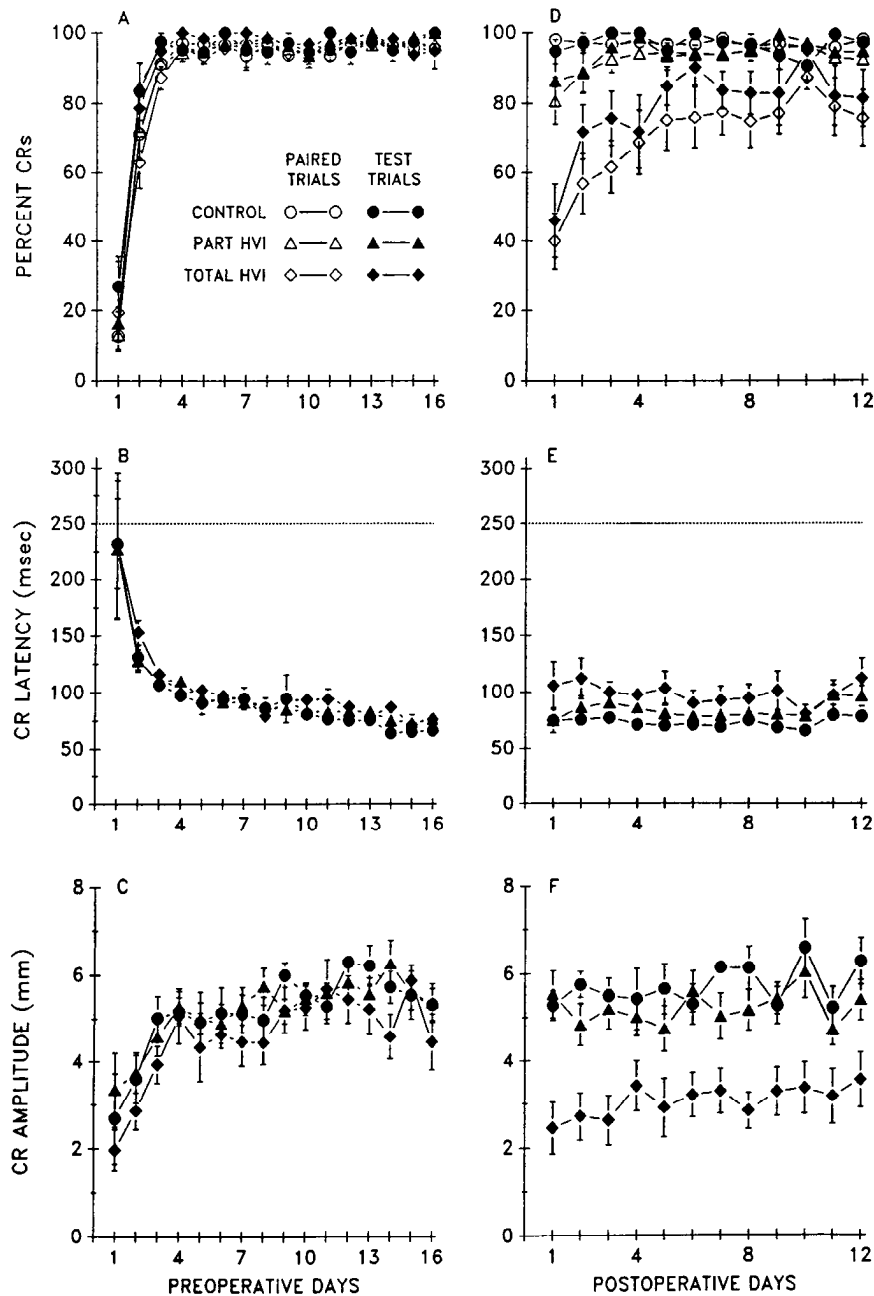
Figure 6. Preoperative acquisition (*A–C*) and postoperative performance (*D–F*) of CRs as measured by their frequency (*A* and *D*), onset latency (*B* and *E*), and amplitude (*C* and *F*). Open *symbols* indicate CR measurements obtained during the 60 daily paired trials, and *solid symbols*, during the six daily test trials. Error bars indicate SEM. In some cases the error bars are not visible because the SE was less than the diameter of the symbol.

in animals of the total HVI group, (2) was not related to any additional damage to surrounding cortical tissue, and (3) was not related to any damage to the interpositus nucleus. The permanent impairment in CR amplitude obtained in this study occurred despite extensive preoperative training. It should be noted that all previous measurements of CR extension after cerebellar cortical lesions in the rabbit (Yeo et al., 1985a; Lavond et al., 1987; Lavond and Steinmetz, 1989; Yeo and Hardiman, 1992) actually employed measures of CR magnitude and not amplitude (see Gormezano, 1966). The measure of CR magnitude was based on the maximum extent of NM movement within the CS–US interval (on paired trials) or during and immediately after CS presentation (on unpaired trials). This peak extension, whether it is less than or greater than the criterion amplitude defined for the estimation of CR frequency, contributes to the expression of CR magnitude. In contrast,

mean CR amplitude is derived only from NM extensions that exceed the amplitude criterion that defines the occurrence of a response. So, for example, a lesioned subject that produced 15 CRs of 5 mm amplitude and 85 "responses" smaller than the 0.5 mm criterion would have a mean CR amplitude of 5 mm but a mean CR magnitude that could be well below 1 mm. Thus, as the percentage of CRs approaches 100, the measure of CR magnitude approaches the measure of CR amplitude, but as the percentage of CRs decreases, the measure of mean CR magnitude becomes significantly smaller than that of mean CR amplitude.

CRs and URs are differentially regulated by HVI

The effects of HVI lesions on the CR were different from those on the UR. In agreement with previous studies (Yeo et al., 1985a; Lavond et al., 1987), the frequency of URs elicited by

Table 1. CR characteristics on test trials

CR measure	Control (N = 7)	Partial HVI (N = 14)	Total HVI (N = 11)
Percentage of CRs			
Preop—last block	97.6 ± 1.1	98.3 ± 1.0	96.4 ± 1.4
Postop—first block	97.3 ± 1.3	90.4 ± 4.1	64.5 ± 6.3*
Postop—last block	96.3 ± 1.9	96.0 ± 1.3	86.6 ± 5.7
Amplitude of CRs (mm)			
Preop—last block	5.5 ± 0.4	5.7 ± 0.4	5.2 ± 0.3
Postop—first block	5.5 ± 0.2	5.1 ± 0.5	2.6 ± 0.4*
Postop—last block	6.0 ± 0.5	5.4 ± 0.4	3.4 ± 0.5*
Onset latency of CRs (msec)			
Preop—last block	65 ± 3	75 ± 3	80 ± 7
Postop—first block	76 ± 4	87 ± 9	110 ± 14
Postop—last block	75 ± 3	91 ± 8	97 ± 10
Latency of peak CR amplitude (msec)			
Preop—last block	256 ± 11	255 ± 8	262 ± 14
Postop—first block	238 ± 8	240 ± 12	301 ± 30
Postop—last block	261 ± 14	276 ± 12	266 ± 24
Rise time of CRs (msec)			
Preop—last block	190 ± 12	180 ± 9	182 ± 11
Postop—first block	162 ± 7	153 ± 6	190 ± 25
Postop—last block	186 ± 13	186 ± 11	169 ± 17

Values are means ± SEM for five measures of conditioned responding on tone-alone test trials before (Preop) and after (Postop) surgery. Values are given for Preop—last block, the mean of the last 3 preoperative days (sessions 14–16); Postop—first block, the mean of the first 3 postoperative days (sessions 1–3); and Postop—last block, the mean of the last 3 postoperative days (sessions 10–12).

* $p < 0.01$ as compared with preoperative score or with surgical controls.

the suprathreshold US normally used for conditioning was not reduced following the removal of HVI. However, using US-alone trials and a range of US intensities, Yeo and Hardiman (1992) found that the amplitude of the UR was enhanced after HVI lesions, a clear demonstration that the cerebellar cortex regulates the motor systems of the unconditioned NM reflex. It is well known that the cerebellar cortex can modulate the threshold of motor systems and that this modulation can be expressed through a facilitation (Snider and Magoun, 1949) or suppression (Snider et al., 1949) of both cerebral and brainstem circuits. The impaired performance of CRs and the enhanced performance of URs after HVI lesions suggest that the cerebellar cortex normally facilitates the brainstem pathways of the CR but normally suppresses the brainstem pathways of the UR. This could be due either to a difference in how the CS and US engage the cerebellar circuitry or to a difference in the projections of the cerebellum to the conditioned as compared with the unconditioned NM pathways.

The influence of the cerebellar cortex upon the NM reflex occurs via the projections of Purkinje cells in HVI to the interpositus nucleus (Yeo et al., 1985b). This projection is exclusively inhibitory (Ito et al., 1964) and produces a phasic decrease in the excitation generated by projections directly upon the interpositus that originate in the brainstem (MacKay and Murphy, 1973, 1974; Llinás and Mühlethaler, 1988). The interpositus, in turn, innervates brainstem motor systems (cf. Jansen, 1969). Auditory stimuli enter the cerebellum through mossy fiber projections that originate in the pontine nuclei (Aitkin and Boyd, 1978), and produce excitation of vermian and paravermian Purkinje cells (Snider and Stowell, 1944). Auditory stimuli also produce an excitation of cells in the deep cerebellar nuclei (Chap-

man et al., 1986; Berthier and Moore, 1990) as a result of the projection of mossy fiber collaterals from the pontine nuclei to the deep cerebellar nuclei (Dietrichs et al., 1983; Brodal et al., 1986; Shinoda et al., 1992). Thus, the auditory CS used in the present study could be expected to excite cellular elements of both cerebellar cortex and the interpositus nucleus. Similarly, corneal stimulation, such as that produced by the air puff US, engages the cerebellar circuitry at both the deep nuclear and cortical levels, through the recruitment of climbing fibers originating in the inferior olive (Matsushita and Ikeda, 1970; Berkley and Hand, 1978; Huerta et al., 1983) and mossy fibers originating in the spinal trigeminal nucleus (Somana et al., 1980; Huerta et al., 1983). Therefore, the output of the interpositus, and thus the entire intermediate cerebellum, following an auditory CS or corneal air puff US is determined by an interaction of excitation, mediated via direct mossy and climbing fiber projections to interpositus, and inhibition, mediated via indirect pathways through HVI. Such an interaction has been clearly demonstrated to occur following proprioceptive stimuli (MacKay and Murphy, 1973, 1974). As proposed by Yeo and Hardiman (1992), the fact that removal of HVI facilitates the ability of the US to elicit URs is consistent with an increased excitation within interpositus. This is most likely due to the removal of the inhibitory influence of the descending Purkinje cell projections. Although such an increased excitation in interpositus can explain the increase in UR amplitudes after cortical lesions, it cannot account for the decrease in CR amplitude.

The effects of cortical lesions on CRs and URs differ in several respects from the effects produced by lesions of the interpositus nucleus. Using procedures identical with those employed in this study, Welsh and Harvey (1989b) demonstrated that lesions of
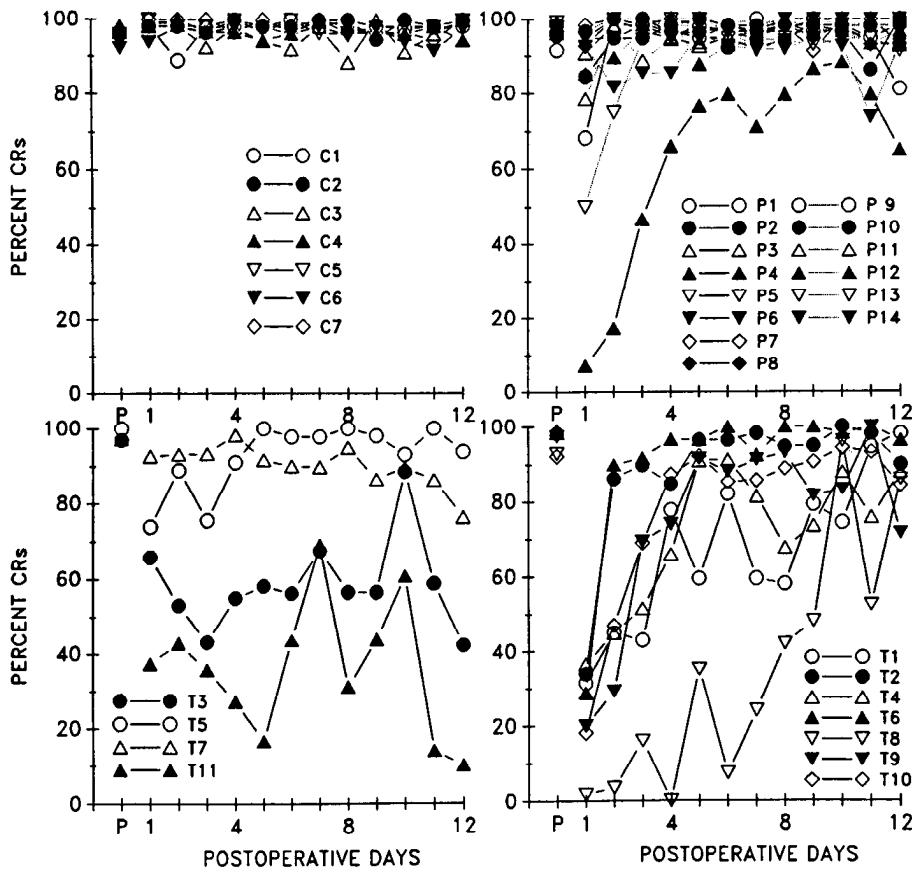
Figure 7. Postoperative frequency of CRs during paired trials for all animals in this study. Surgical controls, *upper left*; partial HVI group, *upper right*; and total HVI group, *both lower* panels. Points above *P* are the mean percentage of CRs on the last 3 preoperative days (sessions 14–16).

interpositus reduced CR amplitude and increased the onset latency, latency of peak amplitude, and rise time of CRs, while cortical lesions in the present study reduced CR amplitude but did not change the temporal characteristics of the CR. Thus, both HVI and interpositus lesions affect CR amplitude while only interpositus lesions affect the temporal characteristics of the CR (see also Welsh, 1992). The greater degradation of CRs following interpositus as compared with HVI lesions might be due to the additional withdrawal of tonic influences that normally maintain background excitation for motor systems (Dow and Morruzzi, 1958). As a corollary, the reason why HVI lesions reduce only CR amplitudes may be due to the presence of a sufficient amount of regulation of brainstem circuits by the interpositus nucleus so as to allow for normal response topographies. Interpositus lesions also increased the stimulus threshold for URs, reduced their amplitude (Welsh and Harvey, 1989a), and degraded the temporal characteristics of the UR (Welsh and Harvey, 1989a,b; Welsh, 1992) while cortical lesions reduced the stimulus threshold for URs and increased UR amplitude (Yeo and Hardiman, 1992). The sluggishness of URs following interpositus lesions would also be consistent with the removal of deep nuclear regulation of motor circuits in the brainstem.

Is cerebellar circuitry involved in associative learning?

We have argued that lesions of the cerebellar cortex change the excitability of the NM motor system and that these changes influence both conditioned and unconditioned NM responses. But a fundamental question remains: do general changes in sensory–motor excitability alone account for deficits in CRs or are there also deficits in associative learning? We present two

interpretations. One interpretation, that cerebellar cortical lesions produce deficits that are restricted to the performance of the CR, is favored by some of us (J.A.H., J.P.W., A.G.R.). The other interpretation, advanced by one of us (C.H.Y), is that learning deficits may be embedded within changes in performance. The key difference between these interpretations is that the former argues against learning mechanisms in the cerebellar cortex and the latter supports them.

Evidence suggesting that cerebellar circuitry is not involved in associative learning. The significant recovery of CR frequency in the presence of a continued decrement in CR amplitude suggests that removal of HVI had not affected learning, but rather had produced a permanent performance deficit. This deficit was most likely due to an increase in the threshold of brainstem motor systems subserving the NM reflex so as to decrease the ability of the CS to elicit CRs of normal amplitude (Snider and Magoun, 1949). It should be recalled that, before learning, the tone CS is a subliminal stimulus in that it is capable of activating the motor systems of the NM reflex but not to the extent of eliciting an overt response (Harvey et al., 1985; Harvey, 1987). Classical conditioning of the NM reflex leads to an increase in the excitatory effects of the CS such that it can elicit an NM response (Guegan and Horcholle-Bossavit, 1981; Harvey et al., 1984; Holstege et al., 1986). However, even after considerable conditioning, the CS remains a weaker stimulus with respect to activation of the NM reflex than the suprathreshold US used during conditioning (Welsh and Harvey, 1989a; Welsh, 1992). Therefore, the recovery of CR frequency in subjects with complete removal of HVI could be due to the CS accruing additional associative strength during the 12 postoperative conditioning

sessions. In this regard, it has been suggested that the underlying neural substrate of the CR comprises two components, the longer latency of which is responsible for at least 30% of the normal amplitude and is selectively impaired by the interruption of afferents of the cerebellar cortex (Welsh, 1992). That HVI lesions reduced CR amplitude by 42% suggests an impairment in this long-latency component of the CR. A learning-related increase in the excitatory properties of the CS following HVI lesions may allow a normal frequency of CRs to be expressed through a system that is constrained to a maximal output of only 70% of normal.

Although the results of this study argue that HVI is not involved in the postoperative acquisition of CRs, some investigators have suggested that CR acquisition after cortical lesions implicates the interpositus nucleus as a site of learning (McCormick et al., 1981; McCormick and Thompson, 1984; Lavond et al., 1987; Thompson, 1989). However, using CS-alone test trials, Welsh and Harvey (1989b) found a significant frequency of CRs after unilateral interpositus lesions, and an impairment of the unconditioned NM response on US-alone trials (Welsh and Harvey, 1989a,b; Welsh, 1992). It was suggested that lesions of the interpositus nucleus produce a performance rather than learning deficit (Welsh and Harvey, 1989a,b). In addition, Kelly et al. (1990) reported that cerebellectomy did not impair acquisition of the conditioned NM response in the decerebrate rabbit. Steinmetz et al. (1992) have recently published a rebuttal of the findings of Welsh and Harvey (1989b) in which they reaffirm their belief that the interpositus nucleus is the site of learning for the rabbit's NM response. In the final analysis, the question as to whether interpositus lesions are altering CR frequencies and amplitudes by affecting learning or performance (or both) cannot be resolved by the use of irreversible lesions. For this reason, Welsh and Harvey (1991) employed reversible inactivation of the interpositus to resolve these issues. They found that animals failed to demonstrate any evidence of CR acquisition during inactivation of the interpositus produced by the intracerebral infusion of lidocaine. However, 2 d later, when the effects of lidocaine had worn off, these animals demonstrated the same frequency of CRs as control animals, indicating that inactivation of the interpositus had blocked the performance but not the acquisition of CRs (Welsh and Harvey, 1991). Taken together, these findings reinforce the long-held view that the cerebellum is necessary for the optimal performance of movements (Luciani, 1915; Holmes, 1917; Brooks, 1986; Welsh and Harvey, 1992) through its regulation of both cerebral and brainstem circuits (Snider and Magoun, 1949; Snider et al., 1949) and argue against a role for the cerebellum in learning.

Evidence suggesting that cerebellar circuitry is involved in the learning and execution of CRs. That cerebellar cortical lesions produced initial deficits in CR frequency but allowed subsequent recovery is consistent with the suggestion that there is learning within the cerebellar circuitry. The initial deficits are due to the loss of this cerebellar learning and recovery is mediated by increases in associative strength in circuits outside the lesioned area. But because CR amplitudes never recover to preoperative levels, these other circuits cannot support normal CRs. What is the evidence to support this view?

The cerebellar cortex receives auditory mossy fiber inputs that can convey information about the CS. In addition to CS-related mossy fiber inputs, there are US-related mossy and climbing fiber inputs to lobule HVI; there is convergence of CS- and US-

related information in lobule HVI. Coactivation of these inputs during conditioning may result in long-term changes in cortical excitability to the CS that produces the CR by phasic modulation of the brainstem motor system. Long-term depression of parallel fiber synapses upon Purkinje cells may be the underlying mechanism (Ito, 1989).

In contrast to lesions of the deep nuclei, lesions of the cerebellar cortex enhance UR amplitudes by disinhibition of brainstem sensory–motor circuits (Yeo and Hardiman, 1992). And yet, in the present study, CR frequencies were temporarily impaired and CR amplitudes were permanently reduced despite an apparent increase in reflex excitability. These findings are consistent with the suggestion that the lesions have damaged associative mechanisms within the cerebellar cortex that assist in the generation of CRs.

In other studies, the effects of cerebellar cortical lesions were more severe (Yeo et al., 1985b; Yeo and Hardiman, 1992). Neither the frequencies nor the amplitudes of CRs recovered to preoperative levels, even after 30 postoperative training sessions. The major difference between those studies and the present one relates to the amount of conditioning before the lesions. In the present study, conditioning was very firmly established to postasymptotic levels over 16 preoperative sessions. All studies indicate that CRs can be generated following unilateral cerebellar cortical lesions, but the rate and degree of recovery may be determined by the strength of conditioning before the lesion. Clearly, if learning can also be mediated by circuits outside lobule HVI, then the effects of HVI lesions will be reduced if these circuits have already undergone modifications by extensive training before the lesion, as in the present study.

These findings are consistent with the view that, at an early stage of learning, there are critical associative changes established within the cerebellar cortex. As training continues, associative changes may be established at other sites outside lobule HVI. These additional sites may include additional ipsilateral eye-blink–related cerebellar cortical regions such as those in the flocculus (Nagao et al., 1984), contralateral cerebellar cortical areas, or brainstem sites (Harvey, 1987; Bracha et al., 1991; Richards et al., 1991; Welsh, 1992).

Conclusions

Lesions of the cerebellar cortex or deep nuclei in otherwise normal animals always disrupt conditioned NM responses. Such lesions also disturb unconditioned NM responses by changing the cerebellar regulation of motor systems involved in the execution of this reflex. To what extent there may be learning impairments embedded within these general performance changes cannot be determined with destructive lesions (Welsh and Harvey, 1989b); this question must be answered using other techniques (Welsh and Harvey, 1991). Nonetheless, the cerebellar cortex cannot be considered as the single locus necessary for NM conditioning.

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