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Cerebellar Lesions and the Nictitating Membrane Reflex: Performance Deficits of the Conditioned and Unconditioned Response

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Unilateral cerebellar lesions abolished the occurrence of ipsilateral conditioned nictitating membrane responses during the 285 msec interval between onset of the conditioned and unconditioned stimuli on paired trials. This effect was obtained in 15 animals sustaining damage to the dorsolateral aspects of the interpositus nucleus and the adjoining white matter. However, conditioned responses did occur during the 800 msec observation interval employed on tone-alone test trials, and these responses exhibited the classic performance deficits normally associated with cerebellar damage: a low frequency of occurrence (14%, as compared with 96% before the lesion); a 3.1 mm decrease in amplitude; a 236 msec increase in onset latency; a 563 msec increase in latency of peak amplitude; and a 327 msec increase in rise time. Four of the 15 animals failed to demonstrate greater than 5% responding during the test trials. These performance deficits were not specific to the learned, conditioned response. Unconditioned responses were also reduced in frequency and increased in latency of peak amplitude and rise time, especially when elicited at lower air-puff intensities. These deficits in the unconditioned response were observed in animals that failed to exhibit conditioned responses on either paired or test trials, as well as in animals demonstrating conditioned responses only during test trials. We conclude that the cerebellum has a general role in regulating the nictitating membrane reflex so that deficits in learned responses observed after cerebellar lesions are secondary to a broader deficit in performance. The performance deficits appear to consist of a sensory component, as reflected by an increase in stimulus threshold for elicitation of the nictitating membrane reflex, and a motor component, as reflected by the altered topography of the evoked response. The results of this study thus reaffirm the role of the cerebellum in regulating the sensorimotor processes necessary for the optimal performance of both conditioned and unconditioned responses and extends this role to the expression of a simple cranial nerve reflex.

A number of recent studies (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a, b) have reported that rabbits with lesions of the cerebellum fail to perform conditioned responses (CRs). These experiments have employed Pavlovian conditioning of the nictitating membrane response (NMR; Gormezano et al., 1962; Gormezano et al., 1983), a corneal–VIth nerve reflex of membrane extension (Prince, 1964; Cegavske et al., 1976; Harvey et al., 1984; Marek et al., 1984). For example, unilateral lesions that encompassed the dorsolateral aspects of the anterior interpositus nucleus were reported to abolish ipsilateral CRs to a tone-conditioned stimulus (CS) without affecting the unconditioned response (UCR) (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a). Additionally, ipsilateral CRs could not be reacquired, while acquisition and performance of CRs contralateral to the lesion remained unimpaired. The unique nature of these findings has led to the conclusion that the abolition of CRs by cerebellar lesions is due to loss of a site that is critical for learning and memory. Such a conclusion presupposes that the cerebellum is not involved in the regulation of sensory or motor systems necessary for the performance of either the unilateral conditioned or unconditioned response (see Thompson, 1986).

Although the above findings are consistent with a hypothesis that a memory may be localized in the cerebellum, the conclusions derived from these data are inconsistent with the known role of the cerebellum in motor coordination (see Luciani, 1915). A wealth of information from human clinical studies and animal laboratory experiments has demonstrated that cerebellar dysfunction produces motor performance deficits. In 1917, Sir Gordon Holmes characterized the primary motor impairments of patients with cerebellar damage (Holmes, 1917), and these descriptions have been verified in a variety of animal models (Brooks et al., 1973; Uno et al., 1973; Conrad and Brooks, 1974; Soechting et al., 1976; Meyer-Lohmann et al., 1977; Llinás and Walton, 1979). Subjects with unilateral cerebellar lesions demonstrated errors of rate, force, direction, coordination, and regularity in movements ipsilateral, but not contralateral, to the side of the lesion. These data have reinforced the view that normal cerebellar function is essential for the optimal execution of movements (Brooks, 1984).

Cerebellar damage in humans and experimental animals produces 200 msec delays in the initiation of movements, and this finding is especially relevant to studies employing classical con-

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ditioning of the rabbit NMR. Acquisition and performance of CRs are under strict temporal control; learning is maximal when CS onset precedes the unconditioned stimulus (UCS) by 200–400 msec (Smith et al., 1969; Harvey et al., 1985). For this reason, the effects of interpositus lesions on the NMR have been typically examined using CS-UCS intervals of 250 msec (McCormick et al., 1981; McCormick and Thompson, 1984). The average CR onset latencies of animals well trained under such conditions is usually never less than 100 msec after CS onset (Gormezano et al., 1983) so that at a CS-UCS interval of 250 msec, their initiation would not occur more than 150 msec before onset of the UCS. If cerebellar damage delayed the initiation of nictitating membrane CRs by more than 150 msec, CRs would not be observable due to the onset of the UCS and consequent elicitation of the UCR.

The present studies examined the effects of damage to the anterior portions of the interpositus nucleus on CRs during the paired presentations of a tone CS and air-puff UCS, as well as during CS-alone test trials. The use of CS-alone trials allowed further characterization of CRs in terms of onset latencies, peak amplitude, latency of peak amplitude, and rise time (the time between CR onset and attainment of peak amplitude). A second aim of these experiments was to obtain equivalent characterizations of the UCR through the use of UCS-alone trials across a range of UCS intensities.

This work was published earlier in abstract form (Welsh et al., 1986).

Materials and Methods

Subjects. This study employed male and female rabbits (New Zealand white albino) weighing approximately 2 kg on arrival from Iowa Ecology Farms (Wilton, IA). All animals were housed individually with free access to rabbit chow and water.

Apparatus and general procedure. The apparatus and procedure for delivering stimuli and recording responses of the nictitating membrane have been described in detail elsewhere (Gormezano, 1966; Gormezano et al., 1983; Schindler et al., 1985). Each rabbit was restrained in a Plexiglas stock, fitted with a headmount, and placed in an individually ventilated and sound-attenuated experimental chamber facing a stimulus panel containing an 11.4 cm speaker located above and directly in front of the animal and two 6 W, 24 V, D.C. houselights, one mounted on each side of the speaker. In general, groups of 12 rabbits were conditioned at one time. The NMR was measured by the direct, physical coupling of a phototransducer to the membrane as previously described (Schindler et al., 1985) and the use of the Apple II/FIRST operating system (Scandrett and Gormezano, 1980) for digitization of analog signals and extraction of dependent variables. The digitized values were stored on a Corvus hard disk (IMI-7710) for subsequent statistical analysis. This method afforded a temporal resolution of 5 msec and an amplitude resolution of 0.06 mm of actual membrane movement. The Apple II/FIRST operating system also controlled the delivery of stimuli to each rabbit. The CS was a 250 msec, 84 dB (0.002 dynes/cm^2 reference), 1 kHz tone produced by a computer-controlled sine wave generator and delivered through the speaker. The UCS was a 100 msec corneal air puff delivered through a 2-mm-diameter metal tube positioned perpendicular to and 4 mm away from the center of the cornea. For conditioning, the UCS was delivered with a source pressure of 2.2 kg/cm^2.

Preoperative conditioning procedure. All animals first received a 66 min adaptation session during which no stimuli were presented. However, in order to obtain a measure of baseline responding, extensions of the right nictitating membrane were recorded at times corresponding to the observation intervals used during the subsequent training sessions. For the following 16 d, animals received daily conditioning sessions. Each 66 min session consisted of 66 trials with a mean intertrial interval of 60 sec (range, 50–70 sec). The 66 trials were divided into 6 blocks of 11 trials each. Within each block, the first 10 trials consisted of paired presentations of the 250 msec tone CS and 100 msec UCS delivered to the right cornea. UCS onset, defined as the time that the UCS impacted the cornea, occurred 35 msec after CS offset. Thus, the time between CS and UCS onsets was 285 msec. The 11th trial in each block was a test trial during which the 250 msec tone CS was presented alone. There were 60 CS-UCS conditioning trials and 6 CS-alone test trials during each daily session.

Surgery. One to two days after the last (16th) conditioning session, animals were anesthetized with 25 mg/kg pentobarbital delivered i.v. and supplemented as needed. Animals were then placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) and positioned according to the orientation of McBride and Klemm (1968) so that the surface of the skull at bregma was 1.5 mm above lambda. Lesions were aimed at the rostral and lateral aspects of the right interpositus nucleus. The rostrocaudal coordinates for such lesions were best approximated by the use of a regression equation: $X = 0.69Y + 4.8$ mm, where Y was the distance (in mm) between bregma and lambda in the horizontal plane (Gray et al., 1981). The rostrocaudal coordinate for the lesion was then X mm posterior to bregma. A 24 gauge nichrome wire electrode, insulated except for 0.5 mm of the tip, was lowered through a burr hole in the skull at a distance caudal to bregma given by the formula described above and a point 5.5 mm lateral to the midline and 15.7 mm below the vertical reading obtained from the skull surface at bregma. Electrolytic, cathodal lesions were produced by passage of a 2 mA current for 40 sec. The burr hole was closed with Gelfoam, the skull restructured with bone wax, and the incision closed with silk sutures. At the end of surgery, animals were injected intramuscularly with 300,000 units of penicillin G procaine (Pfizer Inc., New York) and after recovery from the anesthetic returned to their home cages. All animals appeared healthy and demonstrated normal eating, drinking, and grooming behaviors within 48 hr after surgery.

Postoperative conditioning procedure. At 20–21 d after surgery, all animals were exposed to 12 additional daily conditioning sessions that were identical with preoperative procedures. After these 12 postoperative sessions (17–28), the UCS was delivered to the left cornea under the same conditioning parameters, and responses were recorded from the left nictitating membrane for 4 additional sessions (29–32). For 4 final conditioning sessions (33–36), UCS delivery and response measurement again returned to the right eye.

Determination of UCS thresholds for elicitation of UCRs. After completion of the 20 d of postoperative conditioning sessions, 44 rabbits received 2 additional sessions in which only air-puff UCSs were presented. Each session consisted of 54 trials composed of 9 air-puff intensities of 0.09, 0.18, 0.37, 0.55, 0.73, 1.10, 1.47, 2.20, and 2.93 kg/cm^2 source pressure, randomly presented in six 9-trial blocks with the restriction that each of the 9 UCS intensities was presented once within each of the 6 blocks. Each air puff was 100 msec in duration and the intertrial interval was 60 sec (range, 50–70 sec).

Histology. After completion of postoperative testing, the animals were perfused with 10% formalin under pentobarbital anesthesia. Frozen, 60-μm-thick sections were stained for cell bodies and myelin by the method of Donovick (1974). The extent and locus of damage was confirmed by light-field microscopy. Lesions were outlined on a set of coronal plates spaced at 0.5 mm intervals for 3.5 mm along the longitudinal axis of the cerebellum. The percentage damage to the dentate and interpositus nuclei was then quantified by overlap grid analysis.

Data analysis. On all trials, a response was defined as a 0.5 mm or greater extension of the nictitating membrane. On paired CS-UCS trials, responses were scored as CRs if they occurred (i.e., reached the criterion of 0.5 mm) during the 285 msec interval between CS and UCS onset. Responses occurring after UCS onset were scored as UCRs. On CS-alone test trials, a response was scored as a CR if it attained an amplitude of 0.5 mm within 1800 msec of CS onset and was initiated within 800 msec of CS onset. Similarly, on UCS-alone trials a response was scored as a UCR if its onset latency was within 800 msec of UCS onset. The windows employed for scoring test-trial data are in accordance with those established by previous workers who have employed observation intervals of 1000 msec (Smith et al., 1969), 1200 msec (Smith, 1968), and 3500 msec (Schneiderman, 1966). Except as noted, the onset latency was determined according to the procedures described by Scandrett and Gormezano (1980, p. 25). Thus, the detection of nictitating membrane response latency (CR or UCR) was determined by searching the digitized response to the right, from CS and/or UCS onset, until a displacement from baseline reached or exceeded 0.5 mm, then sampling to the left until the displacement (found by successive averaging of a specific data point with the values of the neighboring point on either side) was reduced to 1/16 mm (0.06 mm), a value approximately 5 SD above the baseline noise level.

The only measures obtained during paired CS-UCS trials were the percentage occurrence of CRs and UCRs and NMR onset latencies. Measures such as the onset and peak latencies, as well as latency of the peak amplitude of the CR and UCR, were only determined during CS-alone or UCS-alone trials. These measures cannot be reliably determined for the CR on paired CS-UCS trials because its full topography is always obscured by the subsequent occurrence of the UCR. Similarly, one cannot obtain an uncontaminated measure of UCR topography on paired CS-UCS trials because of the enhancement of UCR amplitudes that results from the tone-induced facilitation of the nictitating membrane reflex prior to CR acquisition (Thompson et al., 1976; Young et al., 1976; Harvey et al., 1985), as well as to the subsequent occurrence of either observable CRs prior to UCS onset or the possible occurrence of undetectable CRs, i.e., CRs hidden within the response envelope of the UCR.

Statistics. A repeated-measures analysis of variance (ANOVA) was performed on all dependent variables of the CR and UCR. Follow-up analyses of significant effects used the method of Dunnett (Winer, 1971). Relationships between the degree of damage to the posterior or anterior aspects of the interpositus or dentate nuclei and the abolition of CRs during the CS-UCS interval were assessed by 2 × 2 contingency tables (Finney et al., 1963). All data are presented as mean ± SEM. Significance for all statistical comparisons was set at $p \leq 0.05$.

Results

Preoperative acquisition of CRs

Percent baseline responding on the adaptation session prior to CR acquisition was low (<1%; Fig. 1*A*) during either the 285 or 800 msec observation interval. All animals demonstrated a robust and significant ($p < 0.001$) acquisition of CRs across conditioning sessions as measured by percentage of CRs (Fig. 1*A*) or NMR onset latencies (Fig. 1*B*), and had reached postasymptotic values by the last 3 preoperative sessions. CR acquisition was essentially the same whether measured during the 285 msec CS-UCS interval of paired trials or during the 800 msec interval of CS-alone test trials. However, there was an increase in the ability to detect CRs during test compared with paired trials on the first 3 days of conditioning (Fig. 1*A*). It should be recalled that a response was always defined as a 0.5 mm or greater extension of the nictitating membrane. Some responses during initial acquisition were initiated (i.e., departed from resting levels by 0.06 mm) within 285 msec of CS onset but did not reach the criterion extension of 0.5 mm until after 285 msec. On test trials such responses would be counted as CRs, while on paired trials the onset of the UCS at 285 msec would prevent their detection. For example, during the first conditioning session when CR frequency on paired trials was only 4.2 ± 1.0%, the frequency of responding on test trials was 14.4 ± 2.4% (Fig. 1*A*, open circles); the mean onset latency of these CRs was 254 ± 26 msec, a value within the 285 msec CS-UCS interval employed on paired trials (Fig. 1*B*, open circles).

Cerebellar lesions eliminated CRs during paired CS-UCS conditioning trials

In agreement with other investigators (Yeo et al., 1985a), we could distinguish 4 groups of animals demonstrating nonoverlapping behavioral effects of cerebellar lesions as measured by the percentage of CRs occurring during the CS-UCS interval of paired trials (Fig. 2*A*). A group of 15 rabbits formed a group designated *abolished.* No animal in the abolished group showed more than 4% responding on the first day of postoperative testing, and the mean value of 0.5 ± 0.3% was not significantly different from the value for baseline responding (0.1 ± 0.1%) obtained on the preoperative adaptation session with a 285 msec sampling interval. Every animal in this group continued to demonstrate a mean level of responding below 4.0% over the 4 postoperative 3 d blocks (6–9) with an overall mean for the 15 animals being 1.2 ± 0.4% (Fig. 2*A*). The postoperative performance of the abolished group replicated previous findings (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a).

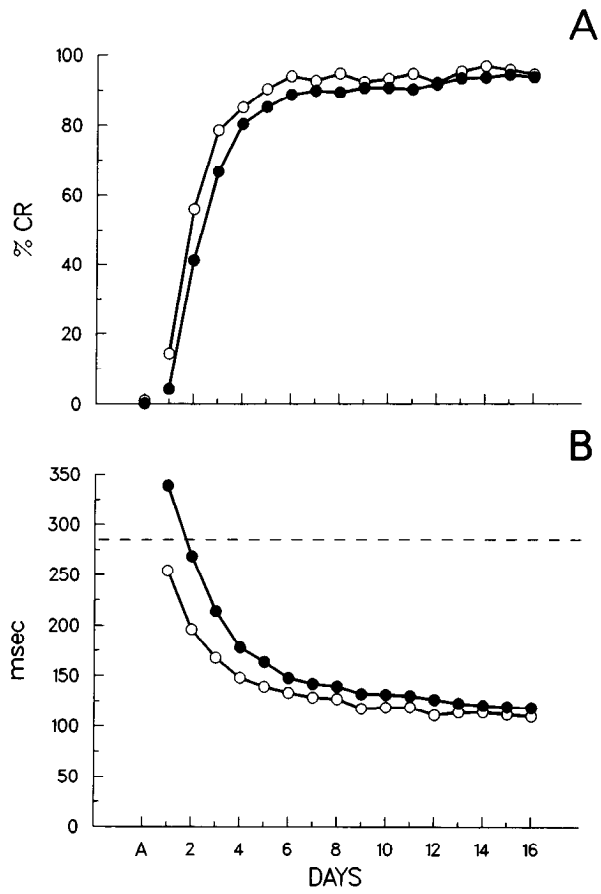

Figure 1. Preoperative acquisition of CRs. Acquisition across 16 daily conditioning sessions is expressed as a percentage of CRs (*A*) and as NMR onset latencies (*B*) of 82 rabbits. The *filled circles* represent data from the paired CS-UCS trials, and *open circles* from the CS-alone test trials. The points above *A* in panel *A* indicate baseline responding during adaptation. The *horizontal dashed line* in *B* indicates the time of UCS onset on paired CS-UCS trials.

A second group of 6 rabbits formed a group designated *impaired.* Animals in this group demonstrated >6% and <65% CRs over the 4 postoperative blocks, with a mean percentage of responding for all 6 animals being 34.1 ± 7.7%. Three of the animals in the impaired group exhibited no CRs on the first postoperative day (session 17), and 3 had <54% CRs with an overall mean for the first postoperative session of 20.1 ± 9.4%. No animal in this group showed substantial recovery during the 4 postoperative blocks (Fig. 2*A*). A third group of 14 animals was designated as *recovered.* These animals demonstrated a transient impairment in CR frequency, with all animals exhibiting <75% CRs on the first postoperative day (session 17), the mean being 38.2 ± 6.4% CRs. However, there was substantial recovery over sessions so that all animals exhibited >73% CRs across the 9th 3 d block (sessions 26–28), the mean being 87.9 ± 1.8% CRs (Fig. 2*A*). Finally, 47 rabbits formed a *no-deficit* group. These animals continued to respond at asymptotic levels of performance despite cerebellar lesions (Fig. 2*A*).

In order to prevent an unequal distribution of power during

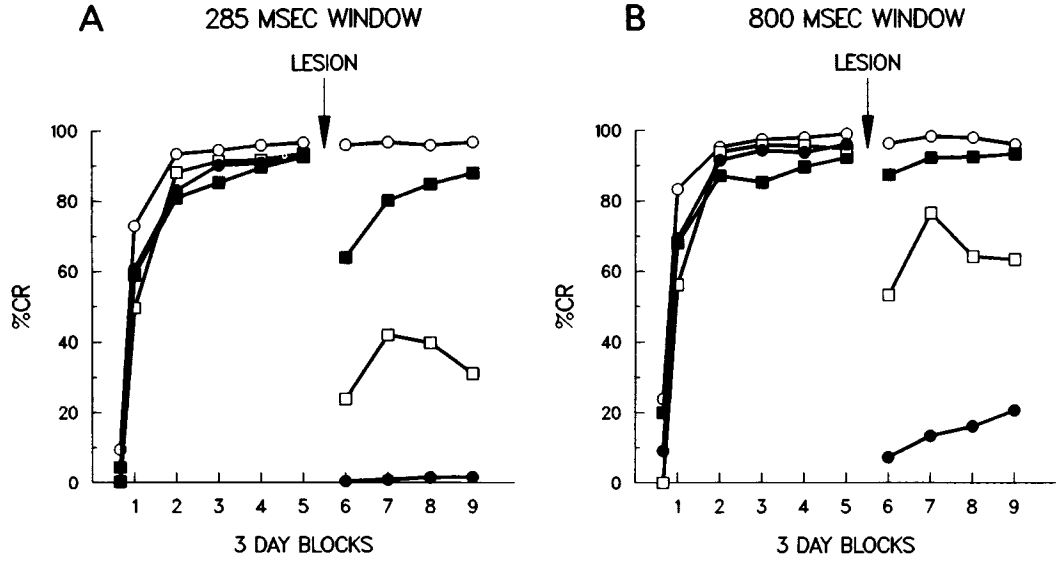

Figure 2. Effect of cerebellar lesions on the frequency of CRs initiated during the 285 msec CS-UCS interval employed on paired trials (*A*) or 800 msec after CS onset as measured on tone-alone test trials (*B*). Data are expressed as mean percentages of conditioned responses for the first day of conditioning and for subsequent 3-d blocks. Blocks 6–9 represent performance during postoperative sessions. Four groups of animals are presented based on the percentage of CRs during paired CS-UCS trials: no deficit, *open circles* (n = 15); recovered, *filled squares* (n = 14); impaired, *open squares* (n = 6); and abolished, *filled circles* (n = 15).

statistical analysis, a random sample of 15 animals was taken from the 47 animals of the no-deficit group. No significant differences could be detected between the randomly selected group (n = 15) and the entire group (n = 47) in the frequency of conditioned responding before or after the lesion with powers of 0.70 and 0.61, respectively, for tests made at the 5% level (Pearson and Hartley, 1951). The frequency of conditioned responding of the randomly selected group did not differ from the group from which they came by more than 6% preoperatively or 2% postoperatively over any 3-d block. The abolished, impaired, recovered, and no-deficit groups did not differ significantly from each other in CR acquisition preoperatively (Fig. 2). Acquisition of CRs was robust, and by the last 3 preoperative sessions the mean percent responding of the 4 groups ranged from 92 to 99% (Table 1).

Relationship of CR impairment to lesion locus

In agreement with previous investigators (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a), abolition of CRs in the CS-UCS interval was associated with damage to the anterior aspects of the interpositus nucleus beginning at the level of lambda and extending 1.0 mm rostrally (from AP0 to A1.0 of Fig. 4). Thus, animals sustaining more than 25% destruction of the anterior interpositus had a greater probability of postoperative abolition of CRs in the CS-UCS interval than those sustaining less than 25% damage ($p < 0.01$). A similar relationship could not be demonstrated for lesions of posterior interpositus or any portion of the dentate nucleus (all $p > 0.05$). In addition, there was no significant relationship between damage to the most anterior pole of interpositus (Fig. 4, A1.5) and reductions in CR occurrence, confirming a previous observation by Yeo et al. (1985a).

There was a wide variation between calculated percentage damage to the anterior interpositus nucleus and loss of CRs. For example, although the 15 animals in the abolished group had exhibited less than 4% CRs postoperatively during the 285 msec CS-UCS interval (mean, 1.2 ± 0.4%), their lesions damaged from 6 to 97% of anterior interpositus. Similarly, damage to anterior interpositus in the 6 animals of the impaired group ranged from 1 to 84%. Damage to anterior interpositus was less than 27% for every animal in the recovered group and less than 18% for every animal in the no-deficit group.

Closer inspection of lesion placement revealed that all animals in the abolished and impaired groups sustained damage to the most dorsolateral aspects of anterior interpositus, including the adjacent white matter. Damage to this dorsolateral region of interpositus is presented as photomicrographs in Figure 3 and as serial reconstructions in Figure 4 for 2 animals in the abolished group that had been calculated to have 97% (Figs. 3*A*, 4*A*) and 91% (Figs. 3*B*, 4*B*) damage to anterior interpositus and for 1 animal in the impaired group with 84% (Figs. 3*C*, 4*C*) damage. This dorsolateral area of interpositus and the adjoining white matter was never damaged in animals of the recovered and no-deficit groups. It should be noted that damage of up to 90% in the anterior aspects of the dentate nucleus failed to have any effect on percentage CRs (Fig. 3*D*).

CS-alone trials reveal CRs in animals with rostral interpositus lesions

Of the 15 animals that exhibited an abolition of CRs on paired trials, 11 exhibited greater than 10% CRs (mean, 18.7 ± 2.1) during the 800 msec after CS onset on test trials. The remaining 4 animals exhibited less than 5% CRs (mean, 2.2% ± 0.8) over the test trials of the 12 postoperative sessions. For the entire group of 15 subjects (Fig. 2*B*, Table 1), responding on test trials averaged 14.4 ± 2.5% (range, 0–33.4%). This level of responding was significantly greater than either the 1.2 ± 0.4% CRs observed during the 285 msec CS-UCS interval of postoperative paired trials (Fig. 2*A*) or the 0.7 ± 0.4% baseline responding measured during 800 msec intervals on the preoperative adaptation session ($p < 0.001$ for each comparison). Further, considered as a group, these animals demonstrated a significant

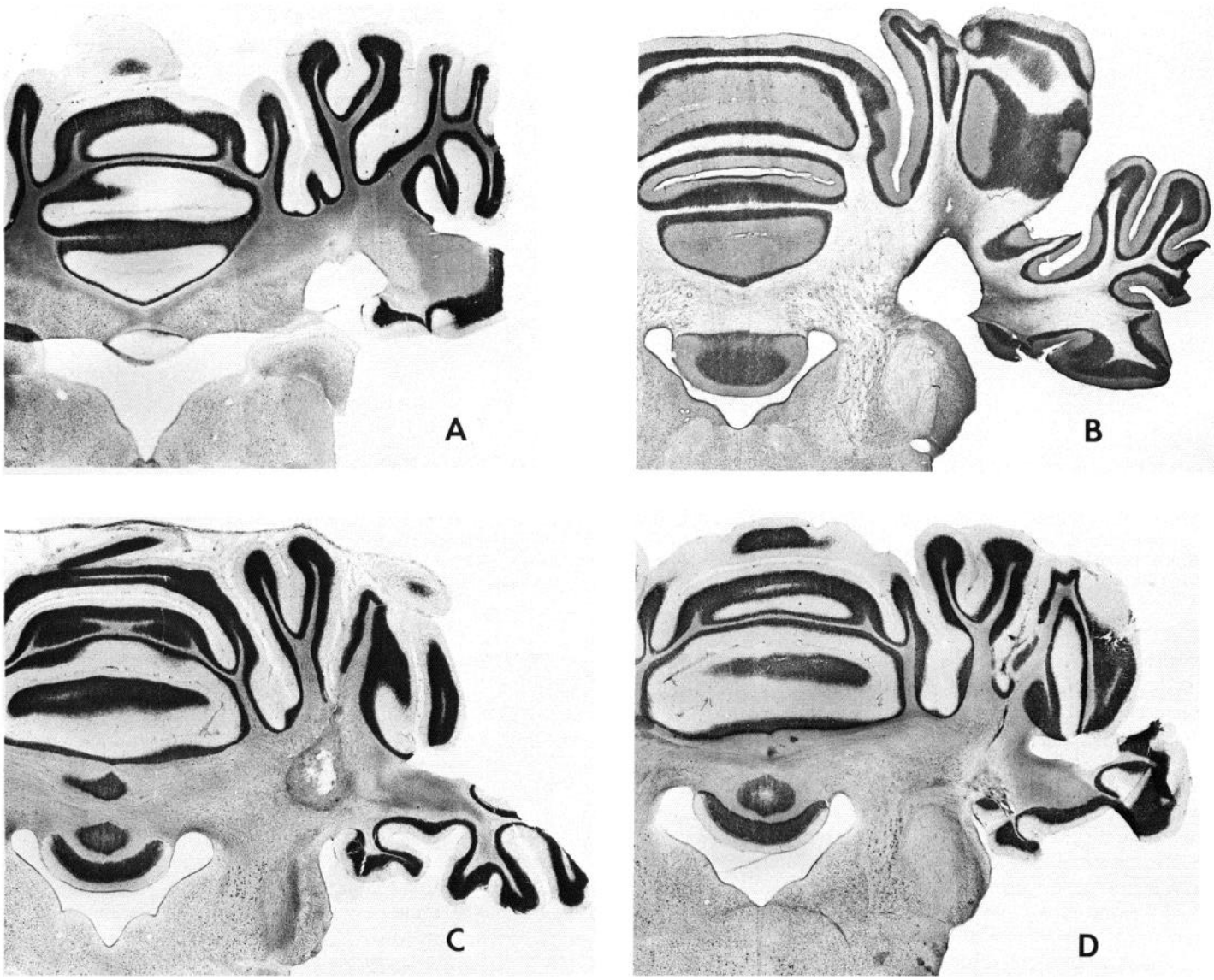

Figure 3. Photomicrographs of electrolytic lesions of 4 rabbits. *A,* Thionin-stained section depicting a lesion that destroyed 97% of anterior interpositus from an animal in the abolished group that demonstrated an average of 0.3% CRs during postoperative CS-UCS trials and 4.6% CRs on CS-alone test trials. The lesion lies just under lobule HVI and consists of a large area of cavitation surrounded by a thin band of reactive gliosis. Some undamaged interpositus neurons are present just medial to the lesion. The dentate nucleus can be seen just lateral to the lesion. *B,* Cresyl violet-stained section through the major extent of a lesion that destroyed 91% of anterior interpositus from an animal in the abolished group that demonstrated 0.0% CRs during CS-UCS trials and 16.1% CRs on test trials. The lesion is located just below the most caudal extent of lobule HVI and is characterized by a large area of cavitation surrounded by reactive gliosis. *C,* Thionin-stained section showing a lesion destroying 84% of anterior interpositus from an animal in the impaired group that demonstrated 6.4% CRs on CS-UCS trials and 27.0% on test trials. The small area of cavitation is surrounded by a large, tear-shaped area of gliosis. Lobule HVI lies just dorsal and the dentate nucleus just lateral to the lesion. Some undamaged neurons of the interpositus nucleus can be seen just medial to the lesion. *D,* Thionin-stained section depicting a lesion that destroyed 90% of anterior dentate from an animal in the no-deficit group that demonstrated 94.2% CRs on CS-UCS trials and 98.6% CRs on test trials. The lesion is characterized predominantly by an area of reactive gliosis that extends dorsally along the electrode tract. Portions of the interpositus nucleus can be seen just medial to the lesion.

increase ($p < 0.02$) in CRs (Fig. 2*B*) from 7.4 ± 1.4 to 20.7 ± 4.6% over the four 3-d blocks (sessions 17–28) of postoperative testing. No consistent differences could be detected between the lesion placements of the 4 animals that did not respond on test trials (range of damage, 35–97%) and the 11 animals that did respond (range of damage, 6–91%). This can be seen by comparing the damage sustained by 2 animals in the abolished group, one of which did not respond on test trials (average of 4.6% CRs, Figs. 3*A*, 4*A*) and one that exhibited 16.1% CRs (Figs. 3*B*, 4*B*).

Animals in the impaired group also demonstrated a significantly ($p < 0.01$) greater frequency of CRs on CS-alone test trials (64.4 ± 4.1%; Table 1) than on paired CS-UCS trials (34.1 ± 7.7%). Animals in the impaired group failed to demonstrate any changes in frequency of CRs during paired or test trials across the four 3-d blocks of testing (Fig. 2). Animals in the

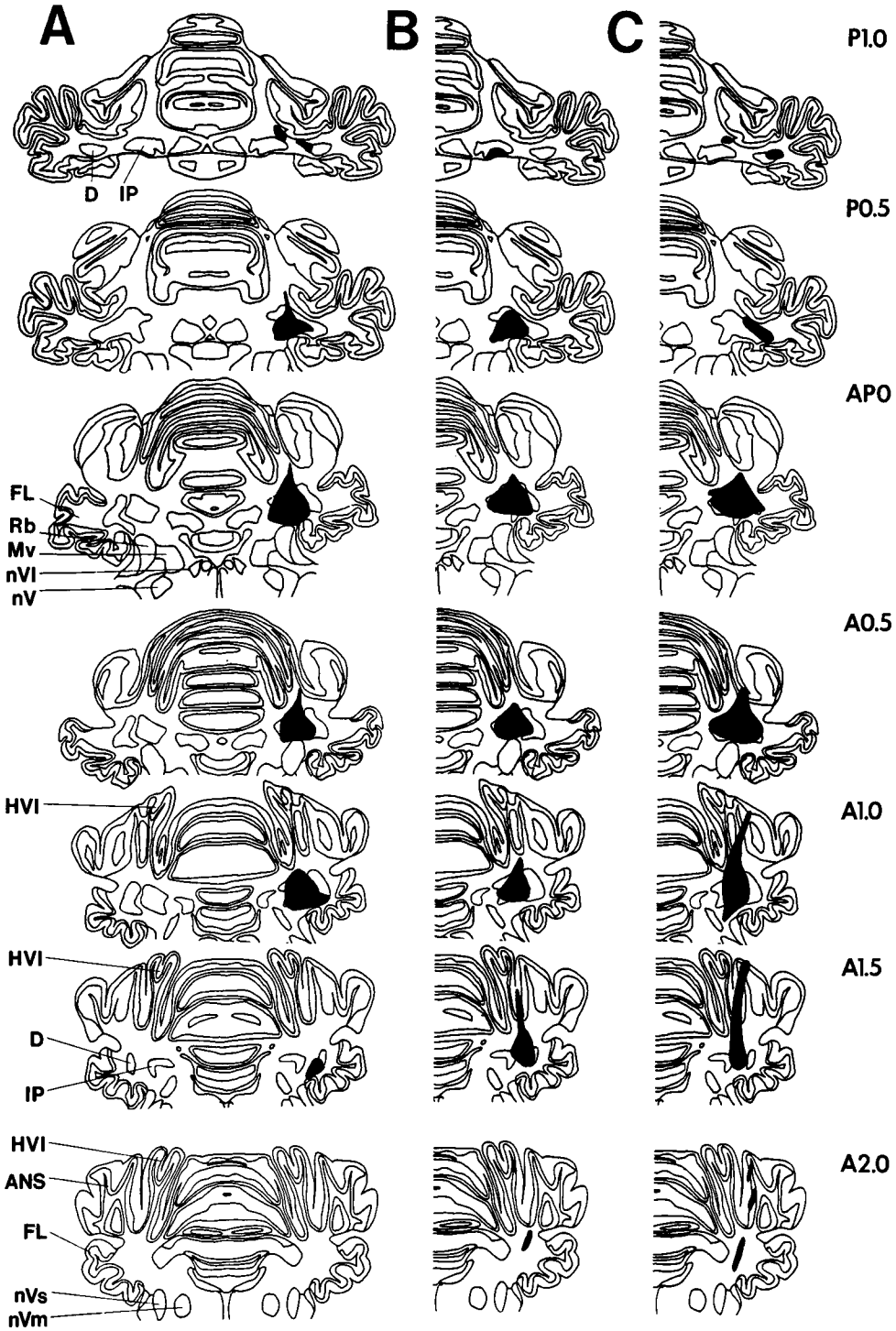

Figure 4. Serial reconstruction of electrolytic lesions of 3 animals sustaining damage to anterior interpositus. Panels *A–C* correspond to the animals depicted in Figure 3, *A–C*, respectively. Sections extend in 0.5 mm intervals from a level 1.0 mm posterior to lambda (P1.0) to 2.0 mm anterior to lambda (A2.0). Abbreviations: *ANS*, ansiform cortex; *D*, dentate nucleus; *FL*, flocculus; *HVI*, hemispheral lobule VI; *IP*, interpositus nucleus; *Mv*, medial vestibular nucleus; *nV*, spinal trigeminal nucleus; *nVI*, abducens nucleus; *nVm*, trigeminal motor nucleus; *nVs*, main sensory trigeminal nucleus; *Rb*, restiform body.

recovered group that had demonstrated a postoperative decrease in CR frequency on paired CS-UCS trials (78.9 ± 4.0%) failed to reveal any significant loss of performance when given the opportunity to respond on CS-alone test trials (91.4 ± 1.2%; Fig. 2, Table 1).

To further characterize the CRs exhibited during test trials for each of the groups over the 12 postoperative conditioning sessions, we calculated CR onset latencies by the 2 methods most commonly employed in studies of the rabbit's NMR and displayed them as frequency histograms (Fig. 5). With both methods a response is defined as a 0.5 mm or greater extension of the rabbit's nictitating membrane. However, one method (described in detail in Materials and Methods) calculates the onset latency of a response as the time at which the membrane extended from resting levels by 0.06 mm (Scandrett and Gormezano, 1980; Marshall-Goodell et al., 1982; Fig. 5, left), while the second method calculates onset latency as the time at which the criterion extension of 0.5 mm occurred (Gormezano, 1966; Cegavske et al., 1976; Fig. 5, right). Using the 0.06 mm criterion for the time of CR onset, the frequency histogram for the no-deficit group had a mode at the 91–105 msec interval, with 99.8% of the responses being initiated within 285 msec after CS

Table 1. CR characteristics on test trials for the 4 lesioned groups[a]

CR measure and condition	Abolished ($n = 15$)	Impaired ($n = 6$)	Recovered ($n = 14$)	No deficit ($n = 15$)
Percentage of CRs				
Preop ipsilateral	96.1 ± 1.3	94.8 ± 1.0	92.3 ± 3.5	99.1 ± 0.6
Postop ipsilateral	14.4 ± 2.5[b]	64.4 ± 4.1[b]	91.4 ± 1.2	97.2 ± 0.5
Postop contralateral	92.0 ± 1.6	87.9 ± 10.0	93.1 ± 1.9	94.5 ± 2.4
Postop ipsilateral	15.7 ± 4.2[b]	71.9 ± 10.4[b]	92.7 ± 1.8	96.4 ± 1.3
Amplitude of CRs (mm)				
Preop ipsilateral	5.2 ± 0.4	3.9 ± 0.4	5.3 ± 0.4	5.1 ± 0.4
Postop ipsilateral	2.1 ± 0.4[b]	1.7 ± 0.2[b]	3.8 ± 0.2[b]	4.8 ± 0.1
Postop contralateral	4.2 ± 0.4	3.8 ± 0.7	3.9 ± 0.3	4.2 ± 0.5
Postop ipsilateral	1.6 ± 0.2[b]	1.9 ± 0.4[b]	3.8 ± 0.3[b]	4.9 ± 0.3
Onset latency of CRs (msec)				
Preop ipsilateral	112 ± 8	116 ± 9	113 ± 7	107 ± 4
Postop ipsilateral	348 ± 19[b]	161 ± 2	160 ± 9[c]	109 ± 3
Postop contralateral	154 ± 7	173 ± 21[c]	137 ± 10	130 ± 5
Postop ipsilateral	285 ± 41[b]	171 ± 10	143 ± 7	106 ± 6
Latency of peak CR amplitude (msec)				
Preop ipsilateral	345 ± 5	347 ± 10	343 ± 4	350 ± 4
Postop ipsilateral	908 ± 14[b]	461 ± 19	386 ± 10	358 ± 1
Postop contralateral	367 ± 12	396 ± 37	347 ± 10	370 ± 16
Postop ipsilateral	717 ± 93[b]	503 ± 75	364 ± 8	359 ± 10
Rise time of CRs (msec)				
Preop ipsilateral	233 ± 8	231 ± 11	230 ± 8	243 ± 5
Postop ipsilateral	560 ± 11[b]	300 ± 17	226 ± 1	249 ± 3
Postop contralateral	213 ± 15	223 ± 18	210 ± 9	240 ± 15
Postop ipsilateral	432 ± 74[b]	332 ± 78	221 ± 7	254 ± 12

[a] Values are means ± SEM for 5 measures of conditioned responding on tone-alone test trials before and after surgery. Values are given for: Preop ipsilateral, the last 3 preoperative days (sessions 14–16); Postop ipsilateral, the 12 d of postoperative testing (sessions 17–28); Postop contralateral, the last 3 d of postoperative testing of the eye contralateral to the lesion (sessions 30–32); and Postop ipsilateral, the last 3 d of postoperative testing of the eye ipsilateral to the lesion (sessions 34–36).

[b] $p < 0.01$, compared with no-deficit controls.

[c] $p < 0.05$, compared with no-deficit controls.

onset (Fig. 5, left). The recovered and impaired groups demonstrated an increase in the frequency of longer-latency responses, and the mode of both groups was at the 151–165 msec interval (Fig. 5, left). Nevertheless, 97% of the onset latencies for the recovered group and 98% for the impaired group fell within 285 msec of CS onset. The abolished group had a much wider distribution of onset latencies that extended across the 800 msec observation interval (Fig. 5, left). A χ^2 test yielded a highly significant difference ($p < 0.001$) between the observed distribution of onset latencies and the distribution one would expect if response occurrence was random, i.e., had an equal probability of occurrence within each interval. It should be noted that the majority (62%) of CRs exhibited by the abolished group had onset latencies within 285 msec of CS onset.

Calculation of onset latencies using the 0.5 mm criterion did not have a great effect on the frequency distributions of the no-deficit and recovered groups compared with the use of the 0.06 criterion (compare left and right sides of Fig. 5). The mode was increased by 45 msec for both the no-deficit and recovered groups, and most of the onset latencies (98 and 88%, respectively) fell within 285 msec of CS onset (Fig. 5, right). The effect of using the 0.5 mm criterion was more pronounced in the impaired group, so that the percentage of responses falling within the 285 msec of CS onset went from 98% using the 0.06 mm criterion to 62% using the 0.5 mm criterion. The change in criterion had the greatest effect on the distribution of onset latencies for the abolished group. In this group, only 14% of the responses fell within 285 msec of CS onset using the 0.5 mm criterion compared with 62% for the 0.06 mm criterion. These results emphasize the basis for the differences in CR frequencies noted during paired CS-UCS and test trials (Fig. 2). In short, while the abolished group could initiate responses within 285 msec of CS onset, most of these responses did not reach the 0.5 mm criterion until well after the point at which the UCS would have been presented on paired CS-UCS trials. In contrast, the no-deficit and recovered groups showed a rapid attainment of the 0.5 mm criterion once the response was initiated (Fig. 5).

As suggested by the frequency histograms of Figure 5, cerebellar lesions had significant effects on the topography of CRs obtained during test trials using the 0.06 mm criterion for measurement of onset latencies (Table 1, Figs. 6 and 7). The amplitude of the CR was significantly ($p < 0.01$) decreased from preoperative values by 57, 56, and 28% in the abolished, impaired, and recovered groups, respectively (Table 1, Fig. 6*A*). The CRs of the abolished group had an average onset latency of 348 ± 19 msec, a value significantly greater ($p < 0.01$) than their preoperative onset latencies (112 ± 8 msec) or the postoperative onset latencies of the no-deficit group (109 ± 3 msec) (Table 1, Fig. 6*B*). The CRs of animals in the abolished group were also characterized by a significant ($p < 0.01$) increase in

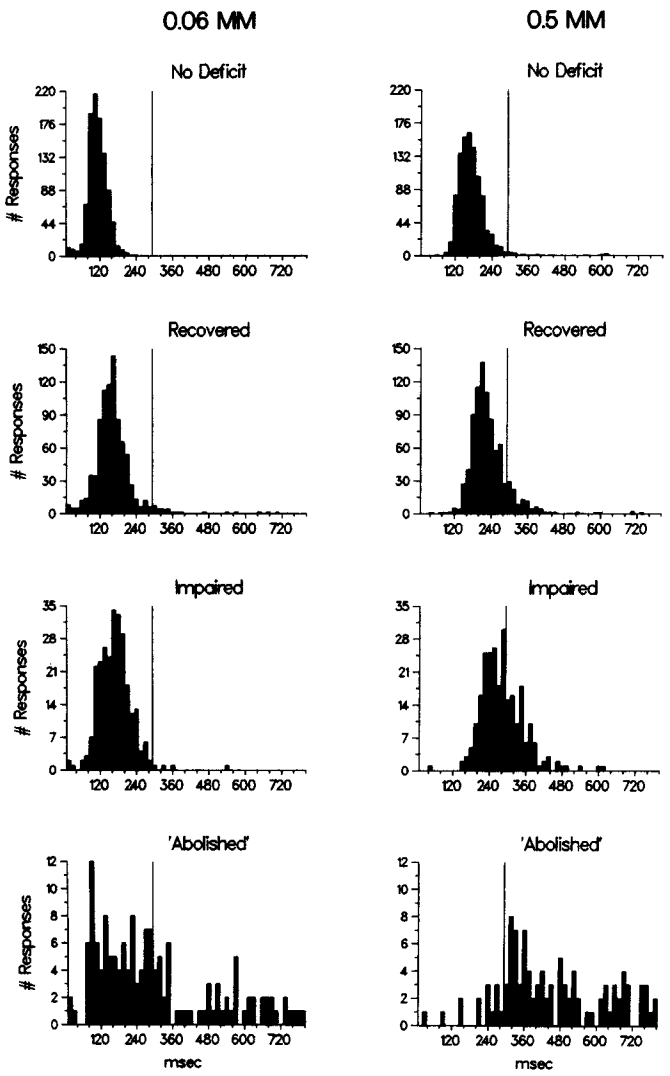

Figure 5. Frequency histogram for onset latencies of the 4 groups of lesioned animals measured during the 800 msec window of CS-alone test trials. Onset latencies are expressed as the time from CS onset. *Right panels* give onset latencies calculated as the time at which membrane extension first reached at least 0.5 mm; *left panels,* calculated onset latencies by working back from the 0.5 mm extension to the point of response initiation as defined as at least a 0.06 mm departure from resting levels. Histograms are based on responses occurring during all of the 12 postoperative sessions (blocks 6–9 of Fig. 2). The number of responses presented were: *No Deficit,* 999; *Recovered,* 863; *Impaired,* 265; and *Abolished,* 149. Bin width, 15 msec.

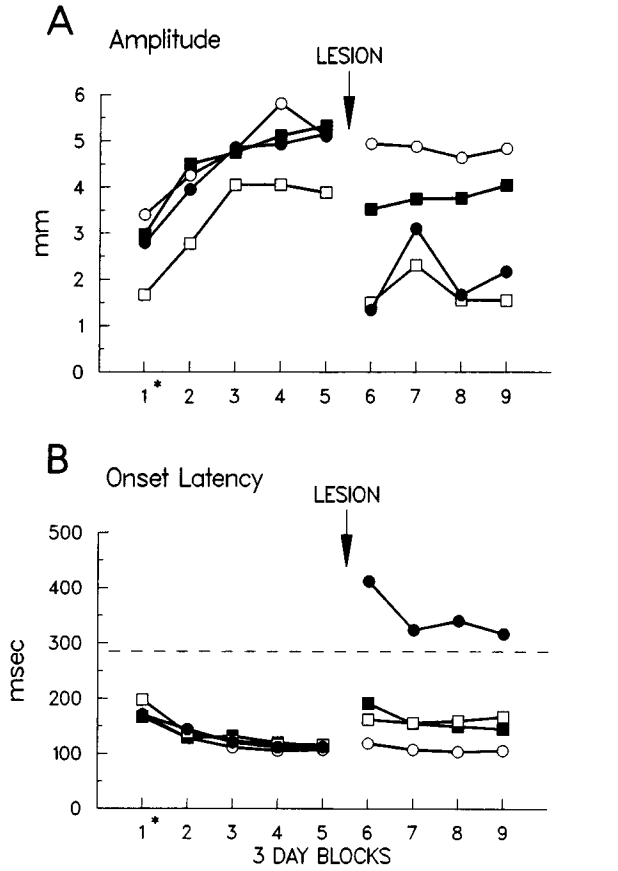

Figure 6. Effect of cerebellar lesions on the mean amplitude (*A*) and onset latency (*B*) of conditioned responses initiated within 800 msec of tone onset as measured on tone-alone test trials before and after surgery. The *asterisk* indicates that block 1 contained the first 4 daily sessions. The symbols refer to the 4 behavioral groups (see Fig. 2).

latency of peak amplitude and rise time (Table 1, Fig. 7). Thus, the performance of the abolished group on CS-alone test trials was characterized by an increase of 236 msec in the time required to initiate a CR to the tone CS as well as a 327 msec increase in the time taken from initiation to maximum amplitude of the CR.

Cerebellar lesions do not impair contralateral CR performance

At the end of the 12 d of postoperative testing (sessions 17–28), all animals received 4 d of conditioning of the NMR contralateral to the lesion. Thus, on sessions 29–32, the tone CS was presented as before but the air-puff UCS was directed at the left cornea and responses were recorded from the left nictitating membrane. There were no significant differences between the 4 groups in their acquisition of contralateral (left) CRs to the tone CS, whether measured during CS-UCS conditioning trials or CS-alone test trials (Table 1). As was found during the initial, preoperative acquisition of CRs (Fig. 1), CS-alone test trials provided a more sensitive measure of left-side CR acquisition, revealing 10.5% more CRs over these 4 conditioning sessions, and CR onset latencies were contained well within the first 285 msec of CS onset even during the first transfer session (mean, 158 ± 6 msec). Moreover, none of the groups differed from each other in CR amplitude, latency to peak amplitude, or rise time as measured on CS-alone trials (Table 1). However, when conditioning of the right NMR was reinstated, all of the significant deficits in percentage of CRs, CR onset latency, latency of peak amplitude, and rise time were again observed in the abolished group on test trials, as were the significant decreases in CR amplitude demonstrated by the abolished, impaired, and recovered groups (Table 1).

Cerebellar lesions affect the frequency and topography of UCRs

For all of the lesion groups, the topography of the UCR varied as a function of UCS intensity. Thus, for all 4 groups, decreases in UCS intensity produced significant ($p < 0.001$) and systematic decreases in UCR frequency (Fig. 8) and amplitude (Fig. 9*A*) and increases in onset latency (Fig. 9*B*) but had no effect on latency of peak amplitude (Fig. 10*A*) or rise time (Fig. 10*B*).

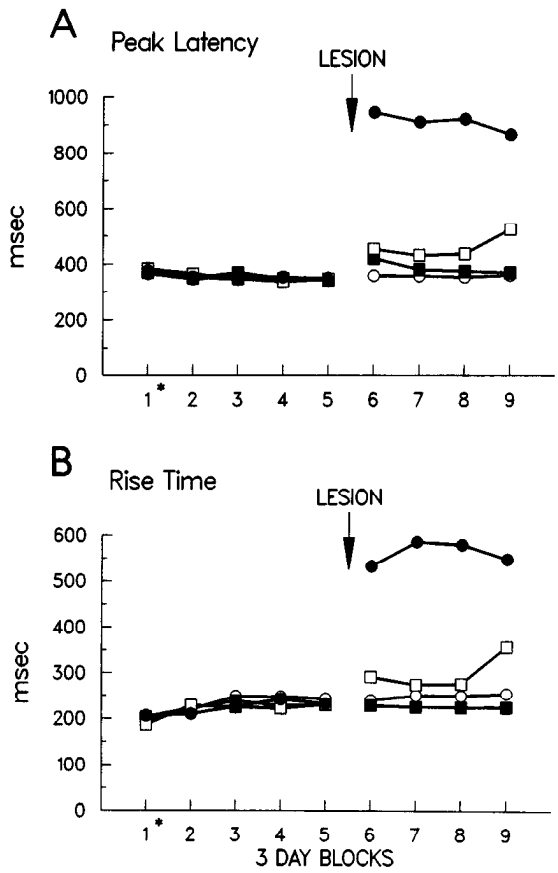

Figure 7. Effect of cerebellar lesions on the latency of peak amplitude (*A*) and rise time (*B*) of conditioned responses initiated within 800 msec of tone onset as measured on tone-alone test trials. All details as in Figure 6.

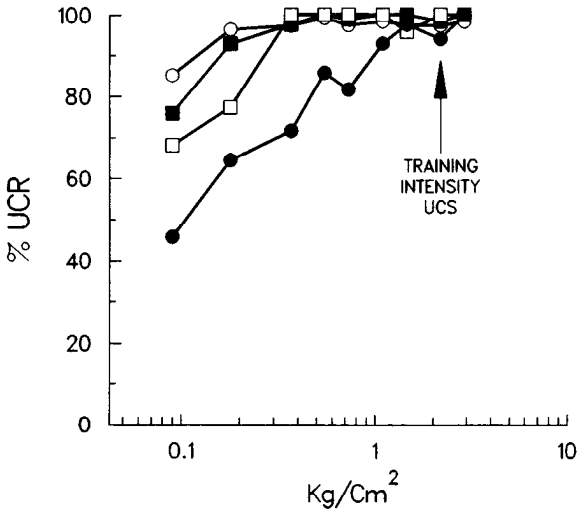

Figure 8. Effect of cerebellar lesions on the frequency of UCRs across a range of air-puff UCS intensities plotted on a log scale. Each data point represents the mean percentage of unconditioned responses for rabbits that demonstrated a complete abolition (*filled circles, n* = 7), a severe impairment with no recovery (*open squares, n* = 2), a slight impairment with recovery (*filled squares, n* = 7), or no deficit (*open circles, n* = 10) of CRs exhibited on paired trials following cerebellar lesions.

The topographical characteristics of the UCR varied in their sensitivity to shifts in UCS intensity as a function of cerebellar damage. In general, the UCRs of animals in the abolished group were far more sensitive to decreases in UCS intensity than were the other 3 groups. For example, there were no significant differences between the abolished and no-deficit groups on the frequency of UCRs, their amplitudes, or onset latencies at the 3 highest UCS intensities (including the 2.2 kg/cm^2 intensity employed during conditioning). However, as UCS intensity was decreased to 0.09 kg/cm^2, animals of the abolished group demonstrated significantly ($p < 0.01$) lower UCR frequency (Fig. 8) and significantly ($p < 0.01$) longer latency of peak amplitude and rise time (Fig. 10) relative to the no-deficit group. Although the latency of peak amplitude and rise time approximated normal values as the UCS intensity increased (Fig. 10), these values remained significantly ($p < 0.01$) elevated. While the abolished group demonstrated consistently lower UCR amplitudes (Fig. 9*A*) and longer onset latencies (Fig. 9*B*) at lower UCS intensities compared with the no-deficit group, these differences were never significant.

The UCS psychophysical testing was performed on 7 animals of the abolished group. Two of these 7 animals had not responded on test trials (<5% CRs). The UCR impairments of these 2 animals were equivalent to the impairments demonstrated by the entire group. For example, the mean percent UCRs of these 2 animals at each of the 9 UCS intensities did not differ by more than 1.4 SE from the mean values plotted for the entire group in Figure 8. Similarly, these 2 animals demonstrated impairments that were within the range of values obtained for UCR amplitude, onset latency, latency of peak amplitude, and rise time of the abolished group as plotted in Figures 9 and 10.

Table 2 presents the values for the various characteristics of the UCR elicited by the UCS intensity of 2.2 kg/cm^2 used during conditioning and by the lowest UCS intensity of 0.09 kg/cm^2 for animals in the abolished group and compares these with values for the same characteristics of the CR elicited by the 84 dB auditory CS. It can be seen that decreases in UCS intensity produced changes in the frequency, amplitude, onset latency, latency of peak amplitude, and rise time of the UCR that approached the values obtained for the CR.

The impaired and the recovered groups did not differ from the no-deficit group in the frequency of temporal characteristics of the UCR. However, an unexpected outcome of this study was the occurrence of a significant ($p < 0.05$) increase in UCR amplitudes by animals of the recovered group compared with the no-deficit group (Fig. 9*A*), with the average difference in UCR amplitudes between these 2 groups, collapsed across all

Table 2. Comparison of UCR and CR characteristics of the abolished group[a]

NMR measure	UCR elicited by 2.2 kg/cm^2 UCS	UCR elicited by 0.09 kg/cm^2 UCS	CR elicited by 84-dB CS
Frequency (%)	94.9 ± 2.8	45.8 ± 7.2	14.4 ± 2.5
Amplitude (mm)	4.2 ± 0.7	2.2 ± 0.4	2.1 ± 0.4
Onset latency (msec)	91 ± 24	202 ± 63	348 ± 19
Latency of peak amplitude (msec)	468 ± 57	754 ± 125	908 ± 14
Rise time (msec)	331 ± 43	507 ± 105	560 ± 11

[a] Values are means ± SEM and are taken from Table 1 and Figs. 7–9.

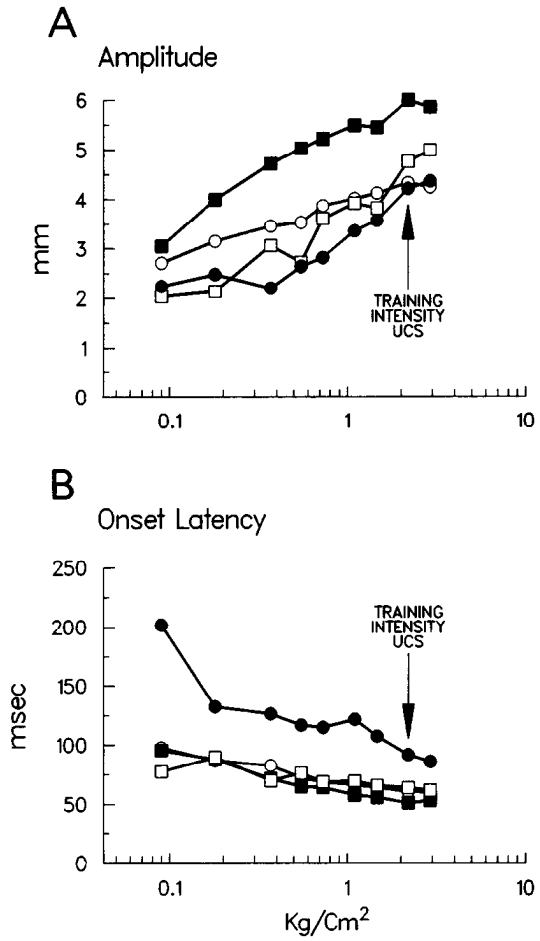

Figure 9. Effect of cerebellar lesions on the amplitude (*A*) and onset latency (*B*) of UCRs across a range of air-puff UCS intensities. All behavioral groups and curve symbols are the same as in Figure 8.

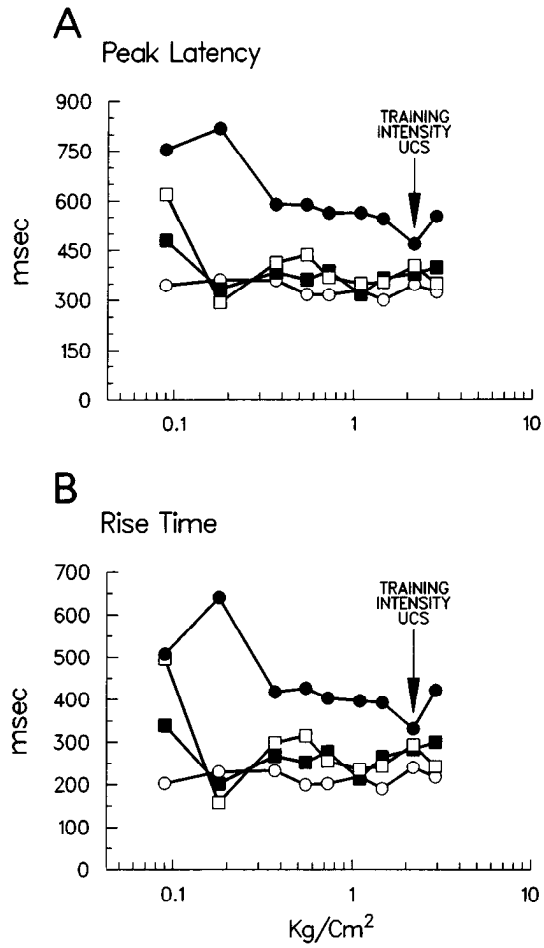

Figure 10. Effect of cerebellar lesions on the latency of peak amplitude (*A*) and rise time (*B*) of UCRs. All details as in Figure 8.

UCS intensities, being 1.3 mm. Of the 6 animals in this group, the 3 with the largest UCR amplitudes had lesions placed in the white matter anterior to interpositus. These lesions would have been expected to interrupt fibers of the middle and inferior cerebellar peduncles that project to cerebellar cortex.

Discussion

CRs could not be observed during the 285 msec CS-UCS interval following cerebellar damage

These experiments confirmed previous reports that one cannot observe conditioned nictitating membrane responses during paired CS-UCS trials after lesions that include the ipsilateral anterior interpositus nucleus (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a). In agreement with McCormick et al. (1981) and McCormick and Thompson (1984), we also failed to note any significant changes in the frequency or topography of ipsilateral UCRs elicited by the suprathreshold intensities of corneal air puff commonly employed in conditioning studies. The ability of all animals to acquire conditioned NMRs contralateral to the lesion was also in full agreement with previous reports (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a).

In agreement with Lavond et al. (1985), effective lesions for abolishing CRs initiated within the CS-UCS interval did not need to completely destroy the anterior aspects of interpositus. Our histological material suggested that the critical locus of damage incorporated both the dorsolateral aspects of the anterior interpositus as well as the adjacent white matter. The behavioral effects of these lesions could have been due to the interruption of the descending cortical influence of lobule HVI upon the brain stem, as suggested by Yeo et al. (1985c), or to damage to interpositus per se, as proposed by McCormick et al. (1981) and McCormick and Thompson (1984). In either case such lesions necessarily disrupt the regulatory influence of the cerebellum on brain-stem motor systems that may be required for optimal performance of both the CR and UCR. Additionally, the consistent damage to adjacent white matter must have interrupted ascending projections from the inferior olivary nucleus (Yeo et al., 1985c), thus partially deafferenting HVI and interpositus from climbing fiber input and ascending projections from the pontine nuclei (Dietrichs et al., 1983; Yeo et al., 1985c; Brodal et al., 1986), thus partially deafferenting HVI from pontine mossy fiber input. Such an effect on sensory input could be a primary factor that results in the decreased ability of the CS to elicit CRs and low UCS intensities to elicit UCRs. Thus, interruptions of both cerebellar afferents and efferents would be consistent with our observation of sensorimotor deficits.

CRs are increased in onset latency following cerebellar lesions

The use of CS-alone test trials and an 800 msec observation window revealed the presence of CRs in animals that had failed to demonstrate CRs during the 285 msec window employed during paired CS-UCS trials (animals in the abolished group). These responses could be identified as CRs (i.e., as resulting from associative factors) rather than representing some high level of nonassociative responding due to an increase in baseline or sensitization. One of the advantages of the rabbit NMR is that responding during unpaired presentations of CS and UCS has been established to be quite low (<2–3%), indicating the virtual absence of such nonassociative contributors to CR production as baseline responding and sensitization (Gormezano, 1966; Gormezano et al., 1983). Furthermore, the level of responding to a tone CS for as long as 1000 msec after CS onset remains stable at low levels over repeated sessions of unpaired presentations of the CS and UCS (Smith et al., 1969). Responding during test trials of animals with lesions including the rostral interpositus failed to fit any of the criteria for nonassociative responding. Test-trial responses had a high frequency of occurrence (approximately 21% over the last 3 postoperative days of testing) compared with baseline responding of less than 1% observed during adaptation in an equivalent (800 msec) observation period. In addition, the onset latencies for baseline responses have been shown to have a rectangular distribution, i.e., to have an equal probability of occurrence across any observation interval (Smith et al., 1969), while the distribution for responses observed during test trials deviated significantly from a rectangular distribution. While sensitized responses are defined as having a shorter onset latency and duration than the CR (Gormezano, 1966), the responses observed during test trials in this study had longer onset latencies and durations than normal CRs, an outcome opposite to what would be expected of sensitized responses. We conclude, therefore, that the responses observed in the abolished group on test trials were CRs occurring as a result of preoperative learning. It is not clear whether the increase in CRs over days of postoperative testing represented some additional learning or a gradual recovery from performance deficits produced by the lesion (see below). It is important to note that the observation of responding at latencies greater than the CS-UCS interval was not specific to subjects with the greatest deficits in CR frequency. As demonstrated by the onset latency histograms (Fig. 5), all deficits in CR frequency were accompanied by foward shifts in the onset latency distribution.

Our measurement of the NMR provided an estimate of onset latencies that may be shorter than that of other investigators. We employed the same criterion for the occurrence of an NMR as do other investigators—an extension of the membrane of 0.5 mm. However, we then employed the procedures of Scandrett and Gormezano (1980) and calculated the onset latency to be the time at which the nictitating membrane first deviated from resting levels by 0.06 mm. Thus, on test trials, a CR might, by our measurement, have an onset latency of less than 285 msec but not reach the criterion of 0.5 mm until after 285 msec. On paired trials such responses would not be counted as CRs. Thus, detection of CRs for animals having problems initiating responses and demonstrating increased rise times becomes more difficult as the observation interval is narrowed. Because some investigators use the 0.5 mm criterion as a measure of onset latency (Smith et al., 1969; Cegavske et al., 1976), the probability of recording the initiation of CRs in narrow windows of observation is further reduced with the presence of performance deficits that increase rise time.

Cerebellar lesions produce a general deficit in the performance of both learned and unlearned responses

The CRs observed during test trials in the abolished group were not only decreased in frequency relative to the other 3 groups, but also significantly altered in topography. Thus, relative to preoperative performance, these CRs were characterized by a 236 msec increase in onset latency, a 563 msec increase in latency of peak amplitude, a 327 msec increase in rise time, and a 3.1 mm decrease in peak amplitude. In agreement with previous studies (McCormick et al., 1981; McCormick and Thompson, 1984; Yeo et al., 1985a), these deficits in CR performance occurred without a detectable effect on the UCR that was elicited by the suprathreshold UCS employed in conditioning.

Differences in the vulnerability of CRs and UCRs to cerebellar damage does not necessarily reflect differences between a learned and unlearned response. The UCR is elicited as a disynaptic reflex but has a second, longer-latency component, which has been suggested to be mediated by premotor systems in the reticular formation, while the CR appears to be predominantly a multisynaptic reflex (Guegan and Horcholle-Bossavit, 1981; Harvey et al., 1984; Holstege et al., 1986). Consequently, the onset latency of the CR of well-trained animals is 2–3 times greater than that of the UCR. These differences in the synaptic organization of the 2 reflexes also determine differences in the intensive properties of the CS and UCS. Conditioning studies employ a suprathreshold UCS in order to obtain robust CR acquisition. However, this UCS is a far more potent stimulus than the CS even when the CS has acquired considerable associative strength and is capable of eliciting near 100% responses (Harvey, 1987). Thus, differences in the effects of cerebellar damage on the CR and UCR are likely to be at least in part a reflection of the disparity between the psychophysical intensity of the 2 stimuli. Indeed, as UCS intensity was decreased, the UCR of all groups exhibited decreases in frequency and amplitude along with an increase in onset latency. Thus, in order to determine that there are generalized performance deficits following cerebellar damage, one must examine the psychophysical function relating UCS intensity to characteristics of the UCR and compare these between experimental groups. One of the advantages of the rabbit NMR is that both the CR and UCR are expressed by the same final common pathway consisting of the retractor bulbi motoneurons in the accessory abducens nucleus and their projections to the retractor bulbi muscle via the VIth cranial nerve (Harvey et al., 1984; Marek et al., 1984; Holstege et al., 1986). Thus, if cerebellar damage produced a generalized performance deficit, one would be able to observe deficits in the UCR that were comparable to those observed in the CR as the UCS was decreased in intensity. We carried out such a procedure and found that as UCS intensity was decreased the animals in the abolished group began to demonstrate deficits in the UCR similar to some of those seen in the CR: a decrease in frequency along with an an increase in latency of peak amplitude and rise time. Thus, when one attempts to equate the CS and UCS as response-eliciting stimuli, the deficits in the CR and UCR become more alike (see Table 2). In agreement with these observations, we have recently found that infusion of the local anesthetic lidocaine into the area of the anterior interpositus produced reversible deficits in the performance of both the conditioned and unconditioned NMR (Welsh and Harvey, 1988).

These results clearly indicate that lesions including the anterior interpositus produce a generalized deficit in the performance of the NMR. There is no experimental evidence to indicate that there might be a learning deficit embedded within this performance deficit. However, an adequate test of this proposition must await the use of reversible cerebellar lesions, since the ablation method by itself does not allow for an independent assessment of learning deficits in the presence of performance deficits.

These alterations in CR and UCR frequency and topography are virtually identical with previous reports of performance deficits in humans and experimental animals following unilateral or bilateral damage to the cerebellum. In summary, since the time of Holmes (1917) cerebellar damage has been characterized by deficits in response frequency, onset latency, rise time, and amplitude (Holmes, 1917; Chambers and Sprague, 1955; Dow and Moruzzi, 1958; Brooks et al., 1973; Uno et al., 1973; Conrad and Brooks, 1974; Soechting et al., 1976; Meyer-Lohmann et al., 1977; Brooks, 1984). In addition, cerebellectomy following Pavlovian conditioning of leg flexion in the dog has been reported not to abolish CRs but to increase their onset latency and reduce their amplitude (Fanardjian, 1961). Finally, our finding that lesions interrupting cortical afferents increased UCR amplitudes was consistent with observations of hyperreflexia following paravermal cortical lesions (see Andersson et al., 1987).

In the case of rabbit nictitating membrane CRs, performance deficits following cerebellar lesions were not specific to subjects that suffered the largest deficits in CR frequency. Animals that were only partially or temporarily impaired in CR frequency suffered permanent reductions in CR amplitudes. Most importantly, CR amplitudes remained impaired over postoperative testing even when CR frequency had returned to normal levels in the recovered group.

The observations of increased onset latency, reduced amplitude, and increased rise time are deficits in the performance of the CR. These performance deficits are in the direction that reduces the probability of observing the learned response. In cases where damage occurred to the anterior interpositus nucleus and neighboring white matter, the probability of measuring a CR on a paired trial was reduced to zero either because CRs did not reach the criterion amplitude during the CS-UCS interval or because they had onset latencies that exceeded the duration of the CS-UCS interval. In the most severe cases of performance deficits, there was an abolition of CRs even on tone-alone test trials despite the increased opportunity to respond at longer latencies. A total abolition of responding, however, sheds little light on the issue of the role of the cerebellum in learning and memory. The distinction between abolition and severe reduction of CRs is arbitrary, only deriving importance within the context of an assumption that the memory for this response is localized to or passes through the cerebellum. On the basis of data presented in this study, the effects of cerebellar damage on the CR may be best viewed as producing a continuum of primary deficits of amplitude and secondary deficits of rise time and onset latency. This continuum was observed as CR amplitude deficits across classes of animals that manifested different degrees of CR frequency impairment and as rise time and frequency deficits across stimulus modality within the class of animals that suffered the greatest impairment in CR frequency. Accordingly, the abolition of CRs following cerebellar damage may lie at the extreme of this continuum, and thereby reflect the greatest withdrawal of cerebellar modulation of sensory and motor systems required for optimal performance of both conditioned and unconditioned NMRs.

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