

Lesions of the Middle Cerebellar Peduncle Disrupt Acquisition and Retention of the Rabbit's Classically Conditioned Nictitating Membrane Response

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Rabbits were classically conditioned to emit a nictitating membrane response (NMR) to either a light or tone conditioned stimulus (CS) paired with an eye shock unconditioned stimulus (UCS). They then received lesions of the middle cerebellar peduncle (MCP) or served as unoperated controls. Following surgery, they were given separate presentations of tone, light, and vibratory CSs, each paired with the eye shock UCS. In this way, conditioned responses (CR) to the previously trained light or tone served as a test of retention, whereas CRs to the remaining two conditioned stimuli (tone and vibratory or light and vibratory) served as a test of acquisition. The results of the study revealed that rabbits with complete lesions of the MCP showed disrupted acquisition and retention of the conditioned NMR to all stimuli, rabbits with partial MCP lesions also showed disrupted acquisition and retention to all CSs, but to a lesser degree, and animals with lesions that missed the MCP and unoperated controls both showed normal acquisition and retention of the conditioned NMR. These data are consistent with the view that the cerebellum is an essential part of the circuit for classical conditioning of the NM response and that information about CSs in the auditory, visual, and tactile modalities reach the cerebellum by way of the MCP.

The rabbit's classically conditioned nictitating membrane response (NMR) has rapidly become the most widely used mammalian model system for investigating neurobiological aspects of associative learning (Gormezano, Prokasy, & Thompson, 1987). The preponderance of research in this preparation has focused on two brain structures: the hippocampus (Moore & Solomon, 1980) and the cerebellum (McCormick, Clark, Lavond, & Thompson, 1982). The emerging view is that whereas the hippocampus plays a modulatory role in the conditioning process (Solomon, Solomon, Vander Schaff, & Perry, 1983), the cerebellum is the essential part of a circuit for acquisition and retention of the learned response (McCormick & Thompson, 1984).

A series of studies by Thompson and co-workers have demonstrated that lesions of the dentate-interpositus area of the cerebellum abolish the previously learned conditioned NMR and prevent acquisition of the CR in naive rabbits. Moreover, this disruption of conditioning does not affect the unconditioned response and is limited to the eye ipsilateral to

the lesion. This suggests that the disrupted CR is due to disruption of central associative processes and not to changes in sensory or motor abilities (McCormick & Thompson, 1984). Based on these data, Thompson has suggested that dentate-interpositus area may be a site of plasticity for NMR conditioning (McCormick & Thompson, 1984).

Others have suggested that areas other than the dentate-interpositus including brain stem (Desmond & Moore, 1982) and cerebellar cortex (Yeo, Hardman, & Glickstein, 1984) are critical sites of plasticity for NM conditioning. Each of these views is generally consistent with earlier theoretical accounts implicating the cerebellum in motor learning (Albus, 1971; Eccles, 1977; Marr, 1969).

If the cerebellum is the site of plasticity for NMR conditioning, it becomes important to characterize the role of cerebellar afferents and efferents in transmitting information about stimuli and responses. The superior cerebellar peduncle (SCP) is the primary cerebellar efferent. Lesions to this structure abolish the conditioned NMR while having no effect on the unconditioned response (McCormick, Guyer, & Thompson, 1982). The middle cerebellar peduncle (MCP) and the inferior cerebellar peduncle (ICP) are the primary cerebellar afferents (Blodel & Courville, 1981). Thompson has suggested that the climbing fibers within the inferior cerebellar peduncle carry information about the unconditioned stimulus (UCS) and the middle cerebellar peduncle transmits information about the conditioned stimulus (CS; Mauk & Thompson, 1984; Steinmetz, McCormick, Baier, & Thompson, 1984). In support of this view, a recent study by Steinmetz et al. (1984) has demonstrated that lesions to the inferior olive, the source of ICP fibers, produce extinction of the CR in previously trained animals. The animals behave as if they are no longer

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experiencing the UCS even though the air puff is still being delivered to the eye on every trial. The role of the MCP remains untested.

The purpose of the present study was to evaluate the role of the MCP in both acquisition and retention of the conditioned NM. In conducting this study we had two major concerns. The first was the possibility that information about CSs in different modalities might enter the cerebellum via different pathways. For example, there is anatomical and physiological evidence to suggest that information about auditory and visual CSs could enter by way of the MCP, whereas information about tactile CSs could enter by way of the ICP (Blodel & Courville, 1981). To test for this possibility, we used CSs in three different modalities: visual, auditory, and tactile. A second issue concerns evaluating the role of the MCP in both acquisition and retention of the conditioned NMR. Because complete lesions of the MCP are difficult to make and thus show significant variability from animal to animal, we felt that it was important to evaluate acquisition and retention of the CR within the same animals (i.e., same amount of tissue damage). To accomplish this, we trained each animal to either the light or tone CS, made the lesions, and then tested to all three CSs: light, tone, and vibratory. In this way, CRs to the previously trained light or tone served as a test of retention, whereas CRs to the remaining two CSs served as a test of acquisition.

Method

Animals

The animals were 18 experimentally naive New Zealand albino rabbits (*Oryctolagus cuniculus*) weighing between 2 and 4 kg at the time of surgery. All rabbits were individually housed in a temperature- and humidity-controlled colony with a 12-hr light/dark cycle. Animals were tested in the light portion of the cycle.

Surgery

Animals were anesthetized with Fluothane (halothane) gas mixture and then injected sc with Xylocaine (lidocaine HCL) along the midline of the scalp. The placement of the lesion electrode was accomplished via a combination of stereotaxic and electrophysiological techniques. Initial electrode placements were made at 3.3 mm anterior to lambda and 6.8 mm lateral to the midline (lambda 1.5 mm ventral to bregma). The dorsal-ventral coordinate was determined by characteristic neuronal activity. Lesions were made via radio-frequency current (Radionics Model RFG-4A), which produced a tip temperature of 70 degrees for 1 min through a 0.25 mm diameter electrode insulated except for 1 mm at the tip. In this way, two lesions were made in each MCP. Animals were given a minimum of 5 days to recover between surgery and the beginning of behavioral testing.

Apparatus

The apparatus used in our laboratory for conditioning the rabbit's NM have been described in detail (Solomon, 1977). Four animals

were run simultaneously in sound-attenuating and ventilated chambers. A panel in front of each chamber contained two lights, each mounted behind a translucent milk-white screen used to deliver the visual (light) CS, and a speaker used to deliver the auditory (tone) CS. The tactile (vibratory) CS was delivered via a speaker mounted on the animal's back.

Each rabbit was restrained in a Plexiglas box, with an adjustable neck plate and ear clamp securing the head and a second plate placed over the animal's back to restrict general movement. This back plate also housed a speaker which made contact with the animal's shaved back and was used to deliver the vibratory CS. A small nylon loop was sutured through the animal's right NM and attached to the shaft of a rotary transducer that was mounted atop the animal's head. In this way lateral movement of the NM was transduced into a dc signal that was subsequently recorded and analyzed. A CR was defined as an NM movement of at least 1 mm occurring in the CS-UCS interval.

The CSs used in this experiment were an 85-dB (SPL) 1000-Hz tone, a 6-V light, or a vibratory stimulus, which consisted of a 5-Hz stimulation to the animal's back. We had previously determined that vibratory CS had no auditory signaling properties. It would not produce conditioning when the speaker was moved 1 in. above the rabbit's back (i.e., its frequency was too low to serve as an auditory CS). The CS duration was 500 ms, and the time between CS onset and UCS onset was a constant 450 ms. The UCS was a 2-mA infraorbital shock of 50 ms duration delivered via two stainless steel wound clips implanted superficially in the skin: one immediately below and the second immediately posterior to the right eye. The intertrial interval was a constant 30 s. The delivery of all stimuli was controlled by a KIM-1 microprocessor and solid state interface (Solomon & Babcock, 1979).

Behavioral Testing

On the day following suturing of the NM, the rabbits were restrained and placed in the conditioning apparatus for 1 hr. On the next day, half the animals received paired presentation of the tone CS (T) and eye shock UCS, and the other half of the animals received paired presentations of the light CS (L) and the eye shock UCS. Each animal received 100 CS-UCS presentations per day until they reached a criterion of 1 day of 80% or more CRs. The animal was given 1 additional day of training. Animals next received bilateral lesions of the MCP or served as unoperated controls (CON). Following a minimum of 5 days of recovery, all animals received individual presentations of three stimuli (tone, light, vibratory) paired with the shock UCS until they reached a criterion of 70% or more conditioned responding to each of the three stimuli or for a maximum of 20 days. Each CS was paired with the UCS 36 times per day. The design is summarized in Table 1.

Table 1
Design and Procedure

CR acquisition	Lesion	Test		
		T ⁺	L ⁺	V ⁺
T ⁺	MCP or UNOP	RET	ACQ	ACQ
L ⁺	MCP or UNOP	ACQ	RET	ACQ

Note: T = tone, L = light, V = vibratory, + = UCS follows CS, MCP = middle cerebellar peduncle, UNOP = unoperated control, ACQ = CR acquisition, RET = CR retention.

Histological

At the completion of behavioral testing, animals were anesthetized with sodium pentobarbital and perfused intracardially with 0.9% saline, followed by 10% formalin solution. The brains were then removed and stored in formalin, followed by sucrose-formalin, and later embedded in albumin gelatin. Coronal sections were taken through the extent of the lesion at 40 μ . The tissue was then mounted and stained. Reconstruction of the lesions was accomplished by placing the stained slides in a photographic enlarger and projecting the image onto the plates of coronal sections through the cerebellum. The extent of the lesions was subsequently confirmed by examining each section microscopically. Reconstructions were done without knowledge of the animal's behavior.

Results

Histological

Figure 1 summarizes the extent of the lesions for all operated animals. Animals were divided into three conditions on the bases of histological findings: (a) misses (MISS)—no damage to the MCP, (b) partial (PAR)—partial damage to the MCP, and (c) complete (COM)—complete bilateral interruption of the MCP. To further quantify the damage in each lesioned animal so that it could be correlated with behavioral impairment, we digitized the amount of damage in animals with partial and complete lesions. To accomplish this, we used an Apple Model A2M0029 graphics tablet interfaced with an Apple II computer. For each animal, we digitized the amount of bilateral damage to the MCP in three coronal sections. We then summed these six numbers to arrive at an estimate of the overall damage. These numbers are shown in Figure 1.

Acquisition of the Conditioned Response

Figure 2 shows acquisition of the conditioned response to the tone, light, and vibratory CS over 20 days of training for animals in the MISS + CON, PAR, and COM lesion conditions. Because animals with damage to areas other than the MCP (Group MISS) and unoperated controls (Group CON) were indistinguishable in terms of their behavioral performance, their data were combined for statistical analysis. As the figure shows, Group MISS + CON acquired the CR most rapidly, followed by Group PAR, followed by Group COM, which showed little evidence of acquiring the learned response to any of the three stimuli over the 20 days of training. No animal in the COM condition reached our criterion for conditioning (1 day of at least 70% CRs to any CS). The highest percent CRs for any animal to any stimulus for a single day was 19%.

Statistical analysis verified these observations. The dependent measure in these analyses was the mean total number of CRs to each stimulus out of a possible 360 (36 per day per stimulus for the 10 days that all animals were run). For each stimulus (tone, light, and vibratory), animals with lesions that

missed the MCP and unoperated controls acquired the CR significantly faster than animals with partial lesions, who in turn acquired the CR significantly faster than animals with complete MCP lesions ($U_s = 0$, $p_s < 0.2$). Moreover, there is no overlap in the range of trials to criterion between Groups MISS + CON, PAR, and COM for any of the three stimuli (Table 2). There was no difference in acquisition of the CR between animals with lesions that missed the MCP and unoperated controls to any of the three stimuli ($U_s > 5$, $p_s > 0.5$).

To better appreciate the relationship between size of the lesion and the deficit in CR acquisition, we correlated the digitized damage estimates with the total number of CRs. The Pearson product correlation for these numbers was negative and significant, which indicated an inverse relation between amount of damage and total CRs, $r(13) = -0.75$, $p < 0.2$.

Retention of the Conditioned Response

Figure 3 shows retention of the CR to the tone and light CSs. Once again, data from unoperated controls and animals with lesions that missed the MCP were combined (Group MISS + CON). The effects of MCP lesions on retention of the CR are similar to those seen for acquisition: animals in Group MISS + CON acquired most rapidly, animals in Group PAR showed disrupted retention, and animals with complete bilateral lesions (Group COM) showed little evidence of retention or reacquisition. None of the animals in Group COM reached criterion for conditioning.

Again, statistical analyses verified these observations. As in the analysis of CR acquisition, the dependent measure was the number of CRs out of a possible 360. For both tone and light, animals in Groups MISS + CON emitted significantly more CRs during retention testing than animals in Group PAR, who emitted significantly more CRs than animals in Group COM ($U_s = 0$, $p_s < 0.2$). Again, there was no overlap in the range of distributions of total CRs between the three surgical groups for either stimulus (see Table 2). There was no difference in the total number of CRs during retention testing between animals in Group MISS and Group CON for either the light or tone ($U_s > 5$, $p_s > 0.5$).

To more carefully analyze the relation between lesion size and retention deficit, we again correlated amount of damage with total CRs. As in the case of acquisition, the correlation was negative and significant, which indicated an inverse relation between amount of MCP damage and CR acquisition, $r(13) = -0.77$, $p < 0.1$.

Discussion

The results of the present study indicate that large bilateral lesions of the MCP severely disrupt retention of the classically conditioned NMR to both a tone and light CS and acquisition of the CR to a tone, light, and vibratory CS. Moreover, the degree of disruption for both acquisition and retention is proportional to the amount of MCP damage.

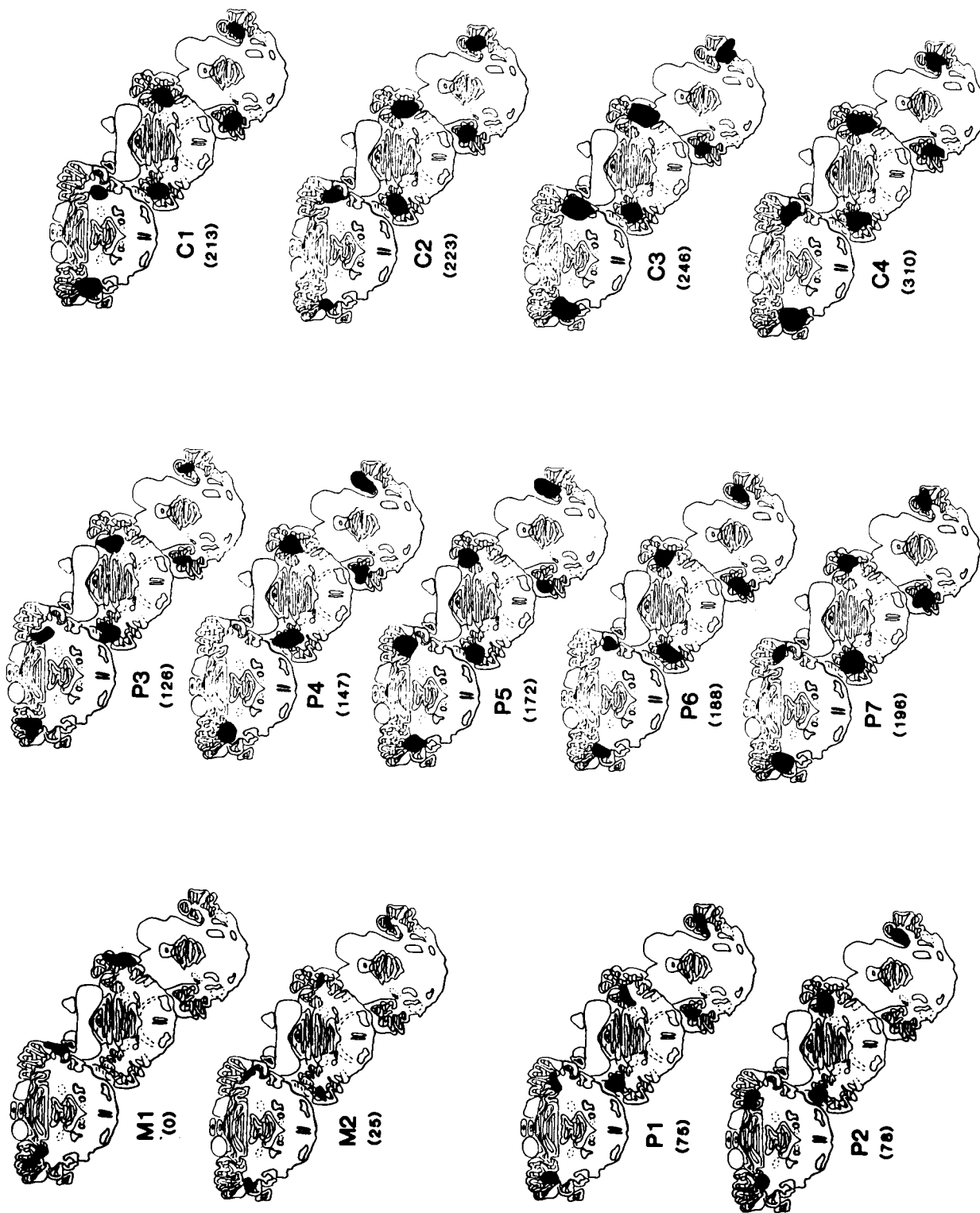


Figure 1 Reconstructed lesions of the middle cerebellar peduncle (MCP) (Animals M1-M2 are cases in which the lesion missed the MCP, animals P1-P7 are cases of partial damage to the MCP, and animals C1-C4 are cases of complete bilateral transection of the MCP. Numbers in parentheses are digitized damage estimates.)

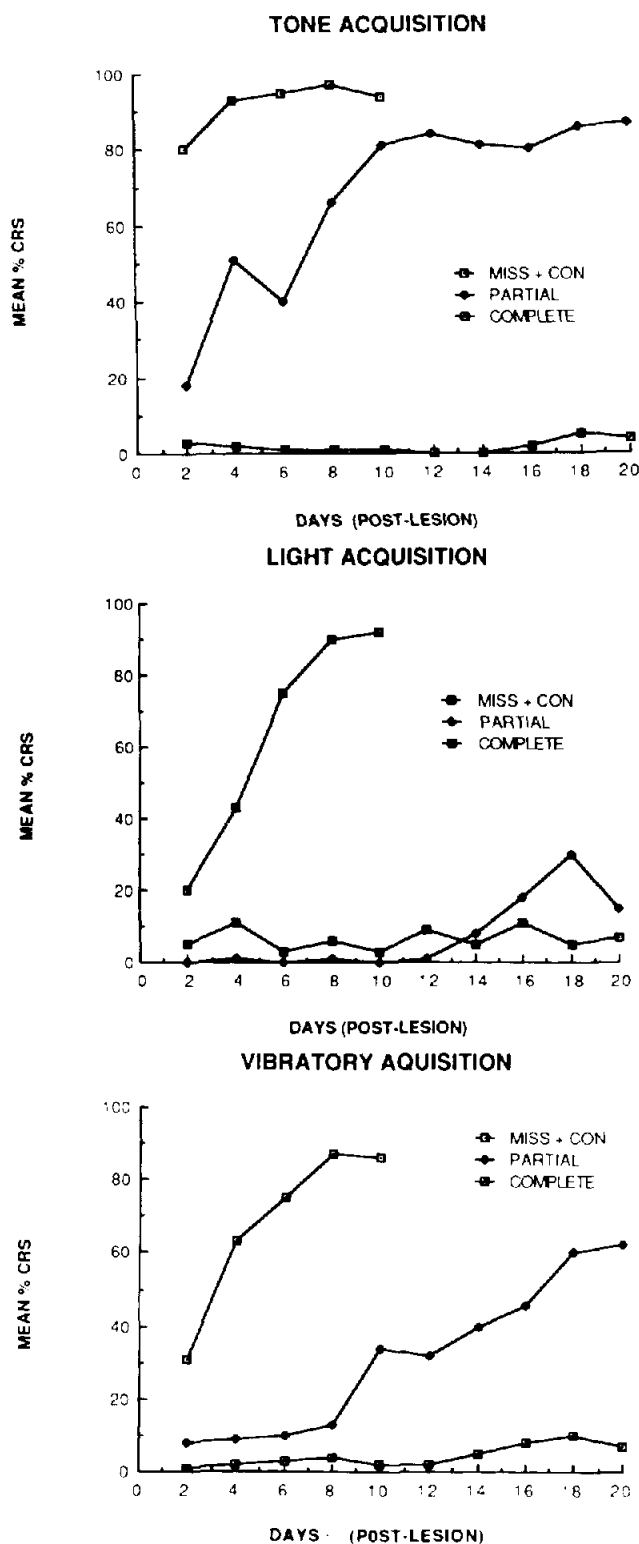


Figure 2. Acquisition of the conditioned response. (Top panel: Mean percentage of conditioned responses [CRs] to the tone conditioned stimulus [CS]; middle panel: Mean percentage of CRs to the light CS; bottom panel: Mean percentage of CRs to the vibratory CS.)

Table 2
Range of Mean Total CRs (360 Possible) to Each of the Three Stimuli for Animals in the Three Surgical Treatment Conditions

Stimulus/treatment	Acquisition	Retention
Tone		
MISS + CON	281-329	311-340
PAR	107-267	32-82
COM	4-9	3-15
Light		
MISS + CON	147-217	217-348
PAR	48-72	44-222
COM	13-19	4-9
Vibratory		
MISS + CON	148-308	—
PAR	22-118	—
COM	3-18	—

Note. MISS + CON = miss plus control; PAR = partial; COM = complete.

The findings of this study are consistent with an accumulating body of literature, indicating that the cerebellum is part of an essential neuronal circuit for acquisition and retention of the rabbit's classically conditioned NMR. These data are also consistent with the view that information about the CS enters the cerebellum by way of the MCP.

Because we used a within-group design that enabled us to measure retention and acquisition within the same animal (i.e., identical tissue damage), it is tempting to speculate that MCP lesions have similar effects on both acquisition and retention of the conditioned NMR. It is important, however, to point out that our complete MCP lesions, which abolished both acquisition and retention of the CR, were comparatively large. It is possible that smaller or more discrete lesions could disrupt one process, acquisition for example, while leaving retention intact. The data from our animals with partial MCP lesions, however, do not support this view. We were unable to detect any differential effects on CR acquisition or retention due to locus or size of the lesion. Nevertheless, it is possible that even our smallest partial lesions were too extensive to selectively affect one process. It will be necessary to test additional animals with lesions of varying size and location before any firm conclusion regarding possible differential effects of MCP lesions on CR acquisition and retention can be drawn.

Although there is some evidence to suggest that auditory and visual information enters the cerebellum by way of the MCP, whereas tactile information enters by way of the ICP (Blodel & Courville, 1981), the results of this study suggest that an intact MCP is essential for all three types of CS information. The disruption of acquisition of the CR was similar for all three CSs. Similarly, retention was disrupted for both the tone and light following MCP lesions. We do not as yet know if MCP lesions also disrupt retention of the tactile CS.

Based on our data, we cannot address the issue of whether different parts of the MCP are essential for different types of CS information. And although the degree of disruption of

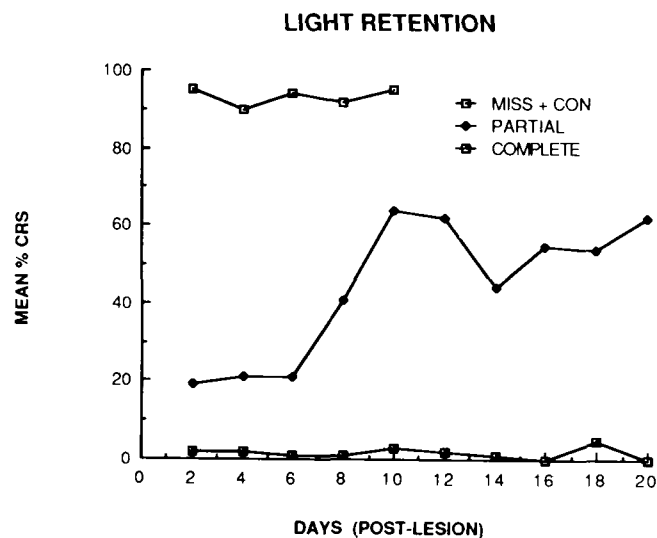
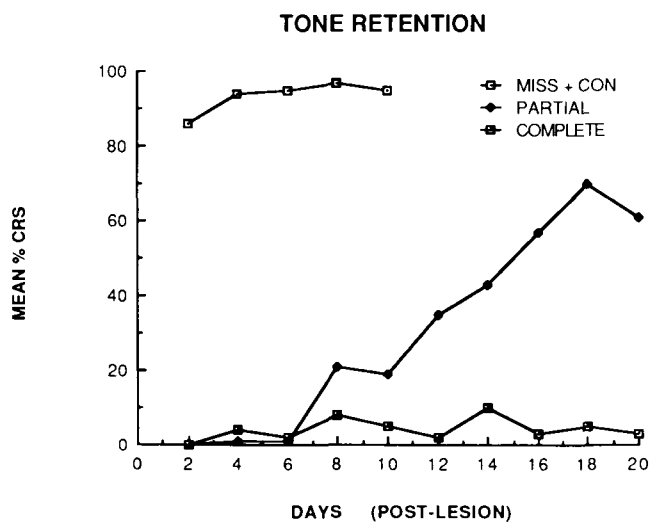


Figure 3 Retention of the conditioned response (Top panel Mean percentage of conditioned responses [CRs] to the tone conditioned stimulus [CS], bottom panel Mean percentage of CRs to the light CS)

conditioning in animals with MCP lesions appears to be consistent across all three modalities, more discrete lesions will be necessary to confirm this point. Our data do, however, suggest that the ICP is not sufficient to transmit information about the CS. This is in general agreement with the view of Steinmetz et al (1984) that the MCP carries information about the CS and the climbing fibers within the ICP carries information about the UCS. It is also consistent with recent data showing that mossy-fiber stimulation can serve as a

sufficient CS for classical conditioning (Logan, Steinmetz, Woodruff-Pak, & Thompson, 1985). The present study extends these findings by indicating that the MCP is necessary for acquisition and retention of the conditioned response.

Although we were unable to detect any sign of CR acquisition or retention in our rabbits with complete MCP lesions over the 20 days that we tested them, it is possible that given enough training, some of these animals might eventually acquire the CR. We would point out, however, that we did not observe any increase in CRs in animals with complete MCP lesions over the 20 days of CS-UCS pairings, nor did we see an increase in CRs over 30 days in one animal with a complete MCP lesion.

In summary, these data indicate that the MCP is necessary for acquisition and retention of the rabbit's classically conditioned NMR. Moreover, they suggest that the role of this cerebellar afferent is to carry information about CS in three modalities to the essential site of plasticity.

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The Publications and Communications Board is seeking nominations for new editors for the following five APA journals: *Journal of Applied Psychology*, *Journal of Comparative Psychology*, *Journal of Experimental Psychology: Human Perception and Performance*, *Professional Psychology: Research and Practice*, and *Psychological Review*. All terms will run from 1989 to 1994.

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