

Cerebellar Involvement in Eyeblink Classical Conditioning in Humans

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The role of the ipsilateral cerebellum in human eyeblink conditioning was investigated using the 400-ms delay paradigm and testing 14 cerebellar patients (7 with unilateral lesions and 7 with bilateral lesions) and 20 control participants. Patients performed significantly worse with the ipsilesional eye than control participants but showed no difference when tested with the contralesional eye. Conditioned responses (CRs) totaled 14% for all patients in comparison with 60% for control participants. Data on timed-interval tapping for 6 patients and 14 control participants showed that clock variability was greater with the ipsilesional hand in patients. Only clock variability correlated significantly with percentage of CRs in control participants. Comparisons of paired associate learning and memory for 8 patients and 14 control participants revealed no significant differences. Results confirm that the ipsilateral cerebellum plays a role in eyeblink classical conditioning.

The primary goal of this study was to investigate the role of the cerebellum in eyeblink classical conditioning by testing human patients with both unilateral and bilateral cerebellar lesions. Unilateral lesions are interesting in that comparisons can be made between conditioning with the ipsilesional and contralesional eyes. Research with nonprimates has consistently shown that eyeblink classical conditioning deficits are restricted to the ipsilesional eye following cerebellar lesions (e.g., Lavond, Hembree, & Thompson, 1985; McCormick & Thompson, 1984a, 1984b; Woodruff-Pak, Lavond, & Thompson, 1985; Yeo, Hardiman, & Glickstein, 1985a). Among eyeblink classical conditioning experiments using human participants, there is only a single case study assessing the ipsilateral cerebellum (Lye, O'Boyle, Ramsden, & Schady, 1988).

Previous studies with neurological patients have addressed the role of the cerebellum in eyeblink classical conditioning,

but most of the patients had bilateral lesions due to atrophic disorders. Topka, Valls-Sole, Massaquoi, and Hallett (1993) investigated eyeblink conditioning in a group of 12 patients: 5 had pure cerebellar cortical atrophy, and 7 had additional brainstem atrophy (olivopontocerebellar atrophy). A tone conditioned stimulus (CS) was paired with a shock unconditioned stimulus (US) in the delay paradigm with a 400-ms interval between CS and US. The motor unconditioned response (eyeblink UR) was normal in these patients, but the ability to acquire conditioned eyeblink responses was impaired in both patient groups in comparison to normal control participants. In particular, the timing of the conditioned responses (CRs) was abnormal. In the case of olivopontocerebellar atrophy, damage is not restricted to the cerebellum and may be rather widespread, affecting brain stem and possibly cortical areas as well. Secondary degeneration of the inferior olive is typically involved in cerebellar cortical atrophy (Adams, Corsellis, & Duchen, 1984). None of the patients in the Topka et al. (1993) study had damage to the deep nuclei that are the essential site for plasticity in nonprimate mammals (Chen, Bao, Lockard, Kim, & Thompson, 1996; Lavond et al., 1985; Skelton, 1988). Topka et al. (1993) provided suggestive but not conclusive evidence that the cerebellum was essential for eyeblink classical conditioning in humans.

Daum, Schugens, et al. (1993) tested 7 patients with cerebellar disorders, 4 of whom had bilateral lesions from atrophy and 3 of whom had unilateral lesions (two ischemic lesions and one unilateral resection of lateral hemisphere). Neuroradiological data provided evidence that in the 4 cases of bilateral lesions from atrophy, degeneration was restricted to the cerebellum. Of course, neuropathological data were not available to confirm that these patients had no degeneration in brain stem or cortex. In comparison with control participants, the cerebellar patients were severely impaired in eyeblink classical conditioning. In contrast to poor conditioning of a somatic eyeblink response, normal autonomic conditioning was observed in the patients as measured by changes in skin conductance. This dissociation parallels findings in the animal literature (Lavond, Lincoln, McCormick, & Thompson, 1984; Supple & Leaton,

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1990) and indicates that the cerebellar lesions produce a selective loss in the memory system required for producing the learned or conditioned eyeblink response.

Daum, Schugens, et al. (1993) tested unilateral patients exclusively on the ipsilesional side, and Topka et al. (1993) limited testing to patients with bilateral cerebellar lesions. A case study report suggested that damage to cerebellar afferents could also disrupt conditioning (Solomon, Stowe, & Pendlebury, 1989). The only investigation comparing ipsilesional and contralesional eyes was reported by Lye et al. (1988), who tested eyeblink classical conditioning in a 62-year-old man who had suffered an infarct, producing a unilateral lesion in the right cerebellar hemisphere 8 years prior to testing. The patient had a normal eyeblink UR in both eyes to the corneal airpuff US. However, even after 396 pairings of a tone CS and the US, few CRs were observed in the ipsilesional eye. When conditioning was shifted to the contralesional eye, CRs were evident by the second trial and persisted for the rest of the 36-trial block. When the US was redirected to the ipsilesional eye, the percentage of CRs dropped dramatically, and this asymmetry in performance was maintained over a series of reversals. Neurological, audiometric, and electrophysiological examinations of the patient suggested that poor eyeblink classical conditioning could not be attributed to primary sensory or motor deficits.

The ipsilateral cerebellum has been identified in rabbits as the critical substrate for classical conditioning of the nictitating membrane (NM)/eyeblink response (McCormick et al., 1981; McCormick & Thompson, 1984a, 1984b; Thompson, 1986; Yeo et al., 1985a; Yeo, Hardiman, & Glickstein, 1985b). Although controversy has been generated over the essential circuitry of eyeblink conditioning in rabbits (e.g., Bloedel, Bracha, Kelly, & Wu, 1991; Welsh & Harvey, 1989; Yeo, Hardiman, & Glickstein, 1984), a preponderance of the evidence supports the role of the ipsilateral cerebellum (e.g., Lavond, Kim, & Thompson, 1993; Steinmetz, Lavond, Ivkovich, Logan, & Thompson, 1992). During acquisition, cerebellar cortical Purkinje cells show conditioning-related firing patterns (Berthier & Moore, 1986; Gould & Steinmetz, 1994; Thompson, 1990), and principal cells in cerebellar interpositus nucleus fire in patterns modeling the CR and UR (Berthier & Moore, 1990; Gould & Steinmetz, 1994; McCormick & Thompson, 1984a). The number of Purkinje cells in cerebellar cortex correlates highly with acquisition of eyeblink classical conditioning (Coffin, Trojanowski, & Woodruff-Pak, 1996; Woodruff-Pak, Cronholm, & Sheffield, 1990). Aspiration of cerebellar cortex ipsilateral to the conditioned eye results in delayed acquisition (Lavond & Steinmetz, 1989), but rabbits eventually acquire CRs. Reversible lesions of the ipsilateral interpositus nucleus such as cooling (Clark, Zhang, & Lavond, 1992) and muscimol blockade (Krupa, Thompson, & Thompson, 1993) prevent acquisition until the nucleus is returned to its normal functioning state. Irreversible interpositus nucleus lesions permanently prevent acquisition (Lincoln, McCormick, & Thompson, 1982) and retention (Lavond et al., 1985; Steinmetz et al., 1992).

The role of cerebellum in eyeblink classical conditioning in humans has been less thoroughly investigated, but mounting evidence supports the role of ipsilateral cerebellum in eyeblink

conditioning in humans as well as in nonprimates. Using positron emission tomography (PET), Logan and Grafton (1995) reported significant activation in the inferior cerebellar cortex and deep cerebellar nuclei in humans engaged in eyeblink classical conditioning. These findings are in accord with other PET studies that have found deactivation in the ipsilateral cerebellar cortex over the course of acquisition, a result taken to reflect decreases in Purkinje cell activity (Molchan, Sunderland, McIntosh, Herscovitch, & Schreurs, 1994; Zeffiro et al., 1993).

Ivry and Keele (1989; Ivry, 1993; Keele & Ivry, 1990) have hypothesized that the cerebellum has the unique capability to represent the temporal relationship between different stimuli and between stimuli and their associated responses. This timing capability may be manifest in a variety of tasks including eyeblink classical conditioning. From this perspective, the reason that eyeblink conditioning is dependent on the cerebellum is because the learned eyeblink response is adaptive only if it occurs at the right point in time, that is, just prior to the onset of the US, allowing the animal to attenuate the negative impact of the US. Some forms of classical conditioning do not depend on the cerebellum, because the CRs are not temporally constrained in a similar manner (but see Ivry, 1993, for different views on this dissociation).

Direct evidence of a role of the cerebellum in tasks requiring precise timing comes from a series of studies with neurological patients including those with cerebellar lesions. Ivry and Keele (1989; Ivry, Keele, & Diener, 1988) tested participants on time production and time perception tasks as well as control tasks that did not depend on temporal processing. The results showed that the patients with cerebellar lesions were selectively impaired on the timing tasks. The patients were more variable in producing periodic intervals on a motor timing task (timed-interval tapping). More surprising, their timing deficit was also evident on a perceptual task in which they had to judge the duration of auditory signals. A second perceptual deficit was reported by Ivry and Diener (1991), who used a velocity perception task. Taken together, these results suggest that the cerebellar timing system is invoked whenever tasks require the precise representation of temporal information, be it for action, perception, or learning. New support for this hypothesis comes from recent PET studies showing cerebellar activation in either duration perception (Jueptner et al., 1995) or velocity perception (Dupont, Orban, DeBruyn, Verbruggen, & Mortelmans, 1994) tasks.

Ivry and Keele (1989) also demonstrated age-related increases in variability on the timing tasks in normal adults. To test the relationship between eyeblink classical conditioning and the cerebellar timing tasks, Woodruff-Pak and Jaeger (1996) analyzed data from a sample of 160 adults aged 20–89 years using multiple regression with four groups of predictor variables. Age accounted for 30% of the variance. The second highest predictor of eyeblink classical conditioning performance was the cerebellar assessment, timed-interval tapping, designed by Ivry and Keele (1989). This component accounted for an additional 8% of the variance and was statistically significant. Reaction time and declarative learning and memory measures were not significant predictors of eyeblink conditioning. These behavioral data are suggestive of a role of the

cerebellum in the age-related deficits in eyeblink classical conditioning (Durkin, Prescott, Furchtgott, Cantor, & Powell, 1993; Solomon, Pomerleau, Bennett, James, & Morse, 1989; Woodruff-Pak & Thompson, 1988). Some of the cerebellar patients in the present study of eyeblink conditioning had been assessed on timed-interval tapping at a 400-ms interval as part of an ongoing research program on timing functions of the cerebellum. Fourteen of the control participants had been assessed on timed-interval tapping at a 550-ms interval as part of an ongoing research program on normal aging. Data on declarative memory was also collected on 8 cerebellar patients and 14 normal control participants. These data are reported as preliminary evaluations of timing and cognitive function in relation to eyeblink classical conditioning in patients with unilateral and bilateral cerebellar lesions.

Method

Participants

A total of 34 participants were tested, including 14 patients with cerebellar lesions and 20 normal control participants (Table 1). The cerebellar patients had lesions resulting from resected tumors, cerebellar infarcts, or degenerative disorders. The age range of patients was 20–73 years, and this age range was closely matched by the age range of the normal control participants (22–75 years). Seven of the cerebellar patients had unilateral cerebellar lesions, and 7 had medial (vermis) or bilateral lesions. All lesions were confirmed with magnetic resonance imaging (MRI) or computerized tomography (CT) scans.

Materials and Procedures

Prior to testing, participants signed a written consent form. Testing consisted of eyeblink classical conditioning on both left and right eyes for all cerebellar patients and for 6 of the normal control participants matched to the unilateral lesioned cerebellar patients. Tapping measures of cerebellar timing functions were collected for most cerebellar patients and normal participants. Memory was tested in 8 cerebellar patients and all control participants using the Verbal Paired Associates Subscale of the Wechsler Memory Scale–Revised (WMS–R; Wechsler, 1987). The Blessed Information Memory and Concentration Test (Blessed IMC; Blessed, Tomlinson, & Roth, 1968) was used as a brief cognitive screening for dementia for all participants over the age of 40 years. The Blessed IMC and WMS–R Verbal were generally administered first, followed by eyeblink classical conditioning. The delayed recall portion of the WMS–R Verbal was given after the eyeblink classical conditioning procedure. Cerebellar timing functions were assessed using the timed-interval tapping task (Ivry & Keele, 1989). In total, testing required approximately 3 hr, carried out in one or two testing sessions, depending on the patient.

Eyeblink classical conditioning. The eyeblink classical conditioning apparatus contained three major components: (a) an adjustable headgear that housed a tubular jet for US presentation and an infrared emitter and receiver for recording CRs and URs, (b) a peripheral hardware device programmed to deliver a 1000-Hz tone CS and an airpuff US (5–7 psi) for each conditioning trial, and (c) a portable 386 IBM compatible microcomputer that controlled the peripheral hardware device and the output of raw data to storage disk.

Participants were asked to direct their attention to a television monitor positioned approximately 1 m in front of them. The experimenter used a VCR to play a videotape with no sound (e.g., Charlie Chaplin's *Gold Rush*). Videos ran continuously during both conditioning sessions. Previous research indicated that the videos help maintain

optimal levels of alertness but that the inclusion of the videos neither facilitates nor interferes with the conditioning process (Woodruff-Pak & Thompson, 1988). Participants were engaged in viewing the video while the experimenter made two baseline measurements. First, an estimate of the participant's natural eyeblink was obtained by physically measuring the distance in millimeters from the upper to the lower eyelid. This measurement provided the maximum blink magnitude for data collection and analyses. Second, an estimate of the participant's natural eyeblink rate was obtained by counting blinks over a 1-min time sample.

The adjustable headgear was then placed on the participant's head. For patients with unilateral cerebellar lesions, the eye ipsilateral to the lesion was tested first. Participants were told that in several seconds they would feel the first of several mild airpuffs directed toward their eye (US-only trials). During this time, the experimenter adjusted the position of the infrared device in relation to the participant's eye until a strong, "noise-free" measure of the UR was obtained. In addition, a minimum airpuff intensity (5–7 psi) was established for each individual participant that consistently resulted in full eyelid closure. After an acceptable eyeblink measurement was obtained, participants were provided with the (neutral) instructions for the conditioning session:

Please make yourself comfortable and relax. From time to time you will hear some tones and feel some mild puffs of air. If you feel like blinking, please do so. Just let your natural reactions take over. If you don't have any further questions, we'll begin now. If at any time during the session you need anything or have additional questions, please let me know.

For cerebellar patients, each conditioning session consisted of 10 blocks with nine trials in each block. Fourteen normal control participants tested on one eye (right eye) also had 10 blocks of nine trials. The 6 normal control participants tested on the left and right eye had 7 nine-trial blocks for each eye, and they were tested in one session. For all participants, the first trial within each block was considered a test trial with the CS presented alone. The remaining eight trials in each block were paired CS–US trials. Only the paired CS–US trials were included in the analyses, since the CS-alone trials represented too small of a sample to be reliable. The CS was a 1000-Hz, 500-ms duration tone of approximately 80 dB SPL, and the US was a 100-ms corneal airpuff. The US was delivered 400 ms after CS onset, and both stimuli coterminated.

Voltage changes from the infrared device were amplified and differentiated, and eyeblink data were entered into the computer for analysis and storage. A CR was scored when an eyeblink of a magnitude of one-twentieth of the total blink magnitude (for an average eye of 10 mm, this would be 0.5 mm) or greater occurred in the interval between 150 and 400 ms after the onset of the CS. Response latency was scored for every trial (whether or not a CR occurred) and was calculated as the first response after CS onset that was one-twentieth or greater of the total blink magnitude. Eyeblinks with a latency of 0–150 ms were not scored as CRs because they were thought to be reflexive responses to the tone (called *alpha* responses; Gormezano, 1966) and not representative of associative learning. The UR was measured as the magnitude of the response after US onset. This was the peak amplitude (in millimeters) during the interval between 401 and 648 ms after the tone CS onset.

Timed-interval tapping. Data were collected on this task for 9 cerebellar patients and 14 normal control participants. A trial was considered unsuccessful if any interresponse interval (IRI) was less than or greater than 50% of the base duration (Ivry et al., 1988). Data from unsuccessful trials were excluded as possibly reflecting tremor or insufficient force to register a response, and 6 cerebellar patients (Patients 1, 2, 3, 4, 12, and 13) and 14 normal control participants provided usable data. Participants were seated in front of a computer monitor with the arm used for tapping (dominant arm for normal

Table 1
Characteristics of Participants

Patient no.	Cerebellar patients	Normal control participants	
		Unilateral eyeblink classical conditioning	Bilateral eyeblink classical conditioning
1	Unilateral left cerebellar tumor resected 3 yrs. ago. Also alcoholism. 48 years old. M. 1-1; 2; 3.	43 years old. M. 1-0; 2; 3.	29 years old. M.
2	Unilateral right cerebellar infarct 2 years ago. Lesion superior to right deep nuclei. 57 years old. M. 1-4; 2; 3.	57 years old. M. 1-3; 2; 3.	55 years old. M.
3	Unilateral left inferior cerebellar lesion: stroke 3 years ago. Deep nuclei spared. 72 years old. F. 1-8; 2; 3.	72 years old. M. 1-1; 2; 3.	64 years old. F.
4	Unilateral left cerebellar aneurysm in deep nuclei region. No hemisphere lesion. 73 years old. M. 1-5; 2; 3.	70 years old. M. 1-0; 2; 3.	66 years old. M.
5	Unilateral large left inferior cerebellar infarct 3 years ago. 73 years old. M. 1-4; 2; 3.	75 years old. M. 1-3; 2; 3.	71 years old. F.
6	Unilateral large right cerebellar tumor, resected 4 years ago. 37 years old. F. 3.	37 years old. F. 1-0; 2; 3.	51 years old. F.
7	Bilateral large medial cerebellar tumor removed 2 years ago. 20 years old. F.	22 years old. F. 1-0; 2; 3.	
8	Bilateral cerebellar degeneration from disease. Identical twin. 23 years old. F. 3.	23 years old. F. 1-2; 2; 3.	
9	Bilateral cerebellar degeneration from disease. Identical twin. 23 years old. F. 3.	26 years old. F. 1-0; 2; 3.	
10	Bilateral cerebellar degeneration from Holmes disease. 23 years old. M.	22 years old. M. 1-0; 2; 3.	
11	Bilateral superior cerebellar lesion extending from left hemisphere to midline. 27 years old. M.	25 years old. M. 1-0; 2; 3.	
12	Bilateral atrophy of cerebellum, alcoholism. 60 years old. M. 1-5; 2; 3.	59 years old. M. 1-0; 2; 3.	
13	Bilateral severe olivopontocerebellar atrophy. 67 years old. M. 1-1; 2; 3.	67 years old. M. 1-0; 2; 3.	
14	Nonessential small right cerebellar infarct in superior hemisphere. 65 years old. M. 1-6; 2; 3.	67 years old. M. 1-5; 2; 3.	

Note. Numbers indicate tests administered. In the case of the Blessed Information Memory Concentration Test, score is given, which is the number of errors made in 33 items. 1 = Blessed; 2 = Wechsler Memory Scale—Revised Verbal Paired Associates; 3 = timed-interval tapping. M = male; F = female.

participants and patients with bilateral lesions; both hands, tested in separate blocks for patients with unilateral lesions) resting on a table, palm down. The participant placed the index finger of the dominant hand on the leftmost microswitch or lever mounted on a wooden block. Pressing the microswitch provided a pulse to an IBM-compatible computer that recorded all responses to the nearest millisecond. Each trial began with a series of 50-ms tones (65 dB) presented at regular intervals of 400 ms (cerebellar patients) or 550 ms (normal control participants).¹ Participants were instructed to begin tapping along with the tones once they had internally established the desired pace. After the participant's first response, 12 more tones were presented during which time the participant attempted to synchronize his or her responses. The participant was instructed to continue tapping at the same rate as the previously presented tones (now without the aid of stimulus presentation) until a message on the computer monitor indicated the end of the trial. The trial terminated after 31 self-paced taps had been emitted. The unpaced portion of the trial was used in the analyses. Three transformed variance measures (clock, motor, and total) were analyzed and derived from a theoretical model proposed by Wing and Kristofferson (1973; also see Wing, 1980). The clock variable is a measure of timing, a determination of when a response should be emitted. The motor variable is a measure assessing the execution of the motor command. By assuming that these processes are independent variables, the variability of the intertap intervals (ITIs) is the sum of variability associated with the clock process and variability associated with implementing these timing commands. The assumptions of the model have been verified in testing with normal participants (e.g., Wing, 1980) and neurological patients (Ivry & Keele, 1989; Ivry et al., 1988).

Memory and screening measures. Data were collected on this task for 8 cerebellar patients (Patients 1, 2, 3, 4, 5, 12, 13, and 14) and 14 normal control participants. The WMS-R Verbal Paired Associates requires participants to remember eight pairs of previously presented

words using a cued-recall procedure. An immediate recall score reflects the number of correctly recalled words (out of 24) in three cued-recall sets (each including one test item for each word pair, in different orders for each set, but consistent across participants) immediately following oral presentation of the word pair list. A delayed recall score was obtained, on average, 40 min later and was based on the number of correctly recalled word pairs out of the eight presented.

The Blessed IMC examines orientation, remote memory, recent memory, and concentration. The number of incorrect responses among 33 items served as the dependent measure. Thus, a score of zero indicates full cognitive capacity, and a score of 7–12 indicates mild dementia.

Results

Eyeblink Classical Conditioning in Cerebellar Patients With Unilateral Lesions

Of the 7 cerebellar patients with unilateral lesions, one (Patient 14) had a lesion extremely lateral in a region that was not critical in rabbits. The other 6 patients had larger and more medial lesions. Eyeblink classical conditioning performance of these 6 patients with unilateral cerebellar damage (tested on

¹ Tapping data for cerebellar patients and normal control participants were collected for different experiments, and hence different intervals were used. Although we could not make direct comparisons between cerebellar patients and normal control participants on this measure, within-cerebellar group comparisons with tapping data and eyeblink conditioning and tapping data comparisons in the control group provided additional perspective on conditioning data.

the lesioned side first) was compared with eyeblink classical conditioning performance on the right and left eyes in 6 normal control participants. The dependent measure was the percentage of CRs in paired conditioning trials. A 2 (group) $\times$ 2 (side) $\times$ 7 (training blocks) repeated measures analysis of variance (ANOVA) revealed significant main effects of group, $F(1, 10) = 9.93, p < .01$, training blocks, $F(6, 10) = 2.61, p < .03$, and Side $\times$ Group interaction, $F(1, 10) = 7.09, p < .02$ (see Figure 1). None of the other effects or interactions attained significance at the .05 level of confidence. The statistically significant Side $\times$ Group interaction occurred because cerebellar patients performed significantly more poorly in comparison with control participants using the eye ipsilateral to their cerebellar lesion, but there was no significant difference between the two groups in the second session when cerebellar patients were tested with the contralesional eye. The significant effect of training block indicated that both groups had a tendency to show increased CRs over training blocks. Because of the significant higher order interaction, we did not interpret further significant main effects.

Individual cerebellar patients with unilateral lesions performed eyeblink conditioning in a comparable fashion to rabbits, rats, and mice with unilateral cerebellar lesions. Cerebellar Patient 4 had a left cerebellar aneurysm that included the globose nucleus (analogue of interpositus nucleus in rabbits). This patient

produced no CRs with the ipsilesional left eye, but he produced a total of 28% CRs with the contralesional right eye (see Figure 2). For a 73-year-old adult, 28% CRs is in the low-normal range (Solomon, Pomerleau, et al., 1989; Woodruff-Pak & Thompson, 1988). Cerebellar Patients 1 and 2 had unilateral lesions that spared the globose nucleus (see Figure 3). Both patients had impaired eyeblink conditioning on the ipsilesional eye, as is indicated by the superior conditioning on the contralesional eye. Because globose nucleus was spared, they were able to produce some CRs with the ipsilesional eye.

Eyeblink Conditioning in Cerebellar Patients With Unilateral and Bilateral Lesions

Total percentage of CRs is the number of CRs made in the entire session. We carried out 3 (group) $\times$ 2 (side) repeated measures ANOVA comparing the total percentage of CRs attained in the first and second sides tested with eyeblink classical conditioning for 6 unilateral and 7 bilateral cerebellar lesion patients and the 6 normal control participants matched with the unilateral patients. The effect of group was significant, $F(2, 16) = 17.77, p < .001$, and the Side $\times$ Group interaction was significant, $F(2, 16) = 4.60, p < .03$. Post hoc investigation of simple effects following the significant interaction effect showed that only patients with unilateral cerebellar lesions

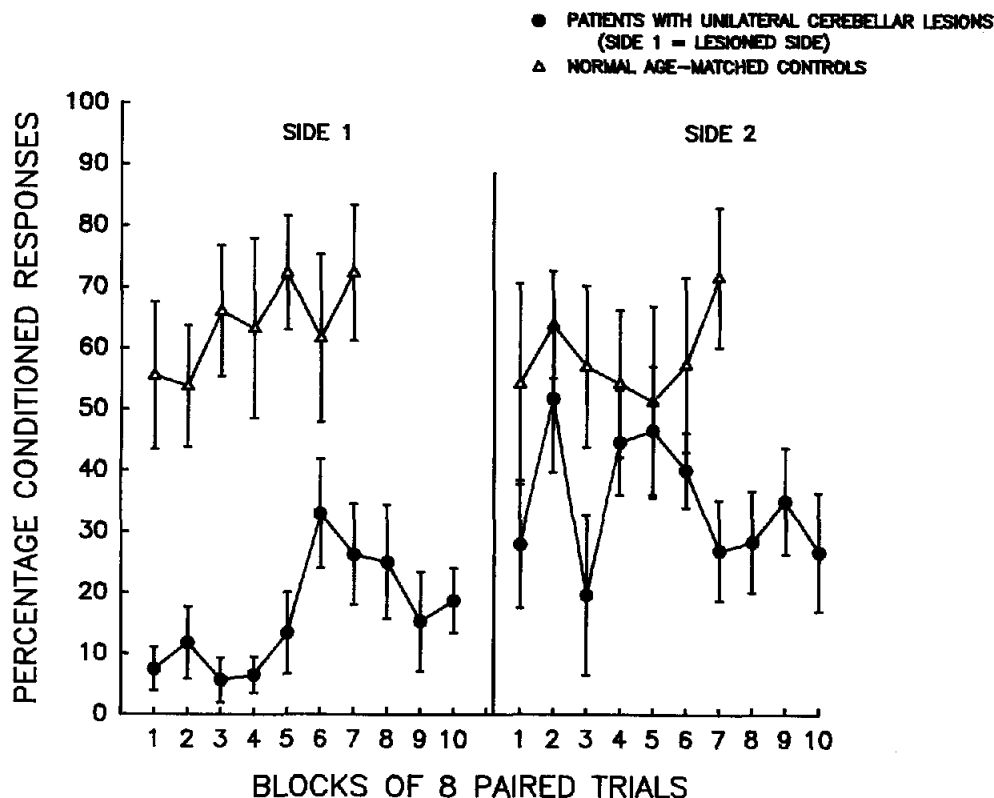


Figure 1. Percentage of conditioned responses in 6 patients with unilateral lesions of the cerebellum and in 6 normal control participants. Cerebellar patients were tested first for 10 nine-trial blocks using the eye ipsilateral to the lesion. Then they were tested for 10 nine-trial blocks using the eye contralateral to the lesion. Normal control participants were tested for 7 nine-trial blocks with one eye and then tested for 7 nine-trial blocks on the other eye. Error bars represent standard errors of the mean.

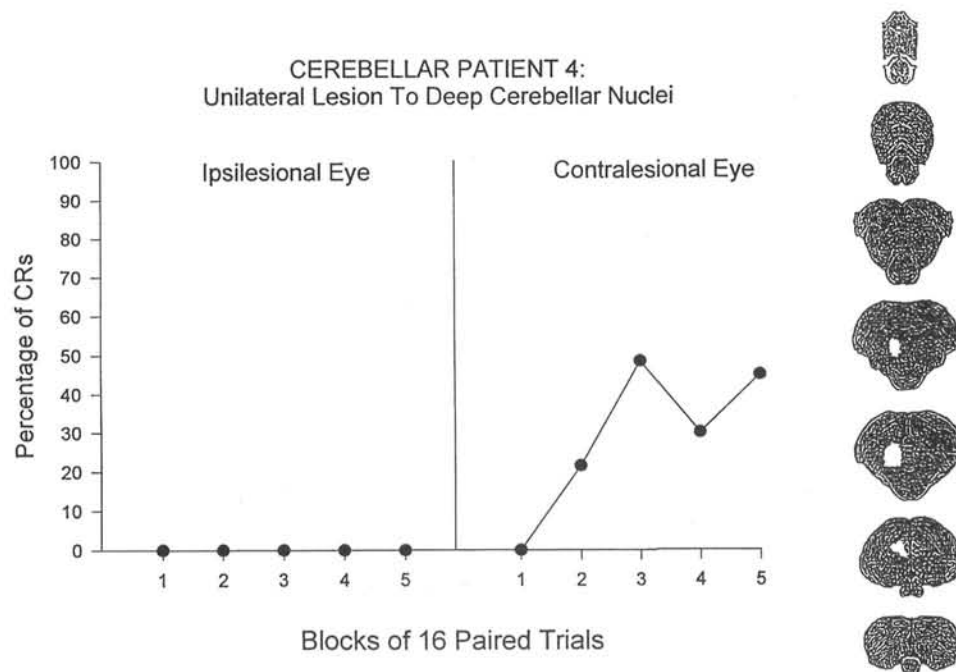


Figure 2. (Left) Percentage of conditioned responses (CRs) on the ipsilesional and contralesional eye in Cerebellar Patient 4, a 73-year-old patient with an aneurysm that lesioned the left globose nucleus (see lesion size and extent depicted on a standardized set of cerebellar templates at right). There was no damage to the cerebellar hemisphere. Data are presented in blocks of 16 paired presentations of tone conditioned stimulus and corneal airpuff unconditioned stimulus. The patient was tested first on the ipsilesional (left) eye, and after a short break he was tested on the contralesional (right) eye. (Right) A vertical series of seven slices of the cerebellum is shown, with the lesion in white. The left side of the template matches the side of the actual lesion. From top to bottom, the templates run from most superior to most inferior. The globose nucleus is located in the fourth and fifth slices from the top.

showed differential conditioning with the right and left eyes, $F(1, 16) = 8.70$, $p < .01$ (see Figure 4). Cerebellar patients with unilateral lesions performed more poorly when conditioned with the ipsilesional eye. However, when the globose nucleus was intact and cerebellar cortex was lesioned, acquisition of CRs was impaired but not abolished.

Of the 13 patients with significant cerebellar damage ipsilateral to the conditioned eye, total percentage of CRs for the first session was 14%. The mean eyeblink conditioning performance of the 13 normal control participants matched to these patients was 60% CRs. The 1 patient with a small cerebellar lesion in the lateral cortex (Patient 14) had 72% CRs on Side 1 and 80% CRs on Side 2 (see Figure 5). A 2 (group) $\times$ 10 (training blocks) repeated measures ANOVA was used to evaluate percentage of CRs in the first session (ipsilesional eye for the six unilateral lesions) for 13 patients with significant cerebellar damage in comparison with their matched normal control participants. The effect of group was significant, $F(1, 24) = 25.80$, $p < .001$, as was the effect of training block, $F(9, 216) = 2.31$, $p < .02$. The Group $\times$ Training Block interaction did not attain statistical significance (see Figure 6). As a group and as individuals, cerebellar patients had significantly fewer CRs than did normal control participants (see Figures 7 and 8 for individual bilateral cerebellar patients and radiology).

One temporal measure in eyeblink classical conditioning is the latency of the first eyeblink after CS onset, with this

response being either the CR or the UR. A 2 (group) $\times$ 10 (training block) repeated measures ANOVA comparing response latency in 12 cerebellar patients (latency data for Patient 6 were not available) and 12 matched normal control participants during the first eyeblink classical conditioning session revealed a significant effect of group, $F(1, 22) = 17.09$, $p < .001$. The effect of training blocks and the interaction did not attain statistical significance. Cerebellar patients had significantly slower responses that on average did not fall within the CR range (less than 400 ms). Normal control participants produced average CR latencies that fell within the CR range from the beginning of Session 1 (see Figure 9). Whereas it would be useful to restrict this analysis to only CRs, many of the patients produced fewer than five CRs per session, making a block by block analysis impossible.

Nonassociative Factors Potentially Affecting Group Differences in Eyeblink Conditioning

It was important to rule out nonassociative explanations for the findings regarding differences in eyeblink classical conditioning acquisition in cerebellar patients and normal control participants. Specifically, we examined the possibility that the group differences in eyeblink classical conditioning favoring normal control participants resulted from (a) differing rates of random blinking between groups, such that conditioning scores

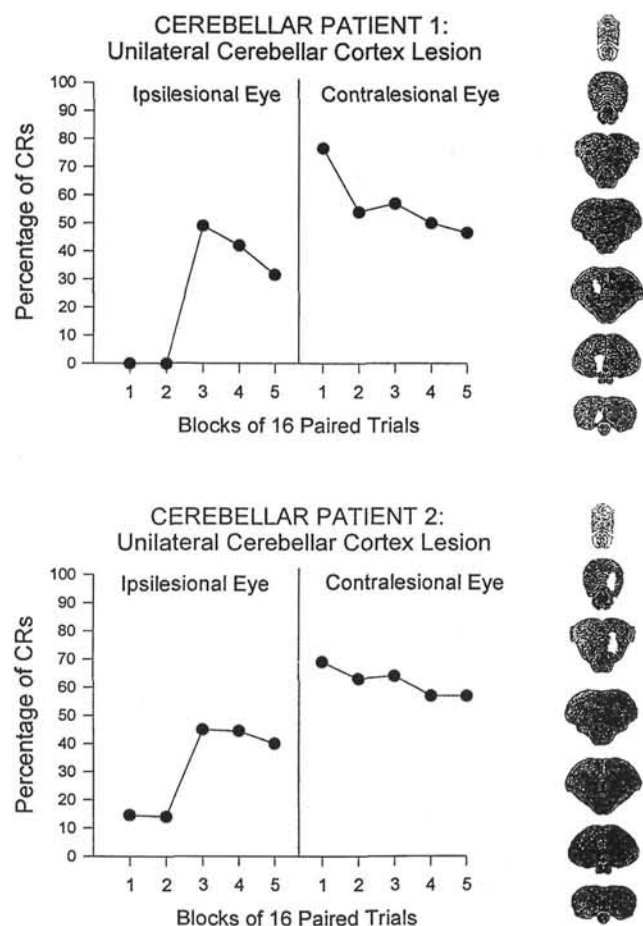


Figure 3. (Top left) Percentage of conditioned responses (CRs) on the ipsilesional and contralesional eye in Cerebellar Patient 1, a 48-year-old patient who was an alcoholic and had a left cerebellar tumor resected 3 years before testing (see lesion size and extent depicted on a standardized set of cerebellar templates at right). Data are presented in blocks of 16 paired presentations of tone conditioned stimulus and corneal airpuff unconditioned stimulus. The patient was tested first on the ipsilesional (left) eye, and after a short break he was tested on the contralesional (right) eye. (Top right) A vertical series of seven slices of the cerebellum is shown with the lesion in white. The left side of the template matches the side of the actual lesion. From top to bottom, the templates run from most superior to most inferior. (Bottom left) Percentage of CRs on the ipsilesional and contralesional eye in Cerebellar Patient 2, a 57-year-old patient who had a right cerebellar infarction 2 years before testing (see lesion size and extent depicted on a standardized set of cerebellar templates at right). Data are presented in blocks of 16 paired presentations of tone conditioned stimulus and corneal airpuff unconditioned stimulus. The patient was tested first on the ipsilesional (right) eye, and after a short break he was tested on the contralesional (left) eye. (Bottom right) A vertical series of seven slices of the cerebellum is shown with the lesion in white. The right side of the template matches the side of the actual lesion. From top to bottom, the templates run from most superior to most inferior.

might be inaccurately represented in one or another group, or (b) a general performance deficit as revealed by a lack of robust eyeblink URs on the part of cerebellar patients.

Random eyeblink rates measured over a 1-min time sample

did not differ significantly between cerebellar patients and normal control participants (both p s > .05). Results suggested that voluntary blinks were not a significant factor in the observed differences. As has been demonstrated above, average response latencies were longer for the cerebellar patient.

A 2 (group) $\times$ 10 (training blocks) repeated measures ANOVA was used to evaluate UR amplitude effects. No main effects were observed for the factors of group or training blocks, nor was the interaction significant. Unconditioned eyeblinks of cerebellar patients were equivalent in magnitude to the eyeblinks of normal control participants (see Table 2). Over the length of the training session, UR amplitude remained relatively constant for both groups.

Timed-Interval Tapping and Eyeblink Conditioning Relationships

Three measures of variability and mean ITI were obtained from cerebellar patients and normal control participants on timed-interval tapping. Variability measures were (a) the total variance of ITIs (total), (b) the amount of variance attributed to the clock mechanism (clock), and (c) the amount of variance attributed to motor delays (motor). Because the patients and normal control participants had been tested at different tapping intervals, only within-participant group evaluations of timed-interval tapping and the relationship between tapping and eyeblink classical conditioning could be made.

Of the 14 cerebellar patients, 6 had twelve trials per hand of timed-interval tapping data at the 400-ms interval that could be analyzed and compared. Five cerebellar patients (Patients 5, 6, 7, 10, and 11) were never tested or were tested with insufficient trials on this task, and a 6th (Patient 14) had a familial tremor that disrupted his tapping. The 7th and 8th patients (identical twins with degenerative cerebellar disease; Patients 8 and 9) had ITIs greater than 50% of the base duration, making their data unusable. For the cerebellar patients with lateralized lesions, there was a consistent within-subject group difference in timed-interval tapping scores between hands. Total variability was greater in the hand ipsilateral to the lesion than in the hand contralateral to the lesion (Table 3).

Ideally, CR production and timed-interval tapping would have been compared in cerebellar patients. However, cerebellar patients were consistently poor in eyeblink conditioning, with 8 patients producing fewer than 10 CRs and with most of the others (with the exception of Patient 14) producing fewer than 20 CRs. In contrast, the mean number of CRs for normal control participants was 54. For cerebellar patients there was not a sufficient sample of conditioning behavior to correlate with timed-interval tapping performance. For the 14 normal control participants, the mean ITI from six trials with the dominant hand was 531.3 ($SD = 26.7$), and mean total variability was 26.2 ($SD = 6.4$). Mean clock variability was 19.7 ($SD = 6.8$), and mean motor variability was 13.2 ($SD = 4.9$). The correlation between total variability and percentage of CRs was $-.42$, and this correlation did not attain statistical significance. However, the correlation with percentage CRs using the clock estimate of variability attributed to timing was $-.59$ ($p < .025$). Greater clock variability predicted poorer

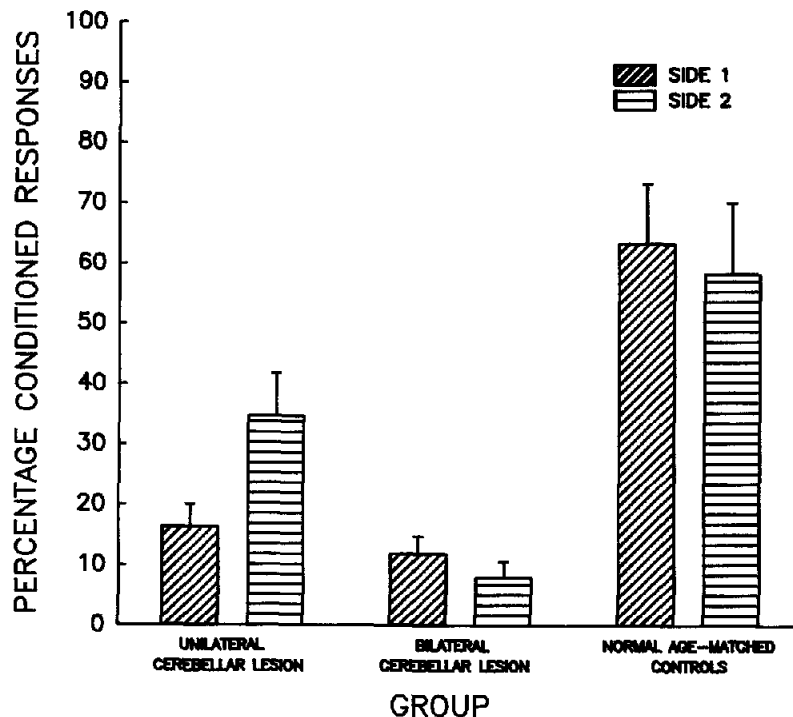


Figure 4. Total percentage of conditioned responses in two sessions of eyeblink classical conditioning using the right and left eyes. Cerebellar patients (with unilateral or bilateral lesions) were tested for 10 nine-trial blocks, and normal control participants were tested for 7 nine-trial blocks. Cerebellar patients with unilateral lesions were tested first with the eye ipsilateral to the lesions. Error bars are standard errors of the mean.

eyeblink classical conditioning in the 14 normal control participants. Motor variability was not related to eyeblink classical conditioning performance: The correlation between percentage of CRs and the motor component of the variance was .14 (*ns*).

Memory in Cerebellar Patients

Declarative memory was assessed in 8 cerebellar patients and 14 control participants using the initial acquisition and delayed recall measures of the WMS-R Verbal Paired Associates. Mean acquisition score (from a total score of 24) for the cerebellar patients was 13.9 (*SD* = 4.4) and for the control participants was 18.5 (*SD* = 5.5). A *t* test comparing these means was not significant, $t(20) = 2.02$, $p = .0571$. Mean delayed recall score (from a total score of 8) for the cerebellar patients was 5.9 (*SD* = 0.8) and for the control participants it was 6.4 (*SD* = 2.5). A *t* test comparing these means was not significant, $t(20) = 0.52$, $p = .610$. A *t* test comparing total percentage of CRs of these same participants revealed a significant effect, $t(20) = 3.11$, $p < .01$. Thus, the groups differed in eyeblink classical conditioning but not memory as assessed by the WMS-R Verbal Paired Associates.

Discussion

There is mounting evidence that the neural circuitry for eyeblink classical conditioning investigated in rabbits, cats,

rats, and mice is similar to that in humans. Previous investigations have demonstrated significant impairment of eyeblink classical conditioning in patients with bilateral cerebellar atrophy (Topka et al., 1993) and bilateral atrophy and unilateral lesions tested on the eye ipsilateral to the lesion (Daum, Schugens, et al., 1993). Results reported in the present study replicate and extend these results in additional patients with bilateral lesions of the cerebellum tested with both eyes. We also tested 7 patients with unilateral cerebellar lesions with the ipsilesional and contralesional eye and extend the results of Lye et al.'s (1988) case study report of a unilateral cerebellar patient. With the exception of 1 patient with a small lesion in the lateral hemisphere (see Figure 5), patients with unilateral cerebellar lesions perform eyeblink classical conditioning significantly more poorly with the eye ipsilateral to the lesion. Performance of patients with unilateral cerebellar lesions using the contralesional eye is comparable to that of normal control participants.

Timed-interval tapping showed greater variability (indicating poorer performance) in cerebellar patients with lateralized lesions in the hand ipsilateral to the lesions. In normal control participants, there was a significant correlation between eyeblink classical conditioning and the clock component, but not the motor component, of the timed-interval tapping score. The clock component assesses variability due to the timing of the response and has been shown to be abnormal in patients with cerebellar cortical lesions (Ivry & Klee, 1989; Ivry et al.,

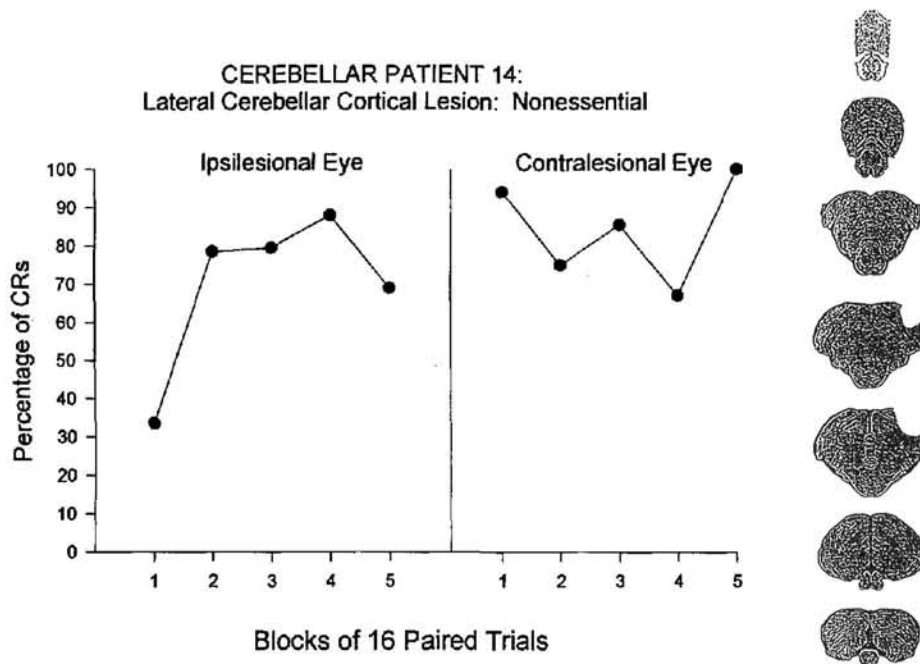


Figure 5. (Left) Percentage of conditioned responses (CRs) on the ipsilesional and contralesional eye in cerebellar Patient 14, a 65-year-old patient with a small right cerebellar infarct affecting the lateral hemisphere (see lesion size and extent depicted on a standardized set of cerebellar templates at right). Data are presented in blocks of 16 paired presentations of tone conditioned stimulus and corneal airpuff unconditioned stimulus. The patient was tested first on the ipsilesional (right) eye, and after a short break he was tested on the contralesional (left) eye. (Right) A vertical series of seven slices of the cerebellum is shown, with the lesion in white. The right side of the template matches the side of the actual lesion. From top to bottom, the templates run from most superior to most inferior.

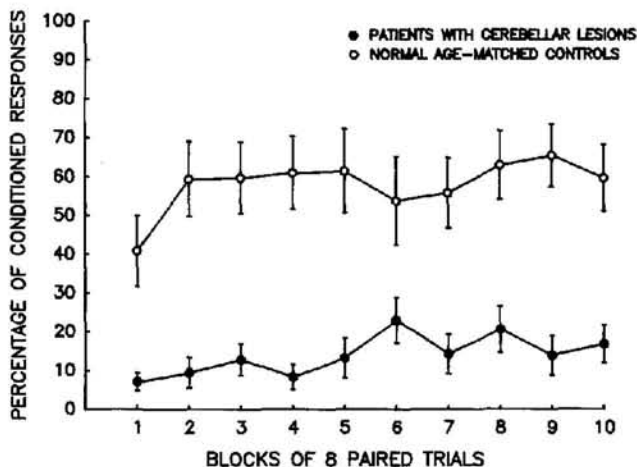


Figure 6. Acquisition as measured by percentage of conditioned responses in 13 patients with cerebellar lesions and 13 normal control participants. Six of the cerebellar patients had unilateral lesions and were tested with the eye ipsilateral to the lesion. Seven of the patients had bilateral cerebellar lesions. Error bars represent standard errors of the mean.

1988). The CR in eyeblink classical conditioning requires precise timing and is optimal when it peaks shortly before US onset. The result that the clock component correlates significantly with percentage of CRs supports the hypothesis that the cerebellum plays a role in the precise timing of the CR in eyeblink classical conditioning (Ivry, 1993; Keele & Ivry, 1990).

In addition to documenting poor eyeblink classical conditioning in cerebellar patients with unilateral lesions in the eye ipsilateral to the lesion and poor eyeblink classical conditioning with both eyes in patients with bilateral cerebellar lesions, we have also documented intact declarative memory as assessed by the WMS-R Verbal Paired Associates. Acquisition and delayed recall on the WMS-R Verbal Paired Associates were similar in cerebellar patients and normal control participants. Comparable verbal memory performance in patients with cerebellar lesions and normal control participants was also reported by Daum, Ackermann, et al. (1993), who administered the Paired Associates subtest of the WMS (German version) to 13 patients with cerebellar lesions and 13 normal control participants. In our study, memory was not impaired in cerebellar patients, although eyeblink classical conditioning was severely impaired.

Individual Cerebellar Lesions and Performance

Performance on eyeblink classical conditioning by individual human cerebellar patients whose lesions resulted from pathol-

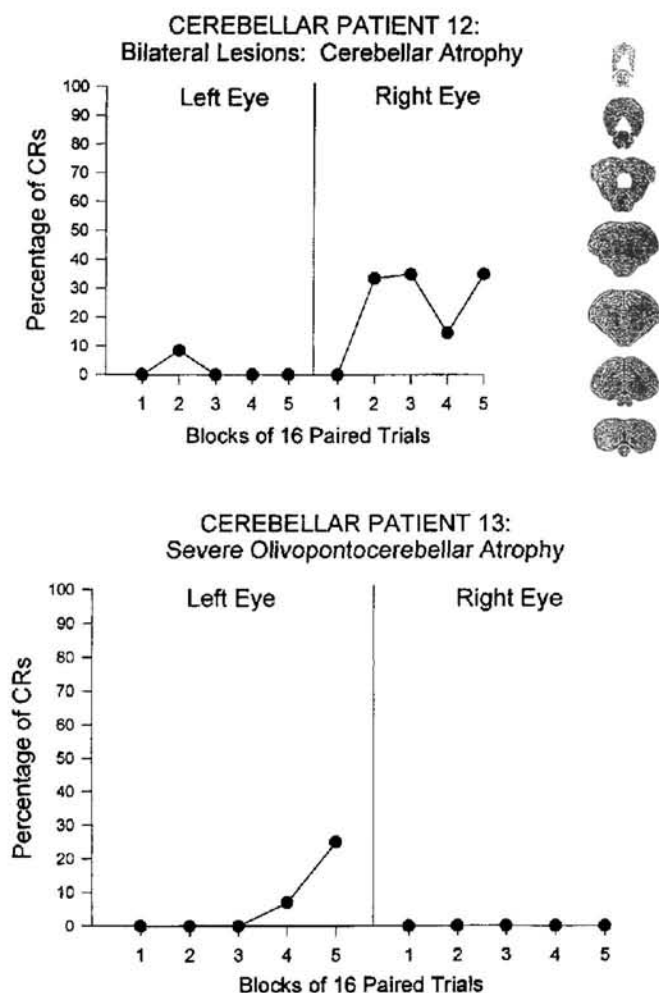


Figure 7. (Top left) Percentage of conditioned responses (CRs) in the left and right eyes in Cerebellar Patient 12, a 60-year-old patient with cerebellar atrophy related to alcoholism (see lesion size and extent depicted on a standardized set of cerebellar templates at right). Data are presented in blocks of 16 paired presentations of tone conditioned stimulus and corneal airpuff unconditioned stimulus. The patient was tested first on the left eye, and after a short break he was tested on the right eye. (Top right) A vertical series of seven slices of the cerebellum is shown, with the lesion in white. The left side of the template matches the left side of the cerebellum. From top to bottom, the templates run from most superior to most inferior. (Bottom) Percentage of CRs in the left and right eyes in Cerebellar Patient 13, a 67-year-old patient with severe olivopontocerebellar atrophy (see magnetic resonance image in Figure 8). Data are presented in blocks of 16 paired presentations of tone conditioned stimulus and corneal airpuff unconditioned stimulus. The patient was tested first with the left eye, and after a short break he was tested with the right eye.

ogy was comparable to eyeblink classical conditioning performance by rabbits with experimental lesions in cerebellar circuitry. Rabbits with permanent lesions (Lincoln et al., 1982) or reversible lesions (Clark et al., 1992; Krupa et al., 1993) to interpositus nucleus are unable to acquire CRs with the eye ipsilateral to the lesion. The deficits are unilateral because the animals demonstrate a high percentage of CRs when tested on

the eye contralateral to the lesion. Patient 4 had a unilateral left cerebellar aneurysm that included the deep nuclear region, involving both the globose and dentate nuclei (Figure 2). As was mentioned previously, the globose nucleus is homologous

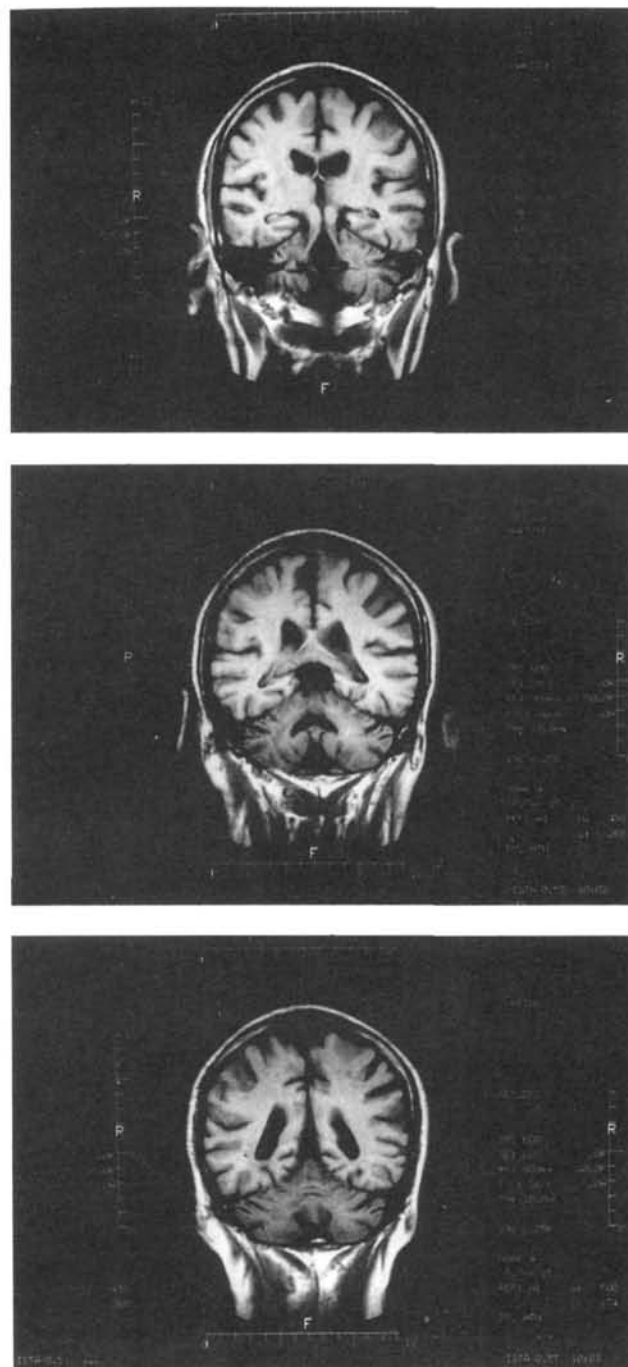


Figure 8. Magnetic resonance imaging scans for Cerebellar Patient 13. This 67-year-old man had severe olivopontocerebellar atrophy. As presented in Figure 7, this patient produced almost no conditioned responses in a total of 180 trials in the 400-ms delay eyeblink conditioning paradigm.

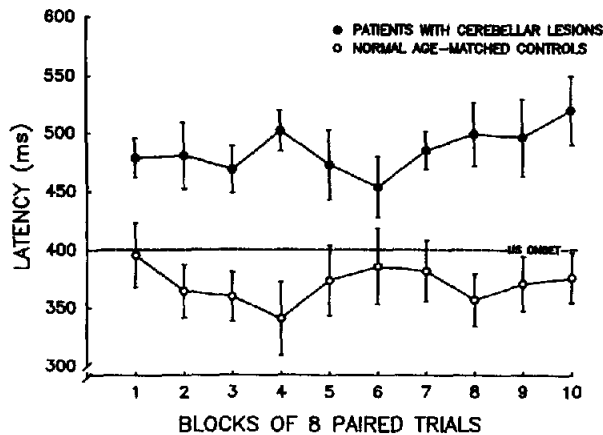


Figure 9. Eyeblink response latency during classical conditioning for 12 cerebellar patients and 12 normal control participants. Response latency data were not available for the 13th cerebellar patient whose lesion was critical for conditioning. Unconditioned stimulus (US) onset was at 400 ms. Error bars are standard errors of the mean.

to the interpositus nucleus in nonhuman mammals. Whereas this patient produced no CRs with the left eye, he produced 28% CRs with the right eye. The other patient with a unilateral lesion that was just superior to the deep nuclear region was Patient 2, who had suffered a cerebellar infarct 2 years before eyeblink classical conditioning testing (Figure 3, bottom). This 57-year-old man had only 12% CRs with the eye ipsilateral to the lesion (right eye) but had 74% CRs with the left eye. It should be noted that, with the method used in this study of testing the ipsilesional eye first, it is possible that these differences reflect an order effect since the contralesional eye was always tested after the ipsilesional eye. Arguing against this interpretation are the facts that (a) normal participants tested with both eyes made no further improvement on the side tested second, and (b) Lye et al. (1988) repeatedly reversed the eyes being conditioned and consistently found poor conditioning with the ipsilesional eye and good conditioning with the contralesional eye.

Patients with severe cerebellar degeneration bilaterally were severely impaired in eyeblink classical conditioning as was indicated by Patient 13 (MRI shown in Figure 8), a 67-year-old

man who produced no CRs with the right eye and only 8% CRs with the left eye. A 23-year-old man with Holmes disease and severe cerebellar degeneration produced 8% CRs with the right eye and 7% CRs with the left eye. When he was tested a second time on eyeblink conditioning the following day, this man performed even more poorly, producing 5% CRs with the right eye and 0% CRs with the left eye.

Many cerebellar lesions do not impair eyeblink classical conditioning in rabbits (McCormick & Thompson, 1984a). However, the animals were young and the lesions were discrete. Many of our patients were older and were suffering from trauma that may have affected a relatively large portion of ipsilateral cerebellum, at a minimum. One exception was Patient 14, a 65-year-old man with a small right cerebellar infarct that was lateral in the superior hemisphere (Figure 5). This patient produced 75% CRs with the right eye and 69% CRs with the left eye. In a manner similar to that of rabbits with lateral cerebellar aspirations tested in the delay paradigm (Woodruff-Pak, Lavond, Logan, Steinmetz, & Thompson, 1993), this patient was unimpaired on eyeblink classical conditioning.

Eyeblink Conditioning and Noncerebellar Neurological Pathology

Any form of brain damage might impair eyeblink classical conditioning, making it imperative to demonstrate that some forms of brain damage or neurodegenerative disease do not destroy the capacity to acquire CRs. Lesions affecting eyeblink conditioning in humans are specific to the neural circuitry involved in this learning and identified in nonprimate mammals. The cerebellum is essential for acquisition and retention of the conditioned eyeblink response. The hippocampus plays a modulatory role in acquisition in that manipulations of the hippocampal cholinergic system can alter the rate of learning.

The hippocampus is activated during eyeblink classical conditioning, but conditioning in the delay paradigm in which the CS and US overlap can occur in the absence of the hippocampus (Schmaltz & Theios, 1972; Solomon & Moore, 1975). Eyeblink classical conditioning in the delay paradigm occurs in humans with hippocampal lesions and amnesia (Daum, Channon, & Canavan, 1989; Daum, Channon, Polkey, & Gray, 1991; Gabrieli et al., 1995; Weiskrantz & Warrington, 1979; Woodruff-Pak, 1993).

Our working hypothesis is that disruption of the septohippocampal cholinergic system impairs acquisition of eyeblink conditioning in probable Alzheimer's disease (AD) beyond the impairment observed in normal aging (Woodruff-Pak, Finkbinder, & Katz, 1989). Eyeblink conditioning was significantly poorer in AD patients and older adults with Down's syndrome and AD (called DS/AD) who showed almost no acquisition in one session (Papka, Simon, & Woodruff-Pak, 1994; Solomon, Levine, Bein, & Pendlebury, 1991; Woodruff-Pak, Finkbinder, & Sasse, 1990; Woodruff-Pak, Papka, & Simon, 1994). We replicated and extended this result with new samples of patients and normal control participants (Woodruff-Pak &

Table 2
Unconditioned Response Amplitude (in Millimeters) in Cerebellar Patients and Normal Control Participants

Block no.	Cerebellar patients	Normal control participants
1	8.31	8.65
2	7.81	8.46
3	8.09	8.53
4	7.63	8.38
5	7.96	8.57
6	8.31	8.20
7	8.51	8.27
8	7.95	8.42
9	7.57	8.36
10	8.13	8.30
<i>M</i>	8.03	8.42

Table 3
Timed-Interval Tapping Performance With Unilesional and Contralesional Hand in Cerebellar Patients With Unilateral or Bilateral Lesions

Patient no.	Ipsilesional hand				Contralesional hand			
	Mean ITI	Total	Clock	Motor	Mean ITI	Total	Clock	Motor
Unilateral lesion								
1	394	45	31	23	386	28	17	16
2	405	35	37	(-8) ^a	377	26	27	(-6) ^a
3	370	26	19	13	376	17	10	10
4	398	26	21	11	385	20	10	12
Bilateral lesion								
12	368	60	58	10				
13	372	37	36	6				

Note. ITI = intertap interval.

^aPatient 2 shows a clear violation of the Wing (1980) model, a result that is observed in about 20% of cerebellar patients.

Papka, 1996a; Woodruff-Pak, Papka, Romano, & Li, in press). In Woodruff-Pak et al. (in press), we also demonstrated that in some cases, eyeblink classical conditioning was effective in differentiating cerebrovascular dementia from probable AD. Cerebrovascular dementia patients with lesions restricted to cerebral cortex perform eyeblink conditioning normally.

Rabbits with disrupted hippocampal cholinergic systems have delayed acquisition of eyeblink classical conditioning but eventually acquire CRs (Solomon, Solomon, Vander Schaaf, & Perry, 1983). On this basis, we predicted that if probable AD and DS/AD patients were given enough training trials, they would eventually produce CRs. Probable AD and DS/AD patients were tested with eyeblink classical conditioning for 5 consecutive days, and most of them eventually attained a learning criterion of eight CRs in 9 consecutive trials (Woodruff-Pak, Romano, & Papka, 1996). Solomon et al. (1995) tested probable AD patients for four consecutive 70-trial sessions and reported eventual acquisition of CRs. The neural substrate supporting eyeblink classical conditioning therefore seems to be impaired by probable AD and DS/AD beyond the impairment observed in normal aging, but it is not destroyed. The fact that probable AD and DS/AD patients eventually acquire CRs argues against lesions of the cerebellar deep nuclei as the cause of the significant impairment of eyeblink classical conditioning in that patient population.

Huntington's disease (HD) is a progressive degenerative neurological disease resulting in abnormalities of voluntary movement and dementia. The basal ganglia are grossly atrophied, and there is thinning and shrinkage of the cerebral cortex. The hippocampus and hippocampal cholinergic system, however, remain relatively intact, as does the cerebellum. Although the eyeblink reflex is hyposensitive in HD, this lack of excitability is overcome with an airpuff directed at the cornea. It was anticipated that HD patients would condition relatively normally in the absence of cerebellar pathology. As predicted, there were no differences in production of CRs between HD patients and normal control participants (Woodruff-Pak & Papka, 1996b).

Role of Cerebellum in Eyeblink Classical Conditioning

At least seven lines of evidence are converging to suggest that the ipsilateral cerebellum is essential for eyeblink classical conditioning in nonprimate mammals and humans. The first and most extensive body of literature comes from 20 years of investigation tracing the neural pathways involved and essential for acquisition and retention of eyeblink classical conditioning in the rabbit and other nonprimate mammals. There has been remarkable success in identifying the brain structures and mechanisms involved in this form of associative learning (for reviews, see Anderson & Steinmetz, 1994; Lavond et al., 1993; Steinmetz & Thompson, 1991; Thompson & Krupa, 1994). A variety of techniques including electrophysiological recording of multiple and single units, electrolytic and chemical lesions, physical and chemical reversible lesions, neural stimulation, and pharmacological manipulation have been used to demonstrate that the dorsolateral interpositus nucleus ipsilateral to the conditioned eye is the essential site for acquisition and retention (Berthier & Moore, 1986, 1990; Clark et al., 1992; Gould & Steinmetz, 1994; Krupa et al., 1993; Lavond et al., 1985; Lincoln et al., 1982; McCormick et al., 1981; McCormick & Thompson, 1984a, 1984b; Steinmetz et al., 1992; Thompson, 1986, 1990; Yeo et al., 1985a). Lesions of the cerebellar cortex of rabbits disrupt acquisition, although the rabbits eventually achieve significant conditioning (Lavond & Steinmetz, 1989). Interestingly, there is some evidence suggesting that lesions of the cerebellar cortex disrupt the timing of CRs (Perrett, Ruiz, & Mauk, 1993).

A second line of evidence implicating the cerebellum's essential role in eyeblink classical conditioning comes from studies of cerebellar lesions in humans. Group studies have documented that patients with cerebellar degeneration perform poorly in eyeblink classical conditioning (Daum, Schugens, et al., 1993; Topka et al., 1993). The case report of Lye et al. (1988) and the current findings extend this work, showing that, as has been found in animal studies, the deficit is most pronounced in the side ipsilateral to the lesion. We have also

demonstrated that eyeblink classical conditioning is normal in a patient with a lesion in a lateral region of cerebellar cortex that is also nonessential in rabbits.

Performance on eyeblink classical conditioning in normal human aging provides a third line of evidence suggesting cerebellar involvement in eyeblink classical conditioning. Studies of eyeblink classical conditioning over the adult human age span documented poorer performance in older participants (Durkin et al., 1993; Solomon, Pomerleau, et al., 1989; Woodruff-Pak & Thompson, 1988). Postmortem studies have shown that cerebellar Purkinje cell number is 25% less in elderly adults than in young adults (Hall, Miller, & Corsellis, 1975). Imaging resolution in humans is too crude to identify individual Purkinje cells, precluding an in vivo correlational analysis of Purkinje cell count and eyeblink classical conditioning study in humans. However, it has been possible to carry out Purkinje cell counts in rabbits after they have been trained in eyeblink classical conditioning. In these studies the correlations between Purkinje cell number and acquisition of CRs are high (Coffin et al., 1996; Woodruff-Pak, Cronholm, & Sheffield, 1990), suggesting that Purkinje cell loss may account for much of the age-related difference in eyeblink classical conditioning.

A fourth line of evidence comes from research using the timed-interval tapping task. Patients with cerebellar lesions are more variable on this task, and the variability increase is associated with a central clock process in patients with lateral lesions (Ivry et al., 1988). This index of cerebellar function was found to be a significant predictor of eyeblink classical conditioning performance in a large sample of adults in the age range of 20 to 89, even after the variance due to age was removed (Woodruff-Pak & Jaeger, 1996). Moreover, in the present study, clock variability was negatively correlated with eyeblink classical conditioning in normal control participants. In cerebellar patients who produced significantly fewer CRs with the ipsilesional eye, clock variability was greater with the ipsilesional hand.

A fifth line of evidence with some implications for the cerebellum in eyeblink classical conditioning is the conditioning performance of autistic children. In autism, there are demonstrated cerebellar abnormalities in cerebellar cortex (e.g., Bauman & Kemper, 1985; Courchesne, 1991; Courchesne, Yeung-Courchesne, Press, Hesselink, & Jernigan, 1988; Ritvo et al., 1986) and in deep nuclei (Bauman, 1991). One of the characteristic features of the abnormal eyeblink classical conditioning in autistic children was the shorter, abnormally timed CR onset and CR peak latency (Scars, Finn, & Steinmetz, 1994). Cerebellar lesions have been associated with abnormal timing of CRs (e.g., Perrett et al., 1993).

Studies of normal young adult participants performing eyeblink classical conditioning during PET assessment provide a sixth line of evidence indicating cerebellar involvement in eyeblink classical conditioning. Three PET investigations of eyeblink classical conditioning concur in their reports of changes in the cerebellum (Logan & Grafton, 1995; Molchan et al., 1994; Zeffiro et al., 1993). During associative learning in eyeblink classical conditioning, Logan and Grafton (1995) observed significant increases in activation in inferior cerebel-

lar cortex/deep nuclei, anterior cerebellar vermis, contralateral cerebellar cortex, and some noncerebellar regions. Molchan et al. (1994) and Zeffiro et al. (1993) reported decreases in cerebellar cortex, and the Zeffiro group also reported increases in ipsilateral deep nuclear regions. Differences among the groups may have occurred as a result of differences in PET scanning technique and the time during acquisition that scanning occurred. Nevertheless, both in terms of foci and direction of metabolic change, all three studies identified the cerebellum as a primary area showing learning-related activity.

Papka, Ivry, and Woodruff-Pak (1995) have taken a novel approach to exploring relationships among the cerebellum, brain memory systems, timing, and eyeblink classical conditioning. Their experiment used a dual-task methodology in which normal young adults underwent eyeblink classical conditioning while engaged in a variety of secondary tasks. Of greatest interest was the result that eyeblink classical conditioning was significantly decreased in participants who were concurrently required to perform the timed-interval tapping task in comparison with a variety of control groups. Simultaneous performance of eyeblink classical conditioning and a difficult declarative memory task, or a choice reaction time task, did not disrupt eyeblink conditioning in comparison with performance of eyeblink classical conditioning while watching a silent video. The pattern of results is consistent with the hypothesis that the cerebellum is critical for both the timed-interval tapping task and eyeblink classical conditioning.

In the present study we have demonstrated that human patients with lateralized cerebellar lesions—as with rabbits, rats, and mice with experimentally created unilateral cerebellar lesions—produce relatively normal CRs with the eye contralateral to the lesion, and few or no CRs with the eye ipsilateral to the lesion. Timed-interval tapping is also more variable in the ipsilesional hand. Patients with bilateral cerebellar lesions or neurodegenerative cerebellar disease produce few conditioned eyeblink responses with either eye. Nevertheless, memory for verbal material is normal in these cerebellar patients. Patients with lesions or neurodegenerative disease not involving the cerebellum can acquire CRs. This investigation adds to the body of research using a number of different strategies and converging to implicate the human cerebellum as essential in classical conditioning of the eyeblink response.

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