

MRI-Assessed Volume of Cerebellum Correlates with Associative Learning

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Richard F. Thompson's cerebellar model of classical eyeblink conditioning highlights Purkinje cells in cerebellar cortex and principal cells in the deep cerebellar nucleus as the integrating cells for acquisition of conditioned responses (CRs). CR acquisition is significantly slower in rabbits with lesions to cerebellar cortex and in Purkinje cell-deficient mice that lose all cerebellar cortical Purkinje cells. Purkinje cells are the largest neurons in the cerebellum and contribute significantly to cerebellar volume. Magnetic resonance imaging (MRI) was used to assess cerebellar volume in humans. Cerebellar volume was related to eyeblink conditioning (400-ms delay procedure) in 8 adults (21–35 years) and compared to 8 older adults (77–95 years) tested previously (Woodruff-Pak, Goldenberg, Downey-Lamb, Boyko, & Lemieux, 2000). In the young adult sample, there was a high correlation between percentage of CRs in a session and cerebellar volume (corrected for total intracranial volume [TIV], $r = .58, p = .066$). There were statistically significant age differences in cerebellar volume, $t(14) = 8.96, p < .001$, and percentage of CRs, $t(14) = 3.85, p < .002$, but no age difference in TIV. Combining the young and older adult sample, the correlation between percentage of CRs and cerebellar volume (corrected for TIV) was $.832 (p < .001)$. Cerebellar volume showed age-related deficits likely due to Purkinje cell loss. Individual differences in classical eyeblink conditioning are associated with differences in cerebellar volume, supporting Thompson's model of a cerebellar cortical role in facilitating this form of associative learning. © 2001

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INTRODUCTION

The initial discoveries of neural correlates of eyeblink classical conditioning in Richard F. Thompson's laboratory revealed hippocampal engagement during acquisition of conditioned responses (CRs) (Berger, Alger, & Thompson, 1976). These observations were followed by investigations documenting the modulatory role of the hippocampus in classical eyeblink conditioning (Berger, Berry, & Thompson, 1986; Thompson, 2000). In the early 1980s, Thompson and colleagues reported a remarkable and unexpected finding. Lesions of the cerebellum ipsilateral to the eye receiving the air puff unconditioned response (UR) prevented naive rabbits from acquiring CRs. These cerebellar lesions also abolished CRs in rabbits trained before the lesions were delivered (McCormick et al., 1981; McCormick & Thompson, 1984). Furthermore, there appeared to be no recovery of responding in the lesioned rabbits. This basic discovery led to wide-ranging experiments in the Thompson laboratory and other laboratories around the world that explored the involvement of the brain stem and cerebellum in classical eyeblink conditioning. A critical role for the cerebellum in associative learning was an idea so controversial as to have generated significant debate and challenge (e.g., Bloedel, 1993; Bower, 1997; Llinas & Sotelo, 1992; Welsh & Harvey, 1989, 1991). However, evidence continues to amass demonstrating that the essential site for acquisition and retention of the conditioned eyeblink response in mammals, including humans, is the cerebellum ipsilateral to the conditioned eye.

Experiments demonstrating an essential role for the cerebellum in the acquisition and retention of CRs have employed a variety of experimental techniques such as brain lesions, reversible brain lesions, brain microstimulation, multiple- and single-unit recording, pharmacological manipulations, and anatomical tract tracing. In total, these studies identified key areas of cerebellar cortex and the deep cerebellar nuclei that appear to encode conditioning. In addition, the conditioned stimulus (CS) and unconditioned stimulus (US) pathways have largely been defined as well as the output pathway from the cerebellum to brain stem motor nuclei responsible for generating the CRs (Lavond & Cartford, 2000; Steinmetz, 1996, 2000; Thompson, 1986; Thompson & Krupa, 1994). These data have indicated that CSs used in eyeblink conditioning are projected to discrete regions of the cerebellar cortex and the interpositus nucleus via mossy fibers that originate in the basilar pontine nuclei. The USs used in eyeblink conditioning appear to be projected to regions of cerebellar cortex and the interpositus nucleus via climbing fibers that originate in the dorsolateral region of the inferior olive complex. Conditioning is thought to be the product of plasticity induced in cerebellar cortical and nuclear regions due to the conjunctive activation of these regions by the CS and the US. It is hypothesized that the pairing-induced alteration of cerebellar activity is then capable of exciting brain stem motor neurons (via red nucleus activation) that are responsible for the generation of the behavioral CR. In essence, this defined neural circuit provides the sufficient and essential substrates for delay eyeblink classical conditioning (Fig. 1).

The cerebellum's role in eyeblink conditioning is essential in humans as well as in rabbits and other nonhuman mammals (Daum & Schugens, 1996; Woodruff-Pak, 1997). Several eyeblink classical conditioning studies have been carried out in human patients with bilateral cerebellar lesions (Daum, et al., 1993; Solomon, Stowe, & Pendlebeury, 1989; Topka, Valls-Sole, Massaquoi, & Hallett, 1993) as well as unilateral cerebellar

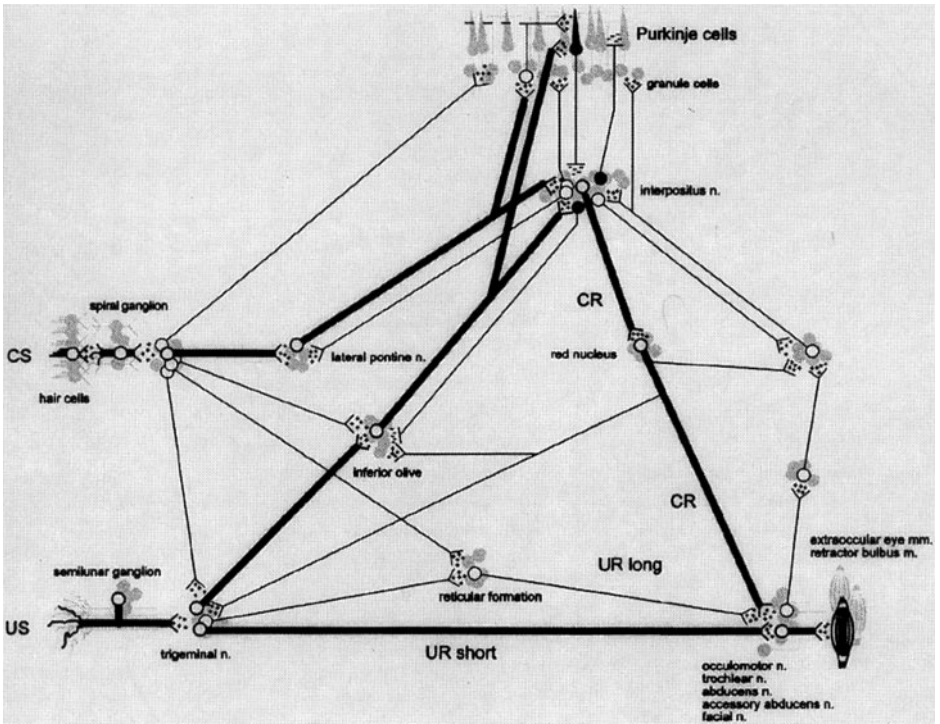


FIG. 1. Simplified schematic of hypothetical memory trace circuit for discrete behavioral responses learned as adaptations to aversive events. The tone conditioned stimulus (CS) pathway consists of auditory projections to the lateral pontine nuclei (lateral pontine n.) and their mossy fiber projections to cerebellar cortical Purkinje cells and also to interpositus nucleus. The corneal air puff unconditioned stimulus (US) pathway consists of somatosensory projections to the dorsal accessory portion of the inferior olive and its climbing fiber projections to cerebellar cortical Purkinje cells and also to interpositus nucleus. The efferent (eyelid closure) conditioned response (CR) pathway projects from the interpositus nucleus (interpositus n.) of the cerebellum to the red nucleus and via the descending rubral pathway to act ultimately on motor neurons. The red nucleus may also exert inhibitory control over the transmission of somatic sensory information about the US to the inferior olive, so that when a CR occurs (eyelid closes), the red nucleus dampens US activation of climbing fibers. Evidence to date is most consistent with storage of the memory traces in the interpositus nucleus and possibly in localized regions of cerebellar cortex as well. Pluses indicate excitatory synaptic action and minuses inhibitory synaptic action. (Original figure, courtesy of David G. Lavond).

lesions (Lye, O'Boyle, Ramsden, & Schady, 1988; Woodruff-Pak, Papka, & Ivry, 1996). Impairment in eyeblink classical conditioning is seen in humans with lesions localized to cerebellar regions ipsilateral to the conditioned eye that are essential for eyeblink conditioning in rabbits. Studies of normal young adults performing eyeblink classical conditioning during positron emission tomography (PET) assessment provide yet another line of evidence indicating cerebellar involvement in human eyeblink conditioning. PET investigations of eyeblink conditioning concur in their reports of changes in the cerebellum (Blaxton, et al., 1996; Logan & Grafton, 1995; McIntosh & Schreurs, 2000; Molchan, Sunderland, McIntosh, Herscovitch, & Schreurs, 1994). Significant increase of regional cerebellar blood flow in the anterior and intermediate parts of the ipsilateral cerebellum during limb flexion reflex conditioning has also been demonstrated in healthy human participants using PET techniques (Timmann, et al., 1996). Functional magnetic resonance imaging

(fMRI) assessment of young adults during eyeblink conditioning using the discrimination procedure demonstrated activations in cerebellar cortex (Ramnani, Toni, Josephs, Ashburner, & Passingham, 2000). Using the delay eyeblink conditioning procedure and event-related fMRI, Lemieux and Woodruff-Pak (2000) reported activations in cerebellar cortical regions HVI and HVIIA and in the deep nuclear region (where the interpositus nucleus in rabbits is represented by two cerebellar deep nuclei in humans: the emboliform and globose nuclei).

Aspiration of cerebellar cortex in rabbits results in a delay in acquisition such that rabbits take up to nine times as many trials to achieve learning criterion (Lavond & Steinmetz, 1989). The magnitude of CRs is also lower in these cerebellar cortically lesioned rabbits. Nevertheless, rabbits with cerebellar cortical lesions eventually acquire CRs. Purkinje cell-deficient (*pcd*) mutant mice that lose all Purkinje cells in cerebellar cortex postnatally during the second month perform similarly in eyeblink conditioning to rabbits with cerebellar cortical aspirations (Chen, Bao, Lockard, Kim, & Thompson, 1996). The *pcd* mice took at least five times as long to acquire CRs as did their wild-type littermates with Purkinje cells, and the magnitude of CR production was lower. These data indicate that the cerebellar cortex is normally engaged but not essential in acquisition.

There are behavioral data that suggest a parallel role in classical eyeblink conditioning for cerebellar cortex in humans. Performance on a task for which the cerebellar cortex is engaged called timed-interval tapping predicted performance on eyeblink classical conditioning (Woodruff-Pak & Jaeger, 1998; Woodruff-Pak et al., 1996). Dual-task studies of eyeblink conditioning in normal young adults who also simultaneously perform tasks such as timed-interval tapping (Papka, Ivry, & Woodruff-Pak, 1995) or sequential finger tapping (Downey-Lamb & Woodruff-Pak, 1998) demonstrate impaired CR acquisition. Tasks sharing the same cerebellar cortical substrate in the case of the two forms of finger tapping and eyeblink conditioning interfere with one another when performed simultaneously. Tasks with noncerebellar cortical substrates such as word stem completion priming (Downey-Lamb & Woodruff-Pak, 1998), rotary pursuit (Green & Woodruff-Pak, 1997), and choice reaction time (Papka et al., 1995) show little or no interference when performed simultaneously with eyeblink conditioning.

The current study was undertaken to evaluate eyeblink conditioning and cerebellar relationships using a neurobiological measure of the cerebellum in young adults in place of the behavioral assessments of cerebellar function that we had used previously (e.g., timed-interval tapping, sequential finger tapping). We measured cerebellar volume with anatomical MRI in young adults who were also tested in the 400-ms delay eyeblink classical conditioning procedure. In a previous study using identical techniques, we had tested older adults with anatomical MRI and 400-ms delay eyeblink conditioning (Woodruff-Pak, Goldenberg, Downey-Lamb, Boyko, & Lemieux, 2000). There was a statistically significant correlation between cerebellar volume (corrected for total intracranial volume [TIV]) and percentage of CRs ($r = .81, p < .02$). We carried out the current study with three aims. The first aim was to determine whether the cerebellar volume–eyeblink conditioning relationship held in a sample of young adults. The second aim was to compare the eyeblink conditioning performance and cerebellar volume between young and older adult age groups. The third aim was to examine cerebellar volume–eyeblink conditioning relationships in the combined sample.

MATERIALS AND METHODS

Participants

A total of 8 young adult participants ranged in age from 21 to 35 years with a mean age of 27.3 years ($SD = 6.0$). They were students in an advanced undergraduate course or acquaintances of these students recruited for participation. There were 5 women and 3 men. The 8 older adults ranged in age between 77 and 95 years with a mean age of 82.5 years ($SD = 5.8$). The older adult participants had been members of a larger longitudinal sample tested on eyeblink classical conditioning and neuropsychological test performance. There were 4 men and 4 women. These individuals scored above average for their age range on neuropsychological tests and were cognitively normal when they were assessed with anatomical MRI. The young adults were tested with the same MRI scanner and eyeblink conditioning equipment 1 year after the older adults had been tested.

This research was approved by the institutional review board (IRB) at the Philadelphia Geriatric Center, where eyeblink conditioning testing took place for the older adults, and by the Temple University Health Sciences Center IRB, where MRI scanning occurred. Young adult participants were tested both with eyeblink conditioning and MRI on the Health Sciences Center campus. All participants read and signed separate IRB-approved informed consent forms for MRI scanning and eyeblink conditioning before participating. The aims of the study were discussed at the conclusion of the test session.

Eyeblink Classical Conditioning Apparatus and Procedure

Prior to testing, a brief hearing test was administered to the older participants with a Maico audiometer at 1000 Hz. Older participants had corrected hearing thresholds lower than 45 dB in both ears. Spontaneous blink rate per minute for each participant was also calculated. Participants were then fitted with an adjustable headpiece that held an infrared photocell transducer that measured the eyeblink and were given "neutral" instructions to blink naturally and make no effort to blink or to suppress blinks. Voltage changes from the photocell transducer were input to a microprocessor that stored and analyzed the data. Also attached to the headpiece was a tube positioned 2 cm from the participant's left cornea that delivered a 5-psi air puff of medical-grade oxygen as the US. Participants were trained using a 400-ms delay procedure. The CS was an 80-dB SPA 1000-Hz tone lasting 500 ms. The corneal air puff US had its onset 400 ms after CS onset with a duration of 100 ms. The CS and US coterminated.

Each participant's preconditioning UR amplitude was calculated by presenting several US-alone trials. After the preliminary measures were administered, a video recorder placed in front of the participant was turned on, and the participant was invited to watch a silent film (*Milo and Otis*). The timing and presentation of the stimuli were controlled by the microprocessor. A response was scored as a CR if it was an eyeblink of 0.5 mm or larger and occurred between 100 and 400 ms after the onset of the CS. Responses that occurred after 400 ms were scored as URs. The performance measure used for analyses was percentage of CRs among the total number of artifact-free trials (minimum of 54 trials).

Magnetic Resonance Imaging Equipment and Procedure

A General Electric Signa 1.5-T MRI echo-speed scanner with standard head coil was used to take two magnetic resonance (MR) sequences. First, the localizer scan was acquired using a T2-weighted sagittal sequence (scan time = 2:15 min, whole brain FSE, $TR = 2000$ ms, $TE = 85$ ms, slice thickness = 5 mm, matrix = 256×256). Second, a volumetric scan was taken using a T1-weighted axial sequence (whole brain 3D-SPGR, scan time = 8 min, $TR = 27$ ms, $TE = 9$ ms, matrix = 256×192 , slice thickness = 1.6, flip angle = 30 degrees). The total scan time for each participant was less than 30 min. All participants were comfortable and cooperative in the MR scanner and were able to remain still.

Volumetric Assessment of the Brain

Cerebellar volume was measured using the volume reformatting image analysis package available on the Advantage Windows Workstation (GE Medical Systems, version 2.0). Trained operators (the third and fourth authors) were supervised by a neuroradiologist (the fifth author). None of these investigators had any knowledge of the eyeblink classical conditioning results when he performed the volumetric analyses. This analysis included manual tracing of 30 sections (1.6 mm thick) of the cerebellum for each of the 16 participants. These operators also carried out the TIV measurement.

For the MR scan of the cerebellum, each slice was outlined and measured from inferior to superior levels in the axial plane, and volumes for 4 cerebella were replicated on the sagittal plane. The cerebellar area was measured three times at each level, and the average value was taken as the area of the cerebellum at that specific level. The area was then multiplied by the thickness of the MR section to yield the volume of the cerebellum at a specific section. Right and left cerebellar hemispheres were measured separately at the inferior levels and together at more superior levels. The window and level was set at each MR section such that the entire cerebellum was seen and the borders between the cerebellum and the surrounding structures were clearly defined. Cerebellar gray and white matter was included in the volume measurements, whereas cerebellar peduncles were not. Distinguishing cerebellar peduncles from cerebellar white matter was difficult in many cases. However, all measurements were consistent with respect to where cerebellar white matter was included and excluded in the volume measurements. The entire measurement process was repeated twice for each brain by two experimenters. The intraclass correlation between the first and second experimenter was .97 (95% confidence interval of .93–.99), indicating a high level of interrater reliability.

Total intracranial volume measurements were made using 40 to 45 axial MR sections that were measured from the inferior to superior level. Sagittal and coronal sections were used to determine the anatomical boundaries for TIV measurements. Total intracranial volume included brain and cerebrospinal fluid (CSF) volumes from the caudal boundary of the foramen magnum to the most superior level where brain and/or CSF were still seen. The measurements excluded diploe, retro-orbital fat, and musculature. The entire brain was traced, total area was measured at each MR level, and this area was then multiplied by slice thickness (3.2 mm) to yield a volume for each level. Window and level were set at each section to ensure clear differentiation among bone, brain tissue, and CSF.

RESULTS AND DISCUSSION

Cerebellar Volume and Eyeblink Conditioning Relationship in a Sample of Young Adults

The first question addressed in this study was whether performance on eyeblink conditioning was related to the volume of the cerebellum in a young adult sample in a restricted age range. In our young adult sample, there were both men and women, and 9 of 13 studies of aging and cerebellar volume reported that women have significantly smaller cerebella (Table 1). Even in our small sample of 8 young adults, the cerebellar volume of the 3 men ($m = 142.43$ ml, $SD = 1.70$) was significantly greater than the cerebellar volume of the 5 women ($m = 132.73$, $SD = 8.36$), $t(6) = 2.51$, $p < .05$. It has been documented repeatedly that there are no gender differences in eyeblink conditioning (e.g., Durkin, Prescott, Furchtgott, Cantor, & Powell, 1993; Papka et al., 1995; Solomon, Pomerleau, Bennett, James, & Morse, 1989; Woodruff-Pak & Jaeger, 1998; Woodruff-Pak & Thompson, 1988). The absence of a significant gender difference in percentage of CRs was also observed in the current investigation, $t(6) = 1.47$, $p = .193$. Good conditioning performance in 1 male and 1 female is illustrated in Fig. 2 along with the gender difference in cerebellar size.

It was essential to correct cerebellar volume for physiognomy to equalize the values for men and women before correlations between cerebellar volume and percentage of CRs could be carried out. We corrected with TIV. In a cross-sectional study of 116 healthy volunteers in the age range of 19 months to 80 years, MRI assessment demonstrated that TIV increased with brain volume during childhood to early adolescence and remained stable throughout adulthood and old age (Courchesne et al., 2000).

When TIV was used to correct for physiognomy, the correlation with percentage of CRs was relatively high and positive ($r = .58$, $p = .066$) (Table 2). As expected, a lower correlation was observed when percentage of CRs was correlated with total raw cerebellar volume ($r = .39$, $p = .169$). Thus, in a group of young adults with a limited range of cerebellar volumes (122.73–142.37 ml, $SD = 7.79$), the correlation between percentage of CRs and cerebellar volume corrected for TIV approached statistical significance.

There is an interesting parallel between Purkinje cell–eyeblink conditioning relationships in young rabbits and MRI-assessed cerebellar volume–eyeblink conditioning relationships in young adults. We counted Purkinje cells in 18 3-month-old rabbits that had been classically conditioned (Woodruff-Pak, Cronholm, & Sheffield, 1990). Among young rabbits, there was a statistically significant correlation between Purkinje cell number and trials to a learning criterion of 8 CRs in nine consecutive trials ($r = -.60$, $p < .01$). The correlation was negative because trials to learning criterion increase when learning is poor. Thus, the more Purkinje cells a young rabbit had, the fewer trials it required to attain learning criterion. In a sample of young adult humans, the eyeblink conditioning and corrected cerebellar volume correlations were of a similar magnitude ($r = .58$ with a correction for TIV).

Present-day imaging techniques do not have the degree of resolution that would enable us to assess human Purkinje cell number *in vivo*. Using human cerebellar tissue at autopsy, Nairn, Bedi, Mayhew, and Campbell (1989) reported a statistically significant correlation ($r = .68$, $p < .02$) between stereological counts of Purkinje cell number and gross cerebellar volume. Brain imaging estimates of cerebellar volume may be an indirect measure of Purkinje cell number.

TABLE 1
Studies Using Magnetic Resonance Imaging Volumetric Assessment of Cerebellar Volume in Relation to Age

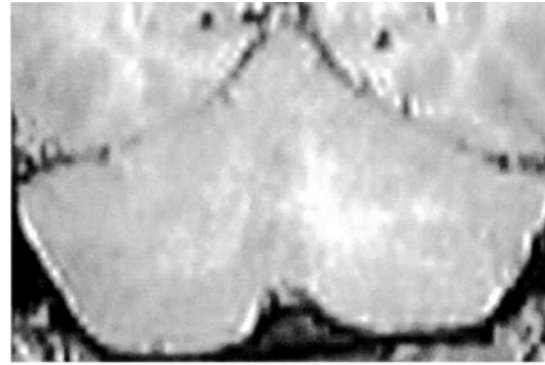
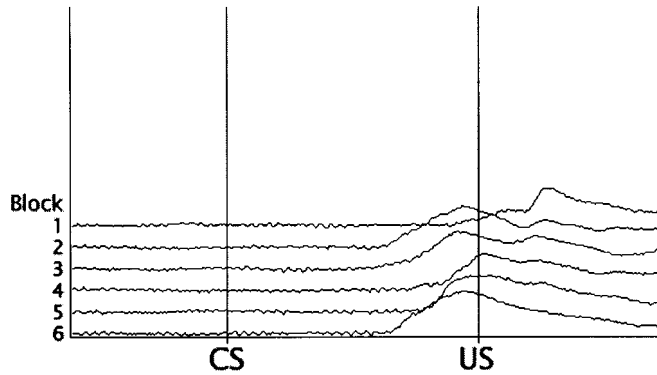
Articles in chronological order of publication	Sample size	Age range of sample (years)	National origin of sample	Magnetic resonance acquisition	Tissue used for volume assessment	Mean cerebellar volume (milliliters)	Range cerebellar volume (milliliters)	Significant M > F cerebellar volume?	<i>r</i> between age and cerebellar volume
1. Escalona et al. (1991)	37	24–79	United States	T2-weighted, 1.5 T	Whole cerebellum	112	81–152	Yes	–.26, <i>p</i> = .11
2. Shah et al. (1991)	36	26–79	United States	1.5 T	Anterior and posterior vermis	—	—	Yes	–.33, <i>p</i> < .05 –.07, <i>p</i> < .68
3. Raz et al. (1992)	59	18–78	United States	0.3 or 1.5 T	Vermis	—	—	No (vermis)	–.54, <i>p</i> < .001 ^a
4. Dupuis et al. (1995)	93	19–77	United States	—	Cerebellar hemisphere	—	—	Yes	–.42 and –.34**
5. Sullivan et al. (1995)	64	22–84	United States	—	Cerebellum	—	—	Yes	–.56 and –.45 ^b
6. Luft et al. (1997)	31	19–73	Germany	1.5 T	Cerebellum/TIV	—	—	No	Not significant
7. Salat et al. (1997)	76	65–95	United States	T1-weighted, 1.5 T	Whole cerebellum	—	—	No	–.04, <i>p</i> = .81
8. Oguro et al. (1998)	152	40–79	Japan	T1-weighted, 0.2 T	Diameter cerebellar vermis/TIV	—	—	Yes	Age difference in men, <i>p</i> < .001
9. Raz et al. (1998)	146	18–77	United States	T1-weighted, 1.5 T	Cerebellar hemisphere and vermis	—	—	Yes	–0.32, <i>p</i> < .01 and –.34, <i>p</i> < .001
10. Luft et al. (1998)	46 ^c	19–73	Germany	T1-weighted, 1.5 T	Total cerebellum and segments	—	—	No	–.46, <i>p</i> < .01 for vermis
11. Luft et al. (1999)	48 ^c	19–73	Germany	T1-weighted, 1.5 T	Total cerebellum and segments	133.8	99.9–170.6	Yes	Age effect beginning at 50 years
12. Rhyu et al. (1999)	124	20–79	Korea	T1-weighted, 1.5 T	Whole cerebellum and vermis	115.4 for females, 126.0 for males	—	Yes	–.02, <i>p</i> > .05 (for whole cerebellum) and –.07, <i>p</i> > .05 (for vermis)
13. Xu et al. (2000)	331	30–79	Japan	T1-weighted, 0.2 T	1 slice, left cerebellum	—	—	Yes	Significant difference between group under 50 years vs group over 50 years

Note. TIV, total intracranial volume.

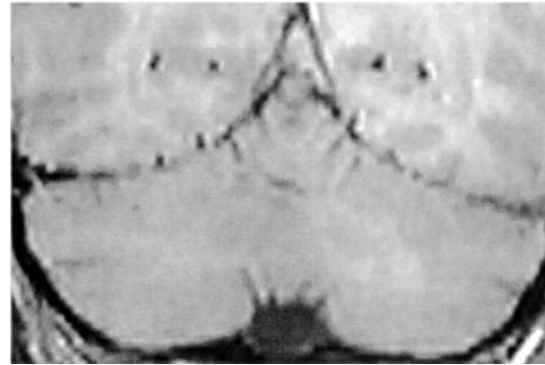
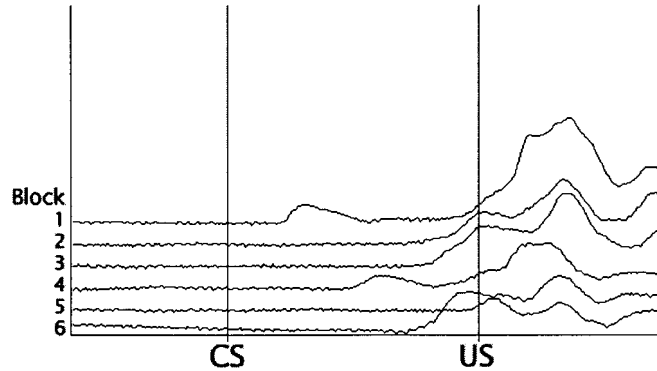
^a For declive, folium, and tuber regions of vermis.

^b Correlation coefficients obtained from Raz's (1997) table.

^c Sample is the same for both studies.



Male, Age 21
141.0 ml



Female, Age 27
130.7 ml

FIG. 2. Performance on eyeblink classical conditioning as indicated by the analog signal assessing eye movement in male and female young adult participants (left) and coronal slices (1.6 mm) of the cerebellum of the same individuals (right). The six lines for each participant show performance averaged over nine paired trials of a tone conditioned stimulus (CS) and corneal air puff unconditioned stimulus (US). A conditioned response (CR) occurs if the participant blinks after the onset of the CS and *before* the onset of the US. In the top record, the participant conditioned rapidly, showing a well-formed CR average in the second block of nine trials. In the bottom record, the first block shows an alpha response that is a startle response to the CS. This is not scored as a CR. In the bottom record, the participant took longer to condition, showing a well-formed CR average in the third block of nine trials.

TABLE 2
Correlations among Cerebellar Volume, TIV, Age, and Percentage of CRs in Young Adult Participants

Subject variable	Raw cerebellum	Cerebellar volume/TIV	TIV
Age	-.18 ($p = .336$)	.12 ($p = .391$)	-.25 ($p = .277$)
Percentage of CRs	.39 ($p = .169$)	.58 ($p = .066$)	-.25 ($p = .275$)

Note. TIV, total intracranial volume; CRs, conditional responses.

Over the adult life span, there is wide variability in performance in eyeblink classical conditioning in the 400-ms delay procedure that shows a significant age-related deficit around the age of 50 years (Durkin et al., 1993; Solomon et al., 1989; Woodruff-Pak & Jaeger, 1998; Woodruff-Pak & Thompson, 1988). Cerebellar volume is relatively stable during young adulthood, but measurable reductions and increasing variability become apparent around the age of 50 years (e.g., Luft, et al., 1998, 1999; Raz, Dupuis, Briggs, McGavran, & Acker, 1998, Table 1). Given the relationship between cerebellar volume and percentage of CRs in a small sample of 8 young adults, it is likely that over the adult life span the correlation between cerebellar volume and percentage of CRs would be high.

Cerebellar Volume and Eyeblink Conditioning Comparisons in Young and Older Adults

Eyeblink conditioning and cerebellar volume data from the young adult participants were compared to data from the older participants tested 1 year earlier (Woodruff-Pak et al., 2000). Absolute cerebellar volumes were corrected using TIV, a physiognomic parameter not related to age (Courchesne et al., 2000; Escalona, et al., 1991; Luft et al., 1998, 1999; Shah, et al., 1991).

The average cerebellar volume in young participants was 135.36 ml, ranging from 122.73 to 142.37 ml. The average cerebellar volume in older participants was 103.81 ml, ranging from 99.3 to 113.9 ml. The distribution of cerebellar volume in our young and older adult samples was nonoverlapping. Young adults had a significantly greater cerebellar volume than did the older adults, $t(14) = 8.96$, $p < .001$ (Table 3 and Fig. 3). Clearly

TABLE 3
Comparison of the Young and Old Groups on Measures of Cerebellar Volume, TIV, and Percentage of CRs

Dependent variable	Young participants ($n = 8$)		Older Participants ($n = 8$)		t test	
	Mean	SD	Mean	SD	t	p
Cerebellar volume	135.36	7.79	103.81	6.21	8.96	.0001
TIV	1502.26	111.18	1442.70	101.16	1.12	.281
Percentage of CRs	56.15	18.51	21.67	17.31	3.85	.002

Note. TIV, total intracranial volume; CRs, conditioned responses.

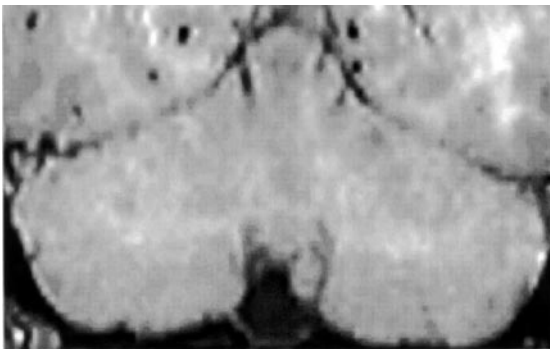
evident in Fig. 3, comparing a coronal slice of young and older adult cerebellum, is the increased size of cerebellar sulci in the older cerebellum due to tissue loss.

Whereas cerebellar volume was nonoverlapping between the young and older samples, TIV was not different between the two groups, $t(14) = 1.12$, $p = .281$ (Table 3). The range of TIV for young adults (1301.39–1586.65 ml) was similar to the range of TIV for older adults (1314.98–1623.52 ml). In this sample of young and older adults, we replicated the results of Courchesne et al. (2000), who evaluated TIV in a large cross-sectional sample covering most of the human life span and demonstrated that TIV remains stable throughout adulthood and old age.

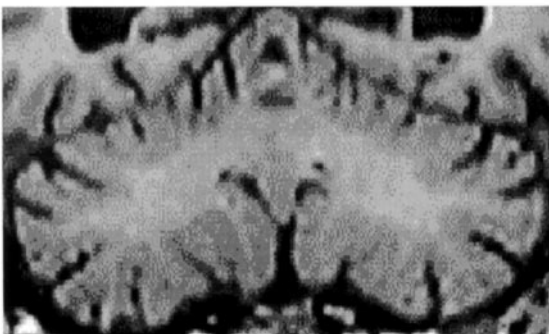
Replicating the well-documented age difference in delay eyeblink classical conditioning, we observed a significant difference between older and younger adults, $t(14) = 3.85$, $p = .002$. The mean percentage of CRs for adults with a mean age of 83 years was 21.70% ($SD = 17.32$), whereas the mean percentage of CRs for adults with a mean age of 27 years was 56.15% ($SD = 18.51$).

Cerebellar Volume and Eyeblink Conditioning Relationships in the Combined Sample

We combined the data of young and older adult participants to a sample of 16 individuals on whom eyeblink classical conditioning in the 400-ms delay procedure and anatomical MRI volumetric data were available. The correlation between percentage of CRs and



Male, Age 33
141.3 ml



Male, Age 78
103.0 ml

FIG. 3. Comparison of a 1.6-mm coronal slice of the MRI of the cerebellum in a young adult (top) and an older adult (bottom). MRIs were assessed with the same General Electric Sigma 1.5-T MRI echo-speed scanner with standard head coil using a T1-weighted axial sequence (whole brain 3D-SPGR, scan time = 8 min, $TR = 27$ ms, $TE = 9$ ms, matrix = 256×192 , slice thickness = 1.6, flip angle = 30 degrees).

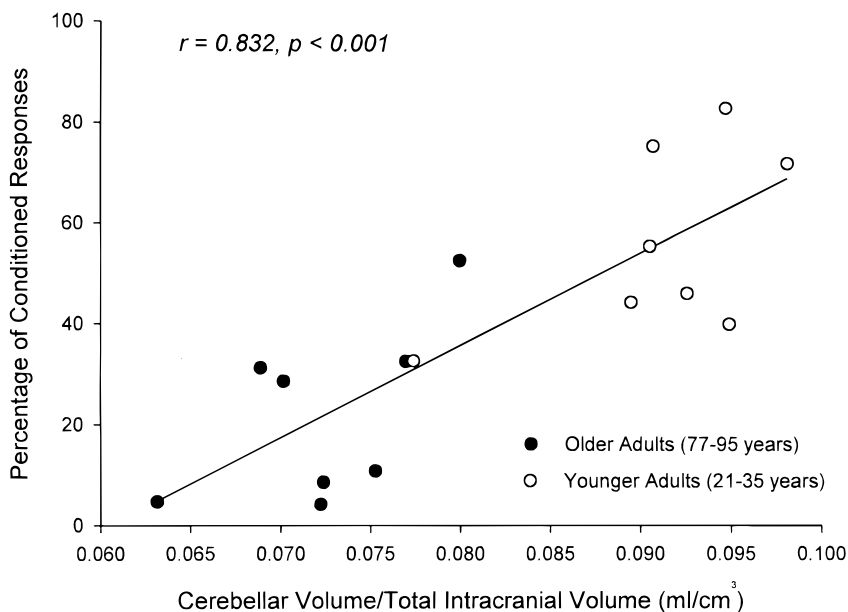


FIG. 4. Association between performance on eyeblink classical conditioning (percentage of conditioned responses) and MRI-assessed cerebellar volume (corrected for total intracranial volume) in a total of 16 participants (8 young adults and 8 older adults).

cerebellar volume corrected for TIV was high and statistically significant ($r = .832$, $p < .0001$) (Fig. 4). In the case of the sample of 16 participants, even the raw cerebellar volume correlated significantly with percentage of CRs ($r = .803$, $p < .0001$).

There is evidence for a role of cerebellar cortex in the rate and magnitude of acquisition of CRs. The largest cells in cerebellar cortex, Purkinje cells, are vulnerable in normal aging. For example, Hall, Miller, and Corsellis (1975) assessed 90 normal cerebella at autopsy in participants ranging from childhood to over the age of 100 years. Wide individual variations were found at all ages, but a mean reduction of 2.5% of the Purkinje cells per decade was found, representing a 25% reduction over the 100-year period of life that was studied. A comparable age-related decline in Purkinje cell numbers was observed in cerebellar vermis by Torvik, Tork, and Lindboe (1986). Scheibel (1996) reported that microscopic studies in his laboratory revealed loss of both Purkinje and granule cells in the older cerebellar cortex. Granule cell loss may occur as a consequence of Purkinje cell loss because granule cells synapse on Purkinje cells (Guerri, 1998). Loss of granule cells would reduce the CS input to Purkinje cells in cerebellar cortex and principal cells in the deep nuclear region. In Scheibel's (1996) opinion, because the Purkinje cell is the primary source of outflow from cerebellar cortex, it follows that age-related alterations must affect cerebellar modulation of many neural systems. Scheibel emphasized the functional consequences of the loss of Purkinje cell axons. Luft et al. (1999) focused on the structural consequences of Purkinje cell loss and suggested that the axons of Purkinje cells comprise the major part of cerebellar white matter. From this perspective, white matter reduction may significantly contribute to the age-associated loss of overall cerebellar volume.

As illustrated in Fig. 1, cerebellar cortical Purkinje cells are one of the central integrating cells in Thompson's model of the cerebellar circuit for eyeblink classical conditioning.

Our results demonstrate that lower cerebellar volume (corrected for physiognomy) in humans is associated with slower CR acquisition in a group of young adults. It has been our working hypothesis that Purkinje cell loss associated with normal aging reduces the central integration of CS–US input that occurs in the cerebellar cortex. Cerebellar Purkinje cells are among the most vulnerable cells in the nervous system. They fire at a high rate throughout both waking and sleep cycles and, thus, are always metabolically active and requiring constant replenishment of oxygen and glucose. Purkinje cells are selectively vulnerable to anoxia (e.g., Myers & Yamaguchi, 1977) and toxins such as ethanol (e.g., Pierce, Williams, & Light, 1999). Cerebellar volume in human adults is significantly reduced after the age of 50 years, as is performance on eyeblink classical conditioning. Given the essential role of the cerebellum in the acquisition of CRs, age-related reductions in the volume of the cerebellum may account for a significant portion of the age-related difference in human eyeblink classical conditioning.

REFERENCES

- Berger, T. W., Alger, B., & Thompson, R. F. (1976). Neuronal substrate of classical conditioning in the hippocampus. *Science*, **192**, 483–485.
- Berger, T. W., Berry, S. D., & Thompson, R. F. (1986). Role of the hippocampus in classical conditioning of aversive and appetitive behaviors. In R. L. Isaacson & K. H. Pribram (Eds.), *The hippocampus* (pp. 203–239). New York: Plenum.
- Blaxton, T. A., Zeffiro, T. A., Gabrieli, J. D. E., Bookheimer, S. Y., Carrillo, M. C., Theodore, W. H., & Disterhoft, J. F. (1996). Functional mapping of human learning: A positron-emission tomography study of eyeblink conditioning. *Journal of Neuroscience*, **16**, 4032–4040.
- Bloedel, J. R. (1993). “Involvement in” versus “storage of.” *Trends in Neuroscience*, **16**, 451–452.
- Bower, J. M. (1997). Control of sensory data acquisition. In J. D. Schmahmann (Ed.), *International Review of Neurobiology*, Vol. 41: *The cerebellum and cognition* (pp. 490–513), San Diego: Academic Press.
- Chen, L., Bao, S., Lockard, J. M., Kim, J. J., & Thompson, R. F. (1996). Impaired classical eyeblink conditioning in cerebellar lesioned and Purkinje cell degeneration (*pcd*) mutant mice. *Journal of Neuroscience*, **16**, 2829–2838.
- Courchesne, E., Chisum, H. J., Townsend, J., Cowles, A., Covington, J., Egaas, B., Harwood, M., Hinds, S., & Press, G. A. (2000). Normal brain development and aging: Quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology*, **216**, 672–682.
- Daum, I., & Schugens, M. M. (1996). On the cerebellum and classical conditioning. *Current Directions in Psychological Science*, **5**, 58–61.
- Daum, I., Schugens, M. M., Ackermann, H., Lutzenberger, W., Dichgans, J., & Birbaumer, N. (1993). Classical conditioning after cerebellar lesions in humans. *Behavioral Neuroscience*, **107**, 748–756.
- Downey-Lamb, M. M., & Woodruff-Pak, D. S. (1998). Dual-task performance and nondeclarative brain memory systems. *Society for Neuroscience Abstracts*, **24**, 1898.
- Dupuis, J. H., McGavran, C., Raz, N., Briggs, S. D., & Acker, J. D. (1995). Aging of the cerebellar hemisphere and vermis observed in vivo. *Society for Neuroscience Abstracts*, **21**, 1563.
- Durkin, M., Prescott, L., Furchtgott, E., Cantor, J., & Powell, D. A. (1993). Concomitant eyeblink and heart rate classical conditioning in young, middle-aged, and elderly human subjects. *Psychology and Aging*, **8**, 571–581.
- Escalona, P. R., McDonald, W. M., Doraiswamy, P. M., Boyko, O. B., Husain, M. M., Figiel, G. S., Laskowitz, D., Ellinwood, E. H., & Krishnan, K. R. R. (1991). In vivo stereological assessment of human cerebellar volume: Effects of gender and age. *American Journal of Neuroradiology*, **12**, 927–929.
- Green, J. T., & Woodruff-Pak, D. S. (1997). Concurrent eyeblink classical conditioning and rotary pursuit performance: Implications for independent nondeclarative systems. *Neuropsychology*, **11**, 474–487.

- Guerri, C. (1998). Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, **22**, 304–312.
- Hall, T. C., Miller, K. H., & Corsellis, J. A. N. (1975). Variations in the human Purkinje cell population according to age and sex. *Neuropathology and Applied Neurobiology*, **1**, 267–292.
- Lavond, D. G., & Cartford, M. C. (2000). Eyeblink conditioning circuitry: Tracing, lesion, and reversible lesion experiments. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning*, Vol. 2: *Animal models* (pp. 51–80). Boston: Kluwer Academic.
- Lavond, D. G., & Steinmetz, J. E. (1989). Acquisition of classical conditioning without cerebellar cortex. *Behavioral Brain Research*, **33**, 113–164.
- Lemieux, S. K., & Woodruff-Pak, D. S. (2000). Functional magnetic resonance imaging studies of eyeblink classical conditioning. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning*, Vol. 1: *Applications in humans* (pp. 71–93). Boston: Kluwer Academic.
- Llinas, R., & Sotelo, C. (1992). *The cerebellum revisited*. New York: Springer-Verlag.
- Logan, C. G., & Grafton, S. T. (1995). Functional anatomy of human eyeblink conditioning determined with regional cerebral glucose metabolism and positron emission tomography. *Proceedings of the National Academy of Science (USA)*, **92**, 7500–7504.
- Luft, A. R., Skalej, M., Schulz, J. B., Welte, D., Kolb, R., Burk, K., Klockgether, T., & Voigt, K. (1999). Patterns of age-related shrinkage in cerebellum and brainstem observed in vivo using three-dimensional MRI volumetry. *Cerebral Cortex*, **9**, 712–721.
- Luft, A. R., Skalej, M., Welte, D., Kolb, R., Burk, K., Schulz, J. B., Klockgether, T., & Voigt, K. (1998). A new semiautomated, three-dimensional technique allowing precise quantification of total and regional cerebellar volume using MRI. *Magnetic Resonance in Medicine*, **40**, 143–151.
- Luft, A. R., Skalej, M., Welte, D., Voigt, K., & Klockgether, T. (1997). Age and sex do not affect cerebellar volume in humans. [letter] *American Journal of Neuroradiology*, **18**, 593–594.
- Lye, R. H., O'Boyle, D. J., Ramsden, R. T., & Schady, W. (1988). Effects of a unilateral cerebellar lesion on the acquisition of eye-blink conditioning in man. *Journal of Physiology (London)*, **403**, 58P.
- McCormick, D. A., Lavond, D. G., Clark, G. A., Kettner, R. E., Rising, C. E., & Thompson, R. F. (1981). The rabbit nictitating membrane and eyelid responses: Correlations and implications. *Physiological Behavior*, **28**, 769–775.
- McCormick, D. A., & Thompson, R. F. (1984). Cerebellum: Essential involvement in the classically conditioned eyelid response. *Science*, **223**, 296–299.
- McIntosh, A. R., & Schreurs, B. G. (2000). Functional networks underlying human eyeblink conditioning. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning*, Vol. 1: *Applications in humans* (pp. 51–69). Boston: Kluwer Academic.
- Molchan, S. E., Sunderland, T., McIntosh, A. R., Herscovitch, P., & Schreurs, B. G. (1994). A functional anatomical study of associative learning in humans. *Proceedings of the National Academy of Science (USA)*, **91**, 8122–8126.
- Myers, R. E., & Yamaguchi, S.-I. (1977). Nervous system effects of cardiac arrest in monkeys. *Archives of Neurology*, **34**, 65–74.
- Nairn, J. G., Bedi, K. S., Mayhew, T. M., & Campbell, L. F. (1989). On the number of Purkinje cells in the human cerebellum: Unbiased estimates obtained by using the “fractionator.” *Journal of Comparative Neurology*, **290**, 527–532.
- Oguro, H., Okada, K., Yamaguchi, S., & Kobayashi, S. (1998). Sex differences in morphology of the brain stem and cerebellum with normal ageing. *Neuroradiology*, **40**, 788–792.
- Papka, M., Ivry, R. B., & Woodruff-Pak, D. S. (1995). Selective disruption of eyeblink classical conditioning by concurrent tapping. *Neuroreport*, **6**, 1493–1497.
- Pierce, D. R., Williams, K., & Light, K. E. (1999). Purkinje cell vulnerability to developmental ethanol exposure in the rat cerebellum. *Alcoholism: Clinical and Experimental Research*, **23**, 1650–1659.
- Ramrani, N., Toni, I., Josephs, J., Ashburner, J., & Passingham, R. E. (2000). Learning- and expectation-related changes in the human brain during motor learning. *Journal of Neurophysiology*, **84**, 3026–3035.

- Raz, N., Dupuis, J. H., Briggs, S. D., McGavran, C., & Acker, J. D. (1998). Differential effects of age and sex on the cerebellar hemispheres and the vermis: A prospective MR study. *American Journal of Neuroradiology*, **19**, 65–71.
- Raz, N., Torres, I. J., Spencer, W. D., White, K., & Acker, J. D. (1992). Age-related regional differences in cerebellar vermis observed in vivo. *Archives of Neurology*, **49**, 412–416.
- Rhyu, J., Cho, T. H., Lee, N. J., Uhm, C.-S., Kim, H., & Suh, Y.-S. (1999). Magnetic resonance image-based cerebellar volumetry in healthy Korean adults. *Neuroscience Letters*, **270**, 149–152.
- Salat, D., Ward, A., Kaye, J. A., & Janowsky, J. S. (1997). Sex differences in the corpus callosum with aging. *Neurobiology of Aging*, **18**, 191–197.
- Scheibel, A. B. (1996). Structural and functional changes in the aging brain. In J. E. Birren & K. W. Schaie (Eds.), *Handbook of the psychology of aging* (4th ed., pp. 105–128). San Diego: Academic Press.
- Shah, S. A., Doraiswamy, P. M., Husain, M. M., Figiel, G. S., Boyko, O. B., McDonald, W. M., Ellinwood, E. H., & Krishnan, K. R. R. (1991). Assessment of posterior fossa structures with midsagittal MRI: The effects of aging. *Neurobiology of Aging*, **12**, 371–374.
- Solomon, P. R., Pomerleau, D., Bennett, L., James, J., & Morse, D. L. (1989). Acquisition of the classically conditioned eyeblink response in humans over the lifespan. *Psychology and Aging*, **4**, 34–41.
- Solomon, P. R., Stowe, G. T., & Pendlebury, W. W. (1989). Disrupted eyelid conditioning in a patient with damage to cerebellar afferents. *Behavioral Neuroscience*, **103**, 898–902.
- Steinmetz, J. E. (1996). The brain substrates of classical eyeblink conditioning in rabbits. In J. R. Bloedel, T. J. Ebner, & S. P. Wise (Eds.), *The acquisition of motor behavior in vertebrates* (pp. 89–114). Cambridge, MA: MIT Press.
- Steinmetz, J. E. (2000). Brain substrates of classical eyeblink conditioning: A highly localized but also distributed system. *Behavioural Brain Research*, **110**, 13–24.
- Sullivan, M. P., De Toledo-Morrell, L., Morrell, F., & Spencer, S. (1995). MRI detected cerebellar atrophy during aging. *Society for Neuroscience Abstracts*, **21**, 1708.
- Thompson, R. F. (1986). The neurobiology of learning and memory. *Science*, **233**, 941–947.
- Thompson, R. F. (2000). Discovering the brain substrates of eyeblink classical conditioning. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning*, Vol. 2: *Animal models* (pp. 17–49). Boston: Kluwer Academic.
- Thompson, R. F., & Krupa, D. J. (1994). Organization of memory traces in the mammalian brain. *Annual Review of Neuroscience*, **17**, 519–549.
- Timmann, D., Kolb, F. B., Baier, C., Rijntjes, M., Muller, S. P., Diener, H. C., & Weiller, C. (1996). Cerebellar activation during classical conditioning of the human flexion reflex: A PET study. *NeuroReport*, **7**, 2056–2060.
- Topka, H., Valls-Sole, J., Massaquoi, S. G., & Hallett, M. (1993). Deficit in classical conditioning in patients with cerebellar degeneration. *Brain*, **116**, 961–969.
- Torvik, A., Torp, S., & Lindboe, C. F. (1986). Atrophy of the cerebellar vermis in ageing. A morphometric and histologic study. *Journal of Neurological Science*, **76**, 283–294.
- Welsh, J. P., & Harvey, J. A. (1989). Cerebellar lesions and the nictitating membrane reflex: Performance deficits of the conditioned and unconditioned response. *Journal of Neuroscience*, **9**, 299–311.
- Welsh, J. P., & Harvey, J. A. (1991). Pavlovian conditioning in the rabbit during inactivation of the interpositus nucleus. *Journal of Physiology*, **444**, 459–480.
- Woodruff-Pak, D. S. (1997). Classical conditioning. In J. D. Schmahmann (Ed.), *International Review of Neurobiology*, Vol. 41: *The cerebellum and cognition* (pp. 341–366). San Diego: Academic Press.
- Woodruff-Pak, D. S., Cronholm, J. F., & Sheffield, J. B. (1990). Purkinje cell number related to rate of eyeblink classical conditioning. *NeuroReport*, **1**, 165–168.
- Woodruff-Pak, D. S., Goldenberg, G., Downey-Lamb, M. M., Boyko, O. B., & Lemieux, S. K. (2000). Cerebellar volume in humans related to magnitude of classical conditioning. *NeuroReport*, **14**, 609–615.
- Woodruff-Pak, D. S., & Jaeger, M. (1998). Predictors of eyeblink classical conditioning over the adult age span. *Psychology and Aging*, **13**, 193–205.

- Woodruff-Pak, D. S., Papka, M., & Ivry, R. B. (1996). Cerebellar involvement in eyeblink classical conditioning in humans. *Neuropsychology*, **10**, 443–458.
- Woodruff-Pak, D. S., & Thompson, R. F. (1988). Classical conditioning of the eyeblink response in the delay paradigm in adults aged 18–83 years. *Psychology and Aging*, **3**, 219–229.
- Xu, J., Kobayashi, S., Yamaguchi, S., Iijima, K.-I., Okada, K., & Yamashita, K. (2000). Gender effects on age-related changes in brain structure. *American Journal of Neuroradiology*, **21**, 112–118.