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Comparison of eyeblink conditioning in patients with superior and posterior inferior cerebellar lesions

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Summary

The aim of the present study was to compare eyeblink conditioning in cerebellar patients with lesions including the territory of the superior cerebellar artery (SCA) and in patients with lesions restricted to the territory of the posterior inferior cerebellar artery (PICA). The cerebellar areas known to be most critical in eyeblink conditioning based on animal data (i.e. Larsell lobule H VI and interposed nucleus) are commonly supplied by the SCA. Eyeblink conditioning was expected to be impaired in SCA, but not in PICA patients. A total of 27 cerebellar patients and 25 age-matched controls were tested. Cerebellar lesions were primarily unilateral (n = 20). Most patients suffered from ischaemic infarctions of the SCA (n = 11) or the PICA (n = 13). The other patients presented with cerebellar tumours (n = 2) and cerebellar agenesis (n = 1). The extent of the cortical lesion (i.e. which lobuli were affected) and possible involvement of the cerebellar nuclei was determined by 3D-MRI. As expected, the ability to acquire classically conditioned eyeblink responses was significantly reduced in the group of all cerebellar patients compared with the controls. In the patients with unilateral cerebellar lesions, conditioning deficits were present ipsilaterally. In SCA patients with lesions including hemispheral lobules VI and Crus I, eyeblink conditioning was significantly reduced on the affected side compared with the unaffected side. No significant difference between the affected and unaffected sides was present in patients with lesions restricted to the common PICA territory (i.e. Crus II and below). Conditioning deficits were neither significantly different in SCA patients with pure cortical lesions compared with SCA patients with additional nuclear impairment nor in SCA patients with unilateral lesions compared with SCA patients with bilateral lesions. To summarize, unilateral cortical lesions of the superior cerebellum appear to be sufficient to reduce eyeblink conditioning in humans significantly.

Keywords: eyeblink conditioning; cerebellum; superior cerebellar artery; posterior inferior cerebellar artery; human

Abbreviations: AC–PC = anterior commissure– posterior commissure; ANOVA = analysis of variance; CR = conditioned responses; CS = conditioned stimulus; fMRI = functional MRI; MNI = Montreal Neurological Institute; PICA = posterior inferior cerebellar artery; SCA = superior cerebellar artery; US = unconditioned stimulus

Introduction

Classical conditioning of the eyeblink reflex has frequently been used as a simple model to study neural substrates involved in motor learning. Numerous studies provide clear evidence for critical involvement of cerebellar structures in this form of associative learning. The parts of the cerebellar cortex and cerebellar nuclei involved in eyeblink conditioning have been assessed carefully in animal models. Animal lesion studies, most of them in the rabbit, indicate that the ipsilateral interposed nucleus and Larsell lobule H VI are of particular importance for acquiring conditioned responses (for a review, see Bloedel and Bracha, 1995; Thompson *et al*., 1997; Yeo and Hesslow, 1998; Steinmetz, 2000). Despite detailed knowledge about cerebellar sites participating in eyeblink conditioning, the exact role of the cerebellar nuclei and cortical structures in learning and how these different sites influence one another are a matter of ongoing discussion. The function(s) of the cerebellum may be to form and store memory traces for associative learning, motor execution and/

or timing (Welsh and Harvey, 1989; Raymond *et al*., 1996; Thompson *et al*., 1997; Mauk *et al*., 2000).

There is evidence from several human lesion and functional brain imaging studies that the human cerebellum is also involved in classical eyeblink conditioning. Eyeblink conditioning has been shown to be impaired in patients with cerebellar lesions (Lye *et al*., 1988; Solomon *et al*., 1989*a*; Daum *et al*., 1993; Topka *et al*., 1993; Woodruff-Pak *et al*., 1996; Timmann *et al*., 1998; Bracha *et al*., 1997, 2000). Studies using PET and functional MRI (fMRI) revealed learning-related changes of activity in the cerebellum during eyeblink conditioning in healthy human subjects (Molchan *et al*., 1994; Logan and Grafton, 1995; Blaxton *et al*., 1996; Schreurs *et al*., 1997; Ramnani *et al*., 2000).

Information about the localization of learning-dependent sites within the human cerebellum, however, is more limited. Patients with diffuse degenerative cerebellar disease were studied by Topka and colleagues (pure cortical atrophy: $n = 5$; olivopontocerebellar atrophy: $n = 7$) (Topka *et al*., 1993). No conclusions could be drawn regarding localization. Daum *et al*. (1993) tested four patients with degenerative cerebellar disorders and three patients with unilateral lesions. The patients with unilateral lesions were tested on the affected side only and no attempt was made to localize conditioning-dependent areas within the cerebellum. Three case reports (Lye *et al*., 1988; Solomon *et al*., 1989*a*; Timmann *et al*., 1998) and two group studies [Woodruff-Pak *et al*., 1996 ($n = 7$); Bracha *et al*., 1997, 2000 (total of five patients, with three participating in both studies)] investigated patients with unilateral lesions and compared the effects of learning on the affected and unaffected side. All but one group observed reduced eyeblink conditioning on the affected side only (Lye *et al*., 1988; Solomon *et al*., 1989*a*; Woodruff-Pak *et al*., 1996; Timmann *et al*., 1998). However, Bracha *et al*. (1997, 2000) found a bilateral conditioning deficit using a cutaneous tap on the forehead midline for unconditioned stimulus (US).

PET studies reported significant changes of blood flow in more widespread areas of the cerebellar cortex bilaterally, and in the cerebellar vermis during eyeblink conditioning (Molchan *et al*., 1994; Logan and Grafton, 1995; Blaxton *et al*., 1996; Schreurs *et al*., 1997). A recent fMRI study in healthy human subjects, which found activation of ipsilateral cerebellar hemispheral lobules VI and Crus I during eyeblink conditioning (Ramnani *et al*., 2000), is in good agreement with findings in animal studies. Activation of the deep nuclei, however, was not detectable.

The aim of the present investigation was to examine in more detail which cerebellar areas are critically involved in eyeblink conditioning in a human lesion study. Classical eyeblink conditioning was investigated in 27 patients with primarily unilateral lesions—in particular infarcts of the superior cerebellar artery (SCA) and the posterior inferior cerebellar artery (PICA). The extent of the cortical lesion (i.e. which lobuli were affected) and possible involvement of the cerebellar nuclei was determined by MRI. The use of a T_1-weighted fast low-angle (FLASH) sequence enabled us to visualize the deep cerebellar nuclei and possible pathology. 3D-MRI data sets were acquired in each patient and transferred in a standard proportional stereotaxic space. The affected lobuli were defined with the help of the 3D-MRI atlas of the cerebellar cortex developed by Schmahmann *et al*. (2000). The location of cerebellar nuclei was confirmed with the help of the 3D-MRI atlas of the human cerebellar nuclei developed by our group (Dimitrova *et al*., 2002*a*). The cerebellar areas known to be most critical in eyeblink conditioning based on animal data (i.e. Larsell lobule H VI and interposed nuclei) are commonly supplied by the SCA (Amarenco *et al*., 1993). Therefore, we hypothesized that conditioning of the eyeblink reflex would be impaired in patients with lesions including the territory of the SCA, but preserved in patients with lesions restricted to the territory of the PICA.

Material and methods

Subjects

A total of 27 cerebellar patients (19 male, eight female, mean age 54.9 years, SD 12.2 years, range 24–75 years) and 25 healthy subjects (16 male, nine female, mean age 53.7 years, SD 14.3 years, range 24–76 years) participated. All patients suffered from isolated cerebellar disease based upon neurological examination and brain MRI scans. Thirteen patients presented with infarctions within the PICA territory. Their average age was 54.4 years, SD 9.5 years. Eleven PICA patients were male and two were female. Fourteen patients presented with lesions including the SCA territory. Their average age was 55.5 years, SD 14.9 years. Eight SCA patients were male and six were female. Eight patients presented with infarctions within the SCA territory, while three had infarctions within the SCA and PICA territory. The remaining three patients suffered from cerebellar angioma, surgical lesion following astrocytoma and cerebellar agenesis. The neurological examination included the ataxia rating scale from Trouillas *et al*. (1997). In the PICA group, 11 patients presented with no or mild signs of cerebellar ataxia (total ataxia score <10 out of 100) and two with moderate signs of cerebellar ataxia (total ataxia score 10–20). In the SCA group, seven patients showed mild signs of cerebellar ataxia, five patients moderate signs and two patients marked signs of cerebellar ataxia (total ataxia score >20). Clinical data for the patients and their matched controls are summarized in Table 1.

None of the control subjects had a history of neurological disease or revealed neurological signs based upon neurological examination. Both controls and cerebellar patients were not receiving any medication modifying nervous system functions. Neither the cerebellar patients nor the control subjects showed clinical evidence of hearing difficulties or reduced visual sight on the routine neurological evaluation. The local ethical committee of the University of Essen,

Table 1 *Summary of cerebellar patients and matched healthy control subjects*

Cerebellar patients											Control subjects		
No.	Cerebellar disorder	Age (years)		Disease duration (months)	Ataxia rating scale*						Box No.	Age (years)	Sex
					Stance/ gait (0–34)	Upper limbs (0–36)	Lower limbs (0–16)	Speech (0–8)	Oculo-motor (0–6)	Total score (0–100)			
cer-01	PICA left	38	F	1	0	0	0	0	0	0	con-01	24	F
cer-02	PICA right	56	M	2	3	0	0	0	0	3			
cer-03	PICA left	57	M	2	6	3	0	0	0	9	con-02	61	M
cer-04	PICA right	64	M	0.5	2	0	0	0	1	3	con-03	73	M
cer-05	PICA right	63	M	3	0	0	0	0	0	0	con-04	61	M
cer-06	PICA bilateral	57	M	0.5	0	0	0	0	0	0	con-05	59	F
cer-07	PICA right	51	M	3	6	0	1	0	0	7	con-06	51	F
cer-08	PICA left	62	M	28	0	1	0	0	0	1	con-07	64	M
cer-09	PICA left	49	M	1	2	1	0	0	2	5	con-08	51	M
cer-10	PICA left	57	M	9	7	2	1	0	1	11	con-09	62	M
cer-11	PICA left	36	M	0.25	2	0	0	0	0	2	con-10	34	M
cer-12	PICA left	50	F	0.5	7	0	0	0	0	7	con-11	48	F
cer-13	PICA left	67	M	1.5	9	4	1	0	0	14	con-12	69	M
cer-14	SCA left	50	F	1.5	0	4	0	0	0	4	con-13	52	F
cer-15	SCA left	53	F	38	2	1	0	0	0	3	con-14	53	F
cer-16	SCA left	65	M	2	2	0	1	0	0	3	con-15	26	M
cer-17	SCA left	75	F	0.5	27	1	2	0	1	31	con-16	74	M
cer-18	SCA/PICA left	45	M	0.25	4	4	2	0	0	10	con-17	46	M
cer-19	SCA left	67	M	1	5	0	3	0	0	8	con-18	66	M
cer-20	SCA right	56	M	5	4	6	0	2	0	12			
cer-21	SCA/PICA left	73	F	23	9	3	0	0	1	13	con-19	76	M
cer-22	SCA/PICA right	38	M	30	0	1	0	0	0	1	con-20	37	F
cer-23	SCA bilateral	39	M	1	21	13	5	5	2	46	con-21	39	F
cer-24	Angioma bilateral	24	M	36	4	3	0	1	3	11	con-22	38	M
cer-25	Astrocytoma bilateral	68	F	42 years	10	1	0	0	3	14	con-23	61	F
cer-26	Cerebellar agenesis	59	F	59 years	7	7	4	2	0	20	con-24	60	F
cer-27	SCA right	65	M	33	2	0	1	0	0	3	con-25	60	F

Scaling of cerebellar symptoms according to International Cooperative Ataxia Rating Scale (Trouillas *et al*., 1997). No. = patient and control subject code; * = range of ataxia scores with zero indicating normal performance.

Germany, approved the study. All subjects gave informed written consent.

Imaging procedures

In cerebellar patients, affected lobuli and possible involvement of deep cerebellar nuclei were defined by individual 3D-MRI data sets, which were normalized spatially to standard stereotaxic brain space according to the Montreal Neurological Institute (MNI) protocol and presented within a Talairach grid (Evans *et al*., 1994). 3D-MRI data sets were available in all but three cerebellar patients. Two patients (cer-13 and cer-27 in Table 1) had a cardiac pacemaker inserted and only cranial CT scans were available. One patient (cer-12) did not participate because of claustrophobia; 2D diagnostic MRIs were available for this patient.

A 3D axial volume of the cerebellum was acquired using a T_1-weighted fast low-angle (FLASH) sequence on a Siemens Sonata 1.5 Tesla MR (FOV (field of view) = 220 mm, number of partitions = 56, voxel size = 1.15 × 0.86 × 1.5 mm^3, TR/TE (repetition time/echo time) = 80/50 ms, flip angle = 8°). This FLASH sequence has been found to be useful in visualizing deep cerebellar nuclei in individual healthy subjects (Dimitrova *et al*., 2002*a*). Each volume was registered, resampled to 1.00 × 1.00 × 1.00 mm^3 voxel size and normalized spatially into a standard proportional stereotaxic space (the MNI 152 space) using SPM99 software (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). Talairach proportional coordinate space is widely used as a standard reference coordinate system for reporting results of functional and structural MRI studies. The MNI 152 space is a new standard template introduced by the Montreal Neurological Institute, which approximately matches the Talairach space and is based on a large series of MRI scans of normal controls (Evans *et al*., 1994). The MNI coordinates of individual cerebellar lesions were determined in the horizontal (x), the sagittal (y) and vertical (z) directions. Measured values correspond to distances in mm from the AC–PC line (anterior commissure–posterior commissure). Negative x-values indicate locations left of the AC–PC line (positive x-values right), negative y-values behind the AC (positive y-values in front of

AC) and negative *z*-values below the AC–PC line (positive *z*-values above). The affected cerebellar lobuli were defined with the help of the 3D-MRI atlas of the cerebellum introduced by Schmahmann *et al*. (2000). Unless otherwise stated, cerebellar lobuli were named according to the nomenclature proposed by Schmahmann *et al*. (2000) (e.g. Larsell lobule H VI = Schmahmann hemispheral lobule VI). The interposed and dentate nuclei and possible pathology were identified visually and the location of the nuclei was confirmed with the help of the 3D-MRI atlas of the cerebellar nuclei proposed by Dimitrova *et al*. (2002*a*). The following MNI coordinates were used to localize cerebellar nuclei: (left) interposed nucleus $x = -8$ to -12 mm, $y = -51$ to -61 mm, $z = -24$ to -31 mm; (left) dentate nucleus: $x = -22$ to -12 mm, $y = -47$ to -68 mm, $z = -32$ to -45 mm.

In addition, cerebellar lesions were traced manually on axial slices of individual non-normalized 3D-MRI data sets and saved as regions of interest using MRIcro software (http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html; Rorden and Brett, 2000). The volumes of each individual lesion and complete 3D-MRI data set were simultaneously normalized stereotaxically by SPM99 as described above. Right-sided lesions were flipped to the left. The same MRIcro software was used to superimpose individual stereotaxically normalized cerebellar lesions. The localization of the centres of overlap in the SCA and PICA groups was defined on the basis of MNI spatial coordinates. Superimposed lesions of the PICA and SCA patients were displayed on 3D-MRI data sets of individual healthy subjects.

Furthermore, axial and sagittal 2D T_2 weighted images of the entire brain were acquired in each patient (sagittal series: FOV = 240 mm, number of partitions: 19, voxel size $0.8 \times 0.5 \times 3$ mm^3, TR/TE = 4870/102 ms; axial series: FOV = 230 mm, number of partitions = 19, voxel size = $1.0 \times 0.9 \times 6$ mm^3, TR/TE = 4800/105 ms). T_2 weighted images were inspected visually for possible extracerebellar lesions within the brainstem and cerebrum. Patients who revealed evidence of additional brainstem involvement or other extracerebellar lesions, such as subcortical arteriosclerotic encephalopathy, were excluded from the study.

Conditioning paradigms

Eyeblink conditioning was performed using the standard delay protocol developed by Gormezano and Kehoe (1975). The conditioned stimulus (CS) preceded the unconditioned stimulus (US) by a fixed time interval and co-terminated with the US. The US consisted of an air puff (duration 100 ms; 4 bar) provided through a nozzle mounted on goggles worn by the subject. The nozzle was set to direct the air puff to the periorbital region near the outer canthus of the eye at a distance of ~10 mm. The CS started 310 ms after onset of each trial and consisted of an initially neutral tone [1000 Hz, 70 dB sound pressure level (SPL), duration 540 ms] given via headphones ipsilateral to the US and preceded the US by 460 ms. The CS was superimposed on a continuous white noise of 60 dB SPL applied bilaterally to mask environmental noise. Five CS-alone trials and five US-alone trials were presented in random sequence at the beginning of the experiment. This was followed by 100-paired CS–US trials. At the end of the experiment, ten CS-alone extinction trials were given. The inter-trial interval varied randomly from 20 to 35 s throughout the experiment. Surface EMG recordings were taken from orbicularis oculi muscles bilaterally. The overlying skin was cleaned with isopropyl alcohol (75%). Signals were fed to EMG amplifiers (sampling rate 1000 Hz, band pass filter frequency between 100 Hz and 2 kHz), full wave rectified and further filtered offline (100 Hz).

After participants were set comfortably in a chair with their eyes open, a silent movie (Charlie Chaplin's *City Lights* or *Modern Times*) was shown using a DVD player to maintain vigilance and attention. Participants were informed about the air puff delivered lateral to their eye, and combined with a tone presented to the ipsilateral ear. The neurophysiological background and essentials of classical conditioning were not explained.

The right and left eyes were tested in all patients and control subjects. In patients with unilateral lesions, the ipsilesional eye was tested first. Testing was performed on two separate days. The second eye was tested within one week in 41% and within two weeks in 19% of the patients (median 9 days). The second eye was tested within one week in 60% and within two weeks in 24% of the controls (median 7 days). In ten patients, the right eye was tested first and in 17 patients, the left eye. In 13 controls, the right eye was tested first and, in 12 controls, the left eye.

Data analysis

EMG recordings of the paired CS–US trials were analysed on a trial-by-trial basis using commercial software (Axograph 3.0, Axon Instruments Inc., USA). Conditioned responses were identified visually. Conditioned responses were defined as EMG responses within the CS–US window that deviated significantly from the pre-CS EMG baseline level. Responses earlier than 150 ms after CS onset were regarded as reflexive responses to the tone (i.e. alpha responses) and not conditioning-related (Woodruff-Pak *et al*., 1996). Trials that showed a spontaneous blink prior to CS onset were excluded from analysis (Bracha *et al*., 1997, 2000). The total number of trials recorded was subdivided into blocks of ten trials each. The number of conditioned responses (CR) was expressed as the percentage of trials containing responses with respect to each block of ten trials (percentage CR-incidence) and the total number of trials (total percentage CR-incidence). To estimate the frequency of spontaneous blinks, responses in the 260 ms time interval prior to CS onset were counted in each trial and the average rate of spontaneous blinks (blinks per minute) calculated across the experiment.

Statistics

Analyses of variance for repeated measures (ANOVA) were calculated with percentage CR-incidence as dependent variable, block (1–10) and side (ipsilesional versus contralesional eye, respectively, first versus second eye) as within subject factors and group (control versus cerebellar and SCA patients versus PICA patients) as between subject factor. For *post hoc* testing, ANOVA were calculated separately for each group (controls, SCA patients and PICA patients). *P* values for effects were set at <0.05. For all effects, the degrees of freedom (DF) were adjusted, if appropriate, according to Greenhouse and Geisser (Bortez, 1999).

Results

MRI findings

Analysis of normalized 3D-MRI data enabled us to define the extent of cerebellar cortical and nuclear impairment in the cerebellar patients. Table 2 summarizes which cerebellar lobuli were affected and whether or not the deep cerebellar nuclei were affected. Most SCA patients (ten out of 14) had a unilateral lesion; the lesion was bilateral in four SCA patients. In eight patients, the lesion was restricted to the SCA territory (superior parts of the cerebellum down to Crus I) and, in six patients, the lesion extended into the PICA territory (posterior and inferior parts of the cerebellum up to Crus II) (Amarenco *et al*., 1993). Fig. 1 shows the lesions of all SCA patients except two superimposed on axial MRIs of the cerebellum of a healthy subject. All unilateral lesions are superimposed on the left cerebellar hemisphere with right-sided lesions being flipped to the left. The data of the patient with cerebellar agenesis (cer-26 in Table 2) were not included because of difficulties in stereotaxic normalization due to changes of the normal anatomy (e.g. the occipital lobes were shifted caudally). In one patient (cer-27), 3D-MRI data were not acquired because the patient had a cardiac pacemaker inserted. The number of overlapping lesions is illustrated by colour, from violet (n = 1) to red (n = 12). The centre of overlap in the SCA group was located in the superior cerebellum within hemispheral lobule VI (light green, n = 9) and adjacent lobules Crus I and V (dark greens, n = 8 and n = 7; see Table 3 for MNI coordinates). The interposed nuclei were affected in eight SCA patients (cer-18, cer-19, cer-20, cer-22, cer-23, cer-24, cer-25 and cer-26). In five patients (cer-18, cer-19, cer-20, cer-22 and cer-23), the interposed nucleus was affected unilaterally with additional involvement of the dentate nucleus in three (cer-20, cer-22 and cer-23). In three patients (cer-24, cer-25 and cer-26), the interposed and dentate nuclei were affected bilaterally. In five SCA patients (cer-14, cer-15, cer-16, cer-17 and cer-21), the interposed nuclei were not affected. In one of these five patients (cer-21), parts of the dentate nucleus were affected. None of the patients had a lesion restricted to the cerebellar nuclei.

In the PICA group (n = 13) lesions were primarily unilateral. In three patients (cer-01, cer-08 and cer-10 in Table 2), there was a small extension across the midline to the contralateral side. One patient had lacunar lesions within the PICA territory of both sides (cer-06). Fig. 2 shows the lesions of all but two PICA patients superimposed on axial MRIs of the cerebellum of a healthy subject. Again, lesions are superimposed on the left cerebellar hemisphere with right-sided lesions being flipped to the left. 3D-MRI data sets were not available in two patients because of a cardiac pacemaker inserted in one (cer-13) and claustrophobia in the other patient (cer-12). The number of overlapping lesions is illustrated by colour, from violet (n = 1) to red (n = 11). The centre of overlap in the PICA group was located within hemispheral lobules VIIB and VIIIA (orange, n = 10) and adjacent lobules Crus II and VIIIB (yellow, n = 9). In three patients (cer-09, cer-10 and cer-11), the lesion extended into Crus I, a region commonly supplied by the SCA (Amarenco *et al*., 1993). Involvement of Crus I is shown in the most cranial axial sections of the superimposed PICA lesions in Fig. 2. Fig. 2 illustrates that the deep cerebellar nuclei were not affected, except for parts of the inferior and posterior dentate nucleus in five patients (cer-07, cer-08, cer-09, cer-10 and cer-11).

Controls versus cerebellar

Eyeblink conditioning on the affected side in the group of all cerebellar patients was compared with eyeblink conditioning on the side tested first in the control group. In patients with bilateral lesions (cer-06, cer-23, cer-24, cer-25 and cer-26 in Table 2), the side tested first was included. Mean total percentage CR-incidences were reduced on the affected side in cerebellar patients compared with the side tested first in the control group (cerebellar patients: 14.4% SD 11.1; controls: 27.0% SD 19.9; see open and filled bars in Fig. 3). Although mean CR-incidences were reduced on the affected side in cerebellar patients, an increase of CR-incidences per block was still present—reflecting some preserved effects of learning.

ANOVA with CR-incidence as the dependent variable, group (cerebellar versus control) as between-subject factors and block (1–10) as within-subject factor was calculated. ANOVA showed a significant group effect (P = 0.006) reflecting reduced conditioning rate in the cerebellar group. Both the block (P < 0.001) and the group by block interaction effects were significant (P = 0.005). Learning effects were present in both groups, but significantly less in the cerebellar group compared with the controls.

Next, eyeblink conditioning was compared in the group of cerebellar patients with unilateral lesions with eyeblink conditioning in the group of their matched controls. Mean total percentage CR-incidences were reduced on the affected side compared with the unaffected side in the cerebellar patients with unilateral lesions (affected side: 16.3% SD 11.4, unaffected side: 30.4% SD 22.2; see open and filled bars in Fig. 4B). However, only a small difference was present when comparing the two sides tested in the matched controls (first

Table 2 *Affected cerebellar lobuli and deep cerebellar nuclei in the cerebellar patients as revealed by stereotaxically normalized 3D-MRI*

Subject	Disorder side	Cerebellar cortex									Cerebellar nuclei	
			x, y, z	Lobuli		*x, y, z*	Lobuli		*x, y, z*	Lobuli	*x, y, z*	Nuclei
cer-01	PICA	Ventral	–30, –64, –46	VIIIA	Cranial	–28, –80, –38	Crus II	Lateral	–42, –75, –48	VIIB		n.a.
	Left	Dorsal	–25, –86, –48	VIIB	Caudal	–26, –75, –63	VIIIA	Medial	04, –83, –51	VIIA		
cer-02	PICA	Ventral	13, –50, –54	IX	Cranial	26, –85, –38	Crus II	Lateral	36, –72, –55	VIIB		n.a.
	Right	Dorsal	20, –93, –38	Crus II	Caudal	24, –70, –68	VIIIA	Medial	01, –63, –46	VIIIB		
cer-03	PICA	Ventral	–20, –36, –53	IX	Cranial	–22, –51, –52	VIIIB	Lateral	–27, –51, –56	VIIIB		n.a.
	Left	Dorsal	–21, –59, –57	VIIIB	Caudal	–27, –67, –60	VIIIA	Medial	–19, –52, –57	VIIIB		
cer-04	PICA	Ventral	14, –58, –58	VIIIB	Cranial	12, –68, –41	VIIIA	Lateral	43, –82, –46	Crus II		n.a.
	Right	Dorsal	14, –89, –48	Crus II	Caudal	17, –75, –62	VIIIA	Medial	03, –74, –36	VIIB		
cer-05	PICA	Ventral	16, –54, –47	IX	Cranial	22, –66, –44	VIIIA	Lateral	25, –68, –48	VIIIA		n.a.
	Right	Dorsal	16, –74, –58	VIIIA	Caudal	16, –65, –62	VIIIB	Medial	07, –58, –51	IX		
cer-06	PICA	Ventral	–23, –72, –56	VIIIA	Cranial	–23, –74, –54	VIIB	Lateral	–28, –77, –57	VIIB		n.a.
	Left	Dorsal	–23, –81, –56	VIIB	Caudal	–25, –78, –58	VIIB	Medial	–17, –78, –54	VIIB		
	+ PICA	Ventral	26, –64, –58	VIIIA	Cranial	26, –68, –56	VIIIA	Lateral	30, –78, –56	VIIB		n.a.
	Right	Dorsal	25, –82, –56	VIIB	Caudal	23, –78, –61	VIIIA	Medial	24, –75, –56	VIIIA		
cer-07	PICA	Ventral	18, –52, –58	VIIIB	Cranial	17, –62, –46	VIIIB	Lateral	27, –72, –56	VIIIA	20, –60, –44	Dentate
	Right	Dorsal	21, –84, –56	VIIB	Caudal	14, –66, –68	VIIIA	Medial	03, –66, –60	VIIIB		
cer-08	PICA	Ventral	–12, –59, –59	VIIIB	Cranial	–18, –88, –31	Crus II	Lateral	–42, –83, –48	Crus II	–14, –64, –45	Dentate
	Left	Dorsal	–11, –96, –50	Crus II	Caudal	–22, –78, –68	VIIB	Medial	08, –80, –47	VIIB		
cer-09	PICA	Ventral	–29, –43, –60	VIIIB	Cranial	–35, –74, –30	Crus I	Lateral	–45, –70, –46	Crus II	–15, –67, –44	Dentate
	Left	Dorsal	–28, –88, –40	Crus II	Caudal	–28, –58, –66	VIIIA	Medial	–02, –62, –44	VIIIB		
cer-10	PICA	Ventral	–28, –47, –59	VIIIB	Cranial	–14, –84, –19	Crus I	Lateral	–46, –68, –48	VIIB	–15, –64, –40	Dentate
	Left	Dorsal	–16, –94, –28	Crus I	Caudal	–30, –70, –58	VIIIA	Medial	09, –73, –54	VIIB		
cer-11	PICA	Ventral	–08, –55, –44	IX	Cranial	–28, –86, –23	Crus I	Lateral	–30, –80, –57	VIIB	–16, –65, –39	Dentate
	Left	Dorsal	–10, –93, –42	Crus II	Caudal	–18, –72, –68	VIIIA	Medial	–05, –74, –47	VIIB		
cer-12	PICA*	Ventral			Cranial			Lateral				
	Left	Dorsal			Caudal			Medial				
cer-13	PICA*	Ventral			Cranial			Lateral				
		Dorsal			Caudal			Medial				
cer-14	SCA	Ventral	–22, –47, –22	V	Cranial	–26, –72, –20	VI	Lateral	–44, –74, –26	Crus I		n.a.
	Left	Dorsal	–32, –84, –22	Crus I	Caudal	–26, –69, –37	Crus I	Medial	–14, –74, –27	VI		
cer-15	SCA	Ventral	–23, –47, –23	V	Cranial	–22, –56, –20	VI	Lateral	–28, –55, –25	VI		n.a.
	Left	Dorsal	–24, –70, –23	VI	Caudal	–21, –58, –32	VI Basis	Medial	–16, –55, –25	V		
cer-16	SCA	Ventral	–26, –53, –19	VI	Cranial	–20, –49, –16	V	Lateral	–26, –45, –24	V		n.a.
	Left	Dorsal	–27, –54, –21	VI	Caudal	–25, –49, –28	VI	Medial	–20, –42, –18	V		
cer-17	SCA	Ventral	–08, –46, –28	III	Cranial	–11, –39, –18	III	Lateral	–10, –44, –26	III		n.a.
	Left	Dorsal	–07, –46, –24	III	Caudal	–08, –46, –34	White matter	Medial	–04, –43, –26	III		
cer-18	SCA	Ventral	–10, –39, –20	III	Cranial	–10, –46, –12	IV	Lateral	–28, –60, –24	VI	–10, –51, –24	Interpos.
	Left	Dorsal	–24, –68, –20	VI	Caudal	–20, –56, –32	VI	Medial	–06, –50, –25	IV		
cer-19	SCA	Ventral	–15, –38, –17	IV	Cranial	–15, –50, –13	V	Lateral	–35, –60, –30	VI	–12, –57, –24	Interpos.
	Left	Dorsal	–28, –72, –30	Crus I	Caudal	–32, –62, –40	White matter	Medial	–12, –56, –25	V		
cer-20	SCA	Ventral	17, –28, –27	III	Cranial	08, –48, –05	IV	Lateral	48, –56, –33	Crus I	10, –58, –24	Interpos.
	Right	Dorsal	24, –81, –25	Crus I	Caudal	32, –58, –47	White matter	Medial	02, –48, –15	IV	20, –56, –44	Dentate
cer-21	SCA	Ventral	–27, –48, –27	VI	Cranial	–20, –58, –15	VI	Lateral	–47, –68, –27	Crus I	–17, –65, –40	Dentate
	Left	Dorsal	–36, –82, –20	Crus I	Caudal	–25, –58, –35	White matter	Medial	–16, –58, –21	V		
	+PICA	Ventral	–25, –60, –55	VIIIB	Cranial	–30, –70, –40	VIIB	Lateral	–46, –73, –42	Crus II		

Table 2 *Continued*

Subject	Disorder side	Cerebellar cortex									Cerebellar nuclei	
			x, y, z	Lobuli		*x, y, z*	Lobuli		*x, y, z*	Lobuli	*x, y, z*	Nuclei
	Left	Dorsal	–20, –90, –48	VIIB	Caudal	–28, –70, –64	VIIIA	Medial	–06, –75, –49	VIIB		
cer-22	SCA	Ventral	21, –36, –28	IV	Cranial	13, –44, –13	IV	Lateral	55, –61, –37	Crus I	12, –56, –24	Interpos.
	Right	Dorsal	26, –92, –34	Crus I	Caudal	35, –56, –47	White matter	Medial	09, –60, –18	V	19, –62, –38	Dentate
	+ PICA	Ventral	14, –60, –55	VIIIB	Cranial	26, –70, –42	White matter	Lateral	38, –70, –52	VIIB		
	Right	Dorsal	17, –88, –47	Crus II	Caudal	30, –76, –66	VIIB	Medial	03, –68, –63	VIIB		
cer-23	SCA	Ventral	–23, –71, –33	Crus I	Cranial	–27, –78, –23	Crus I	Lateral	–31, –78, –28	Crus I		n.a.
	Left	Dorsal	–28, –85, –25	Crus I	Caudal	–26, –75, –35	Crus I	Medial	–18, –75, –30	Crus I		
	+ SCA	Ventral	08, –55, –29	V	Cranial	17, –73, –17	VI	Lateral	40, –65, –30	Crus I	10, –60, –28	Interpos.
	Right	Dorsal	29, –80, –30	Crus I	Caudal	20, –70, –44	White matter	Medial	04, –60, –28	White matter	20, –62, –38	Dentate
cer-24	Angioma	Ventral	–26, –45, –28	V	Cranial	–35, –64, –26	VI	Lateral	–50, –58, –30	Crus I	–12, –51, –24	Interpos.
	Left	Dorsal	–26, –77, –36	Crus I	Caudal	–26, –60, –46	White matter	Medial	–08, –64, –34	White matter	–20, –57, –44	Dentate
	+ Right	Ventral	18, –50, –23	V	Cranial	20, –62, –20	VI	Lateral	31, –58, –30	VI	08, –51, –24	Interpos.
		Dorsal	22, –72, –30	VI	Caudal	24, –65, –37	VI Basis	Medial	06, –56, –14	V	14, –60, –40	Dentate
cer-25	Astrocytoma	Ventral	–14, –62, –28	VI	Cranial	–14, –77, –22	VI	Lateral	–27, –69, –33	Crus I	–08, –55, –24	Interpos.
	Left	Dorsal	–19, –92, –30	Crus II	Caudal	–13, –72, –44	VIIB	Medial	00, –79, –28	VIIAt	–20, –59, –38	Dentate
	+ Right	Ventral	35,–43, –34	VI	Cranial	05, –43, –09	IV	Lateral	32, –65, –42	Crus II	10, –53, –24	Interpos.
		Dorsal	29, –90, –34	Crus I	Caudal	05, –53, –64	IX	Medial	16, –58, –25	V	20, –62, –43	Dentate
cer-26	Agenesis	Ventral			Cranial			Lateral				
	**	Dorsal			Caudal			Medial				
cer-27	SCA*	Ventral			Cranial			Lateral				
	Right	Dorsal			Caudal			Medial				

MNI coordinates (*x*, *y* and *z*) are given for (i) lesion extent in the horizontal (lateral/medial), vertical (cranial/caudal) and sagittal (ventral/dorsal) planes and (ii) position of affected cerebellar nuclei (see Methods and Fig. 10 for details). Cerebellar lobuli are named according to Schmahmann *et al*. (2000). n.a. = not affected; dentate = dentate nucleus; interpos. = interposed nucleus; *no 3D-MRI-data available due to cardiac pacemaker (cer-13 and cer-20) and claustrophobia (cer-4); ** complete absence of cerebellum.

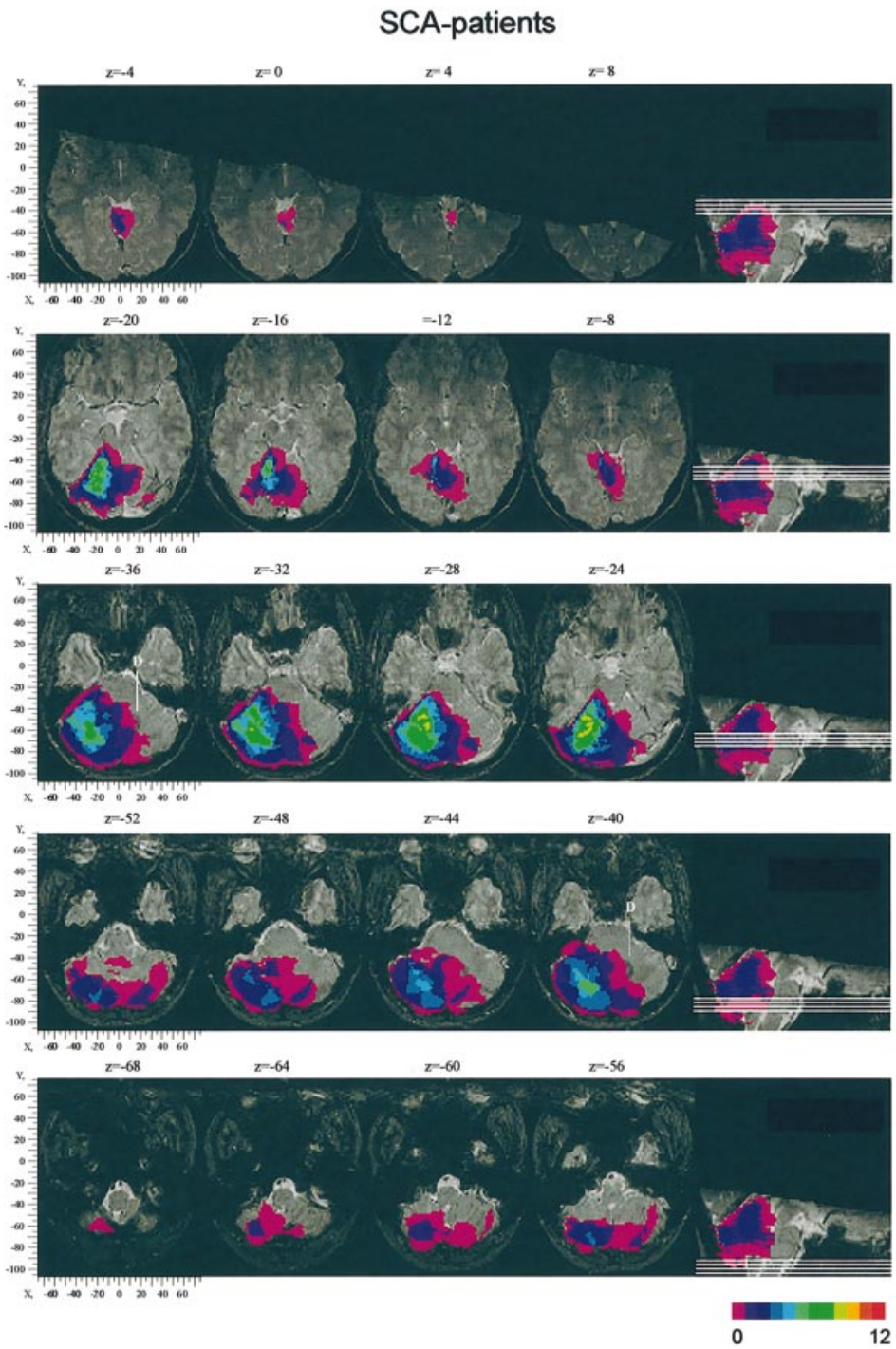

Fig. 1 Lesions of SCA patients superimposed on axial stereotaxically normalized MRIs of the cerebellum of a healthy 26-year-old female subject. Slices are 4 mm apart with the most caudal slice in the left lower corner (z = –68 mm) and the most cranial slice in the right upper corner (z = +8 mm). All unilateral lesions are superimposed on the left cerebellum with right-sided lesions being flipped to the left. The number of overlapping lesions is illustrated by colour, from violet (n = 1) to red (n = 12). Data of two patients (cer-26 and cer-27) are not included for technical reasons (see Results: MRI findings). Note centre of overlap (light green, n = 9; darker greens, n = 8 and 7) in the superior cerebellum within the hemispheral lobules VI and Crus I. MRIs of the healthy subject were acquired using the same FLASH 3D-MRI sequence as in the patients. D = dentate nucleus.

side: 27.2% SD 20.0, second side: 30.6% SD 20.9; Fig. 4A). Inspection of changes of CR-incidences per block revealed that the controls reached their final level of CR-incidences within less blocks on the second tested side compared with

Table 3 *Centres of lesion overlap in the PICA and SCA groups*

Patient group	Overlap lesions	Cerebellar cortex						Cerebellar nuclei		
		x, y, z	Lobuli		*x, y, z*	Lobuli		*x, y, z*	Lobuli	
PICA	*n* = 10	–20, –68, –50	VIIIA	Cranial	–20, –72, –48	VIIB	Lateral	–24, –72, –52	VIIIA	n.a.
	(orange)*	–14, –78, –52	VIIB	Caudal	–18, –68, –64	VIIIA	Medial	–06, –72, –52	VIIB	
	n = 9	–16, –56, –56	VIIIB	Cranial	–14, –80, –44	Crus II	Lateral	–28, –74, –58	VIIB	n.a.
	(yellow)	–20, –86, –45	Crus II	Caudal	–18, –64, –60	VIIIA	Medial	–02, –62, –52	VIIIB	
SCA	*n* = 9	–24, –46, –24	V	Cranial	–24, –56, –20	VI	Lateral	–30, –58, –26	VI	n.a.
	(light green)**	–28, –60, –26	VI	Caudal	–24, –54, –30	VI	Medial	–20, –56, –22	VI	
	n = 7	–22, –42, –28	V	Cranial	–22, –56, –18	VI	Lateral	–38, –64, –28	Crus I	–12, –56, –26 Interpos.
	(dark green)	–20, –84, –28	Crus I	Caudal	–26, –68, –38	Crus I	Medial	–12, –72, –30	Crus I	

MNI coordinates (*x*, *y* and *z*) are given for (i) extent of each centre of overlap in the horizontal (lateral/medial), vertical (cranial/caudal) and sagittal (ventral/dorsal) planes and (ii) position of affected nuclei. *See colour bars in Fig. 2; **see colour bars in Fig. 1; interpos = interposed nucleus; n.a. = not affected.

the first (Fig. 4A). Again, although mean CR-incidences were reduced on the affected side in cerebellar patients, an increase of CR-incidences per block was still present reflecting some preserved effects of learning (Fig. 4B).

ANOVA with CR-incidence as the dependent variable, group (unilateral cerebellar versus matched controls) as between-subject factors, and block (1–10) and side (affected versus unaffected, respectively, first versus second) as within-subject factors was calculated. Statistical analysis showed a significant block effect ($P < 0.001$) reflecting effects of learning in patients and controls. There was a significant difference between the sides being tested (affected versus unaffected, respectively, first versus second) ($P < 0.001$). The group by side interaction effect ($P = 0.013$) was significant indicating that the difference in conditioning rate between sides was different in the patient and control groups. In addition, the group-by-side-by-block interaction effect ($P = 0.006$) was significant, indicating that the amount of change of CR-incidences across blocks was different when comparing sides in the two groups. All other effects (including group, group by block interaction, side by block interaction) did not reach significance (all P values >0.2).

In sum, eyeblink conditioning was impaired in the group of all cerebellar patients compared with control subjects. In the cerebellar patients with unilateral lesions eyeblink conditioning was impaired on the ipsilateral side.

PICA patients versus SCA patients

Our hypothesis was that eyeblink conditioning is reduced in patients with lesions including the superior parts of the cerebellum, but preserved in patients with lesions restricted to the posterior and inferior parts of the cerebellum. To test this hypothesis, CR-incidences were compared between patients with lesions including the territory of the SCA and patients with lesions limited to the territory of the PICA. Patients with unilateral lesions and their matched controls were included. Mean total CR-incidences were most clearly reduced on the affected side compared with the unaffected side in cerebellar patients with lesions including the territory of the SCA (affected side: 13.0% SD 6.31, unaffected side: 33.9% SD 17.7; see filled and open bars in Fig. 4D). In cerebellar patients with lesions restricted to the territory of the PICA, the differences in CR-incidences comparing the affected and unaffected side were less pronounced (mean total percentage CR-incidence affected side: 19.0% SD 14.3, unaffected side: 27.6% SD 25.7; Fig. 4C).

Examples of characteristic EMG recordings of individual subjects further illustrate the group findings. EMG recordings of the orbicularis oculi muscle ipsilateral to the air puff (US) are shown for the 100 paired CS–US trials with the first trial at the top and the last trial at the bottom. CR are specified by EMG responses occurring within the CS–US window indicated by the two vertical lines. An example of a healthy 66-year-old control subject (con-18 in Table 1) is shown in Fig. 5. The total percentage CR-incidence was 69% on the first and

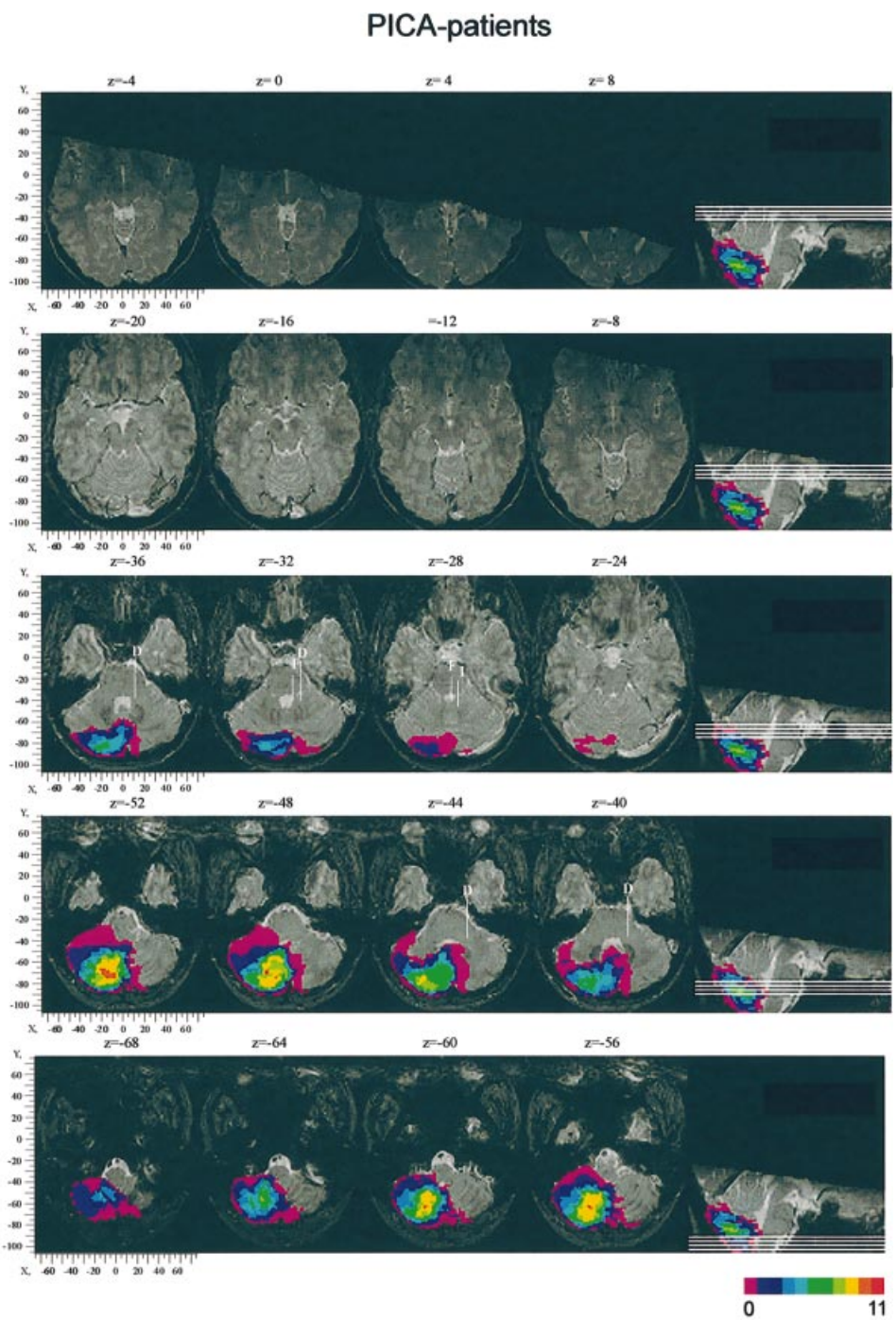

Fig. 2 Lesions of PICA patients superimposed on the same axial MRIs of the cerebellum of a healthy subject as shown in Fig. 1**.** The positions of the deep cerebellar nuclei are indicated by white lines (D = dentate nucleus, I = interposed nucleus, F = fastigial nucleus). All unilateral lesions are superimposed on the left cerebellum with right-sided lesions being flipped to the left. The number of overlapping lesions is illustrated by colour, from violet (n = 1) to red (n = 11). Data of two patients (cer-12 and cer-13) are not included for technical reasons (see Results: MRI findings). Note centre of overlap (orange, n = 10) in the posterior inferior cerebellum within lobule VIIB and VIIIA, and that some lesions affected the lower and inferior part of the dentate nucleus (dark blue, n = 4, light blue, n = 5), with the interposed nucleus being preserved.

71% on the second side. Note that CRs were present earlier on the second (left side) compared with the first (right) side. An example of a 63-year-old patient (cer-05 in Tables 1 and 2) with a PICA infarction is shown in Fig. 6. Total CR-incidences were within the normal range on both sides (affected side: 54%; unaffected side: 78%). Comparable with

the control, CRs were present earlier on the second (unaffected side) compared with the first (affected) side. Fig. 7 shows an example of a 50-year-old patient (cer-14 in Tables 1 and 2) with an SCA infarction. Total CR-incidence was reduced on the affected side (12%), the patient showed a normal conditioning rate (total CR-incidence = 59%) on the unaffected side.

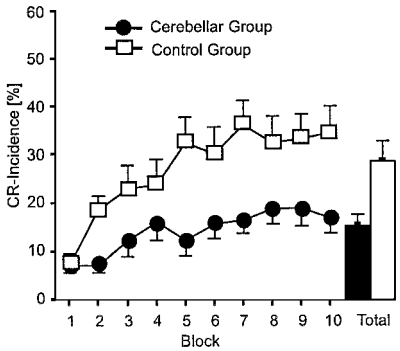

Fig. 3 Mean percentage CR-incidences ± SE of the ipsilesional eye in the group of all cerebellar patients and of the eye tested first in the group of all control subjects. In patients with bilateral lesions, data from the eye tested first are included. Mean percentage CR-incidences are shown for each of the ten blocks (controls: open squares; cerebellar: filled circles) and across all blocks (controls: open column; cerebellar: filled column). Total = mean total percentage CR-incidence.

ANOVA with CR-incidence as the dependent variable, lesion location (restricted to the PICA territory versus including the SCA territory) as between-subject factor, and block (1–10) and side (affected versus unaffected) as within-subject factors was calculated. ANOVA showed a significant block effect ($P < 0.001$) reflecting effects of learning in both PICA and SCA patients. There was a significant difference between the sides being tested (affected versus unaffected) ($P = 0.001$). The lesion location by side interaction effect ($P = 0.107$) and all other effects (including lesion location, lesion location by block interaction, side by block interaction, lesion location by side by block interaction) did not reach significance (all P values >0.2).

Analysis of individual MRI scans revealed that in three PICA patients (cer-09, cer-10, cer-11 in Table 2; see above MRI findings), the lesion extended into Crus I, a region commonly supplied by the SCA. Crus I has been shown both in animal lesion studies and a recent fMRI study in humans to

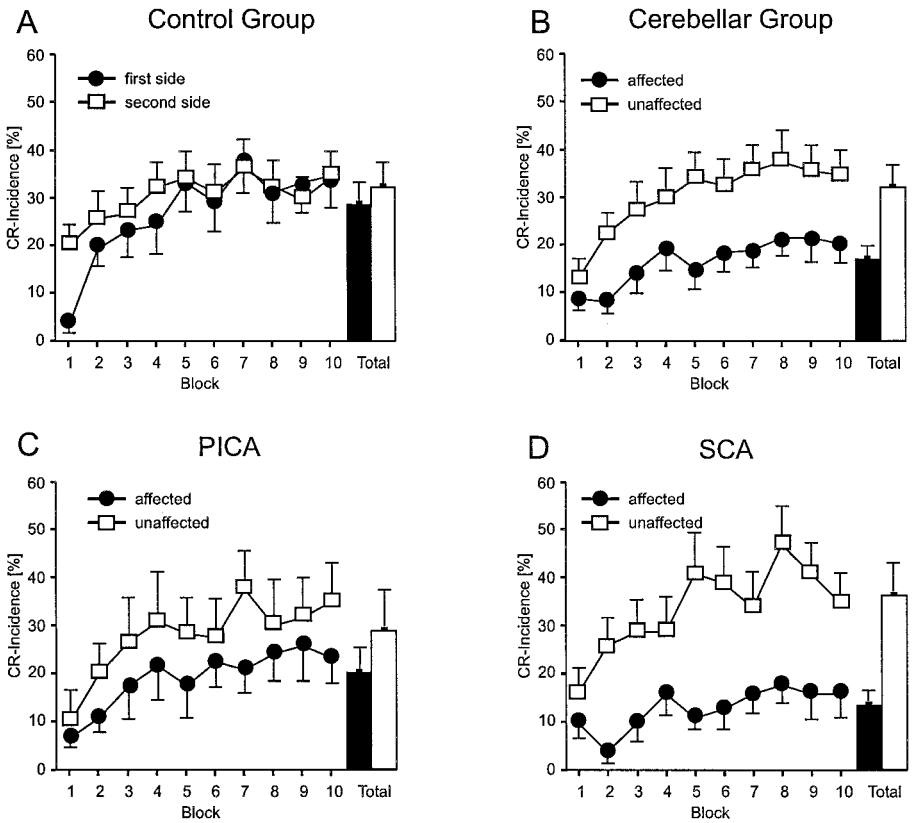

Fig. 4 Mean percentage CR-incidences ± SE for each of the ten blocks and across all blocks (Total) in (**B**) the group of cerebellar patients with unilateral lesions, (**A**) their age- and sex-matched controls, (**D**) in patients with lesions including the SCA territory and (**C**) in patients with lesions restricted to the PICA territory. Filled circles and columns represent the eye tested first in controls and the ipsilesional eye in patients. Open squares and columns represent the eye tested second in controls and the contralesional eye in cerebellar patients. Total = mean total percentage CR-incidence.

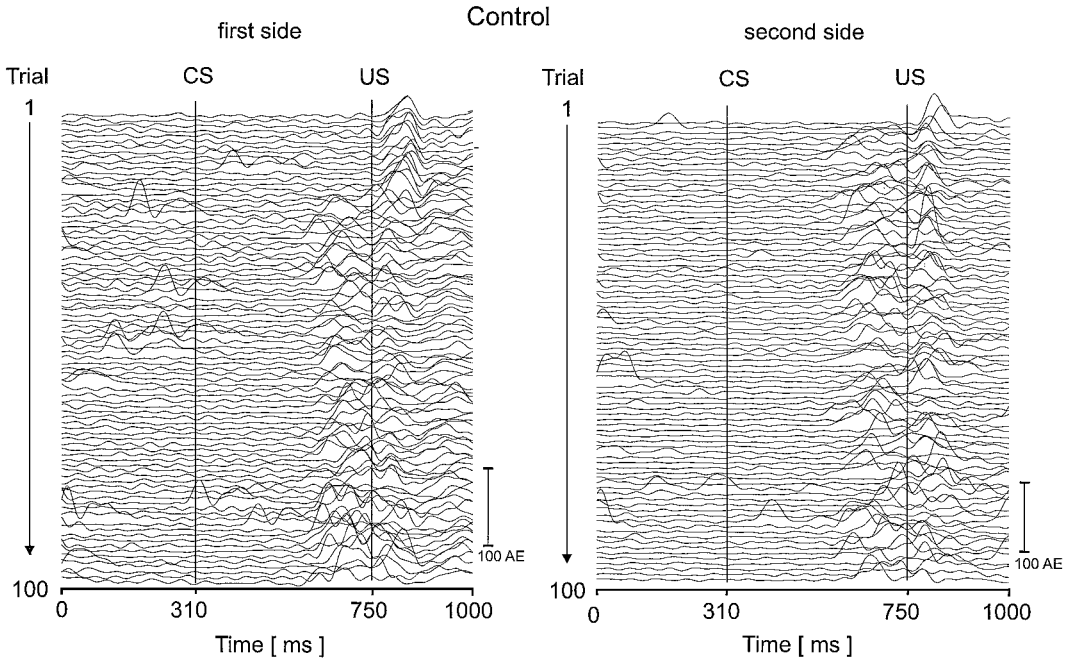

Fig. 5 Eyeblink conditioning in a 66-year-old male control subject (con-18). Rectified and filtered (45 Hz) EMG data of the orbicularis oculi muscles of 100 paired CS–US trials (first trial on the top, last trial on the bottom) are shown for the eye tested first (right eye) on the left and the eye tested second (left eye) on the right. The first vertical line indicates the beginning of the tone (CS) and the second vertical line the beginning of the air puff (US).

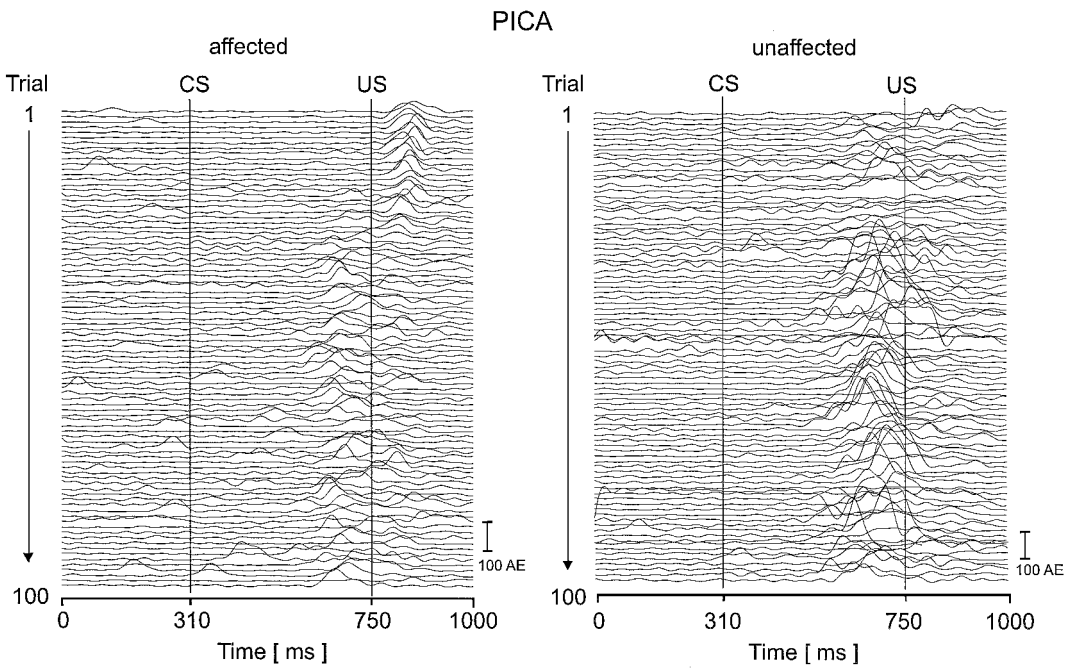

Fig. 6 Eyeblink conditioning in a 63-year-old male PICA patient (cer-05). Rectified and filtered (45 Hz) EMG data of the orbicularis oculi muscles of 100 paired CS–US trials (first trial on the top, last trial on the bottom) are shown for the ipsilesional (right) eye on the left and the contralesional (left) eye on the right. The first vertical line indicates the beginning of the tone (CS) and the second vertical line the beginning of the air puff (US).

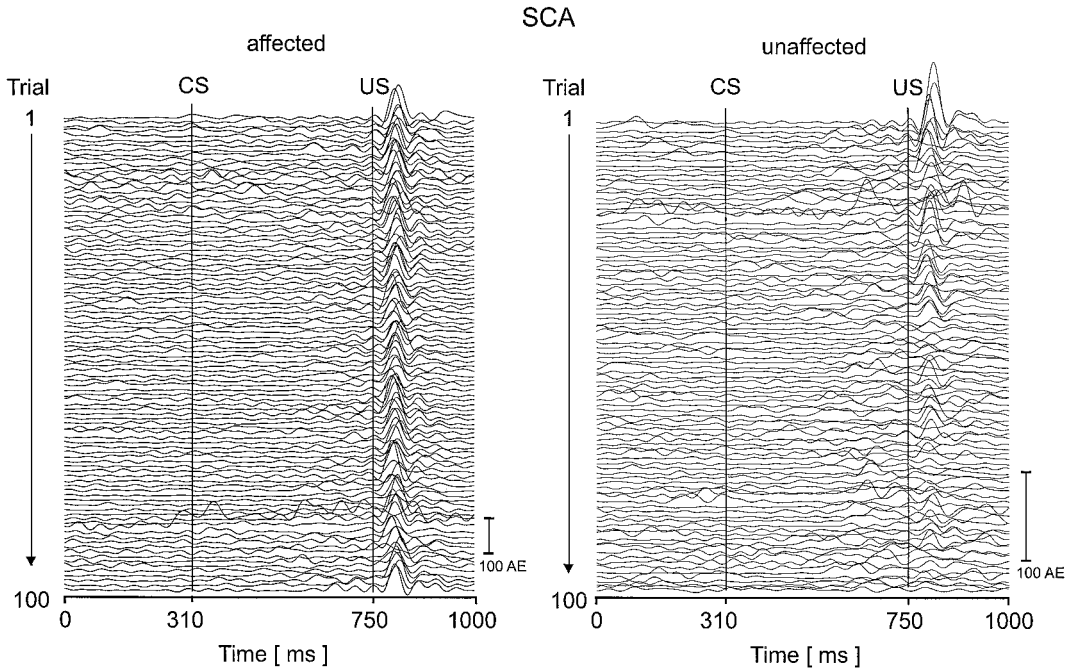

Fig. 7 Eyeblink conditioning in a 50-year-old female SCA patient (cer-14). Rectified and filtered (45 Hz) EMG data of the orbicularis oculi muscles of 100 paired CS–US trials (first trial on the top, last trial on the bottom) are shown for the ipsilesional (left) eye on the left and the contralesional (right) eye on the right. The first vertical line indicates the beginning of the tone (CS) and the second vertical line the beginning of the air puff (US).

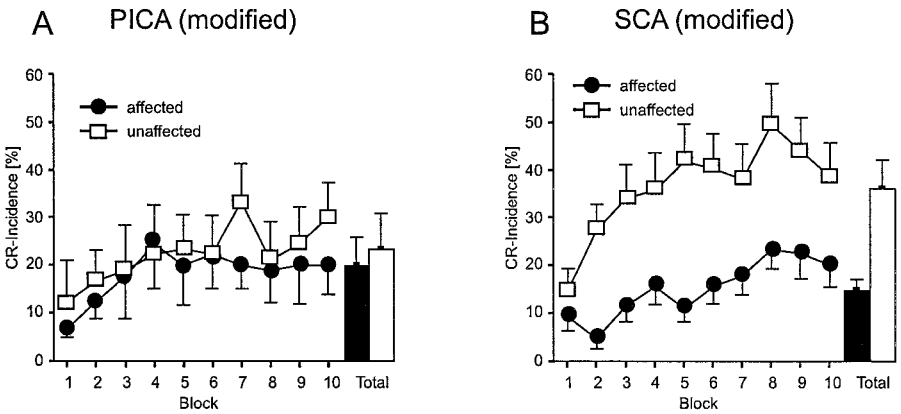

Fig. 8 Mean percentage CR-incidences ± SE for each of the ten blocks and across all blocks (Total) of the ipsilesional eye (filled circles and columns) and contralesional (open squares and columns) eye in (**A**) patients with lesions restricted to the common PICA territory (Crus II and below, i.e. excluding the three PICA patients with additional Crus I lesion: cer-09, cer-10 and cer-11) and (**B**) patients with lesions including the common SCA territory (Crus I and above, i.e. including the three PICA patients with Crus I lesions). Total = mean total percentage CR-incidence.

be involved in eyeblink conditioning (Hardiman and Yeo, 1992; Ramnani *et al*., 2000). Therefore, analysis was redone with these three patients considered as SCA patients. The mean total percentage CR-incidence was clearly reduced on the affected compared with the unaffected side in the modified SCA group (affected side: 15% SD 7.7; unaffected side: 36.1% SD 21.2; see open and filled bars in Fig. 8B). No clear difference was present in the modified PICA group (affected side: 18.2% SD 15.7; unaffected side: 22.2% SD 22.0; Fig. 8A). ANOVA revealed significant block ($P < 0.001$) and side effects ($P = 0.001$). In addition, the location of the lesion by side interaction effect was significant ($P = 0.023$), indicating a difference in conditioning rate when comparing the affected and unaffected side in patients with lesions including the common SCA territory compared with patients with lesions restricted to the common PICA territory.

For *post hoc* testing, ANOVA were calculated separately for the matched control subjects, PICA and SCA patients with CR-incidence as dependent variable, and block and side as within-subject factors. All three groups showed significant effects of learning (block effect: all P values <0.01). In the controls, the side effect did not reach statistical significance (P = 0.11). The side by block interaction was significant (P = 0.045), reflecting the faster acquisition of CR responses on the second side compared with the first. Further *post hoc* analysis revealed a significant difference in CR-incidences in the first block of ten conditioning trials between the two eyes (P = 0.001; paired t-test), but not in the last nine blocks (all P values >0.1). In the SCA group, there was a significant difference when comparing the affected and unaffected side (P = 0.005; modified SCA group: P = 0.002), reflecting the significantly reduced conditioning effect on the affected side. There was no significant side by block interaction (P = 0.19; modified SCA group: P = 0.11). In the PICA group, the side effect did not reach significance (P = 0.085; modified PICA group: P = 0.21) and there was no significant side by block interaction (P = 0.84; modified PICA group: P = 0.8).

To summarize, reduced eyeblink conditioning in cerebellar patients was particularly prominent in patients with lesions which included the superior parts of the cerebellum down to Crus I (i.e. common SCA territory).

SCA patients

Further analysis took into account the results of individual 3D-MRI data sets. First, SCA patients with lesions restricted to the cerebellar cortex were compared with SCA patients with additional involvement of the interposed nucleus. A cortical lesion with no impairment of the interposed nucleus was present in SCA patients cer-14, cer-15, cer-16, cer-17 and cer-21 (see Table 2). The cerebellar cortex and interposed nucleus were affected in cer-18, cer-19, cer-20, cer-22, cer-23, cer-24, cer-25 and cer-26. CR-incidences of the affected sides were compared. Data of the side tested first were included in the four patients with bilateral lesions (cer-23, cer-24, cer-25 and cer-26). The mean total CR-incidence was 11.6% (SD 6.6) on the affected side in the group with pure cortical lesions and 10.2% (SD 6.5) in the group with additional lesions of the interposed nucleus (Fig. 9). ANOVA with CR-incidence as dependent variable, nuclear lesion (affected versus unaffected) as between-subject factor and block (1–10) as within-subject factor revealed no significant effect of nuclear impairment (P = 0.72), no significant block effect (P = 0.31) and no significant nuclear lesion by block interaction (P = 0.78).

Results in individual patients further illustrate these findings. In subject cer-15, the lesion was restricted to hemispheral lobule VI without involvement of the cerebellar nuclei (Fig. 10A). Eyeblink conditioning was reduced on the affected side (total CR-incidence 16%) compared with the unaffected side (43%). Findings on the affected side merely reflected spontaneous blinking because the spontaneous blink rate was high and no increase of CR-incidence from the first to last block was present (spontaneous blink rate affected side: 23/min; unaffected side: 14/min). Figs 10B and C show findings in two other patients (cer-14 and cer-19), who had a lesion of lobule VI with some extension into Crus I and lobule V. Cerebellar nuclei were affected in cer-19, but not in cer-14. Both patients showed a reduced conditioning rate on the affected compared with the unaffected side (total CR-incidence affected/unaffected side: cer-14 = 2%/59%, cer-19 = 16%/25%). Fig. 10D shows findings in a patient (cer-23) with a lesion affecting lobules IV–VIIIA of the right cerebellum and Crus I of the left cerebellum. The interposed nuclei were affected on the left side but not on the right side. In this patient, eyeblink conditioning was clearly reduced on both sides (total CR-incidence left/right: 10%/7%).

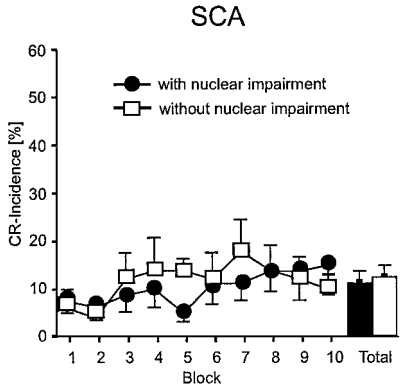

Fig. 9 Mean percentage CR-incidences ± SE for each of the ten blocks and across all blocks (Total) of the ipsilesional eye in SCA patients with impairment of the interposed nucleus (filled circles and columns) and without impairment of the interposed nucleus (open squares and columns). In patients with bilateral lesions, data of the eye tested first are included. Total = mean total percentage CR-incidence.

Next, the centre of overlap of the superimposed lesions of SCA patients was analysed more closely. Nine of the 12 SCA patients showed an area of overlap within hemispheral lobule VI. For illustration, the centre of overlap has been superimposed on sections of MRI scans of healthy subjects using two different MR acquisition protocols (Fig. 11). Figs 11A und B show that this area of overlap was located within lobule VI, while Fig. 11C shows that the deep cerebellar nuclei were not affected. The lesions in two subjects included lobule VI, but did not overlap with the centre of overlap (cer-18 and cer-25; see Table 2). In one patient (cer-17), the lesion was restricted to lobule IV. In this 75-year-old patient, the total CR-incidence was low on both sides (affected: 5%, unaffected: 15%). These values are within the range of mean total percentage CR-incidences reported in elderly healthy subjects (e.g. 32.0% SD 22.9, see Table 1, p. 222 in Woodruff-Pak and Thompson, 1988). These data suggest that hemispheral lobule VI is at least one of the important regions for eyeblink conditioning in humans, i.e. a lesion in this cortical region appears to be sufficient to impair eyeblink conditioning.

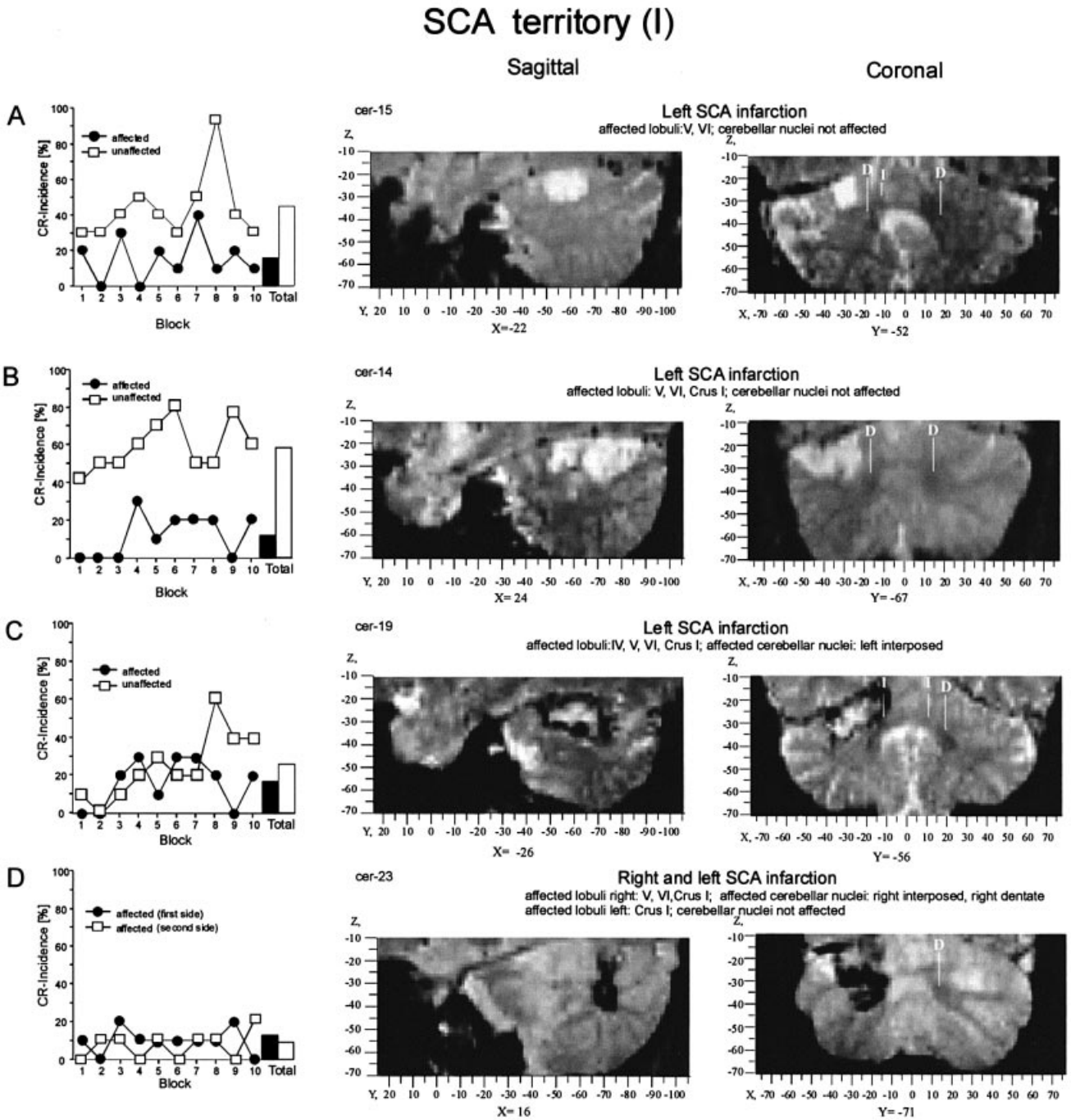

Fig. 10 Eyeblink conditioning and MRI findings in four representative SCA patients (**A**–**D**). Lesions were restricted to the common territory of the SCA. In (**A**) cer-15, (**B**) cer-14 and (**C**) cer-19, lesions were unilateral; in (**D**) cer-23, they were bilateral. Percentage CR-incidences for each of the ten blocks and across all blocks (Total) are shown on the left. Filled circles and columns indicate the ipsilesional eye (the eye tested first in bilateral lesions) and open squares and columns the contralesional eye (the eye tested second in bilateral lesions). Sagittal and coronal sections of the stereotaxically normalized 3D-MRI data are shown on the right. *x*, *y* and *z* represent MNI coordinates in MNI-space with *z* defining the vertical direction (- = mm below the AC–PC line), *x* the horizontal direction (+ = mm right of the AC–PC line, – = mm left of the AC–PC line) and *y* the sagittal direction (- = mm behind the anterior commissure). A FLASH sequence was used to visualize the cerebellar nuclei (see Methods). Therefore, structures of cerebellar cortex are shown in less detail compared with other, e.g. T_1 weighted, MRI sequences. The cerebellar nuclei are indicated by white lines. D = dentate nucleus; I = interposed nucleus.

Finally, findings in patients with unilateral and bilateral lesions were compared. Unilateral lesions were present in cer-14, cer-15, cer-16, cer-17, cer-18, cer-19, cer-20, cer-21, cer-22 and cer-27. Bilateral lesions were present in cer-23, cer-24, cer-25 and cer-26. The mean total CR-incidence was 13% (SD 6.3) on the affected side (unaffected side: 33.9%,

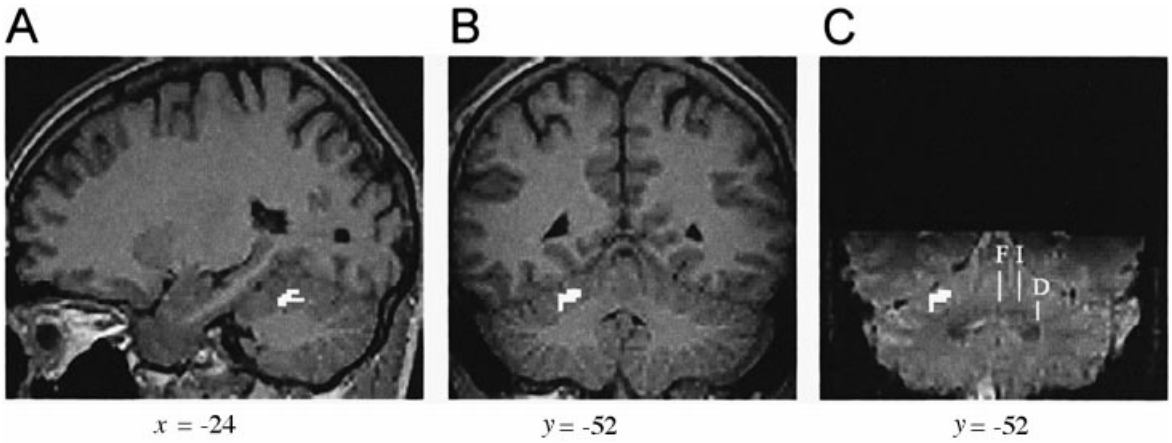

Fig. 11 Centre of overlap (white) of the superimposed lesions of the SCA patients superimposed on sagittal and coronal sections of healthy control subjects using an MPRAGE sequence (**A**, **B**) and a coronal section using a FLASH sequence (**C**). In (**A**) and (**B**), the lobuli of the cerebellar cortex are shown in detail. In (**C**), the cerebellar nuclei are shown, indicated by white lines (D = dentate, I = interposed, F = fastigial). The localization of the centre of overlap within hemispheral lobule VI is illustrated in (**A**) und (**B**). (**C**) shows that the cerebellar nuclei were not affected.

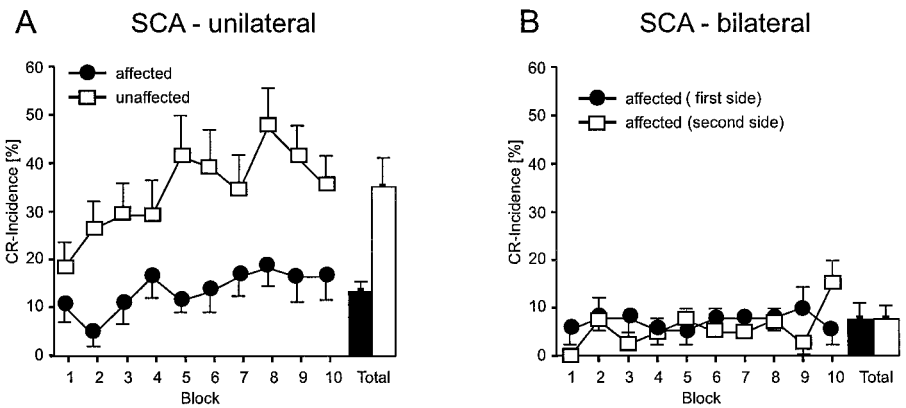

Fig. 12 Mean percentage CR-incidences ± SE for each of the ten blocks and across all blocks (Total) in (**A**) SCA patients with unilateral lesions and (**B**) SCA patients with bilateral lesions. Filled circles and columns represent the eye tested first in bilateral SCA patients and the ipsilesional eye in unilateral SCA patients. Open squares and columns represent the eye tested second in bilateral SCA patients and the contralesional eye in unilateral SCA patients. Total = mean total percentage CR-incidence.

SD 17.7) in SCA patients with unilateral lesions and 6.7% (SD 3.9) on the side tested first and 5.7% (SD 2.5) on the side tested second in SCA patients with bilateral lesions (Fig. 12A and B). There was a tendency for mean total CR-incidences to be less in patients with bilateral lesions. However, analysis of variance with CR-incidence as dependent variable lesion extent (unilateral versus bilateral) as between subject factor and block (1–10) as within subject factor revealed no significant effect of lesion extent (P = 0.094), block and block by lesion extent interaction (P values >0.2). ANOVA was calculated for the affected side in unilateral lesions and the first side tested in bilateral lesions.

Figs 13A and B show findings in two patients (cer-21 and cer-22) with extended unilateral lesions. The interposed nuclei were affected in cer-22 but not in cer-21. Eyeblink conditioning was clearly reduced on the affected side in both patients (total CR-incidence cer-21: 5%, cer-22: 14%). On the unaffected side, the conditioning rate was within normal limits (cer-21: 58%, cer-22: 54%). Three patients with bilateral lesions (cer-24, cer-25 and cer-26)—including bilateral lesions of the interposed nuclei—are shown in Fig. 13C–E. Eyeblink conditioning was diminished on both sides in the three patients (total CR-incidence affected/unaffected side: cer-24: 2%/2%; cer-25: 10%/7%; cer-26: 5%/10%). Fig. 13E shows the findings in the patient (cer-26) with cerebellar agenesis. Findings in cer-26 probably reflected spontaneous blinking (spontaneous blink rate first side: 19/min; second side: 44/min).

To summarize, our findings of patients with lesions including the territory of the SCA suggest that a unilateral

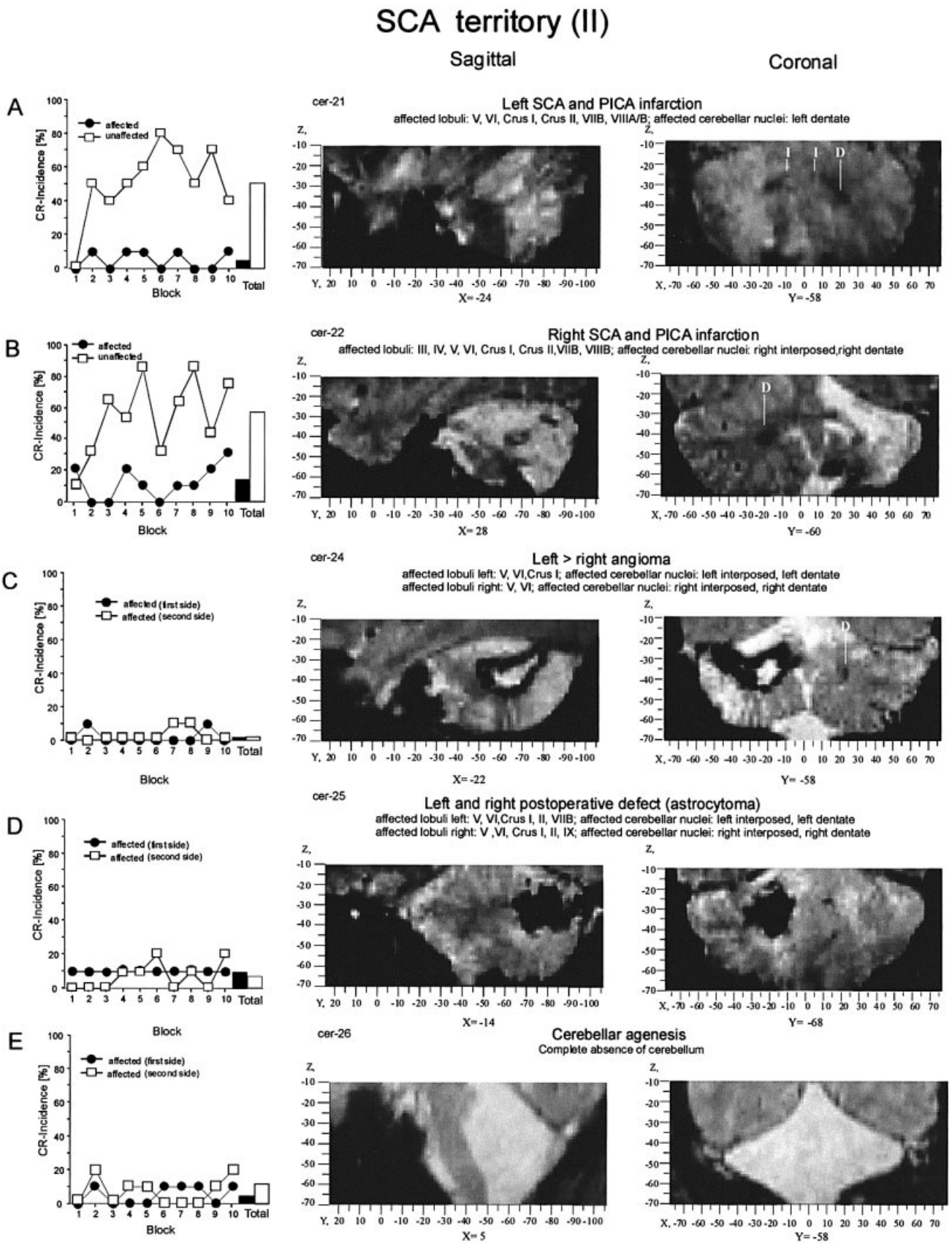

Fig. 13 Eyeblink conditioning and MRI findings in five representative SCA patients. Lesions included the common territory of the SCA with additional lesions of the common PICA territory. Lesions in (**A**) cer-21 and (**B**) cer-22 were unilateral; those in **C**–**E** (cer-24, cer-25 and cer-26) were bilateral. For further details, see Fig. 10.

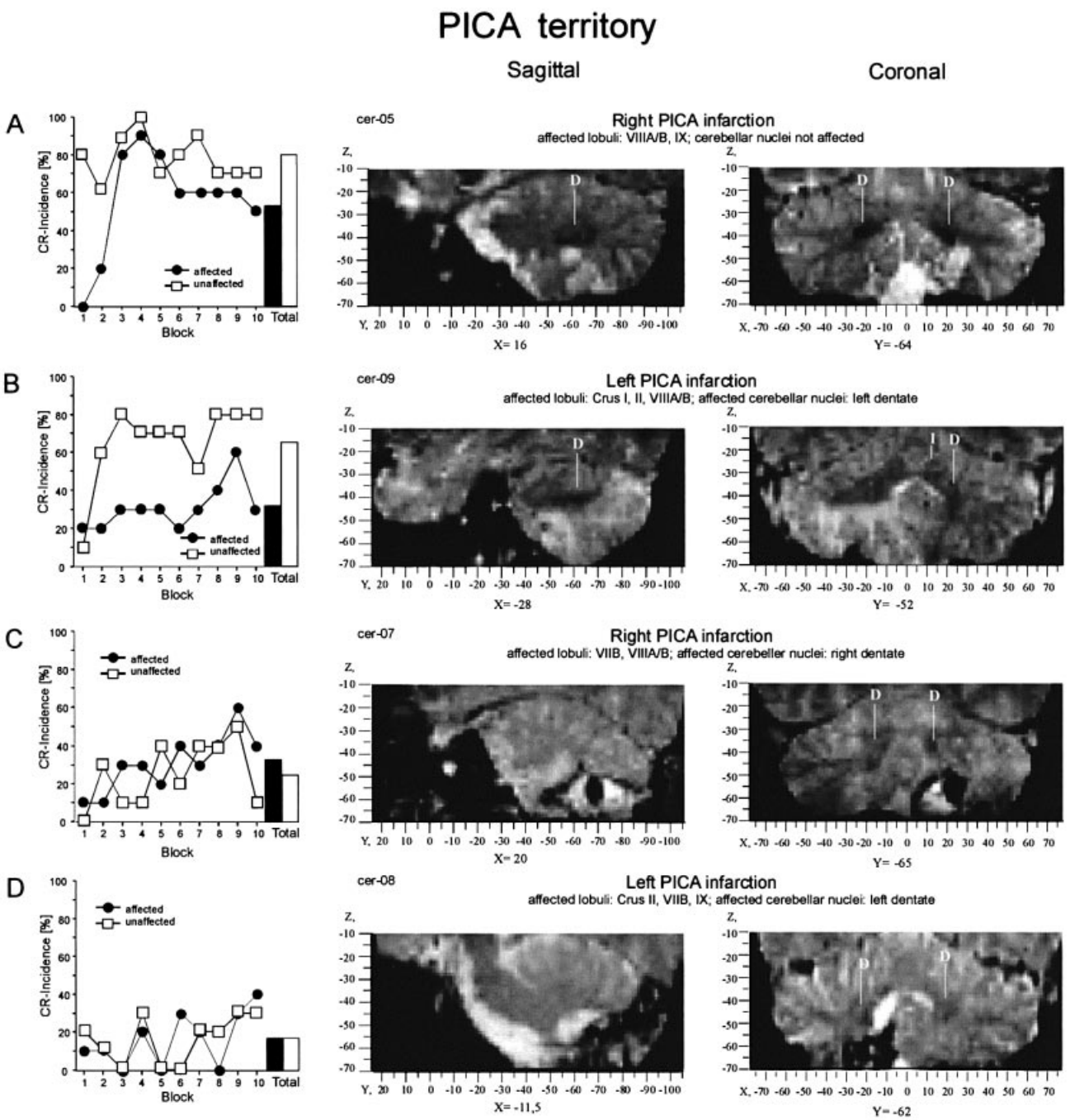

Fig. 14 Eyeblink conditioning and MRI findings in four representative PICA patients. Lesions included the common territory of the PICA in (**A**) cer-05, (**C**) cer-07 and (**D**) cer-08. In (**B**) cer-09, the lesion extended into Crus I, commonly supplied by the SCA. For further details, see Fig. 10.

cortical lesion in hemispheral lobule VI is sufficient to impair eyeblink conditioning in humans significantly.

PICA patients

Inspection of individual data revealed that three PICA patients (cer-05, cer-09 and cer-10) showed clear differences in mean CR-incidences with smaller mean values on the affected compared with the unaffected side. In cer-05, total CR-incidences were within normal limits on both sides (54% and 78%; see also Fig. 6). As revealed by Fig. 14A, CR-incidences per block were different in the first two blocks with smaller values on the affected side (which was tested first) compared with the unaffected side (which was tested second). There were no clear differences comparing the two sides in blocks 3–10. Achievement of maximal individual CR response rates within less blocks on the side tested second compared with the first was observed in control subjects (see

Fig. 4A), and probably reflects some transfer of learning (see Discussion). In the two other PICA patients with reduced CR-incidences on the affected side (affected/unaffected side: cer-09: 31%/65%; cer-10: 22%/62%), MRI scans showed that the lesion of the PICA infarction extended cranially into Crus I, which is commonly supplied by the SCA (see MRI findings above and SCA versus PICA). The findings for cer-09 are shown in Fig. 14B. Crus I involvement was present in one other PICA patient (cer-11) but there was no difference in conditioning rate between the two sides tested. However, total CR-incidences were low on both sides (affected: 12%, unaffected: 4%).

In all other PICA patients, lesions were restricted to regions caudally of the horizontal fissure (i.e. Crus II and below). For example, the lesion in cer-07 extended cranially into VIIB and in cer-08 into Crus II (Figs 14C and D). No clear differences comparing total CR-incidences on the affected and unaffected side were present (affected versus unaffected: cer-07: 31%/25%, cer-08: 16%/16%).

To summarize, the inferior and posterior territory of the cerebellum supplied by the PICA appeared not to be critical for eyeblink conditioning. Eyeblink conditioning seemed to be affected in PICA patients with lesions extending into Crus I, which is commonly supplied by the SCA.

Spontaneous blink rate

Comparison of the spontaneous blink rate revealed no significant difference comparing control subjects and cerebellar patients ($P = 0.944$), the affected and unaffected side (the first and second side, respectively) ($P = 0.87$) and no significant group by side interaction effect ($P = 0.51$). The mean number of spontaneous blinks across the experiments was 17.5 blinks/min (SD 15.6) on the first side and 18.7 blinks/min (SD 13.8) on the second side in the control group, and 18.3 blinks/min (SD 9.6) on the affected side and 17.6 blinks/min (SD 9.0) on the unaffected side in the patient group.

Discussion

There are five main results of the present study.

(i) The ability to acquire classically conditioned eyeblink responses was reduced in cerebellar patients.

(ii) Conditioning deficits were present ipsilaterally in patients with unilateral cerebellar lesions.

(iii) Deficits of eyeblink conditioning were most prominent in patients with lesions of the superior cerebellum, including hemispheral lobule VI and/or Crus I.

(iv) Eyeblink conditioning deficits were neither significantly different in patients with pure cortical lesions compared with patients with additional nuclear impairment; nor

(v) in patients with unilateral and bilateral lesions.

The first two results strengthen findings described in the previous human literature in a larger patient sample ($n = 27$) with more accurate MRI-based descriptions of the cerebellar lesion. The three other results provide evidence that some additional findings described in the previous animal literature are transferable to humans. In brief, the present data indicate that a unilateral cortical lesion within hemispheral lobule VI or Crus I is sufficient to reduce eyeblink conditioning in humans significantly.

Eyeblink conditioning is impaired in cerebellar patients

Involvement of the human cerebellum in eyeblink conditioning is a robust finding in various human lesion and functional brain imaging studies, despite differences in the cerebellar patient's pathology and differences in the experimental design (Lye *et al*., 1988; Solomon *et al*., 1989*a*; Daum *et al*., 1993; Topka *et al*., 1993; Molchan *et al*., 1994; Logan and Grafton, 1995; Blaxton *et al*., 1996; Woodruff-Pak *et al*., 1996; Bracha *et al*., 1997, 2000; Schreurs *et al*. 1997; Timmann *et al*., 1998; Ramnani *et al*., 2000). The present findings add to the compelling evidence that the human cerebellum is required for the acquisition of classically conditioned eyeblink responses. In the cerebellar group, eyeblink conditioning was significantly reduced [mean total percentage CR-incidence (SD): 14.4% (11.1)] compared with the age-matched control group [27.0 % (19.9)]. Given that the majority of patients presented with ischaemic cerebellar infarction, most cerebellar patients and their age-matched controls were middle-aged or elderly [mean age (SD): cerebellar: 54.9 (12.2), controls: 53.7 (14.3)]. In the control group, eyeblink conditioning resembled findings reported in the literature for a similar age range {Woodruff-Pak and Thompson, 1988 [mean age 52.8 years: 36.5 % (27.7)]; Woodruff-Pak and Jaeger, 1998 [50–59 years: 15.84% (16.9)]; Solomon *et al*., 1989*b* [50–59 years; mean total number of CRs (SD) in 70 conditioning trials: 31.4 (21.1)]}.

The ipsilateral cerebellum plays a role in eyeblink classical conditioning

Animal models have consistently shown that eyeblink classical conditioning deficits are restricted to the ipsilesional eye following cerebellar lesions (McCormick *et al*., 1982; McCormick and Thompson, 1984; Yeo *et al*., 1985; Bracha *et al*., 1999). Both the studies by Lye *et al*. (1988) and Woodruff-Pak *et al*. (1996) found that eyeblink conditioning was impaired on the ipsilesional eye but preserved on the contralesional eye. The present results confirm these findings in a larger group of cerebellar patients. In the 20 patients with unilateral lesions, eyeblink conditioning was significantly reduced on the affected side, but within normal limits on the unaffected side. However, Bracha *et al*. (1997, 2000) found impaired eyeblink conditioning on both sides in four out of five patients with unilateral lesions. Bracha *et al*. (1997, 2000) elicited the UR by a midline forehead tap and not by unilateral stimulus, which is commonly used in animal and

human lesion studies. Furthermore, lesions may have crossed the midline because MRI data were limited and available for two patients only. Their findings need to be confirmed when comparing conditioning using the same midline US in animal models and in a larger group of unilateral cerebellar patients with more detailed MRI information about lesion extent.

As in the study by Woodruff-Pak *et al*. (1996), the contralesional eye was always tested after the ipsilesional eye. It has been shown in animal lesion studies that the rate of learning is more rapid on the contralesional side when training is switched to the contralesional eye after training on the ipsilesional side (Yeo *et al*., 1985; Lavond *et al*., 1994). Comparable order effects were observed in the present control group. Normal participants reached their final level of CR-incidences within less trials on the side being tested second compared with the first. There was, however, no significant difference between both eyes in the final level of CR-incidences. Similarily, Woodruff-Pak *et al*. (1996) found no significant difference between the total mean percentage CR-incidences when comparing the two sides tested in their control group.

Although more pronounced order effects in cerebellar patients compared with controls cannot be excluded and may account for some parts of the differences between the affected and unaffected side in cerebellar patients, it is unlikely that they account for most parts. First, if learning had transferred significantly to the contralesional side following training of the ipsilesional side, learning should be faster on the unaffected side. However, no significant differences in the rate of conditioning (i.e. side by block interaction effect) were present when comparing the affected and unaffected side in the cerebellar patients. Furthermore, further analysis revealed that side effects were significant in patients with lesions of the superior cerebellum, but not in patients with lesions limited to the inferior and posterior cerebellum (see below). Significant side differences should be present in the both patient groups if side effects were caused by order effects alone. In addition, Lye *et al*. (1988) repeatedly reversed the eyes being tested in their patient and consistently found poor conditioning with the ipsilesional eye and good conditioning with the contralesional eye.

To summarize, the present study supports the findings of two previous human lesion studies that eyeblink conditioning is impaired on the affected but not the unaffected side in patients with unilateral cerebellar lesions.

Eyeblink conditioning is impaired in lesions of the superior but not the inferior cerebellum

Our working hypothesis was that eyeblink conditioning should be impaired on the affected side in patients with lesions including the territory of the SCA, but preserved in patients with lesions restricted to the territory of the PICA. As expected, eyeblink conditioning was significantly reduced on the ipsilesional side in SCA patients but within normal limits on the contralesional side. The area of maximum lesion overlap in the SCA group was within hemispheral lobule VI. In patients with lesions restricted to the common PICA territory (i.e. Crus II and below), no significant difference in eyeblink conditioning was found when comparing the affected and unaffected side. If the PICA lesion, however, extended cranially into Crus I, eyeblink conditioning was significantly impaired on the ipsilesional eye. The importance of Crus I is further supported by an SCA patient (cer-23) with a large infarction on one side and a lesion restricted to Crus I on the other side. Conditioning was similarly reduced on either side. In addition, most of the SCA lesions overlapping in lobule VI showed some extension into adjacent lobule Crus I.

The present findings suggest that lesions of the superior cerebellum, particularly of lobules VI and Crus I, are sufficient to impair eyeblink conditioning in humans. These findings are in good agreement with animal lesion data and findings of functional brain imaging in healthy human subjects. Numerous animal lesion studies (mainly by Yeo and colleagues) show that acquisition of eyeblink conditioning is critically dependent on normal function in cerebellar cortical lobule H VI (Yeo and Hesslow, 1998; Attwell *et al*., 2001), which contains the major eyeblink control regions (Hesslow, 1994*a*, *b*). Although ipsilateral Larsell lobule H VI has been shown to be most critical, adjacent areas of the ansiform lobe (i.e. Crus I and II) have been shown to play an additional role (Hardiman and Yeo, 1992). It should be noted that Yeo's group studied conditioning of the nictitating membrane response and not the eyelid blink in the rabbit. The former is a sixth nerve response and the latter a seventh nerve response. Although it is unlikely that these two responses differ very much in their central and cerebellar control, there might be minor differences.

In their fMRI study in healthy human subjects, Ramnani *et al*. (2000) found significant haemodynamic response changes during conditioning in the ipsilateral cerebellar cortex overlapping a medial portion of lobule VI and in an adjacent area of Crus I. The maximally active voxel was located within Crus I. Like the findings in animal models, these areas overlap with regions involved in (unconditioned) eyeblink control in humans. A recent fMRI study by our group showed that areas within ipsilateral lobules Crus I and VI are most active during evocation of the unconditioned eyeblink in healthy human subjects (Dimitrova *et al*., 2002*b*). Results of previous PET studies of eyeblink conditioning are also consistent with the present human lesion data. Although detailed information about the involved cerebellar lobuli is not given in the original papers, localization of activated cerebellar regions with the help of the atlas developed by Schmahmann *et al*. (2000) revealed activations both within ipsilateral lobule VI (Molchan *et al*., 1994; Logan and Grafton, 1995; Blaxton *et al*., 1996) and Crus I (Schreurs *et al*., 1997).

In their animal lesion studies, however, Mauk and colleagues (Perrett *et al*., 1993; Perrett and Mauk, 1995;

Garcia *et al*., 1999) found that the anterior lobe (i.e. lobule V and above) was essential for eyelid blink conditioning while hemispheral lobule VI was not. Although the present findings support the importance of hemispheral lobules VI and Crus I in eyeblink conditioning in humans, they do not exclude a role for other cerebellar lobules. Most of the SCA lesions affected the anterior lobe to various degrees. Although the area of maximal lesion overlap was within lobule H VI, it showed some extension into adjacent hemispheral lobule V. Based on the present human lesion data alone, it is not possible to differentiate finally between the role of particular lobules of the superior cerebellum in eyeblink conditioning. A study in more patients with lesions restricted to various parts of the superior cerebellum would be necessary.

There is only a limited possibility to compare the present findings with previous reports in patients with focal cerebellar lesions because information about the lesion location is less detailed or missing. Two of the seven cerebellar patients tested by Daum *et al*. (1993) presented with SCA infarctions. Consistent with the present results, eyeblink conditioning was reduced on the affected side in both SCA patients. Four of the seven patients with unilateral lesions included in the study by Woodruff-Pak *et al*. (1996) suffered from stroke (two within the SCA and two within the PICA territory). No attempt was made to compare findings in the PICA and SCA patients. Individual findings were presented for only one of the four stroke patients. This patient with a partial SCA infarction showed eyeblink conditioning within normal limits on the ipsilesional and contralesional sides. Although drawings of the lesion on axial slices of a cerebellar template are given, one cannot be certain which cerebellar lobuli were affected. If the lesion was restricted to hemispheral lobule V and above, the findings would be consistent with our present results. Likewise, one of the patients (cer-17) revealed a lesion restricted to lobule IV and showed no significant differences in conditioning between the affected and unaffected side. Bracha *et al*. (2000) reported abolished conditioning on the ipsilesional side in a patient with a PICA infarction and normal conditioning on the contralateral side. No information on CT or MRI findings are given. Based on the present findings, it is likely that the PICA infarction included Crus I. The cerebellar patient tested by Lye *et al*. (1988) had a cerebellar infarction, but no information about the affected cerebellar artery is given. Based on the present findings, an SCA infarction is most likely.

Pure cortical lesions are sufficient to impair eyeblink conditioning in humans

Lesions both of the cerebellar cortex and the deep cerebellar nuclei have been shown to be sufficient to impair acquisition of CR responses in the rabbit (Thompson *et al*., 1997, 2000; Yeo and Hesslow, 1998; Garcia *et al*., 1999). There is general agreement that small lesions of the anterior interposed nucleus are sufficient to abolish eyeblink conditioning (Bloedel and Bracha, 1995; Thompson *et al*., 2000). There is, however, some controversy if cortical lesions can completely and permanently abolish eyeblink conditioning in the rabbit (McCormick and Thompson, 1984; Lavond and Steinmetz, 1989; Harvey *et al*., 1993). Published data in cerebellar patients comparing the effects of cortical and nuclear lesions are very limited because detailed MRI-based information about lesion extent are so far missing. The 3D-MRI technique used in the present study enabled us to visualize directly the deep cerebellar nuclei in many cerebellar patients and to confirm their position (particularly that of the interposed nucleus) with the help of an MRI atlas of the human cerebellar nuclei (Dimitrova *et al*., 2002*a*). Based on MRI findings, SCA patients were divided in a group with lesions restricted to the cerebellar cortex and in a group with additional lesions of the interposed nucleus. No evidence was found that deficits in eyeblink conditioning were more severe in SCA patients with additional lesions of the deep cerebellar nuclei compared with SCA patients with pure cortical lesions. None of the patients presented with a pure nuclear lesion. Therefore, the effects of exclusive cortical and nuclear lesions could not be compared.

The present findings suggest that a lesion of the critical cortical areas of the cerebellum is sufficient to impair eyeblink conditioning in humans significantly. Results agree with findings of reduced or abolished eyeblink conditioning in patients with degenerative cerebellar disorders (Daum *et al*., 1993; Topka *et al*., 1993; Woodruff-Pak *et al*., 1996). Degenerative cerebellar disorders are thought to primarily affect the function of the cerebellar cortex leaving the cerebellar nuclei relatively intact. In the study by Woodruff-Pak *et al*. (1996), data are presented for one patient with a lesion predominantly of the cerebellar nuclei and two patients with cortical lesions. In the patient with the nuclear lesion, conditioning was abolished on the ipsilesional side but within normal limits on the contralesional side. In the two patients with cortical lesions, conditioning was reduced on the ipsilesional side compared with the contralesional side; CRs were still present. In their view, these two patients were able to produce some CRs with the ipsilesional eye because the interposed nucleus was spared. Based on the present findings, we propose that some effects of conditioning may equally be preserved because some of the critical cortical areas were not affected. In the first patient, drawings of the lesion on axial slices of a cerebellar template indicate a lesion in the PICA territory (Fig. 3 top in Woodruff-Pak *et al*., 1996). Our findings would suggest conditioning within normal limits on both sides. In fact, inspection of CR-incidences across blocks shows that CR-incidences were nearly the same within the last three of five blocks of the ipsilesional and contralesional eyes. CR conditioning was faster on the contralesional eye, which was tested second, suggesting an order effect. In the second patient, a small lesion of the superior cerebellum was present, which may not have affected all critical areas within lobules VI and Crus I (Fig. 3 bottom in Woodruff-Pak *et al*., 1996).

In a recent case study, Fortier *et al*. (2000) found preserved delay and trace eyeblink conditioning, but impaired discrimination learning, in a degenerative cerebellar patient. The authors suggest that delay conditioning was supported by intact deep cerebellar nuclei. Again, it cannot be excluded that the cerebellar cortical areas most critical for eyeblink conditioning might have been relatively preserved. No detailed MRI analysis of different sections of the cerebellar cortex is given and the most critical areas might be spared. In addition, the amount of cellular dysfunction does not necessarily correlate with the degree of cortical atrophy on MRI scans.

To summarize, the present data suggest that acquisition of conditioned eyeblinks is equally affected in patients with cortical cerebellar lesions and in patients with additional involvement of the interposed nucleus. In patients with cortical cerebellar lesions, intact deep cerebellar nuclei do not appear sufficient to acquire conditioned eyeblinks. Final conclusions, however, about the relative roles of the cerebellar cortex and nuclei in eyeblink conditioning in humans cannot be drawn. First, some limitations apply in differentiating patients with and without nuclear lesions despite the use of special MRI techniques. Localization of cerebellar nuclei was confirmed with the help of an atlas which—as many other atlases—is based on findings in an individual subject. Therefore, individual variability was not accounted for. Secondly, the present results are based on findings of a single session of 100 paired trials. In animal models, transitory deficits of eyeblink conditioning following cortical lesions have been described that recovered with further conditioning trials (McCormick and Thompson, 1984; Lavond and Steinmetz, 1989; Harvey *et al*., 1993). It cannot be excluded that patients with pure cortical lesions, but not with additional nuclear lesions, would have shown increasing effects of conditioning after several training sessions. In a few cerebellar patients only, eyeblink conditioning has been tested in sequential sessions. Three of the patients tested by Bracha *et al*. (1997) were tested in a total of four conditioning sessions. They showed a tendency toward an increased CR-incidence, which was not statistically significant. The patient in the study by Lye *et al*. (1988) was tested three times on the affected side and maintained relatively poor conditioning on this side. There is, however, no detailed information about whether the cerebellar nuclei were affected or not. Finally, in humans, other than in rabbits, conditioned responses may also be mediated by extracerebellar, cortical pathways. One may argue that effects of nuclear lesions are partially hidden because extracerebellar structures step in.

Deficits in eyeblink conditioning do not differ in unilateral and bilateral lesions

Although there was a tendency for patients with bilateral cerebellar lesions to show more severe deficits of eyeblink conditioning than patients with unilateral lesions, this did not reach statistical significance. There is some evidence in the animal literature that the contralateral side of the cerebellum may support eyeblink conditioning on the ipsilateral side (Yeo *et al*., 1997; Yeo and Hesslow, 1998). A role of the contralateral cerebellum has also been discussed in human PET studies (Logan and Grafton, 1995; Blaxton *et al*., 1996; Schreurs *et al*., 1997). Similarily, trigeminal afferents are known to be represented bilaterally (Snider and Stowell, 1944; Miles and Wiesendanger, 1975) and a bilateral representation of the unconditioned blink reflex has been shown in our recent fMRI study in humans (Dimitrova *et al*., 2002*b*). The present study does not exclude an additional, although smaller, role of the contralateral cerebellum in eyeblink conditioning. Small differences comparing a limited number of middle-aged and older patients with unilateral and bilateral lesions might not become significant because of the known variability of response acquisition in individual healthy subjects independent of age and the age-dependent decline (Woodruff-Pak and Thompson, 1988; Solomon *et al*., 1989*b*; Merrill *et al*., 1999). Again, differences in patients with unilateral and bilateral lesions might have become more obvious in sequential training sessions.

Conclusions

The present results confirm previous findings of disturbed eyeblink conditioning in cerebellar patients and on the ipsilesional side in patients with unilateral cerebellar lesions. Results extend previous findings in human lesion studies by showing that cortical lesions of the superior cerebellum (particularly of hemispheral lobules VI and Crus I) are sufficient to reduce eyeblink conditioning significantly. The present data cannot exclude the fact that other cerebellar cortical regions (e.g. on the contralateral side) play an additional although smaller role in eyeblink conditioning in humans.

Acknowledgement

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