Eyeblink conditioning is impaired in subjects with essential tremor

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Several lines of evidence point to an involvement of the olivo-cerebellar system in the pathogenesis of essential tremor (ET), with clinical signs of cerebellar dysfunction being present in some subjects in the advanced stage. Besides motor coordination, the cerebellum is critically involved in motor learning. Evidence of motor learning deficits would strengthen the hypothesis of olivo-cerebellar involvement in ET. Conditioning of the eyeblink reflex is a well-established paradigm to assess motor learning. Twenty-three ET subjects (13 males, 10 females; mean age 44.3 ± 22.3 years, mean disease duration 17.4 ± 17.3 years) and 23 age-matched healthy controls were studied on two consecutive days using a standard delay eyeblink conditioning protocol. Six ET subjects exhibited accompanying clinical signs of cerebellar dysfunction. Care was taken to examine subjects without medication affecting central nervous functioning. Seven ET subjects and three controls on low-dose beta-blocker treatments, which had no effect on eyeblink conditioning in animal studies, were allowed into the study. The ability to acquire conditioned eyeblink responses was significantly reduced in ET subjects compared with controls. Impairment of eyeblink conditioning was not due to low-dose beta-blocker medication. Additionally, acquisition of conditioned eyeblink response was reduced in ET subjects regardless of the presence of cerebellar signs in clinical examination. There were no differences in timing or extinction of conditioned responses between groups and conditioning deficits did not correlate with the degree of tremor or ataxia as rated by clinical scores. The findings of disordered eyeblink conditioning support the hypothesis that ET is caused by a functional disturbance of olivo-cerebellar circuits which may cause cerebellar dysfunction. In particular, results point to an involvement of the olivo-cerebellar system in early stages of ET.

Keywords: cerebellum; action tremor; ataxia; olivo-cerebellar pathways; motor learning

Abbreviations: CS = conditioned stimulus; ET = essential tremor; US = unconditioned stimulus; MRI = Magnetic Resonance Imaging; CR = conditioned response; SPL = sound pressure level; bmp = blinks per minute; EMG = electromyography; CRST = Clinical Rating Scale for Tremor; ICARS = International Cooperative Ataxia Rating Scale; SPT = symptomatic palatal tremor


Introduction

Essential tremor (ET), characterized by upper limb action tremor or head tremor, is one of the most common movement disorders (Deuschl et al., 1998; Louis, 2005). Evidence from lesioning studies (Dupuis et al., 1989; Nagaratnam and Kalasabail, 1997; Elble, 1998; Wilms et al., 1999) and PET studies (Hallett and Dubinsky, 1993; Wills et al., 1994; Boecker et al., 1996) led to the hypothesis that ET comes from oscillatory activity in olivo-cerebellar pathways (Elble, 1998; Deuschl and Elble, 2000). This hypothesis is supported by clinical studies demonstrating cerebellar motor dysfunction-like deficits in ET subjects. Intention tremor (Deuschl et al., 2000), disturbed tandem gait (Stolze et al., 2001) and eye movement deficits (Helmchen et al., 2003) were found in subjects with marked ET similar to subjects with cerebellar malfunction. Functional MRI and MR-spectroscopy studies in ET subjects are in accordance with these observations (Bucher et al., 1997; Louis et al., 2002a, 2004).

In addition to motor coordination, the cerebellum is critically involved in motor learning (for reviews see Thach, 1998; Barlow, 2002; Doyon et al., 2003). Evidence of
impaired motor learning would strengthen the hypothesis of olivo-cerebellar involvement in the pathogenesis of ET.

Eyeblink conditioning is a well-established model to study motor learning (for reviews see Green and Woodruff-Pak, 2000; De Zeeuw and Yeo, 2005). In contrast to other motor learning paradigms, for example learning of a motor sequence or adaptation of arm movements, tremor is unlikely to affect execution of the to be learned movement, that is the conditioned eyelink, as tremor of the eyelid is not a feature of ET. A behaviourally neutral conditioned stimulus (CS), such as an auditory tone, is presented and followed by an unconditioned stimulus (US), such as an air-puff that reliably elicits an eyelink response. Repeated paired presentations of US and CS leads to the gradual development of conditioned eyelink responses (CRs) to the CS. CRs are accurately timed, so that the eye is closed at the time of the air-puff. Extensive studies in animals, most of them in the rabbit, have successfully delineated the neuroanatomical circuities that are required for the CR-acquisition and retention of classically conditioned eyelink responses (for reviews see Thompson et al., 1997; Yeo and Hesslow, 1998; Bracha, 2004). Cerbellar cortex, cerebellar nuclei and inferior olives are essential for normal CR-acquisition. The cerebellar cortex appears to be important for timing of the conditioned eyelink response (Perrett et al., 1993; Koekkoek et al., 2003). Human lesion studies and functional brain imaging studies provide evidence that the human cerebellum is similarly involved in this form of associative learning (Daum et al., 1993; Topka et al., 1993; Woodruff-Pak et al., 1996; Schreurs et al., 1997; Ramnani et al., 2000; Gerwig et al., 2003). Both acquisition and timing of conditioned responses have been shown to be impaired in subjects with focal and degenerative cerebellar disorders (Gerwig et al., 2005; Timmann et al., 2005).

The aim of the present study was to examine whether ET patients exhibit eyelink conditioning deficits that are consistent with olivo-cerebellar pathology. To allow a larger number of acquisition trials and to investigate retention, subjects were tested on two consecutive days. ET subjects taking centrally acting drugs, which may affect cerebellar function (in particular primidone), were excluded.

### Material and methods

#### Subjects

Twenty-three ET subjects (13 males, 10 females; mean age 44.3 ± 22.3 years, mean disease duration 17.4 ± 17.3 years) and 23 neurologically healthy control subjects (12 males, 11 females; mean age 44.5 ± 21.1 years) participated (Table 1). At the time of study, all ET subjects fulfilled established criteria for ET (Deuschl et al., 1998). Six ET subjects exhibited accompanying cerebellar signs. Four ET subjects (ET-1, ET-9, ET-12 and ET-17 in Table 1) had intention tremor of at least one arm based on scores of two or more on an intention tremor score (Helmschen et al., 2003). Two ET subjects had cerebellar postural instability (ET-6 and ET-15). Eight ET subjects had a family history of ET, with at least two affected relatives in two generations (Deuschl et al., 2000). Six subjects with ET reported a beneficial response of alcohol to tremor.

Special care was taken that ET subjects with treatment (that is, primidone and other antiepileptics, anticholinergics, benzodiapezines) affecting central nervous system functions were excluded (Bahro et al., 1995). Seven ET subjects and three controls on low-doses of beta-blockers were included as animal studies indicate that propranolol up to 1 mg per kg body weight does not affect eyelink conditioning (Cartford et al., 2002). Additionally, two ET subjects and six controls were taking medication to treat hypertension, thyroid dysfunction, benign prostatic hyperplasia or elevated blood cholesterol levels.

With the exception of one ET subject (ET-17) and one control subject (CON-15), who had hearing difficulty due to tinnitus of the right ear, neither the ET subjects nor the control subjects showed clinical evidence of hearing difficulties.

ET subjects were evaluated with the Clinical Rating Scale for Tremor (CRST), part I (Fahn et al., 1988) and the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al., 1997). The local ethical committee approved this study. All participants gave informed written consent prior to the study.

### Conditioning paradigm

All participants were studied on two consecutive days. Parameters of the eyelink conditioning delay protocol (Gormezano and Kehoe, 1975) were similar to previous studies of our group where subjects with cerebellar lesions have been assessed (Gerwig et al., 2003, 2005; Timmann et al., 2005). In brief, a tone [1000 Hz, 70 dB sound pressure level (SPL), duration 540 ms] provided to the right ear was used as the CS and an air-puff [duration 100 ms; intensity 400 kPa at source, 110 kPa at nozzle] provided to the right eye as the US. The CS preceded the US by a fixed time interval of 440 ms and co-terminated with the US. In the two participants with hearing impairment of the right ear, the CS was presented to the left ear where hearing was normal. Ten CS-alone trials and 10 US-alone trials were presented in random sequence at the beginning of each experiment. This was followed by 100 paired CS–US trials. Hippocampus-dependent latent inhibition and learned irrelevance were unlikely to affect expected group differences. Because the hippocampus is not known to be involved in ET, possible retardation of CR-acquisition should be the same in controls and ET subjects. At the end of each experiment, 30 CS-alone extinction trials were performed. The inter-trial interval varied randomly from 20 to 35 s throughout the experiment. A silent movie was shown to maintain vigilance and attention.

Surface electromyography (EMG) recordings were taken from orbicularis occuli muscles on the right side. 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).

Before beginning of the experiment, hearing thresholds were assessed on both ears separately at 1000 Hz, the tone used as the CS. Thresholds of the stimulated ear were within normal age limits in all participants and there was no significant difference between groups (ET subjects 29.6 ± 4.9 dB, controls: 30.2 ± 5.5 dB, \( P = 0.6, \) unpaired \( t \)-test).
Table 1 Summary of essential tremor subjects and control subjects

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ET = essential tremor, * Clinical Rating Scale for Tremor (Fahn et al., 1988) with maximum of 24 points for tremor at both arms and maximum of 80 points for total part I; ICARS = International Cooperative Ataxia Rating Scale (Trouillas et al., 1997) with maximum of 100 points, No. = ET or control subject code; # Intention tremor score according to Helmchen et al. (2003), with maximum of 3 points per arm, ET Subjects in bold and italics printing are subjects with clinical cerebellar signs, m = male, f = female, UE = upper extremities, LE = lower extremities, H = head, V = voice
The number of spontaneous blinks was determined within 1 min before and after each experiment. The mean number of spontaneous blinks per minute (bpm) did not differ between groups (all P-values >0.2, ANOVA). In ET subjects it was 21.0 ± 7.9 bpm before and 23.8 ± 10.6 bpm after the first day of testing, and 22.8 ± 9.8 bpm before and 22.1 ± 12.9 bpm after the second day of testing; in controls it was 20.9 ± 11.5 bpm, 21.5 ± 10.2 bpm, 24.7 ± 13.2 bpm and 24.2 ± 11.4 bpm, respectively.

Data analysis
EMG recordings were analysed on a trial-by-trial basis using commercial software (Axograph V4 for Macintosh, Axon Instruments Inc., USA) with the examiner (M.K.) blinded to diagnosis and day of testing. EMG activity lasting at least 50 ms or merging into superimposed UR of at least twice the amplitude of mean EMG baseline activity and clear rising slope was defined as CR (Gerwig et al., 2003). Responses occurring up to 150 ms after CS onset or trials that showed a spontaneous blink prior to CS onset were excluded (Woodruff-Pak et al., 1996; Bracha et al., 1997, 2000). The total number of trials was subdivided into blocks of 10 trials. The number of CRs was expressed as the percentage of trials containing responses with respect to each block of 10 trials (percentage CR-incidence) and the total number of trials (total percentage CR-incidence).

The onset and peak-time latencies of unconditioned responses (URs) in the US-alone trials and CRs within the CS–US interval were visually identified as described previously (Gerwig et al., 2005). EMG amplitudes were not further considered because of methodological considerations in surface EMG recording.

To evaluate retention, CR-incidences in the first block of paired trials (block 1) were compared between day 1 and day 2. In addition, to evaluate retention without confounding effects of relearning, the number of CRs was compared in the CS-alone trials at the beginning of each experiment between day 1 and day 2.

To assess extinction, the CR-incidence within the last block of the paired trials (block 10) was compared with the CR-incidence in the last block of the extinction trials (extinction block 3). Additionally, the change of CR-incidence over the three extinction blocks was analysed. To ensure a remaining ability of CR-acquisition, only those participants who exhibited at least one CR in the first extinction block on day 1 were analysed for extinction (Gerwig et al., 2006). Based on this criterium CR-acquisition was above the spontaneous blink rate in subjects included in the extinction-analysis.

Results
Acquisition of conditioned eyeblink responses: ET subjects versus controls
Eyeblink conditioning was reduced in ET subjects on both days of testing as compared with controls (mean total percentage CR-incidence of ET subjects day 1: 14.0 ± 14.7% and day 2: 20.3 ± 17.42%, versus controls day 1: 32.0 ± 20.9% and day 2: 40.9 ± 22.5%; Fig. 1). Analysis of variance with repeated measures showed a significant group effect (P ≈ 0.001).

Both groups showed an increase of CR-incidences across the 10 blocks, that is learning (block effect P <0.001). Learning rate, however, was significantly decreased in ET subjects compared to controls (block by group interaction effect P <0.001). The group difference in learning rate was most marked on day 1. On day 2, both ET subjects and controls showed a small further increase of CR-incidence and reached their final level of CR-incidences in less blocks compared to day 1. Accordingly, day (P <0.001) and day by block interaction (P = 0.02) effects were significant, with no significant day by group (P = 0.46) or day by block by group interaction effects (P = 0.8).

Examples of EMG recordings further illustrate the findings. EMG recordings are shown for the 100 paired CS–US trials with the first trial at the top and the last trial at the bottom. CRs are specified by EMG recordings occurring within the CS–US window indicated by the two vertical lines. An example of a healthy 24-year-old male control subject (CON-22) is shown in Fig. 2A and B.

Fig. 1 Mean percentage CR-incidences ±SE in the group of all ET subjects and in the group of all controls on the first (A) and second day (B) of testing. Mean percentage CR-incidences are shown for each of the ten blocks (controls: open squares; ET subjects: filled circles) and across all blocks with paired CS-US presentations (controls: open columns; ET subjects filled columns). Total = mean total percentage CR-incidence ±SE.
Fig. 2  Eyeblink conditioning in a 24-year-old male control subject (CON-22 in Table I) on the first (A) and second day (B) of testing and in a 26-year-old male ET subject (ET-22 in Table I) on the first (C) and second day (D) of testing. Rectified and filtered (45 Hz) EMG data of the orbicularis oculi muscle of 100 paired CS-US trials (first trial on the top, last trial on the bottom) are shown. The first vertical line indicates the beginning of the tone (CS) and the second vertical line the beginning of the air-puff (US). AU = arbitrary units.
The total percentage CR-incidence was 67% on the first day and 79% on the second day. Note that CRs started after the second block of paired CS–US trials on the first day and CRs were present right at the beginning of the second day. In comparison, an example of a 26-year-old male ET subject (ET-22) is shown in Fig. 2C and D. The total percentage CR-incidence was 9% on the first day and 12% on the second day. Note that almost no CRs occurred throughout the 2 days of testing, with some CRs at the end of day 2.

Controls, but not ET subjects showed signs of retention. Comparing the first block of paired trials on day 1 and day 2 showed a significant increase of CRs in the control but not the ET group (Fig. 1; day by group effect $P = 0.048$). Differences in retention are further illustrated by comparing the number of conditioned responses in the 10 CS-alone trials at the beginning of each day of testing. Controls showed an increasing number of CRs from day 1 (mean absolute CRs: 0.6 ± 0.7) to day 2 (mean absolute CRs: 1.5 ± 1.7), but ET subjects showed no increase of CRs from day 1 (mean absolute CRs: 0.9 ± 1.7) to day 2 (mean absolute CRs: 0.9 ± 1.3). ANOVA revealed a significant day by group effect ($P = 0.045$) but no significant day ($P = 0.09$) and group effects ($P = 0.7$).

**Effect of low-dose beta-blocker therapy on conditioned eyelink acquisition**

To exclude effects of low-dose beta-blocker therapy on eyelink conditioning, only ET subjects ($n = 16$) and age-matched controls ($n = 16$) without beta-blocker medication were compared. The same results as for all participants studied were observed (mean total percentage CR-incidence of ET subjects day 1: 17.5 ± 16.3%, day 2: 24.9 ± 18.8% versus controls day 1: 38.8 ± 19.9%, day 2: 46.4 ± 21.5%; Fig. 3) with ANOVA showing similar effects (most notably block by group interaction $P = 0.005$, group effect $P = 0.002$). Additionally, the seven ET subjects on beta-blocker therapy (mean age 64 ± 15.6 years) were compared with the 16 ET subjects without any medical treatment (mean age 35.8 ± 19.5 years). Direct comparison between the two groups is difficult because of significant age differences ($P = 0.003$, unpaired t-test). On average, the ET subjects with no treatment were younger. Because eyelink conditioning is known to significantly decrease with age in particular after the age of 40 years (Woodruff-Pak and Thompson, 1988) age was included as a covariate in the statistical analysis. ANOVA showed a significant age effect ($P = 0.002$) and block effect ($P = 0.045$), while all other effects did not reach significance (all $P$-values >0.2).

These findings indicate that low-dose beta-blocker therapy does not explain decreased acquisition of conditioned eyelink responses in ET subjects.

**Effect of cerebellar signs on conditioned eyelink acquisition**

To answer the question if eyelink conditioning is more markedly impaired in ET subjects with clinical signs of cerebellar dysfunction, the six ET subjects with cerebellar signs (mean age 52.8 ± 26.6 years) were compared with the 17 ET subjects without cerebellar signs (mean age 41.41 ± 21.0 years). Age difference between the two groups was not significant ($P = 0.29$, unpaired $t$-test). In both groups, a small increase of CR-incidences across blocks was present on day 1 and day 2, with low total CR-incidences on both days (ET subjects with cerebellar signs day 1: 18.2 ± 23.1%, day 2: 17.8 ± 21.2%; ET subjects without cerebellar signs day 1: 12.5 ± 11.1%, day 2: 21.2 ± 16.6%; Fig. 4). On day 1 an increase of CR-incidences across blocks appeared to be more marked in the group with additional cerebellar signs in comparison to the group without cerebellar signs. This was due to two young ET subjects.
with cerebellar signs (18 and 21 years; ET-6 and ET-15) who exhibited relatively high-conditioning rates on both days (ET-6: day 1: 36%, day 2: 21%; ET-15: day 1: 57%, day 2: 59%). ANOVA revealed no significant group ($P=0.87$) and group by block effect ($P=0.88$). The block effect ($P=0.01$) was significant, while all other effects were not significant ($P$-values $\leq 0.2$).

The high-conditioning rate in subjects ET-6 and ET-17 may in part be due to the mentioned age effects on conditioning rates with significantly higher conditioning rates before the age of 40 years in healthy subjects (Woodruff-Pak and Thompson, 1988). The present group findings suggest that conditioning rates were probably higher before disease onset.

Next, the two ET subject subgroups were compared with their subgroups of age-matched controls (controls for ET subjects with cerebellar signs: mean age 49.5 ± 23.3 years; controls for ET subjects without cerebellar signs: mean age 42.7 ± 20.7 years). Both ET subject groups showed less CRs compared to their matched control groups (controls for ET subjects with cerebellar signs mean CR-incidence day 1: 23.5 ± 16.4%, day 2: 33.3 ± 19.9%; controls for ET subjects without cerebellar signs day 1: 35.0 ± 22.0%, day 2: 43.6 ± 23.3%). However, the difference in total CR-incidence between ET subjects with cerebellar signs and their controls was smaller than between ET subjects without cerebellar signs and their controls. Comparing ET subjects with cerebellar signs and their age-matched controls revealed a block by group effect close to significance ($P=0.06$), but no significant group effect ($P=0.38$), while these effects for the comparison of the ET subjects without cerebellar signs and their age-matched controls were significant (group effect $P=0.001$, block by group interaction effect $P<0.001$).

In sum, deficits in eyelink conditioning were not more marked in ET subjects with cerebellar signs compared to ET subjects without cerebellar signs.

### Timing of unconditioned and conditioned eyelink responses

Timing of onset and time to peak of the URs in the unpaired trials did not differ between groups (ET subjects UR-onset day 1: 67.5 ± 13.9 ms, day 2: 64.6 ± 10.1 ms, UR-peaktime day 1: 108.2 ± 24.0 ms, day 2: 104.2 ± 24.9 ms; controls UR-onset day 1: 62.7 ± 11.8 ms, day 2: 61.6 ± 10.6 ms, UR-peaktime day 1: 109.7 ± 20.0 ms, day 2: 105.2 ± 16.9 ms; all $P$-values $\leq 0.2$ as assessed by ANOVA).

Moreover, timing parameters of CRs in paired trials showed no significant difference between ET subjects and controls (ET subjects CR-onset day 1: –141.8 ± 57.8 ms, day 2: –130.3 ± 36.9 ms, CR-peaktime day 1: –89.7 ± 56.9 ms, day 2: –84.4 ± 32.2 ms; controls CR-onset day 1: –135.0 ± 30.8 ms, day 2: –122.8 ± 37.6 ms, CR-peaktime day 1: –87.9 ± 36.6 ms, day 2: –74.6 ± 29.8 ms; all $P$-values $>0.2$ as assessed by ANOVA). Negative values of CR-timing parameters refer to the time prior to US-onset, as US-onset was set to zero. Please also see the figure in supplementary material.

### Extinction

Fourteen ET subjects (mean age 36.3 ± 20.2 years) and 21 controls (mean age 46.0 ± 22.0 years) had at least one CR in the first extinction block on day 1 and entered the analysis. Both groups decreased the number of CRs comparing the last block of paired trials and the last block of extinction trials (Fig. 5A and B). In addition, both groups showed a decrease of CRs across the three extinction blocks (Fig. 5C and D). For the comparison of block 10 of paired trials with extinction block 3, ANOVA showed a significant extinction effect ($P<0.001$), but no significant group by extinction effect ($P=0.2$). The group effect reached significance ($P=0.055$) reflecting the lower CR-incidence of ET subjects in block 10. The day by extinction effect was close to significant ($P=0.06$), while all other effects were not (all $P>0.2$). Additionally,
comparison of CR-incidence across the three extinction blocks revealed similar results (extinction effect \( \text{P} < 0.001 \), group by extinction effect \( \text{P} = 0.3 \), group effect \( \text{P} = 0.074 \), all other effects \( \text{P} > 0.2 \)). Thus, effects of extinction were present in both groups. The absolute decrease appeared larger in controls compared with ET subjects. This difference, however, was largely due to the larger number of acquired CRs in the last block of paired trials. In fact, both groups showed a similar CR-rate in the last extinction block, which was close to the spontaneous blink rate.

**Correlation of conditioned eyeblink acquisition with clinical features of ET**

The comparisons of the total CR-incidence in paired trials and CRST, part I score [Pearson’s correlation coefficient day 1: \(-0.114, \text{P} = 0.6\); day 2: \(-0.04, \text{P} = 0.86\) (two-tailed); Fig. 6A and B] or subscores of CRST, part I (arm tremor or axial tremor: Pearson’s correlation coefficients ranging between \(-0.2 \) and \(0.2\); all \(\text{P}\)-values \(>0.2\)) were not significant. Additionally, there was no significant correlation between total percentage CR-incidence and total ICARS score [Pearson’s correlation coefficient day 1: \(0.12, \text{P} = 0.57\); day 2: \(-0.016, \text{P} = 0.94\) (two-tailed); Fig. 6C and D] or subscores (Pearson’s correlation coefficients ranging between \(-0.17 \) and \(0.2\); all \(\text{P}\)-values \(>0.3\)). Considering the six ET subjects with cerebellar signs alone (grey circles in Fig. 6C and D) showed equally no significant correlations.

Furthermore, neither beneficial response to alcohol nor a family history of ET showed a significant group effect (\(\text{P} = 0.9 \) and \(\text{P} = 0.14\), respectively) or group by block effect (\(\text{P} = 0.9 \) and \(\text{P} = 0.18\), respectively).

Total percentage CR-incidence and duration of ET showed a tendency to correlate on day 1 [Pearson’s correlation coefficient \(-0.38, \text{P} = 0.07\)], but not on day 2 [Pearson’s correlation coefficient \(-0.09; \text{P} = 0.6\)]. Next, the decline of eyeblink conditioning rates with age in both groups was examined. Figure 7A and B illustrate mean total percentage CR-incidences in three age groups (16–30,
31–60, 61–79 years) in ET subjects and controls on day 1 and day 2. As expected, eyeblink conditioning rates decreased with age, but in any age group, ET subjects had lower total CR-incidences than controls. The comparison of differences in total percentage CR-incidence between ET subjects and age-matched controls showed no significant difference between the three age groups (One-way ANOVA day 1: $P = 0.69$, day 2: $P = 0.77$).

**Discussion**

The present findings of disordered acquisition and retention of the classically conditioned eyeblink response provide further evidence that the olivo-cerebellar system is involved in the pathogenesis of ET. The unconditioned response was unaffected and therefore disorders in eyeblink response could not explain reduced eyeblink conditioning. Furthermore, impairment was not due to medication. Finally, acquisition of the conditioned eyeblink response was reduced in ET subjects regardless of the presence of cerebellar signs.

**Impaired acquisition of eyeblink conditioning points to olivo-cerebellar dysfunction in ET**

Results of animal studies reveal that normal activity around the olivo-cerebellar-cortical-nuclear loop is essential for normal acquisition and retention of eyeblink conditioning. The information about the unconditioned stimulus (that is, air-puff) is mediated through the inferior olive to the cerebellum via the climbing fibres. The information about the conditioned stimulus (that is, tone) is mediated via the pontine nuclei and their mossy fibre system. Both the intermediate cerebellar cortex and the interposed nucleus are likely places for plastic changes that contribute to eyeblink conditioning. In the cerebellar cortex, the simultaneous activation of US-related climbing fibres and CS-related mossy fibre-parallel fibre input is thought to

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**Fig. 6** Scatter plots comparing total percentage CR-incidences in paired trials of ET subjects (filled circles) with CRST, part I tremor score on the first (A) and second (B) day of testing, and with ICARS ataxia score on the first (C) and second (D) day of testing. Please note that total ICARS score was elevated in most ET subjects because some subitems are affected by the presence of tremor. Grey circles in (C) and (D) indicate ET subjects with clinical signs of cerebellar dysfunction. CRST = Clinical Rating Scale for Tremor; ICARS = International Cooperative Ataxia Rating Scale.
result in long-term depression (LTD) of excitatory parallel fibre-Purkinje cell synapses, which are active just before the expected US and may inhibit the cerebellar nuclei from generating a response until just before the US-onset. The learned association in turn is believed to influence the brainstem reflex centers of the eyeblink reflex via the red nucleus (for reviews see Christian and Thompson, 2003; Bracha, 2004; De Zeeuw and Yeo, 2005).

Accordingly, animal lesion studies showed that acquisition and retention of the conditioned eyeblink response are permanently abolished following lesions of the lateral pontine nuclei, inferior olive, cerebellar-interposed nucleus, the red nucleus and the inferior, middle and superior cerebellar peduncles (McCormick et al., 1982; Welsh and Harvey, 1998; Welsh, 1998). Animal studies examining the effects of lesioning to the cerebellar cortex reported either a complete disruption of eyeblink conditioning (Yeo et al., 1985) or its weakened but not abolished rate (Lavond and Steinmetz, 1989).

Studies examining eyeblink conditioning have been performed in humans with cerebellar lesions or lesions of the inferior olive. Despite differences in cerebellar pathology and differences in experimental design, the involvement of the human cerebellum in eyeblink conditioning is a robust finding in various human lesion studies (Daum et al., 1993; Topka et al., 1993; Woodruff-Pak et al., 1996; Bracha et al., 1997, 2000; Gerwig et al., 2003; Timmann et al., 2005). Reduction in CR-acquisition in the present group of ET subjects was within the range of impairment described in cerebellar subjects with focal lesions or degenerative cerebellar disorders (Gerwig et al., 2003; Timmann et al., 2005).

Thus, one possible explanation for impaired eyeblink conditioning in ET subjects is cerebellar dysfunction. However, dysfunction in the brainstem circuits outlined above, which are known to be important in eyeblink conditioning in animals, are other or additional options. Dysfunction of the inferior olives, which leads to cerebellar dysfunction, may be the most likely possibility.

Acute inactivation of the inferior olive in rabbits blocks associative learning (Welsh and Harvey, 1998; Welsh, 1998). In subjects with symptomatic palatal tremor (SPT), who show pseudohypertrophy of the inferior olives, eyeblink conditioning was found to be diminished (Deuschl et al., 1996). It has been proposed that SPT and ET share the pathophysiology of rhythmic activity in olivo-cerebellar circuits (for reviews see Deuschl et al., 2001; Deuschl and Bergman, 2002). A central oscillator may be located within the inferior olive with rhythmic activity being transferred through the cerebellum and reticulospinal system to the motoneurons.

This view is supported by the harmaline animal model of tremor (for review see Elble, 1998; Wilms et al., 1999; Deuschl et al., 2001). The tremorogenic effects of harmaline are thought to be mediated through an activation of the inferior olives that result in an enhancement of rhythmic bursting neurons in the olivo-cerebellar pathway (Elble, 1998; Wilms et al., 1999; Deuschl et al., 2000). Additionally, harmaline treatment severely diminished acquisition of CRs in rabbits possibly by the same mechanism of action as harmaline causes tremor (Turker and Miles, 1984; Harvey and Romano, 1993).

Lesions above the brainstem level are unlikely to explain significantly reduced CR-acquisition in ET subjects. In particular the basal ganglia, hippocampus and prefrontal cortex are active during simple delay eyeblink conditioning as revealed by animal recording studies and functional brain imaging studies in humans (Blaxton et al., 1996; Schreurs et al., 1997; for review see McIntosh and Schreurs, 2000). However, both animal and human lesion studies suggest a modulatory, but not an essential role of these brain structures (Daum et al., 1996; Woodruff-Pak and Papka, 1996; for a reviews see McGlinchey-Berroth, 2000; Powell et al., 2000). That is, brain tissue above the level of the midbrain is not necessary to acquire or express conditioned eyeblinks (Oakley and Russell, 1977; Mauk and Thompson, 1987), but timing and amplitudes of CR may change (Green et al., 2000; Delgado-Garcia and Gruart,
affected compared to ET subjects without cerebellar signs. Notwithstanding, a marked further reduction on a group level appears unlikely because CR-rates in the ET group were already within the range found in subjects with cerebellar lesions. Additionally, due to the known functional compartmentalization within the cerebellum, cerebellar areas related to gait and upper limb ataxia and eyeblink-conditioning-related areas are likely affected to different degrees in individual ET subjects. Both human and animal studies show that paravermal Larsell lobule HVI is most important in the acquisition of eyeblink conditioning (Yeo et al., 1985; Gerwig et al., 2003). This area overlaps with the known facial representation within the cerebellum (Grod et al., 2001). In humans, lesions of superior paravermal areas are followed by dysarthria (Urban et al., 2003). Accordingly, one study in subjects with spinocerebellar ataxia type 6 (SCA6) showed the strongest and most consistent correlation between reduction in eyeblink conditioning and dysarthria (Timmann et al., 2005). None of the ET subjects, however, presented with dysarthria.

Deficits in eyeblink conditioning did not correlate with clinical tremor severity, which is also true for neuropsychological deficits (Lombardi et al., 2001; Troster et al., 2002), postural instability (Bove et al., 2006) and olfactory dysfunction (Louis et al., 2002b). However, kinematic analysis of hand movements (Deusch et al., 2000), gait abnormalities (Stolze et al., 2001), eye movement abnormalities (Helmchen et al., 2003) and hearing impairment (Ono et al., 2003) did correlate with tremor severity. Because of the small range of tremor severity in ET subjects assessed in the present study, correlations may have been missed. Studies including ET subjects with more severe signs of tremor (but without centrally acting medication) need to be performed to validate the present observations.

Timing and extinction of conditioned eyeblink responses is preserved in ET

In subjects with cerebellar disorders, disordered timing of conditioned eyeblink responses and impaired extinction has been described (Gerwig et al., 2005, 2006). None of these abnormalities were present in the ET subjects.

The most robust marker of impaired eyeblink conditioning both in human and animal cerebellar lesion studies, however, is reduced or abolished CR-acquisition (Yeo and Hesslow, 1998; Bracha, 2004). Timing disorders have also been described, but findings both in animal and human studies appear contradictory.

Whereas shortened CRs have been reported by some authors (Perrett et al., 1993; Koekkoek et al., 2003; for review see Maek et al., 2000), Yeo et al. (1984, 1985) found an increased variability but no shortening of CR-latencies. Likewise, the possible role of the cerebellum in CR-timing is an issue of ongoing debate. Maek et al. (2000) suggest that appropriate timing depends on the
cerebellar cortex of the anterior lobe, whereas Attwell et al. (2002) argue that changes in CR-timing may equally be explained by extracerebellar premotoneuronal disinhibition. Two human studies reported a tendency of conditioned responses to be delayed in subjects with cerebellar disorders (Topka et al., 1993; Woodruff-Pak et al., 1996). In contrast, short-latency conditioned responses have been found by our group (Gerwig et al., 2005).

In our past study, effects of disordered timing were small, that is CRs occurred on average 20 ms earlier than in controls (Gerwig et al., 2005). In contrast to animal studies, findings are based on the remaining CRs in subjects with significantly reduced CR-acquisition. Accordingly, timing disorders were present only in a large number of cerebellar subjects (n = 43). CR-incidences in ET subjects were also significantly reduced and group size (n = 23) may have been too small to observe small CR-timing differences compared with controls. Our previous study in cerebellar subjects suggests that CR-acquisition and CR-timing depend on different areas within the cerebellar cortex (Gerwig et al., 2005). CR-timing deficits were found to be most prominent in subjects with cortical lesions of the anterior lobe including Larsell lobule HV, whereas deficits in CR-acquisition were related to lesions of the more caudal parts of the superior cerebellar cortex including Larsell lobule HVI. These areas may be affected differently in ET subjects.

Taking the reduced CR-incidences in paired trials into account, ET subjects were able to extinguish remaining CRs to a similar extent as controls. Animal studies show that extinction is an active learning process that is distinct from acquisition (Perrett and Mauk, 1995; Medina et al., 2002; for review see Robleto et al., 2004). It is a matter of ongoing research as to what extent brain substrates differ from those involved in acquisition and extinction. Both the cerebellum and hippocampus appear to be involved in extinction. ET may cause dysfunction in the olivo-cerebellar circuits involved in CR-acquisition, but not CR-extinction. It cannot be excluded, however, that in more severely affected ET subjects, the olivo-cerebellar structures necessary for extinction are more substantially affected.

Conclusions

The present findings of disordered eyelid conditioning support earlier reports of olivo-cerebellar dysfunction in ET. Results extend previous findings as they point to an involvement of the olivo-cerebellar system in early stages of ET without the presence of clinical cerebellar signs.

Supplementary material

Supplementary material is available on Brain online.

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