Cerebellar responses evoked by nociceptive leg withdrawal reflex
as revealed by event-related fMRI.

Dimitrova A¹, Kolb FP³, Elles H-G¹, Maschke M¹, Forsting M², Diener HC¹, Timmann D¹

¹Departments of Neurology and ²Neuroradiology, University of Essen, 45122 Essen
³Institute of Physiology, University of Munich, 80336 Munich; Germany

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Corresponding author
Dagmar Timmann, MD
Department of Neurology
University of Essen
Hufelandstraße 55
45122 Essen
Germany
Phone  +49 201 723 3816
Fax  +49 201 723 5901
Email  Dagmar.Timmann@uni-essen.de

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**Abstract**

The aim of the present study was to examine nociceptive leg withdrawal reflex-related areas in the human cerebellum using event-related functional brain imaging (fMRI). Knowledge about cerebellar areas involved in unconditioned limb withdrawal reflex control has some relevance in understanding data of limb withdrawal reflex conditioning studies. Sixteen healthy adult subjects participated.

Nociceptive leg withdrawal reflexes were evoked by electrical stimulation of the left tibial nerve behind the medial malleolus. An event-related fMRI-paradigm was applied with a total of 30 stimuli being delivered pseudorandomly during 500 consecutive MR scans. Surface electromyographic (EMG) recordings were performed from the left anterior tibial muscle. Only trials with significant reflex EMG activity were used as active events in fMRI statistical analysis. The specified contrasts compared the active event condition with rest. Leg withdrawal reflex-related areas were located within the vermis, paravermis and lateral posterior cerebellar hemispheres bilaterally. Vermal and paravermal areas in lobules III/IV in the anterior lobe and in lobule VIII in the posterior lobe agree with the cerebellar representation of climbing and mossy fiber hindlimb afferents and voluntary leg movements. They are likely related to efferent modulation of the leg withdrawal reflex and/or sensory processing of afferent inputs from the reflex and/or the noxious stimulus. Additional activation within vermal lobule VI and hemispherical lobules VI/Crus I may be related to other pain-related processes, e.g. facial grimacing, fear and startle-like reactions.

**Keywords:** Limb flexion reflex, human, unconditioned response.
Introduction

Classical conditioning of specific aversive reactions has frequently been used to study the role of the cerebellum in motor learning in both animals and humans. Numerous cerebellar studies have investigated classical conditioning of the eyeblink response (for reviews: Bloedel and Bracha 1995; Yeo and Hesslow 1998; Thompson et al. 1997; Mauk et al. 2000; Woodruff-Pak and Steinmetz 2000a,b). Other studies have examined involvement of the cerebellum in conditioning of the nociceptive limb withdrawal reflex (Kolb et al. 1997; Timmann et al. 1996, 2000; Voneida 2000; Apps and Lee 2002). In order to understand the role of the cerebellum in reflex conditioning knowledge about the cerebellar areas involved in control of the unconditioned responses have some relevance. Firstly, lesion studies are likely to show the clearest deficits in learning if all areas involved in unconditioned reflex control are affected (Yeo and Hesslow 1998). Likewise, one possible reason for controversial results of lesion studies may be that cerebellar cortical areas involved in unconditioned reflex control are affected to various extents. In addition, demonstration of cerebellar areas involved in control of unconditioned reflexes raises the controversial issue of impaired motor performance (i.e. impaired unconditioned reflexes) and its possible relation to deficits in conditioning (pro: Welsh and Harvey 1989; Harvey et al. 1993; contra: Steinmetz et al. 1992).

Cerebellar areas involved in control of unconditioned eyeblink response have been investigated in detail in animals (Hesslow 1994a,b; Pellegrini and Evinger 1997; Morcuende et al. 2002) and humans (Dimitrova et al. 2002a). Knowledge about cerebellar areas involved in control of the nociceptive limb withdrawal reflex is less detailed. There is, however, experimental evidence that the cerebellum is involved in performance of the limb withdrawal reflex. Early animal studies report changes of limb withdrawal reflex following lesions and stimulation of the cerebellum (Chambers and Sprague 1955; Denny-Brown et al. 1929) and inferior olive (Rabacchi et al. 1986). More recent studies describe limb withdrawal reflex changes following lesions of the paravermal cortex (Yu 1972) and interposed nuclei
Although involvement of the intermediate cerebellum has been shown in limb withdrawal reflex control, no attempts have been made to map the cerebellar cortical lobules involved. The number of human studies examining the cerebellar role in nociceptive limb withdrawal reflex control is more limited. Impaired leg withdrawal reflexes have been reported in patients with Friedreich’s ataxia (Shahani and Young 1971) and in a patient with an unilateral surgical cerebellar lesion (Timmann et al. 1998a). In a recent PET study of our group changes of cerebellar activity evoked by nociceptive leg withdrawal reflex have been observed within vermal regions primarily of the anterior lobe in healthy human subjects (Maschke et al. 2002a).

The aim of the present study was to use the high spatial and temporal resolution of event-related fMRI to examine nociceptive leg withdrawal reflex-related cerebellar areas in humans. More detailed knowledge of unconditioned withdrawal reflex-related cerebellar areas are thought helpful to interpret data of limb withdrawal reflex conditioning studies both in cerebellar patients and in healthy human subjects using functional brain imaging.
Methods

Subjects

Sixteen healthy adult subjects participated. Their average age was 30.8 ± 6.0 (range 22 - 41) years. Five subjects were female and eleven male. None of them had a history of neurological disease or revealed neurological signs based upon neurological examination. All experiments were undertaken with the understanding and written consent of each subject. The local ethical committee of the University of Essen approved the study.

EMG recordings

Subjects were lying supine with the knee supported by a pillow and with their eyes closed. Nociceptive leg withdrawal reflexes were evoked by a train of bipolar current pulses (f=100 Hz, pulse duration 0.65 ms) lasting for 100 ms applied through surface electrodes to the left tibial nerve behind the medial malleolus. The electrical stimulator (Elektrischer Stimulator ES, Jaeger Toennies, Germany) was placed outside the MR scanning room. Before the recording session the sensory and motor thresholds were determined followed by successively increase of the current intensity until significant leg flexion responses were evoked. Mean sensory threshold was 1.2 mA (SD 0.5, range 0.4 -1.5), mean motor threshold was 4.9 mA (SD 1.9, range 2-7) and mean leg withdrawal reflex stimulation strength was 14.3 mA (SD 7.5, range 6-30 mA).

An event-related fMRI-paradigm (see below) was applied with a total of 30 electrical stimuli being delivered pseudorandomly and with long inter-trial intervals (range 28-56 seconds) during 500 consecutive MR scans. For each subject electrical stimuli were delivered at the end of the 15th, 35th, 50th, 67th, 83rd and 95th MR scan, with the same order being repeated during the following 400 scans (115th, 135th, 150th, 167th, 183rd, 195th, 215th, 235th, 250th ...). Intervals of 600 ms were interposed between individual scans. Electrical stimulation occurred 186 ms after the beginning of the last of 22
slices of each scan and limb flexion reflexes were recorded within the interval without echo-planar imaging.

Electromyographic (EMG) activity was recorded from the left anterior tibial muscle (sampling rate 1000 Hz). A pair of disposable surface carbon electrodes (ConMed, USA), commonly used for ECG recording in the MR environment, was placed over the muscle belly. The overlying skin was cleaned with isopropyl alcohol (75%) and shaved if needed. A velcro fasting ground strip was fixed between the site of stimulation and the recording site close to the ankle. The EMG signals were fed via carbon fiber cables (D120RL radiolucent cables, 3M Medica, USA) to a battery powered EMG amplifier (LWL-EXX-amplifier, Institut für Explorative Datenanalyse, Germany) custom built for use in the MR environment (gain: 50-100, filter: 10 (2) Hz < f < 2000 Hz). Electrical signals were transformed into optical signals and transmitted via fiber-optic cables to a fiber-optic receiver placed outside the scanning room. EMG signals were fully rectified, further filtered (100 Hz) and stored for off-line analysis.

**EMG analysis**

The EMG data were analyzed on a trial-by-trial basis using a commercial software (AxoGraph 3.5, Axon Instruments, USA). Visual inspection of each trial allowed identification of trials without significant flexion reflex activity. Further analysis of leg withdrawal reflex was limited because of superimposed artifacts. The nociceptive leg withdrawal reflex consists commonly of an early (F1) and a late (F2) reflex component which are separated by a silent period (S) (Shahani and Young 1971; Meinck et al. 1983). Because onset of the early F1 component frequently overlapped with the stimulation artifact, onset of the F2 component only was determined. Onset was defined where EMG activity significantly raised from baseline activity. The end of the reflex response was frequently difficult to determine largely due to cable movement artifacts. Data inspection revealed that a fixed
interval of 50 ms was free of artifacts in most cases. Therefore, integrated EMG was quantified for a fixed interval of 50 ms (50 ms-IEMG). Mean EMG baseline activity was acquired in a 100 ms interval prior electrical stimulation. Mean baseline activity was subtracted from 50 ms-IEMG. To allow for comparison between subjects, leg withdrawal reflex amplitudes were normalized to the mean amplitudes across all 30 trial in each individual subject. Each withdrawal reflex amplitude was expressed as a percentage of this value.

Means and SDs of F2 onsets and fixed 50 ms-IEMGs were calculated. To quantify possible changes across time (i.e. habituation and sensitization effects) linear regression analyses with 50ms-IEMG as dependent factor were performed across trials for the group of all subjects and each individual subject.

**Imaging**

All fMRI scans were taken with a 1.5 T Siemens Sonata scanner (Dept. of Neuroradiology, University of Essen) with standard head coil. A multislice echo planar imaging sequence (EPI) was used to produce 22 continuous 3 mm thick axial slices covering the volume of the cerebellum and adjacent brainstem with TR = 2.2 s, TE = 60 ms, flip angle = 90°, 64 x 64 matrix and voxel size = 3.59 x 3.59 x 3 mm³. An event-related fMRI-paradigm was used for the experiment. An electronic triggering signal was used to achieve synchronization between the time of initiation of the active event (electrical stimulus) and the MR acquisition. Each series consisted of 500 scans with a total duration of 23.3 minutes. Structural images were acquired for each subject using a T1 3D sequence with TR = 11.1 ms, TE = 4.3 ms, flip angle = 15°, 136 partitions, effective thickness = 1.2 mm and voxel size = 1 x 1 x 1.2 mm³.
**Image analysis**

The stereotaxical transformations and statistical analysis were carried out on a Sun Sparc Ultra 80 computer with statistical parametric mapping software (Wellcome Department of Cognitive Neurology, London, UK), version SPM99, implemented in MATLAB (Mathworks, Sherborn, MA). The functional images were first subjected to a slice time-alignment process. This correction procedure is necessary to minimize image intensity inhomogeneity arising from differences in slice image acquisition timing. All individual volumes were realigned after the six head-movement parameters were estimated (3 translations and 3 rotations) from rigid body transformations. The realignment procedure minimizes the displacement effects between each volume of the time series and the first. Images were then spatially normalized (Friston et al. 1995) into the reference system of Talairach and Tournoux (1988), using a representative standard EPI template from the Montreal Neurological Institute (MNI; Evans et al. 1994). The functional images were subsampled to a voxel size of $2 \times 2 \times 2$ mm$^3$ and smoothed using an isotropic Gaussian kernel of 6 mm.

The statistical analysis was performed for each time series after specifying the appropriate design matrix. The active event was modeled with hemodynamic response functions (HRF). The six head movement parameters were included as trial specific covariates in the design matrix to take into account effects of head movement related changes following electrical stimulation of the foot. To minimize the effects of magnetization relaxation artifacts and to reduce the effects of accompanying startle-like reactions (see Results, Nociceptive withdrawal reflex recordings) scans corresponding to the first two stimuli (i.e. the first 40 scans of each time series) were excluded from statistical analysis. The significance of effects was assessed using Z statistics for every voxel from the brain, and these sets of Z values were used to create statistical parametric maps (SPMs). A high-pass filter was used to remove low-frequency drifts and fluctuations of the signal (Friston et al. 1996), and proportional scaling was applied to remove global changes in the signal.
Data were analyzed for each subject individually and for subjects as a group. Group effects were calculated using random effects models. Contrast images, one for each single subject, were taken to the second level analysis and entered into the one sample t-test model (Friston et al. 1999). The statistical test of variance of these single contrast images from subject to subject consists of contributions from both the between and within subject components of variance and can be used to extend the inference to population.

The specified contrasts compared the active event condition with rest. Only trials with significant EMG activity were used as active events in fMRI statistical analysis (see Results, Nociceptive withdrawal reflex recordings). In three subjects no significant flexion reflexes were observed in EMG recordings. Data from these three subjects were excluded from statistical analysis. For the group study a threshold of $p < 0.0001$ (uncorrected; extended threshold 20 voxels) was adopted. Thresholds for statistical analysis of individual subjects were set as $p < 0.001$ (uncorrected; extended threshold 20 voxels).

Anatomical localization of MNI coordinates were defined based on the 3-D MRI atlas of the human cerebellum in proportional stereotaxical space developed by Schmahmann et al. (2000) and the 3-D MRI atlas of the human cerebellar nuclei developed by Dimitrova et al. (2002b). The lateral extent of the vermis is difficult to define, particularly within the anterior lobe (Schmahmann et al. 2000). As an approximation, vermal activation was defined as activation within an x-range of -10 mm and 10 mm. Activation more laterally than 10 mm from the AC-PC line were defined as activation within the cerebellar hemispheres.

For localization of cerebral activation the Talairach and Tournoux atlas (1988) was used. To correct for a certain mismatch between MNI and Talairach and Tournoux space MNI coordinates were transferred to the Talairach and Tournoux space using the algorithms described in http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html.
Results

Nociceptive withdrawal reflex recordings

Visual inspection of surface EMG recordings of the anterior tibial muscle revealed that leg withdrawal responses were present on average in 84 % (SD 21 %; range 50 –100%) of the trials in 13 of the 16 subjects. In three subjects no significant limb flexion response activity was observed. These three subjects were excluded from fMRI analysis. Onsets and 50ms-iEMGs of the limb flexion response were determined in nine of the 13 subjects with provable withdrawal responses. In the remaining four subjects prominent artifacts prevented more detailed EMG analysis.

Individual, non-rectified EMG recordings of one subject (s-9 in Tab. 1 and 2) are shown in Fig. 1A. The beginning of electrical stimulation within the MR scanning free interval is indicated by a vertical line. The electrical stimulation is followed by EMG bursts in all 30 trials, with the first trial at the top and the last trial at the bottom. The beginning of subsequent MR scanning is indicated by the artifacts at the end of each trace. EMG responses were largest within the initial trials with no prominent change of response size in the remaining trials.

Mean changes of responses size (normalized 50ms-IEMG) across trials in the group of all subjects are shown in Fig. 1B. Response reduction was most prominent within the first three trials. Largest response size of the first two trials suggested accompanying startle-like responses. Linear regression analysis of group data showed significant changes across trials (R = 0.63, p < 0.001). All but two of the individual subjects showed a tendency to decrease responses across trials (i.e. negative slope in individual linear regression analysis) which was significant in four (p < 0.05; s-6, s-8, s-9, s-13 in Tab. 1 and 2). Because quantitative EMG data were not available in all subjects, amount of EMG reductions across trials was not considered covariate in statistical fMRI analysis. The first two trials, however, were excluded from fMRI analysis to reduce effects of startle-like responses.
In the group of all subjects, mean onset of leg withdrawal response (i.e. the F2 component) was 160 ms (SD 47 ms). Latencies were within the range described in the literature for nociceptive leg withdrawal reflex recordings without preinnervation of the anterior tibial muscle (e.g. Shahani and Young 1971; Torring et al. 1981).

**FMRI data**

SPM (t) maps of random model group analysis (p < 0.0001, uncorrected) are shown in Fig. 2. Nociceptive leg withdrawal reflex-related areas of cerebellar activations were present within the vermis, paravermal areas and the lateral posterior hemispheres. Local maxima were found within the anterior vermis (lobules III/IV) and the posterior vermis (lobules VI and VIII). Activation extented into the fastigial nuclei. Paravermal areas were activated bilaterally within the anterior lobe (lobules III/IV) and posterior lobe (lobule VIII). Within the lateral posterior hemispheres local maxima were found within lobules VI and Crus I on the left and lobules V and VI on the right. Hemispheral activations were more prominent on the left.

Group analysis revealed the largest cluster of activation (cluster size = 308) within the vermis with an absolute maximum in lobule VI (-2, -64, -26; Z = 4.96) extending into lobule V (-2, -64, -12; Z = 4.55). Additional local maxima were present within lobule III (-6, -44, -20; Z = 4.15), extending into lobule IV (-4, -48, -16; Z = 4.17), and lobule VIII A (-2, -68, -42; Z = 4.75; -2, -66, -34; Z = 4.58), extending into lobule VIIIB (-2, -58, -30; Z = 4.77). Fig. 3 illustrates that the cluster of activation included the fastigial nuclei. The statistical group data from the random model are displayed on MR templates taken from the 3-D atlas of the human cerebellum nuclei developed by our group (Dimitrova et al. 2000b).

An area of significant activation is seen in the roof of the fourth ventricle (2, -52, -28) which is consistent with the known location of the fastigial nuclei (Angevine et al. 1961; Duvernoy 1995). There was no significant activation of the interposed and dentate nuclei (I and D in Fig. 3). However, because
the exact borders between fastigial and interposed nuclei are difficult to define, activation of parts of
the interposed nuclei cannot be excluded.

The second largest cluster of activation (cluster size = 121) was present in paravermis of the left
anterior lobe with an absolute maximum in paravermal lobule IV (-22, -32, -36; Z = 4.85) and an
additional maximum in lobule VI (-30, -44, -34; Z = 4.71). The third largest cluster (cluster size = 77)
was located within left Crus I (-42, -64, -32; Z = 4.89). Smaller clusters of activations were present
within right paravermal lobules III/IV (24, -34, -34; Z = 4.63), hemispheral lobules V (28, -38, -28; Z =
4.12) and VI (28, -48, -32; Z = 4.87). In addition, small clusters of activation were located within left
paravermal lobule VIIIa (-22, -72, -52; Z = 4.78) and right paravermal lobule VIIIb (24, -44, -56; Z =
4.67). On the group level, no significant extracerebellar activations (e.g. within the temporal lobes or
brain stem) were found. The statistical group data from the random model are displayed on axial
sections of a typical canonical MNI brain in Fig. 4. Table 1 summarizes the Z-scores and MNI
coordinates for the vermal activation for the random model group analysis as well as for each single
subject. Table 2 summarizes the data for the hemispheral activation.

Analysis in individual subjects showed a similar pattern of activation within the vermis and cerebellar
hemispheres. In six subjects (s-1 – s-6) withdrawal reflex related activation was present in vermal
lobule III and/ or IV. In two subjects vermal activation was present in lobules I/II (s-5, s-7). Vermal
activation in the seven subjects are displayed on sagittal sections of the individual normalized
anatomical data sets in Fig. 5. Activation in vermal lobule VI was present in nine subjects with
additional activation in vermal lobule V in two subjects. Vermal activation in lobule VI is shown in six
individual subjects in Fig. 5 (s-1, s-2, s-3, s-5, s-7 and s-10). Note that in s-2 nearest local maximum
was within vermal lobule VIIa and activation in VI is therefore not listed in Table 1. Vermal lobule
VIIIa was activated in five subjects, with extension into lobule VIIIb in one subject. Vermal lobule VII
was activated in seven subjects. Activation of vermal lobules VII and/ or VIII is shown in seven of these
subjects (s-1 – s-7) in Fig. 5. Vermal activation extended into the region of the fastigial nuclei in six subjects. Activation overlapped with MNI coordinates of fastigial nuclei based on Dimitrova et al. (2002b) in three subjects (s-1, s-6, s-8 in Tab. 1) and was in close neighborhood in the other three subjects (s-2, s-3, s-7). Activation extending into the roof of the fourth ventricle is seen in four of the individual subjects in Fig. 5 (s-1, s-2, s-3, s-7). Activation overlapping with the location of the dentate or interposed nuclei was present in five subjects [s-2: right interposed (4,-68,-38; Z = 4.46), s-3: left interposed (-14,-56,-26; Z = 4.8), s-4: left dentate (–18,-66,-38; Z=5.34), s-7: left dentate (-14, -58, -36; Z = 3.86), s-8: left interposed (-8,52,-24; Z = 4.72)].

Activation of paravermal lobules III/IV was present in one subject on the left, in five subjects bilaterally and in three subjects on the right. Activation of paravermal lobules VIII A/B was present in four subjects on the left, in four subjects bilaterally and in one subject on the right. Two subjects showed activation in left lobule VII. Finally, activation of hemispheral lobule VI was present in five subjects on the left and in four subjects bilaterally. Activation of lobule Crus I was present in three subjects on the left, in two subjects bilaterally and in four subjects on the right.

In ten individual subjects additional activation was found within the temporal lobes. Temporal activation was on the left in three subjects and bilateral in seven subjects. The location of activation within the temporal lobes varied between individual subjects. Locations included gyrus temporalis superior and medius, gyrus parahippocampalis, uncus and amygdala.
Discussion

Different areas within the cerebellum showed a significant change of activity related to the nociceptive leg withdrawal reflex. Areas were located within anterior and posterior parts of the vermis and paravermis as well as both posterior lateral cerebellar hemispheres. Vermal and paravermal areas (i.e. lobules III and IV in the anterior lobe and lobule VIII in the posterior lobe) match with areas known to be involved in leg control and are likely related to the withdrawal of the limb from the noxious stimulus (Sherrington 1910; Kugelberg et al. 1960; Hagbarth and Finer 1963). The other areas within the posterior lobe (i.e. hemispheral lobules VI and Crus I, vermal lobule VI) may be related to other pain-related processes (e.g. pain-induced facial grimacing and/or fear; Craig 1984) and startle-like reactions (Dowman 1992; Bromm and Scharein 1982).

Findings confirm and extend results of our previous PET study of the nociceptive leg withdrawal response (Maschke et al. 2002a). Activations of the anterior vermis and more anterior parts of the posterior vermis (i.e. vermal lobules III-VI) were present in both studies. In the present fMRI study additional activations were observed in the posterior vermis and posterior cerebellar hemispheres. The more widespread cerebellar activation are likely due to the better temporal and spatial resolution of event-related fMRI compared to PET.

Cerebellar activation related to the spinal nociceptive leg withdrawal reflex

Nociceptive leg withdrawal reflex related areas within the vermal and paravermal areas of the anterior lobe (i.e. lobules III/IV) and the more posterior parts of the posterior lobe (i.e. lobule VIII) agree with the known cerebellar representation of the hindlimb and leg. Somatotopic representation in the cerebellum with two homunculi, one located upside down in the anterior lobe and a second one in the posterior lobe, has first been decribed by Adrian (1943) and Snider and Stowell (1944). Although subsequent anatomical and electrophysiological studies have revealed a more complex spatial
distribution of mossy and climbing fiber afferents, a rough somatotopic pattern appears to be present (Brodal 1975; Oscarsson 1976; Welker 1987; Ekerot et al. 1991a,b; for reviews: Bloedel and Courville 1974; Voogd and Glickstein 1998). Consistent with the present findings, climbing fiber hindlimb afferents have been found to the more rostral vermal and paravermal parts of the anterior lobe (Garwicz 1997) and to the more posterior vermal parts of the posterior lobe (i.e. vermal lobule VIII) (Atkins and Apps 1997).

More recent fMRI studies have confirmed the existence of a similar somatotopic representation in humans. In agreement with the present findings, representation of foot movements has been found both in the more anterior parts of the anterior lobe (i.e. in hemispheral lobules II-IV with extension into the vermis; Nitschke et al. 1996; Rijntjes et al. 1999; Grodd et al. 2001) and within the more posterior parts of the paravermal hemispheres (hemispheral lobules VII-VIII; Rijntjes et al. 1999) and vermis of the posterior lobe (Rijntjes et al. 1999; Grodd et al. 2001).

Paravermal activation was present bilaterally. Contralateral cerebellar activation may reflect movement of the contralateral limb and/or bilateral representation of sensory afferents. Electrical stimulation of one limb results in withdrawal of the stimulated limb and concomitant postural adjustments of the contralateral limb, e.g. extension during standing (Sherrington 1910; Kugelberg 1960; Hagbarth and Finer 1963). In addition, cerebellar afferents have been shown to have a large ipsilateral and a smaller contralateral cerebellar representation (Bloedel and Courville 1974; Brodal 1981).

No voluntary foot movement and pure sensory stimulation conditions were performed in the present study. Therefore, the present study does not allow to differentiate if the reflex-related cerebellar activity reflects cerebellar modulation of the spinal reflex pathways, afferent input of the noxious stimulus and/or afferent feedback of the limb withdrawal. It appears, however, likely that the afferent input is used to modulate the spinal nociceptive leg withdrawal reflex. As outlined in the Introduction changes of reflex amplitude have been found in a human case study (Timmann et al. 1998a) and animal lesion
studies of the cerebellum (Denny-Brown et al. 1929; Chambers and Sprague 1955; Yu 1972; Kolb et al. 1997) and inferior olive (Rabacchi et al. 1986). Findings of animal lesion studies, however, are not unequivocal. Despite clear deficits of classical conditioning, others have found no changes of the unconditioned limb withdrawal reflex following lesions of the interposed nucleus (Donegan et al. 1983), brachium conjunctivum (Voneida 2000), red nucleus (Smith 1970; Tsukahara et al. 1981), rubrospinal tract (Voneida 1999) and inferior olive (Voneida et al. 1990). Electrophysiological animal studies, on the other hand, provide detailed evidence that the spino-olivo-cerebellar system is involved in nociceptive limb withdrawal reflex control (Oscarsson 1973; Ekerot et al. 1991a,b; Garwitz et al. 1992, 2002; Kalliomäki et al. 1992; Schouenborg and Weng 1994; Schouenborg et al. 1994). The organization of cutaneous receptive fibers for spino-olivo-cerebellar pathways to cerebellar zones controlling detailed limb movements has been shown to resemble that of the nociceptive withdrawal reflexes (Ekerot et al. 1991a,b; Garwicz et al. 1992). Partial overlap of cerebellar areas activated during limb flexion reflex in the present fMRI study and during voluntary leg movements in previous fMRI studies are consistent with this finding. Overlap of cerebellar areas during voluntary and reflexive leg movement is further supported by the recent PET study of our group. Limb flexion reflex related areas were found within the upper vermis of the anterior lobe including lobules III and IV. These areas partially overlapped with vermal activation areas in a voluntary foot movement condition (Maschke et al. 2002a). Similar cerebellar areas may be involved in control of reflexive and voluntary limb movements by modulating spinal neuronal networks which implement the withdrawal reflexes and also mediate signals from supraspinal motor centers involved in coordination of voluntary motor behaviour (Lundberg 1982; Schomburg 1990; Jankowska 1992; Kalliomäki et al. 1992; Schouenborg and Weng 1994; Kolb et al. 1997). Future studies need to investigate possible impairment of performance of the nociceptive leg withdrawal reflex in a larger group of cerebellar patients.
Cerebellar activation related to other pain-related reactions

In addition to areas which overlap with the known cerebellar representation of the leg, nociceptive leg withdrawal reflex related activation was present within the anterior parts of the posterior vermis (lobule VI), with extension into lobule V and the fastigial nuclei and the anterior parts of the posterior cerebellar hemispheres bilaterally (lobules VI/Crus I). Consistent with the present findings, withdrawal reflex related activity extended into vermal lobules V and VI in our recent PET study (Maschke et al. 2002a). These areas may be related to functions other than the reflexive withdrawal of the leg.

Electrical stimulation is accompanied by sensation of pain, facial expressions of pain, fear and startle-like reactions (Craig 1984; Craig and Patrick 1985; Dowman 1992; Ploghaus et al. 1999). There is evidence from the animal and functional human brain imaging literature that the cerebellum may be involved in these pain-induced reactions.

Firstly, painful stimuli are accompanied by facial actions including eye closing or blinking (Craig and Patrick 1985). Cerebellar somatotopic representation of facial afferents and movements are known to be within the posterior cerebellar lobe, i.e. vermal and paravermal lobules VI and VII (Snider and Stowell 1944; Hesslow 1994a,b; Nitschke et al. 1996; Grodd et al. 2001). Likewise, a recent fMRI study of our group showed blinkreflex-related areas within bilateral hemispheral lobules VI and Crus I with extension into the vermis (Dimitrova et al. 2002a). Reflex eyeblinking as part of startle-like responses may also account for at least part of activation in areas representing facial movements.

Responses to the first two stimuli have been excluded from analysis to minimize effects of startle-like reactions. Whole-body startle habituates within few trials. Startle-related eyeblinks, however, take a slower course of habituation and their occurrence cannot be excluded in later trials (Koch 1999; Maschke et al. 2000). Furthermore, the cerebellar vermis, particularly lobule VI, and adjacent parts of the hemispheres have been shown to be involved in modulation of the startle reflex (i.e., habituation
and fear-induced potentiation) (Leaton and Supple 1991; Lopiano et al. 1990; Timmann et al. 1998b; Frings et al. 2002; Pissiota et al. 2002).

Thus, cerebellar activation of the anterior part of the posterior cerebellum may reflect pain-induced facial movements, e.g. grimacing and/or startle-related eyeblinking. An increasing number of studies, however, suggest that the cerebellum is involved in non-motor functions including affect and behaviour (Schmahmann 1997 for review). Areas within the neocerebellum, i.e. the neovermis (i.e. lobules VI/VII) and the posterior cerebellar hemispheres (i.e. lobule VI and below) appear to be of particular importance (Akshoomoff and Courchesne 1994; Schmahmann and Sherman 1998; Thach 1998).

Likewise, cerebellar activation of the vermis and posterior cerebellar hemispheres have been demonstrated in fMRI studies of neural substrates involved in pain (Becerra et al. 1999; Svensson et al. 1997; Coghill et al. 1999). Based on Schmahmann et al.‘s (2000) atlas of the human cerebellum activations were found primarily within lobule VI. Ploghaus et al. (1999) showed that different areas in the cerebellum are involved in different aspects of pain-processing. In their study painful stimuli of the hand evoked responses of the anterior cerebellum (i.e. within the known hand areal) whereas expectation of the same stimuli evoked activations within the posterior cerebellar hemisphere. Vermal and paravermal areas of the anterior lobe and more posterior parts of the posterior lobe (corresponding to the somatotopic representation of the stimulated limb) may be involved with integration of nociceptive information to other motor areas, whereas neocerebellar areas within the posterior hemispheres may be involved in other, e.g. affective or cognitive, dimensions of pain.

Finally, pain is frequently accompanied by fear. Both the cerebellar vermis and the posterior cerebellar hemispheres have been shown to be activated due to externally evoked emotions (Lane et al. 1997; Reiman et al. 1997; Beauregard et al. 1998). In most fMRI studies areas of activation were located within vermal lobules V/VI and in hemispherical lobules VI/Crus I (based on Schmahmann et al.‘s atlas).
Cerebellar activations due to pain-related reactions other than the actual limb withdrawal is further supported by the likely activation of the fastigial nuclei. Fear is known to be accompanied by changes of autonomic functions (for review: LeDoux 1993; Maschke et al. 2002b). The fastigial nuclei have been shown to participate in emotions and autonomic functions (e.g. cardiovascular control), in addition to processing of vestibular signals (Heath et al. 1974; Holmes et al. 2002; for review: Schmahmann 2000).

Hemispheral activations on the group level were more prominent on the left. Although this may simply reflect effects of stimulation side, another explanation appears worth mentioning. The oldest model of emotion lateralization holds that the right cerebral hemisphere is dominant for emotional processing. An alternative view posits that the left cerebral hemisphere is preferentially involved in positive emotions and approach, whereas the right hemisphere is preferentially involved in negative emotions and withdrawal (Zald 2003). These models focus on the prefrontal cortex. Because of the known connections of the left cerebellum to the right prefrontal cortex (Middleton and Strick 1994) fear-related activation of the cerebellum may be expected on the left.

In sum, in addition to actual limb withdrawal, a variety of other motor and non-motor reactions are induced by pain which appear able to induce changes of cerebellar activity in the posterior cerebellar lobe. Quantitative assessment of facial movements, startle-like reactions and fear-induced autonomic changes (i.e. skin conductance response, heart rate) may be helpful to differentiate between the possibilities in future studies.

**Conclusions**

Different areas within the vermis and cerebellar hemispheres appear to be related to the nociceptive leg withdrawal reflex. Vermal and paravermal areas overlapping with the known representation of the leg are likely related to the leg withdrawal reflex in itself. Areas within the posterior lateral hemispheres
and neovermis appear to be related to other pain-induced reactions. Studies of conditioning of the nociceptive limb withdrawal reflex should take these findings into consideration. Conditioning of the electrically evoked leg withdrawal reflex will induce conditioning of specific (i.e. the leg withdrawal) and non-specific (e.g., fear, changes of heart rate) aversive reactions (Lavond et al. 1993). Accordingly, in fMRI studies in healthy human subjects different cerebellar areas are likely to be activated. Likewise, in patients with focal cerebellar lesions concomitant conditioning of specific and non-specific aversive reactions is likely to be differentially affected depending on the side of the lesion.

**Acknowledgments**

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References


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Figure legends

Figure 1
EMG data of nociceptive leg withdrawal recordings during fMRI-experiments. (A) Non-rectified and filtered (45 Hz) EMG data of the anterior tibial muscle of the 30 trials in an individual subject (s-9 in Tab. 1 and 2). First trial on the top, last trial on the bottom. Vertical line indicates beginning of electrical stimulation. Note artifacts of MR scanning at the end of each trace. (B) Mean changes of leg withdrawal response size (normalized 50ms-IEMG) across the 30 trials in the group of all subjects. Error bars indicate standard deviations.

Figure 2
Group data (n = 13) are shown based on the random effects model (p < 0.0001, uncorrected). SPM (t) maps are displayed in coronal, axial and sagittal projections. The volume scanned covered the entire cerebellum and parts of the adjacent occipital, temporal and frontal lobes. Laterality is indicated by L (left) and R (right).

Figure 3
SPM (t) maps of group data (random effects model; p < 0.0001, uncorrected) are displayed on sagittal, coronal and axial sections of a template from the 3-D MRI atlas of the human cerebellar nuclei (Dimitrova et al. 2002b). Note activation in the roof of the fourth ventricle, i.e. the location of the fastigial nuclei. I = interposed nucleus, D = dentate nucleus.
Figure 4

SPM (t) maps of group data (random effects model; p < 0.0001, uncorrected) are displayed on axial sections of a typical canonical brain from the MNI series. The z coordinate varies between -14 mm and -58 mm with a step of 4 mm. Laterality is indicated by L (left) and R (right).

Figure 5

Data of eight single subjects are shown. SPM (t) maps (p < 0.001, uncorrected) are displayed on sagittal sections of the individual normalized T1-weighted MR data sets.
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**Table 1**

Coordinates in MNI standard anatomical space for the peaks (i.e. local maxima) of cerebellar vermal activation. Error probabilities for group analysis (random model): p < 0.0001 (uncorrected), for individual subjects: p < 0.001 (uncorrected). x, y, z = coordinates in mm; Z = Z-score; lob. = lobule; + activation overlap with fastigial coordinates; * activation in close neighborhood of fastigial coordinates (based on Dimitrova et al. 2002b). Note that coordinates of fastigial activation correspond to the nearest local maximum of the cluster and may differ from localization of fastigial nuclei.
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Table 2

Coordinates in MNI standard anatomical space for the peaks (i.e. local maxima) of cerebellar hemispheral activation. Error probabilities for group analysis (random model): p < 0.0001 (uncorrected), for individual subjects: p < 0.001 (uncorrected), x,y,z = coordinates in mm; Z = Z-score; lob. = lobule; Cr. 1 = Crus I
Fig. 1, Dimitrova et al.
Fig. 2, Dimitrova et al.
Fig. 3, Dimitrova et al.
Fig. 4, Dimitrova et al.
Fig. 5, Dimitrova et al.