The study addresses the issue of the role of the cerebellum in human withdrawal-reflex conditioning by comparing data from patients with pure cerebellar diseases (CBL, n=10) and from cerebellar patients showing additional extracerebellar symptoms (CBL+, n=10) with those from 11 control subjects (CTRL). During recording sessions, the standard delay-conditioning paradigm with paired-trials was used with tone as the conditioned stimulus (CS). Parameters of the conditioned muscle responses are analyzed in an accompanying paper. Here, we focus on the unconditioned muscle response. A train of current pulses (unconditioned stimulus, US) evoked a lower-limb withdrawal reflex (unconditioned response, UR), which was recorded electromyographically from leg muscles. During the recording sessions with CTRL subjects, UR amplitudes decayed from initially 100% to approximately 50% at the end of the session. This type of decay was clearly less pronounced in the CBL group and minimal in the CBL+ group. Furthermore, the CBL group exhibited UR onsets that were delayed by 20 ms compared with those from CTRL subjects. Although the ranges of measurements characterizing the URs of a given cerebellar patient tested in the paired-trial paradigm overlapped with those of control subjects, the statistically significant differences observed at the group level suggest deficits in the performance of the reflex responses. The delayed URs in patients and the different type of decay of UR amplitudes in repetitively evoked withdrawal reflexes constitute evidence that the cerebellum is critically involved in the control of these UR parameters.

Key words Classical conditioning · Withdrawal reflex · Unconditioned response · Motor learning · Cerebellum

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Abbreviations CS Conditioned stimulus · US Unconditioned stimulus · CR Conditioned response · UR Unconditioned response · TA M. tibialis anterior · EMG Electromyogram/graphic

Introduction

The cerebellum is generally accepted as a structure involved in the control of body balance and in the coordination of movements. Clinical observations in cerebellar patients showed ataxia to be a main symptom, i.e., a lack of coordination and precision in the performance of movements unrelated to pareses, distinct deficits of sensation, or other deficits in involuntary movements (Lechtenberg 1993). These deficits may affect the trunk, stance, and gait, may evoke a tremor and dysmetric reaching, and may decompose speech and eye movements (e.g., Holmes 1917, 1939; Goldstein 1927; Dow and Moruzzi 1958; Timmann and Diener 1998).

Besides the cerebellar involvement in balance and in the control of voluntary movement of an extremity (e.g., Thach 1970a, 1970b; Bauswein et al. 1983; Hore et al. 1991; van Kan et al. 1993; Bastian et al. 1996; Martin et al. 1996a), cerebellar control of different parameters of various reflexes has also been reported (see reviews in MacKay and Murphy 1979; Bloedel and Bracha 1995). The role of the cerebellum in limb-withdrawal reflexes has been studied particularly in dogs by Popov (1929), Gambaryan (1960), and Fanardijan (1961). The common denominator of these studies is a change in the characteristics of the reflex following an experimental lesion of the cerebellum or its ablation. Specifically, the normal performance was impaired, exhibiting delayed responses and a tendency to extend the limb, in contrast to a normally flexed extremity.

Human lower-limb withdrawal reflexes in healthy subjects without a conditioning stimulus have also been tested in numerous studies (e.g., Shahani and Young 1971; Hugon 1973; Meinck et al. 1981, 1983; Timmann et al. 1994). The effects of standardizing electrical stimu-
loration (Torrington et al. 1981) and the influence of stimulus parameters (Meinck et al. 1985) on the reflex response have been reported. For repetitively applied electrical stimuli, a habituation of the muscle response has been observed. Specific pre-training (e.g., Bromm and Treede 1980) or the intermittent application of higher stimulus strengths (e.g., Hagbarth and Finer 1963) have been employed in attempts to avoid or minimize the decay of the reflex response. However, as will be shown in this paper, this decay of reflex amplitudes seems to be characteristic for a given subject and is most likely due to an underlying plastic process. The decay of amplitudes in the experiments of Bromm and Treede (1980) and Hagbarth and Finer (1963) is typical for a habituation process. However, since we were using paired-trials in a classical conditioning paradigm, the decay of amplitudes in our experiments could not be unequivocally related to a habituation process.

Classical conditioning of the human lower-limb reflex was first introduced as a method in a group of young, healthy subjects (Kolb and Timmann 1996). Timmann et al. (1996) tested the hypothesis of a cerebellar involvement in this specific withdrawal reflex in humans by a positron-emission study. A significant increase of the intermediate cerebellar blood flow during the acquisition supported the assumption. The aim of the present study was to test further the involvement of the human cerebellum in classically conditioned lower-limb reflexes using electrophysiological techniques. Whereas in an accompanying study Timmann et al. (1999) reported abnormalities related to the conditioned withdrawal reflex in cerebellar patients, the present report focuses on the change in parameters of the unconditioned reflexes during paired-trials in the same patients.

Materials and methods

Subjects

The study was performed with the permission of the local ethical committee. A total of 20 cerebellar patients and 11 healthy subjects participated after giving their written informed consent. A complete description of the subjects tested in this study is described in the accompanying paper (Timmann et al. 1999) and is repeated here only briefly. The cerebellar group consisted of a "pure cerebellar group" (CBL in Table 1, n=10, six male, four female; mean age: 49.6±15.8 years, range: 19–67 years; eight right-handed, two left-handed) and a "cerebellar group with additional extracerebellar signs" (CBL+, Table 1, n=10, three male, seven female; mean age: 50.8±13.2 years, range: 38–75 years; all right-handed). In the CBL group, five patients presented with a cerebellar ischemic infarct (subjects nos. 1–5, Tables 1, 2), two had been operated on for a pilocytic cerebellar astrocytoma (subjects nos. 6, 7 in Tables 1, 2), two presented with a form of degenerative ataxia with an idiopathic cerebellar ataxia (IDCA) and an autosomal dominant cerebellar ataxia (ADCA) type III (subjects nos. 8, 9 in Tables 1, 2; Harding 1993), and one had an anterior lobe syndrome (subject no. 10 in Tables 1, 2). Subjects with cerebellar infarcts had no clinical evidence of additional brainstem involvement, neither at the onset of the disease nor at the time of the present investigation. In the CBL+ group, one patient had a hemorrhagic infarct (subject no. 11 in Tables 1, 2), eight patients presented with a form of degenerative ataxia: three had IDCA with extracerebellar signs (olivo-ponto-cerebellar atrophy (OPCA) subjects nos. 12–14), three had ADCA type I (subjects nos. 15–17 in Tables 1, 2), one early onset cerebellar ataxia with retained tendon reflexes (EOCA, subject no. 18 in Tables 1, 2), and one had Friedreich’s ataxia (subject no. 19 in Tables 1, 2). One patient had been operated on for a cerebellar epidermoid tumor (subject no. 20 in Tables 1, 2). Patients with peripheral neuropathy were included because the only difference between these and the CBL patients was the prolongation of responses. A more elaborate description of subscores of the cerebellar rating scale is provided in the accompanying paper (Timmann et al. 1999).

The control group (CTRL in Table 2, n=11, six male, five female; mean age: 48.8±14.2 range: 19–67 years; all right-handed) had no neurological or orthopedic histories and were not receiving any medication. Neither the cerebellar patients nor the control subjects presented any clinical evidence of hearing difficulties on a routine neurological evaluation.

Stimulation procedure, recordings, and data analysis

The paradigm, stimulation procedure, recording techniques, and data analysis are described elsewhere (Timmann et al. 1999) and are repeated only briefly here. Using the standard delay paradigm, the human withdrawal reflex was classically conditioned and studied in control subjects and cerebellar patients. All recordings were performed with subjects lying supine and comfortably on a daybed.

Stimulation

According to the rules of classical conditioning suggested by Gormezano et al. (Gormezano and Kehoe 1975), the stimulus protocol consisted of a sequence of a conditioned stimulus (CS) and a co-terminating unconditioned stimulus (US). The US consisted of a 100-ms train of bipolar current pulses (200 Hz, pulse duration 0.2 ms) applied to the medial plantar nerve. This stimulus was given to CTRL on their side of handedness and to cerebellar patients ipsilateral to their affected side. The CS consisted of an initially neutral tone (1000 Hz, 75 dB sound-pressure level, 550 ms), given via head phones to the same side as the US and preceding the US by 450 ms. A continuous white noise of 50 dB SPL was applied bilaterally to mask environmental noise. At the beginning of the session, 5–20 US-alone and 5–20 CS-alone trials were given followed by at least 100 paired-trials.

Recording

With standard EMG techniques, the activities of different muscles of both legs were recorded differentially. For this study, only results from the anterior tibial muscle (TA) of the stimulated leg are presented. The EMG signals were amplified, filtered, full-wave rectified, and fed to a multichannel recording system (Microlinc 1000) connected to a standard personal computer. Subjects were asked to generate a level of background activity, i.e., a weak tonic voluntary contraction of the TA muscle within a range of 2–8% of the maximal force of contraction (tested initially) and which was controlled by a previously reported method (Kolb et al. 1997b).

Data analysis

Recorded EMG data were analyzed on a trial-by-trial basis using a computer program developed for this study. Muscle responses occurring more than 65 ms after the US and meeting specific dynamic criteria were accepted as unconditioned responses (UR). Latency of a muscle response was defined as the time between the stimulus and the time at which a change in the TA muscle activity exceeded a predefined level of 5 mV/s (see also Fig. 1 in Timmann et al. 1999).