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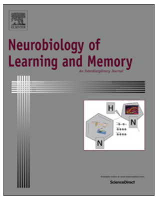

Comparison of the classically conditioned withdrawal reflex in cerebellar patients and healthy control subjects during stance: 2. Biomechanical characteristics ☆

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ABSTRACT

This study addresses cerebellar involvement in classically conditioned nociceptive lower limb withdrawal reflexes in standing humans. A preceding study compared electromyographic activities in leg muscles of eight patients with cerebellar disease (CBL) and eight age-matched controls (CTRL). The present study extends and completes that investigation by recording biomechanical signals from a strain-gauge-equipped platform during paired auditory conditioning stimuli (CS) and unconditioned stimuli (US) trials and during US-alone trials. The withdrawal reflex performance—lifting the stimulated limb (decreasing the vertical force from that leg, i.e. 'unloading') and transferring body weight to the supporting limb (increasing the vertical force from that leg, i.e. 'loading')—was quantified by the corresponding forces exerted onto the platform. The force changes were not simultaneous but occurred as a sequence of multiple force peaks at different times depending on the specific limb task (loading or unloading). Motor learning, expressed by the occurrence of conditioned responses (CR), is characterized by this sequence beginning already within the CSUS window. Loading and unloading were delayed and prolonged in CBL, resulting in incomplete rebalancing during the analysis period. Trajectory loops of the center of vertical pressure—derived from vertical forces—were also incomplete in CBL within the recording period. However, exposing CBL to a CS resulted in motor improvement reflected by shortening the time of rebalancing and by optimizing the trajectory loop. In summary, associative responses in CBL are not absent although they are less frequent and of smaller amplitude than in CTRL.

1. Introduction

Sherrington (1910) first demonstrated the classical withdrawal reflex during nociceptive stimulation of skin receptors in animals. The corresponding spinal network has been studied extensively by Eccles and Lundberg (1959), the interneuronal relay by Jankowska (review: Jankowska, 1992) and the circuitry by Pierrot-Deseilligny and Burke (review di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). Forssberg (1979) demonstrated a stumbling corrective reaction in cats, depending on the swing or stance phase when the stimulus was applied. This indicates a modification of the withdrawal reflex which may be due partly to the spinal network itself. This network, however, also receives descending commands allowing the segmental condition of the withdrawal to be modified (Schomburg, 1990). The withdrawal reflex in humans has been addressed e.g. Shahani and Young (1971). Torring, Pedersen, and Klemar (1981) have standardized the electrical elicitation, and a series of publications has provided principles of flexor reflex generation (Meinck, Benecke, Küster, & Conrad, 1983; Meinck, Küster, Benecke, & Conrad, 1985; Meinck, Piesiur-Strehlow, & Koehler, 1981). The plasticity of the human lower limb withdrawal reflex has been shown by Hagbarth and Finer (1963). A reversal of the reflex has been observed during the transition from the swing to

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Abbreviations: CBL, cerebellar patients; CTRL, healthy control subjects; TA, tibial anterior muscle; EMG, electromyography; CVP, center of vertical pressure; cpo, contralateral push-off peak; ipo, ipsilateral push-off peak; clp1, contralateral load peak 1; clp2, contralateral load peak 2; iup, ipsilateral unloading peak (in CSUS trials); iup1, ipsilateral unloading peak 1; iup2, ipsilateral unloading peak 2 (both in US-alone trials).

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stance phase compared with the transition from stance to swing phase (Duysens, Tax, Trippel, & Dietz, 1993; Nakajima et al., 2008).

The contribution of modulatory effects onto the withdrawal reflex by the cerebellum has been assumed by MacKay and Murphy (1979) and Welsh and Harvey (1992). The adaptive effect of the cerebellum in protective reflexes is generally accepted although the specific aspect in motor learning is still under discussion (e.g. Bloedel & Bracha, 1995; Bracha et al., 2009; Gruart & Yeo, 1995; Harvey & Welsh, 1996; Thompson & Steinmetz, 2009). Plastic changes in protective reflexes have been studied using classical conditioning, a method introduced by Pavlov (1927) and established as a test protocol by Gormezano and Kehoe (1975). Amongst protective reflexes the eye blink reflex has been studied most intensively in animals (e.g. Bracha, 2004; McCormick & Thompson, 1984) and in humans (e.g. Manto et al., 2012; Krupa, Thompson, & Thompson, 1993; Lindquist, Steinmetz, & Thompson, 2013; Thompson & Krupa, 1994). A recent elaborate study in cats has suggested, that the contribution of the cerebellum seems to be a re-evaluation of its prior steps and functions, i.e. a retrospective analysis of the functional history in the course of a motor learning process (Sanchez-Campusano, Gruart, & Delgado-Garcia, 2009).

Our laboratory has studied the withdrawal reflex as another modifiable protective reflex employing the method of classical conditioning (Kolb & Timmann, 1996; Timmann, Baier, Diener, & Kolb, 2000). The use of imaging techniques has provided evidence that the cerebellum is involved in this type of procedural learning (PET: Maschke et al., 2002; Timmann et al., 1996; fMRI: Dimitrova et al., 2004). The comparison of different types of reflexes in groups of patients suffering of cerebellar disease with sex and age-matched groups of healthy controls suggests a critical involvement of the cerebellum in plastic changes of reflex pattern (Kolb, Lachauer, Maschke, & Timmann, 2004; Kolb, Timmann, Baier, & Diener, 2000; Kolb et al., 2007; Timmann et al., 2000). Studies of the lower limb withdrawal reflex have been performed with subjects lying supine on a day bed. This may be misleading with respect to the role of the reflexes in movement (Stein & Thompson, 2006).

With electrophysiological techniques the activities of a small number of individual muscles that are accessible from the surface can be detected, and which contribute to the movement of the reflex. Even when a large array of recording electrodes is employed (e.g. Schumann, Bongers, Guntinas-Lichius, & Scholle, 2010) the result is still restricted with respect to the total number of muscles involved in the reflex. To obtain the reflexive movement in its entirety as the final output of the corresponding motor system, biomechanical techniques must be employed. Consequently, the aim of the present study was to identify characteristic biomechanical differences in the unconditioned (UR) and conditioned responses (CRs) of the classically conditioned lower limb reflex in standing subjects. The complete study consists of three parts, in which the initial part was focused on establishing the method for testing the withdrawal reflex in standing young and healthy controls, providing electrophysiological and biomechanical data (Kaulich et al., 2010). In the second part the electrophysiological muscle responses of the withdrawal reflex obtained in patients with cerebellar disease were compared with a sex and age-matched group of healthy control subjects (Timmann et al., 2013). The present and final part provides corresponding biomechanical data from the identical group of subjects from which the electrophysiological properties of the withdrawal reflex were obtained.

2. Materials and methods

2.1. Participants

This study was performed with the permission of the ethics committee of the Ludwig-Maximilians-University of Munich (Nr. 310/00). A total of eight cerebellar patients (CBL) and eight age- and sex matched healthy subjects (CTRL) participated after giving written informed consent. The cerebellar group (mean age: 42.1 ± 10.3 years, range: 21.5–54.2 years) consisted of three females and five males (Table 1). Two subjects (#1, #2) suffered from an autosomal dominant cerebellar ataxia (ADCA) type III (according to Harding, 1993), one of which presented additionally a sensible polyneuropathy (#2, Table 1). Patient #3 presented with an early onset cerebellar ataxia (EOCA), patient #4, Friedreich ataxia (FRDA), patients #5, #6, and #7 suffered from a sporadic adult-onset ataxia (SAOA), and patient #8 presented with a cerebellar version of multi system atrophy (MSA cbl vs, Table 1). At the time at which our observations were made, patients #2, #3, and #4 showed primarily atrophy of the cerebellum. In particular, patient #3 presented an uncommon form of FRDA with predominant cerebellar atrophy. The degree of ataxia was defined as the total of specifically defined subscores of the cerebellar rating scale according to the International Cooperative Ataxia Ratings Scale (World Federation of Neurology adapted from Trouillas et al. (1997)). Three groups of subscores were established: postural and gait-related ataxia, limb ataxia (separated into lower limb ataxia and right and left upper limb ataxia) and oculomotor- and speech-related ataxia. The neurological examination was performed by one of the authors, a trained neurologist. Table 1 summarizes the patients' clinical characteristics with patients sorted according to subgroup and diagnosis. The control group (CTRL in Table 1, $n = 8$, three female, five male, mean age: 41.4 ± 8.6, range: 24.5–54.2 years) had no history of neurological or orthopedic disturbance. They were not receiving any medication. CTRL subjects in Table 1 are listed according to their corresponding cerebellar patient. The groups of subjects and patients are identical to those described in our preceding publication (Timmann et al., 2013).

2.2. Paradigm

Classical conditioning is an accepted method for providing evidence for motor-related association processes in the cerebellar circuitry. The standard delay paradigm was used according to the protocol suggested by Gormezano and Kehoe (1975) resulting in a time-locked sequence of a preceding conditioning stimulus (CS) and an unconditioned stimulus (US), both coterminating. The CSUS window was fixed at 450 ms, whereas the inter-trial interval varied from 15 to 45 s. In the current study this method was applied to subjects standing on a force-platform (Stopper, Burladingen, Germany, Fig. 1). A withdrawal reflex of the lower leg was elicited by the US which consisted of an electrical current train. The sequence of trials consisted of a block of 70 paired trials followed by a block of 50 US-alone trials. Within the block of paired trials subjects were exposed to 8 single stimuli (4 CS-alone trials (trial #20,# 40, #60, #80) and 4 US-alone trials (trial #10, #30, #50, #70)), resulting in a total of 130 trials. Initially subjects were exposed to a small number of US to allow familiarisation with the procedure. Detailed descriptions of methodological procedures appear elsewhere (Kaulich et al., 2010) and are given here only briefly. The vertical forces due to the weight and the US-elicited movement exerted by a standing subject were recorded by strain gauges placed in each corner of the platform. The latter consisted of two units, one for each leg. The forces were processed online to yield a trajectory of the center of vertical pressure (CVP). The x- and y-components were fed back to an oscilloscope screen situated in front of the subject at a distance of 1.2 m and at the height of the subject's head (Fig. 1A). Subjects were asked to keep their initial CVP close to the origin of the coordinate system and to position each leg in the middle of each platform-half. The resulting distance between the heel-centers was 23 cm, with the feet forming a