Cutaneous reflex modulation and self-induced reflex attenuation in cerebellar patients

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Abstract:

Modulation of cutaneous reflexes is important in the neural control of walking, yet knowledge about underlying neural pathways is still incomplete. Recent studies have suggested that the cerebellum is involved. Here, we evaluated the possible roles of the cerebellum in cutaneous reflex modulation and in attenuation of self-induced reflexes. First it was checked whether leg muscle activity during walking was similar both in patients with focal cerebellar lesions and in healthy controls. We then recorded cutaneous reflex activity in leg muscles during walking. Additionally, we compared reflexes after standard (computer-triggered) stimuli with reflexes after self-induced stimuli for both groups. Biceps femoris and gastrocnemius medialis muscle activity was increased in the patient group compared to the controls, suggesting a co-activation strategy to reduce instability of gait. Cutaneous reflex modulation was similar between healthy controls and cerebellar patients, but the latter appeared less able to attenuate reflexes to self-induced stimuli. This suggests that the cerebellum is not primarily involved in cutaneous reflex modulation but that it could act in attenuation of self-induced reflex responses. The latter role in locomotion would be consistent with the common view that the cerebellum predicts sensory consequences of movement.

Keywords:

Cerebellum; ataxia; phase-dependent modulation; locomotion
Introduction

‘Cutaneous reflexes’ are seen as (changes in) muscle activity in reaction to non-noxious stimulation of a cutaneous nerve. During gait such reflexes are partly, but certainly not solely, influenced by the ongoing (background) activity in the same muscle (for review: Zehr and Duysens 2004). This is most clearly seen in the case of a phase-dependent reflex reversal (Duysens et al. 1990; Yang and Stein 1990). In the tibialis anterior muscle (TA), stimulation of the sural nerve during the early swing phase results in facilitatory reflex activity, while a similar stimulus during the late swing phase results in a suppression of the background activity, despite similar background levels in these two phases (Duysens et al. 1990; Yang and Stein 1990). Although, this phase-dependent reflex modulation is studied extensively (for review: Zehr and Duysens 2004), precise knowledge about the underlying neural pathways is still incomplete (Bagna and Bouyer 2011; Behrendt et al. 2013; Ruff et al. 2014).

In this paper, we aim to evaluate the role of the cerebellum in the modulation of cutaneous reflexes. The cerebellum has an important role in the control of (adaptability of) gait in humans (for review: Ilg and Timmann 2013; Morton and Bastian 2007) and observations in rats suggest a role in phase-dependent modulation of cutaneous reflexes as well (Bronsing et al. 2005; Pijpers et al. 2008). Selective impairment of the C1-module in the cerebellum of rats severely affected the modulation of cutaneous reflexes during walking. Whether humans also rely on the cerebellum for this modulation is unknown. Therefore, we analyzed and compared cutaneous reflex activity both in cerebellar patients with stable focal lesions after cerebellar tumor resection and in healthy controls. We hypothesized that phase-dependent reflex modulation patterns would be less pronounced in patients.
Additionally, the cerebellum is thought to be involved in the prediction of sensory consequences of actions (for review: Bastian, 2011; Wolpert et al. 1998). A well-known example is the proposition that the cerebellum is involved in the central cancellation of tickle sensation (Blakemore et al. 1998, 2001). Such predictions are important in the control of movement since afferent feedback has a delay and disruption of cerebellar activity results in movement errors (Miall et al. 2007). Furthermore, this predictive control is also important in active proprioception (Bhanpuri et al. 2013). For locomotion, it has also been suggested that sensory input resulting from one’s own movements can be suppressed. In particular, it was found that sensory stimuli from the foot are not easily perceived in the period just after landing, when one may expect an abundance of afferent input from the foot (Duysens et al. 1995). Reflexes during gait are also suppressed when their occurrence can be predicted because they are elicited voluntarily (Baken et al. 2006). It was hypothesized that the cerebellum would be important for this suppression. To test these hypotheses, we directly compared cutaneous reflexes of cerebellar patients with those of healthy controls in reaction to both externally-triggered and self-induced stimuli.

Materials and Methods

Participants

Eleven patients with stable focal lesions after cerebellar tumor resection (CBL; age: 24.0±7.1; 3 males; Table 1) and ten healthy participants (age: 23.9±3.7; 5 males) participated in this study. All CBL suffered from cerebellar tumors [medulloblastoma (n = 4), pilocytic astrocytoma (n = 5), Lhermitte Duclos disease (n = 1) or hemangioblastoma (n = 1)]. Seven CBL received adjuvant radiotherapy and three of them received adjuvant chemotherapy (treatment details in Table 2). In most CBL, no extra-cerebellar damage was seen, neither on
MR imaging, nor on clinical examination. In a few CBL (Table 2) there were mild signs of extra-cerebellar damage, as assessed on MRI images, but in no case was there a clinical repercussion: three CBL had mild residually enlarged supratentorial ventricular system and a ventriculoperitoneal shunt catheter passing through the right frontal lobe, and three had small sized, asymptomatic cavernous angiomas (Table 2). No deficits in muscle force or sensation were observed in any of the CBL during the neurological screening. The reflexes were normal and there were no long tract signs. In the three CBL who had received chemotherapy, there were no signs or symptoms of polyneuropathy (and no data suggestive of polyneuropathy in the medical files at the time chemotherapy was given). CBL were in a stable condition ( > 2 years post-op; range 8.7-30.2 yrs) and were able to walk independently. Severity of ataxia was rated using the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al. 1997) and scores ranged from 0 to 19 (6.6±5.6; Table 1). All participants gave written informed consent. The experiments were conducted in accordance with the Declaration of Helsinki and were approved by the local ethics committee.

**Experimental set-up and protocol**

Procedures and set-up were similar to earlier experiments (e.g. Hoogkamer et al. 2012). Participants walked for ~10 minutes at 1.11m/s (4.0 km/hr) on an instrumented dual-belt treadmill (Forcelink, Culemborg, The Netherlands). An electrical stimulus was repeatedly applied at the sural nerve near the ankle of the right leg. The stimulus consisted of a train of 5 rectangular pulses of 1ms duration with a frequency of 200 Hz (Grass S48 stimulator, in series with an SIU5 isolator and a CCU1 constant-current unit, Grass Instruments).

The stimulation electrode was attached near the lateral malleolus, where the sural nerve is closest to the skin surface (approximately halfway between the lateral malleolus and the
Achilles’ tendon). We determined the exact position of the electrode according to the optimal irradiation of the stimulus, corresponding to the innervation area of the sural nerve. The stimulus electrode was attached to the skin with tape and stabilized by a strap around the ankle, to keep conditions stable throughout the experiment. We set the stimulus intensity to twice the perception threshold (Duysens et al. 1996). We recorded bipolar EMG in the biceps femoris (BF), tibialis anterior (TA) and gastrocnemius medialis (GM) of both legs by using surface electrodes to sample at 1000 Hz (ZeroWire, Aurion). Additionally, ground reaction forces were recorded at 1000 Hz.

Participants were tested in two different conditions. In the ‘externally-triggered’ condition the stimuli were automatically triggered by the software. In the ‘self-induced’ condition the stimuli were manually triggered by the participants, similar to the study by Baken et al. (2006). The order in which participants performed the ‘externally-triggered’ and ‘self-induced’ condition was random. In both conditions, custom-written MATLAB software was used to enable a reproducible stimulation at 16 equidistantly distributed phases in the gait cycle. Stimulation was timed relative to the instant of heel strike of the right (stimulated) leg, determined by a vertical force threshold of 10% of body weight. In the ‘externally-triggered’ condition the software directly triggered the electrical stimuli; in the ‘self-induced’ condition auditory beeps were generated. We instructed participants to push a handheld button in reaction to the beep. This action triggered a stimulus. We asked the participants to aim for a constant interval between the beep and the button response, and we emphasized it was not necessary to respond as fast as possible (as in a reaction time test) (Baken et al. 2006). This was important since the aim was to have the participants perform a voluntary movement, unaffected by start-react effects. The algorithm presented stimuli/beeps in each phase in random order, such that there was at least one complete stride without a stimulus between
consecutive stimuli. To reach the target of 10 stimuli in each of the 16 phases, 15 beeps were presented per phase (to account for variability in reaction time).

Data analyses

Data analysis procedures were similar to Hoogkamer et al. (2012). Raw force data was filtered with a fourth-order recursive, zero phase-shift, Butterworth low-pass filter with a cutoff frequency of 10 Hz. Instants of heel strike and toe-off were determined, based on anterior-posterior and medio-lateral maxima in the center of pressure trajectory (Roerdink et al. 2008). Stride time and stance percentages were calculated based on the instants of heel strike and toe-off. Variability of these parameters was assessed using the coefficient of variation (CV); the ratio between the standard deviation and the mean values. We defined gait cycles from right heel strike (0%) to the next right heel strike (100%). We excluded strides when a foot incidentally was placed on two belts or when two feet were on the same belt.

The EMG signals were amplified and high-pass filtered (cutoff frequency 3 Hz), full-wave rectified and low-pass filtered (cutoff frequency 300 Hz). To evaluate muscle activity and TA-GM co-activation over the gait cycle, EMG traces were time-normalized into 100 samples per gait cycle. For these analyses only the strides without stimuli and reflex responses were included. For each muscle the average muscle activity over these strides was calculated and then normalized to its maximum value during the gait cycle. Finally, the normalized traces of both legs were averaged. TA-GM co-activation was calculated sample by sample based on the normalized muscle activity in these muscles using:

\[
\text{TA-GM co-activation} = \left[ \frac{a_H + a_L}{2} \right] \times \frac{a_L}{a_H}
\]
where $a_H$ represents the activity of the muscle that has the highest activity during the considered sample (i.e. either TA or GM) and $a_L$ represents the activity of the other muscle at the same time sample (Mari et al. 2014).

We quantified the reflex responses by calculating the mean of the EMG data over the reflex time window. Reflex time windows were manually set around the middle latency reflexes (or ‘P2 reflexes’), starting 70–80 ms after the stimulation (Baken et al. 2005; Duysens et al. 1993; Haridas et al. 2005; Yang and Stein 1990). The time windows were estimated based on visual inspection of the subtracted EMG traces (see below) (Duysens et al. 1996). A single time window, relative to the stimulus, per muscle was used for all conditions. For muscles showing little or no response, we set a time window based on the response in other muscles (e.g. Duysens et al. 1996, 2010; Tax et al. 1995; Van Wezel et al. 1997). Time window mean values were designated to the appropriate phases based on the onset of the response (start of the time window).

For each of the 16 phases of the gait cycle we performed the following calculations to get the reflex and background EMG data of that phase: we averaged the values of 10 stimulated strides (Fig. 1). For each stimulus, we estimated the background EMG activity by calculating the mean EMG over the same relative time window of the preceding stride (without stimulation). Next, we calculated the average value of the 10 unstimulated strides. Finally, we averaged the background values from the two conditions.

Then, background and reflex EMG data was normalized to the maximum background EMG activity (phase-average) for each participant and muscle. In a last step, we then calculated the “net” reflex amplitude by subtracting the background EMG activity from the reflex EMG
activity (Fig. 1). The range of this subtracted reflex curve was used as an index of reflex modulation.

To assess habituation in the reflex responses, we analyzed the mean absolute reflex responses to self-induced stimuli over all phases of the gait cycle, per stimulus number. For each participant, these responses were then normalized with respects to their average. Eventually, group means were calculated and ordered according appearance during the trial.

**MRI data acquisition and processing**

Image acquisition was performed using a Philips 3T Achieva MRI scanner (Philips, Best, The Netherlands) with a 32-channel matrix head coil. For all but one CBL, a 3D MPRAGE high resolution T1-weighted image (repetition time = 970ms, echo time = 4.60ms, flip angle = 8°, 230 1-mm slices, in-plane resolution = 0.97×0.98, 384×384 matrix) was acquired.

Lesions on the MPRAGE images were manually traced using MRIcroN software (http://www.mccauslandcenter.sc.edu/micro/mricron/index.html). Lesion traces were spatially normalized to the atlas of the cerebellum (Diedrichsen et al. 2009) using the SUIT toolbox (http://www.icn.ucl.ac.uk/motorcontrol/imaging/suit.htm; (Diedrichsen 2006; Diedrichsen et al. 2011)) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). When spatial normalization with the SUIT toolbox was inaccurate (some cases with large lesions at the outer border of the cerebellum), lesions were spatially normalized based on the whole brain image with manual corrections in atlas space when needed. Total lesion size was assessed (Table 1) and lesions in the interposed (Table 1) and other deep cerebellar nuclei (Table 3) were listed (see Fig. 2 for superposition image of all lesions).
threshold of 20% was used to determine whether the normalized lesions overlapped with the
specific nuclei (Diedrichsen et al. 2011) and lobules.

Statistical analyses

Results in the text are presented as mean values ± SD. To compare muscle activity and co-
activation during the normal (unstimulated) strides between groups, we calculated the mean
values of these parameters during 4 periods of the gait cycle: initial double stance, single
stance, terminal double stance and swing. A group×period ANOVA was performed for each
muscle and for the TM-GM co-activation. To evaluate potential differences in phase-
dependent modulation of subtracted reflex responses between groups, we used Mann-Whitney
U tests (Baken et al., 2006; Duysens et al. 2010; Van Wezel et al. 1997). To evaluate the
cerebellar role in attenuation of self-induced reflex responses, we compared subtracted reflex
EMG in the TA muscle after externally-triggered and self-induced stimuli within groups
(Wilcoxon Matched Pairs Test). In addition to these tests (uncorrected for multiple
comparisons), we performed a group×condition ANOVA on average values over the period
where suppression was relevant (43.75 – 93.75% of the gait cycle). To assess correlations
between muscle activity, reflex attenuation, temporal gait parameters and ICARS
(sub) scores, Spearman’s rank correlation coefficients were calculated. Additionally, we used
Wilcoxon tests to assess whether muscle activity and reflex attenuation was different between
patient subgroups (subdivisions based on lesion characteristics; Table 1). A traditional level of
significance was used for all statistical tests (\(\alpha = 0.05\)).

Results

Muscle activity during gait
Stride time (CBL: 1.05±0.03s vs. healthy controls: 1.07±0.07s, p = 0.55) and relative stance time (64.6±0.7% vs. 64.6±0.5%, p = 0.60) were similar between groups, but variability of those parameters was higher in the CBL group than in the control group (stride time variability: 2.6±0.8% vs. 1.6±0.2%, p < 0.001; relative stance time variability: 2.3±0.7% vs. 1.4±0.2%; p < 0.001). CBL walked with increased activity in the BF muscles, specifically during the single stance period (Fig. 3; main effect for group: p = 0.002; group×period interaction: p = 0.027; post hoc comparisons: initial double stance p = 0.67, single stance p = 0.002, terminal double stance p = 0.08, swing p = 1.0). For the GM muscle a significant main effect for group indicated that the CBL grouped walked with a higher activation than the healthy control group (p = 0.042). The group×period interaction was not significant (p = 0.07). For the TA muscle the main effect for group and the group×period interaction were not significant (p = 0.43 and p = 0.61, respectively). The main effect for period was significant for all muscles (p < 0.001).

Co-activation between the TA and GM muscles appeared higher in the CBL group, specifically during the double stance periods and during the swing, but no significant main effect for group (p = 0.06) or group×period interaction (p = 0.32) was observed (Fig. 3). Overall co-activation was small in both groups (<0.2). To assess whether the increased co-activation or the increased BF activity in the CBL group was related to ataxia severity or gait variability, we correlated these muscle activation measures with ICARS (sub) scores and variability of either stride time or relative stance time. None of those parameters were significantly correlated (|rho| < 0.5; p > 0.10, for all). In addition, we compared TA-GM co-activation and BF activity between subgroups based on lesion location (affected nuclei) or radiation therapy application. No differences were observed (all p > 0.10).
Phase-dependent reflex modulation

Based on the difference in background EMG one would expect to see larger BF responses in the cerebellar group. This appears to occur for the contralateral BF (cBF; Fig. 4), but not for the ipsilateral side where there was no increase in BF responses during most of the stance phase despite the consistent increase in background activity. However, specifically for the BF inter-individual variability was large (see also individual indexes of reflex modulation; Fig. 4, right panels). As such no significant group differences were observed in subtracted reflex traces of the BF muscles. In the GM one would have expected some larger responses in the second half of the step cycle (increased background) in the patients, but this was not observed, at least not in the ipsilateral GM (iGM; Fig. 4). On the contralateral side there was significantly less suppression during the late single stance in the patient group while background did not differ (87.5 – 93.75% of the gait cycle of the stimulated limb). In TA the subtracted reflex EMG traces were similar between groups (Fig. 4). In both groups the iTA traces showed a reflex reversal with clear facilitatory reflex activity during the early swing phase and suppressive activity around heel strike. For all muscles the indexes of reflex modulation were similar between groups (Fig. 4, right panels).

Attenuation of self-induced reflex responses

To evaluate attenuation of self-induced reflex responses (Fig. 5), we compared subtracted reflex EMG in the iTA muscle after externally-triggered and self-induced stimuli, since the mean reduction in reflex responses is known to be the strongest in the iTA (Baken et al. 2006). Background EMG activity was similar between groups for this muscle (as mentioned above; Fig. 3). However the suppression of reflex responses showed a tendency to differ between the two groups (Fig. 5). In the control group, reduced subtracted reflex responses were seen in the stance swing transition and during the swing phase. Specifically, this reflex
activity was attenuated for several phases when self-induced (56.25 – 62.5, 68.75 – 81.25 and 87.5 – 93.75% of the gait cycle). This attenuation was less pronounced in the CBL group where reflex activity to externally-triggered and self-induced stimuli only differed during the early stance phase of the contralateral leg (50 – 56.25% of the gait cycle; Fig. 5). To evaluate whether this attenuation was different between groups we calculated the mean subtracted reflex activity over the period where suppression could occur (namely 50 – 100% of the gait cycle) for both conditions in both groups (Fig. 5) and performed a repeated measures ANOVA. The majority of the CBL and all healthy controls showed attenuation of self-induced reflex responses (Fig. 5, bottom panel) and a significant main effect for condition was observed (p < 0.001), indicating that in general reflexes to self-induced stimuli were smaller than reflexes to externally-triggered stimuli. However, the main effect for group was not significant (p = 0.69) and also the group×condition interaction did not reach significance (p = 0.14). Hence, attenuation of self-induced reflex responses appeared only different between groups when considering individual phases but not when considered over the whole period. The amount of suppression varied between CBL, but there was no correlation with ICARS (sub) scores, lesion location (affected nuclei) or radiation therapy application for this relatively small group (all p > 0.10).

Habituation

To assess whether differences in attenuation of self-induced reflexes were related to different habituation characteristics for both groups, we plotted the mean responses with respect to order of presentation (Fig. 6). Habituation was similar between groups (Fig. 6) and habituation of responses was very modest, conform to previous studies (Bastiaanse et al, 2006; see also figure 6 in Tax et al. 1995).
Discussion

In this study we aimed to address the role of the cerebellum in modulation or attenuation of cutaneous reflexes during gait. We observed that patients with focal lesions in the cerebellum walk with an overall higher BF and GM muscle activity than healthy controls. Our results from the reflex activity analyses show relatively minor differences (with respect to the differences in background, some expected increases in reflexes were not seen in some muscles such as BF and GM). Hence the data do not suggest that the cerebellum has a major role in phase-dependent modulation of cutaneous reflexes in walking humans. On the other hand, the suppression of reflexes, which was consistently seen in controls, was much less apparent in the patients (less phases with significant suppression). Hence these data do suggest that the cerebellum might play a role in attenuating cutaneous reflexes after self-induced stimuli.

Increased GM and BF activity

Although the evaluation of the muscle activity during the steps without stimulation was not a main goal in this study, the first major finding was that the CBL group walked with an overall higher GM and BF muscle activity than healthy controls. The increased GM muscle activity was accompanied with an overall increased TM-GM co-activation. BF muscle activity was specifically higher during the single stance period. Muscle activity has been observed to be increased in more severely affected cerebellar ataxia patients with diffuse cerebellar damage (Mari et al. 2014; Mitoma et al. 2000). In those patients, the increased activity in the GM and BF muscles was accompanied by an increased activity in their antagonists, the TA and vastus lateralis muscles, respectively. Such antagonist co-activation could increase leg stiffness, which can be a compensation strategy when postural threat is elevated. It is often observed in walking elderly (Franz and Kram 2013; Hortobagyi et al. 2009; Peterson and Martin 2010).
our CBL group TA-GM co-activation was higher overall, similar to observations by Mari et al. (2014) for more severely affected cerebellar ataxia patients with diffuse cerebellar damage. As our main purpose was to evaluate cutaneous reflex activity, we did not measure EMG activity in the vastus lateralis. Accordingly, we could not directly assess co-activation between the BF and the vastus lateralis in our patient group.

Phase-dependent reflex modulation

Increased co-activation could theoretically lead to increased reflex activity, due to increased excitability of the motoneuron pool. Indeed minor differences were observed for the GM muscles, but for the BF and TA muscles no significant differences were observed in the subtracted reflex traces of both legs. Overall, the reflex modulation patterns of both groups were similar to those observed in earlier studies (Baken et al. 2006; Bastiaanse et al. 2006; Duysens et al. 2010; Hoogkamer et al. 2012; Lamont and Zehr 2007; Van Wezel et al. 1997). Hence overall the results point towards subtle changes in some muscles but overall there is no strong indication that the cerebellum plays a dominant role in the modulation of reflexes.

At first sight the present results appear to be inconsistent with animal studies. In rats, Pijpers et al. (2008) observed reduced reflex activity in the BF after selective impairment of the C1-module in the cerebellum. However, it should be mentioned that the present group consisted of mildly affected patients with long standing lesions. Lesions in our CBL group were due to tumor resection at young age (7.1±4.3yrs) and their immature brains had a high potential for neural reorganization and compensation (Caeyenberghs et al. 2009; Gramsbergen 2007; Kolb et al. 2001). In contrast, the rats in the study mentioned had acute lesions and had no chance to show plastic changes. Furthermore, it should be noted that in the rat study the differences in reflexes between lesioned rats and control rats became less apparent and non-significant at
postoperative day 7. Finally, it could be that the locations of the lesions differed between the rats and the humans. In the rats the hind-limb part of C1-module (paravermal zones of lobules I-IV, V and VIII; medial part of the anterior interposed nucleus) was impaired (Pijpers et al. 2008). In none of the CBL these paravermal zones of lobules I-IV, V and VIII were fully affected (Table 3). However in most CBL the interposed nuclei were affected (Table 1). Hence, if these areas were important for this function one would have expected to see at least some effect.

A second point is that, in humans, differences between populations are difficult to demonstrate because there are considerable inter-individual differences with respect to the modulation of this type of reflexes (Bagna and Bouyer 2011; Baken et al. 2006; Duysens et al. 1992; Hoogkamer et al. 2012; Ruff et al. 2014). Due to the nature of our patient sample, one should be cautious in drawing too strong conclusions. Related to the potential for compensation mentioned above, and the limited size of the lesions and the variation in their location, it could be argued that cerebellar damage in our sample was too mild to observe major effects on phase-dependent reflex modulation.

In this respect it would be interesting to evaluate phase-dependent modulation of cutaneous reflex in more severe ataxic patients, such as patients with degenerative cerebellar diseases. However, gait pattern and muscle coordination in these patients might be too much affected, indirectly changing reflex modulation, and making it less feasible to assess the direct effects of the cerebellar damage on reflex modulation. Future research should also focus on other brain structures to reveal the underlying neural pathway for phase-dependent reflex modulation. So far, the most likely neural structures involved are the spinal cord (Central Pattern Generator) and the motor cortex (for review: Duysens et al. 2004). Results from
several transcranial magnetic stimulation studies suggest an important role for the motor
cortex and the transcortical pathway (Christensen et al. 1999; Nielsen et al. 1997; Pijnappels
et al. 1998). Recent observations of phase-dependent reflex modulation during passive
viewing of walking (Behrendt et al. 2013) and during visually guided stepping (Ruff et al.
2014) further support the importance of the motor cortex in reflex modulation.

Attenuation of self-induced reflex responses

Our third finding entails the observation that some CBL were less able to attenuate reflex
activity after self-induced stimuli than healthy controls. There was a clear reduction in the
number of phases where such suppression was observed. Attenuated reflexes after self-
induced stimuli during walking were first observed by Baken et al. (2006). In their main
group, self-induced reflexes were attenuated over 50% of the gait cycle, from mid stance to
mid swing (31.25 – 81.25% of the gait cycle). Healthy participants in our study showed
attenuated reflexes over 25% of the gait cycle, from late stance to mid swing (56.25 – 62.5,
68.75 – 81.25 and 87.5 – 93.75% of the gait cycle; Fig. 5). In the patient group attenuation
occurred in a single phase of the gait cycle during double stance (50 – 56.25% of the gait
cycle). However, on a group level (considering the period where suppression could occur) the
reflex attenuation was not significantly different between groups. Hence, one has to conclude
that the data are suggestive for a contribution of cerebellar structures but that a definite role
cannot be assigned based on the present sample of mildly affected cerebellar patients. Note
that we specifically refer to these reflexes as “self-induced” since it was observed in earlier
work that auditory cues preceding the stimuli do not result in reflex attenuation, suggesting
that the attenuation is not just caused by anticipation of the stimuli in general (Bastiaanse et
al. 2006).
Cerebellar involvement in reflex suppression would be in line with findings from other studies, supporting the idea that the cerebellum provides signals to cancel the sensory response to self-induced stimulation. According to this idea, predictions of the sensory consequences of motor commands are used to partly cancel the actual sensory consequences. This would make the system more sensitive to external (unexpected) perturbations as they result in unpredictable sensory inputs. Furthermore, such predictions are an important control feature. Without predictive control a system only depends on feedback information, which comes with delays, making correction of movements in real time impossible (Miall et al. 2007; Wolpert and Flanagan 2001). Reduced responses after self-induced stimuli or self-initiated actions have been observed in multiple different domains, such as self-induced tickling sensations (Blakemore et al. 1998, 2001), active head movements (McCrea et al. 1999), self-induced muscle stimulation (Gerilovsky et al. 2002), self-induced sounds (Knolle et al. 2013) and self-induced eye blink reflexes (Meincke et al. 2003). In the latter study it was found that the electrically evoked R2 blink reflex is suppressed by using self-stimulation (in particular the later sections of R2 were affected). The current study shows that such self-induced suppression can be consistently evoked in the context of cutaneous reflexes during locomotion. Note that habituation was similar between groups, which suggests that differences in attenuation of self-induced reflexes were not related to different habituation characteristics.

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References


Bronsing R, van der Burg J, Ruigrok TJ. Modulation of cutaneous reflexes in hindlimb muscles during locomotion in the freely walking rat: A model for studying cerebellar


Zehr EP, Duysens J. Regulation of arm and leg movement during human locomotion. 

Legend

Fig. 1. Schematic overview of the EMG data analysis procedures. Top panels display rectified, low pass filtered EMG activity. In each panel the time axis is based on the timing of the stimulus (stimulated step) or the same relative timing in the preceding gait cycle (unstimulated steps). Full lines represent data for stimulated steps; dashed lines represent data for unstimulated steps. Grey shading on EMG traces represents the time window, over which average EMG activity values were calculated. For each phase of the gait cycle we averaged the values of 10 steps. Background EMG traces were then calculated as the average of the background traces of the ‘Externally-triggered’ condition (left hand side) and the ‘Self-induced’ condition (right hand side). Then, background and reflex EMG data was normalized to the maximum background EMG activity; displayed in the three panels with Normalized activity. Grey lines represent traces for the ‘Externally-triggered’ condition (left hand side); black lines represent traces for the ‘Self-induced’ condition (right hand side). In a last step, we then calculated the “net” reflex amplitude by subtracting the background EMG activity from the reflex EMG activity.

Fig. 2. Superposition of the regions of cerebellar lesions of all patients. Maximum overlap (7 patients) was within the left paravermal lobule VIIb.

Fig. 3. Comparisons of muscle activity and co-activation traces between cerebellar patients and healthy controls. Muscle EMG activity for cerebellar patients (CBL; grey line) and healthy controls (black line). Shaded areas show SE. Duration of the stance period for the ipsilateral (i) and contralateral (c) legs are displayed in the top frames. BF, biceps femoris; TA, tibialis anterior; GM, medial gastrocnemius. Bottom panel: Co-activation between TA
and GM for cerebellar patients (grey line) and healthy controls (black line). Co-activation was calculated sample by sample based on the normalized muscle activity (mean of both legs) using: TA-GM co-activation = \( \left( \frac{a_H + a_L}{2} \right) \times \frac{a_L}{a_H} \), where \( a_H \) represents the activity of the muscle that has the highest activity during that phase (i.e. either TA or GM) and \( a_L \) represents the activity of the other muscle during that phase (Mari et al. 2014).

**Fig. 4. Subtracted reflex traces and modulation indexes are similar between cerebellar patients and healthy controls.** Normalized background EMG activity (left) and normalized subtracted reflex EMG activity (middle) for cerebellar patients (CBL; grey line) and healthy controls (black line). Error bars show SE. Bold data points and error bars indicate phases when CBL differ significantly from healthy controls in a given phase (uncorrected for multiple comparisons). Durations of the stance period for the ipsilateral (i) and contralateral (c) legs are displayed in the top frames. Reflex modulation index (right) in different muscles for CBL (grey circles) and healthy controls (black circles). BF, biceps femoris; TA, tibialis anterior; GM, medial gastrocnemius. Note that subtracted reflex traces and modulation indexes are similar between CBL and controls, irrespective of differences in background EMG.

**Fig. 5. Attenuation of self-induced reflex responses in cerebellar patients and healthy controls.** Normalized subtracted reflex EMG activity for cerebellar patients (left) and healthy control (right). Durations of the stance periods for the ipsilateral (i) and contralateral (c) legs are displayed in the top frames. Middle frames: Self-induced reflex are shown in black, externally-triggered reflexes in grey. Error bars show SE. Bold data points and error bars indicate phases when self-induced reflex activity is significantly attenuated. iTA, ipsilateral.
tibialis anterior. Bottom frames: Individual data for externally-triggered and self-induced
reflexes.

Fig. 6. Limited habituation across trials. Total mean reflex responses due to self-induced
stimuli as a function of the order of appearance during the trial for cerebellar patients (grey
line) and healthy controls (black line). We analyzed the mean absolute reflex responses to
self-induced stimuli over all phases of the gait cycle, per stimulus number. For each
participant, these responses were then normalized with respect to their average. Eventually,
group means were calculated and plotted as a function of the order of appearance during the
trial. Error bars show SE.
Externally-triggered

- 10 steps per phase
- Stimulation step
- Preceding step

Self-induced

- 10 steps per phase
- Stimulation step
- Preceding step

Average

Background EMG activity

Normalized to maximum background EMG activity

Normalized Reflex (ext. trig.)

Normalized Reflex (self-ind.)

Subtracted Reflex (ext. trig.)

Subtracted Reflex (self-ind.)
Patients

Stance Phase

- Externally triggered
- Self-induced

Healthy controls

- Externally triggered
- Self-induced
Normalized Subtracted reflex EMG

Stimulus number within the phase

Healthy controls

Patients
Table 1: Patients were mildly ataxic and in a stable condition (> 2 years post-op)

<table>
<thead>
<tr>
<th>#</th>
<th>Age (years)</th>
<th>Post-op (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Interposed Nuclei Lesioned</th>
<th>Adjuvant Therapies</th>
<th>Lesion Volume (cm³)</th>
<th>Total /100</th>
<th>ICARS P&amp;G /34</th>
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For patient 11 no MRI data was acquired; f = female, m = male; Y = yes; P&G = Posture & Gait sub score; Kin Fin = Kinetic Functions sub score. Empty fields indicate that no lesions were present or that no adjuvant therapy was received.
Table 2: Treatment details

<table>
<thead>
<tr>
<th>#</th>
<th>Diagnosis</th>
<th>Time Post RT (years)</th>
<th>Target Areas</th>
<th>Dose Hypothalamic Post RT</th>
<th>Time Post CT</th>
<th>Total Duration CT (months)</th>
<th>Scheme</th>
<th>VP shunt cerebellar sequelia</th>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

f = female; m = male; Y = yes; RT = radiotherapy; CT = chemotherapy; CSP = craniospinal; SP = spinal; FP = fossa posterior; CT = chemotherapy; VP = ventriculo-peritoneal; HIT-2000 = cisplatinum, vincristine, CCNU; HIT-91 = ifosfamide, etoposide (VP16), metotrexate, ara-C, cisplatinum.

* Thalamic cavernous angioma, asymptomatic;
** Hydrocephalus;
*** Cavernous angioma parietal white matter, asymptomatic; cavernous angioma intramedullary spinal cord, level D12, 1.8x2.6 mm, asymptomatic.
Table 3: Overview of lesioned lobules and nuclei

<table>
<thead>
<tr>
<th>#</th>
<th>Vermis</th>
<th>Paravermis</th>
<th>Hemispheres</th>
<th>Nuclei</th>
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</table>

For patient 11 no MRI data was acquired; Y = yes; L = left; R = right; B = both; F = fastigial nuclei; I = interposed nuclei; D = dentate nuclei.