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Motor learning induces time-dependent plasticity that is observable at the spinal cord level

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Key points

- The spinal cord is an important contributor to motor learning
- It remains unclear whether short-term spinal cord adaptations are general or task-specific
- Immediately after task acquisition, neural adaptations were not specific to the trained task (i.e. were general)
- Twenty-four hours after acquisition, neural adaptations appeared to be task-specific
- The neural reorganization and generalization of spinal adaptations appears to be time-dependent.

Abstract Spinal cord plasticity is an important contributor of motor learning in humans, although its mechanisms are still poorly documented. In particular, it remains unclear whether short-term spinal adaptations are general or task-specific. As a marker of neural changes that are observable at spinal level, we measured the Hoffmann reflex (H-reflex) amplitude in the soleus muscle of 18 young healthy human adults before, immediately after (acquisition), and 24 h after (retention) the learning of a skilled task (i.e. one-legged stance on a tilt board). H-reflexes were elicited 46 ± 30 ms before touching the tilt board. Additionally, and at the same time points, we measured the H-reflex with the subject sitting at rest and when performing an unskilled and untrained task (i.e. one-legged stance on the floor). After task acquisition, there was a decrease of the H-reflex amplitude measured at rest but not during the skilled or the unskilled task. At retention, there was a decrease of the H-reflex when measured during the skilled task but not during the unskilled task or at rest. Performance increase was not associated with changes in the H-reflex amplitude. After the acquisition of a new skilled task, spinal changes appeared to be general (i.e. observable at rest). However, 24 h after, these changes were task-specific (i.e.

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observable only during performance of the trained task). These results imply that skill training induces a time-dependent reorganization of the modulation of spinal networks, which possibly reflects a time-dependent optimization of the feedforward motor command.

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Introduction

A multitude of studies demonstrate that neural plasticity associated with motor learning not only occurs within supraspinal structures, but also can be observed at the spinal cord level as well (Carp & Wolpaw, 1994; Feng-Chen & Wolpaw, 1996; Perez *et al.* 2005; Mazzocchio *et al.* 2006; Gruber *et al.* 2007; Meunier *et al.* 2007; Geertsen *et al.* 2008; Vahdat *et al.* 2015; Giboin *et al.* 2019*c*). The results of these studies appear to argue against the assumption of a hard wired spinal cord that does not change with motor learning. Furthermore, because the spinal cord is often referred to as the 'final common path' (Sherrington, 1906), this would imply that any general change that occurs at the spinal level would require an adaptation of all descending motor commands of previously adopted motor behaviours (Rothwell, 2012; Wolpaw, 2018).

To explain this phenomenon, Wolpaw (2018) proposed the 'negotiated equilibrium model of spinal cord function'. This theory states that the substrates of motor learning are distributed and hierarchized within the CNS (i.e. brain plasticity induces and maintains spinal cord plasticity), and that all motor behaviours are continuously maintained and in concurrent negotiation with each other (Wolpaw, 2018). In line with this theory, it has been demonstrated that general changes in the spinal cord, induced by Hoffmann reflex (H-reflex) operant conditioning, could be compensated by other networks at the spinal or supraspinal level to prevent undesired changes in locomotion (Chen *et al.* 2017). This theory further implies that for the spinal cord to remain a 'reliable final common pathway for all behaviours', changes occurring at the spinal cord must not occur rapidly and must be a 'gradual process with similarly gradual functional consequences'. In other words, this model may not necessarily explain short-term plasticity that can be observed at the spinal level (Wolpaw, 2018). This is an important point to consider because many H-reflex experiments in humans have demonstrated rapid changes at the spinal cord level, including immediately after motor skill learning (Perez *et al.* 2005), and even at the very beginning of the motor learning process (Lungu *et al.* 2010).

The H-reflex has been extensively used as a marker of motor learning-related changes occurring within the spinal cord. It corresponds to the sum of the muscle action potentials elicited by the activation of motoneurons after electrical stimulation of afferent fibres (mainly Ia afferents) and is sensitive to both pre- as well as post-synaptic mechanisms (Pierrot-Deseilligny & Burke, 2012). It has been repeatedly shown that the H-reflex is decreased when measured immediately after a skill learning session in the muscle at rest but not when measured immediately after a similar but unskilled training session (Perez *et al.* 2005; Mazzocchio *et al.* 2006; Meunier *et al.* 2007; Perez *et al.* 2007). Such results might indicate that the observed spinal cord changes are associated with skilled motor learning rather than general motor activity. Furthermore, because H-reflexes have been measured in the resting muscle, these changes may indeed reflect general changes occurring at the spinal level (e.g. synaptic efficiency changes, changes in the excitability of the recruited neurone, changes in the baseline activity of other modulatory networks) or general changes mediated by higher centres (e.g. presynaptic inhibition of Ia afferent fibres).

It appears plausible that, within a short-term timeframe and despite its very quick plasticity, the reliability of the spinal cord could be explained by spinal adaptations extremely specific to the task trained. For the purposes of the present study, we have used the phrase 'adaptations observable at the spinal level' to refer to changes that can be observed with methods inferring about spinal networks excitability, such as the H-reflex method. However, it must be acknowledged that these adaptations could stem from local spinal plasticity (i.e. actual spinal plasticity), or from the modulation of spinal networks by supraspinal descending pathways (e.g. presynaptic inhibition, modulation of pre-motoneuronal spinal networks), with the actual plasticity occurring above the spinal cord (Capaday & Stein, 1987; Giboin *et al.* 2012). The concept of task-specific training adaptations at the spinal level is appealing because it would allow, at least in the short-term, for a large accumulation of task-specific motor command optimization without disturbing old behaviours. This would limit the requirement of short-term 'negotiations among behaviours', that could be deleterious to the reliability of the final pathway that is the spinal cord (Wolpaw, 2018).

The idea that changes at the spinal level could also be task-specific is supported by several studies. It has been observed that four weeks of explosive strength

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training in ankle dorsiflexors alter the modulation of a spinal inhibitory network, although this change was only observed when performing the trained task and not at rest (Geertsen *et al.* 2008). More recently, we demonstrated that 6 weeks of slackline training was accompanied by a reduced H-reflex amplitude when performing the trained task (i.e. stepping on a slackline) but not when performing an untrained task (i.e. stepping on a tilt board) (Giboin *et al.* 2019*c*). These results suggest the presence of task-specific changes that are observable at the spinal cord level. Interestingly, the studies indicating general changes at the spinal level in humans have made their observations immediately after the acquisition of the task (Perez *et al.* 2005; Mazzocchio *et al.* 2006; Perez *et al.* 2007). On the other hand, studies showing task-specific changes in the H-reflex have made this observation after several training sessions (Geertsen *et al.* 2008; Giboin *et al.* 2019*c*). This difference of results may suggest the existence of a temporal dynamic in the generalization of spinal adaptations (i.e. from general to more specific). Interestingly, studies performed at the supraspinal level suggest that sleep or more training sessions after the acquisition of a skilled task induce neural reorganization that increases the specificity of the neural adaptation (Fischer *et al.* 2002; Xu *et al.* 2009; Yang *et al.* 2014). In the present study, we hypothesized that, during the learning of a balance task, similar reorganization mechanisms take place and cause time-dependent plasticity that is observable at the spinal level.

For this, we measured the H-reflex in the soleus muscle before, just after and 24 h after the acquisition of a skilled balance task at the same time as performing the skilled trained task or a similar but unskilled balance task and at rest. We hypothesized that the H-reflex amplitude would be decreased in all tasks (i.e. skilled task, unskilled task and at rest) when measured just after the acquisition of the balance task, indicating general training-induced adaptation at the spinal level. However, we hypothesized that 24 h after the acquisition period, the H-reflex would be decreased only when performing the skilled trained task, indicating a task-specific reorganization of the neural adaptation observable at the spinal level.

Methods

Participants

Twenty-one subjects participated in the study after providing their written informed consent. Exclusion criteria were age <18 years at the time of the experiment, a leg injury during the past 6 months and any experience in the balance task tested during the experiment. Three subjects were excluded during the first experimental session because we could not elicit a stable H-reflex during movement. Thus, data from 18 subjects (height 172 ± 11 cm, body mass 68 ± 12 kg, aged 27 ± 8 years old, 10 women) were collected and analysed. As a result of to time limitations, it was not possible to test one subject at rest during the post-acquisition. The experiment was approved by the ethical committee of the University of Konstanz (IRB 36/2018) and conducted in accordance with the latest revision of the *Declaration of Helsinki*, except for registration in a database.

Overall experimental protocol

Subjects participated in two experimental sessions, which were separated by 24 h. Figure 1 summarizes the overall experimental protocol. In the first session (i.e. the acquisition session), subjects learned to perform a one leg stance on a tilt board. In the second session, i.e., the retention session, the balance performance on the tilt board was tested. In both sessions, we elicited H-reflexes in the soleus muscle of the stance leg. During the acquisition session, we first obtained an H-reflex and M-wave recruitment curve at rest (pre-acquisition recruitment curve). Then, we measured the H-reflex at the same relative intensity under three different conditions (pre-acquisition conditions) and in the order: at rest, when subjects executed the floor task and when subjects executed the tilt board task. Fifteen trials or stimulations were collected for each condition. Next, subjects learned the tilt-board task for four blocks of 15 trials separated by breaks of 90 s (acquisition phase). These trials were completed without peripheral nerve stimulation. Immediately after the acquisition phase, the H-reflex was measured in three different conditions (post-acquisition conditions) and in the order: when executing the tilt board task, when executing the floor task and at rest. Fifteen trials or stimulations were collected for each condition. The next day, during the retention session, we obtained an H-reflex and M-wave recruitment curve at rest (retention recruitment curve). Then, we measured the H-reflex under different conditions (retention conditions) and in the order: at rest, when executing the floor task and when executing the tilt-board task. Fifteen trials or stimulations were collected for each condition. Before measuring the H-reflex for any condition (at rest, when executing the floor task or the tilt board task) and at any time point (pre-acquisition, post-acquisition and retention), we measured the maximal M-wave (M_{max}) at rest or when performing the relevant task.

Balance tasks

Subjects performed two balance tasks: the tilt board task and the floor task. Both tasks were done identically until reaching the stable (floor) or unstable (tilt board) surface. Participants were barefoot, instructed to put their hands

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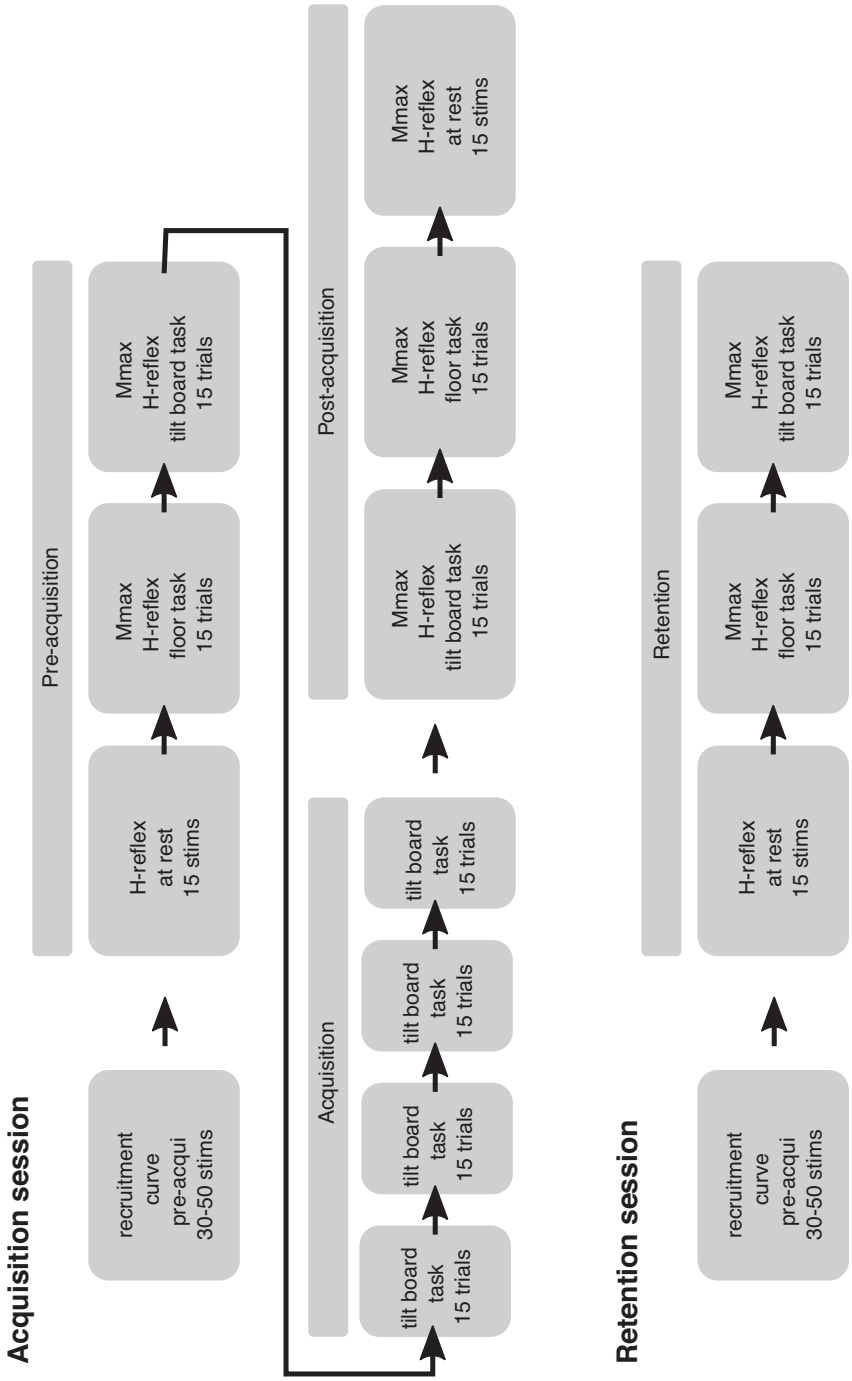

Figure 1. Overall experimental protocol
The overall protocol consisted of two experimental sessions: the acquisition session and the retention session, separated by 24 h. Each session started with the collection of the H-reflex and M-wave recruitment curves at rest. Then, 15 H-reflexes were measured at rest, when performing the floor task and when performing the tilt board task. For the latter two tasks, M_{max} was measured before eliciting H-reflex. In the acquisition session, these measurements (pre-acquisition measurements) were followed by four blocks of 15 tilt board task trials separated by 90 s breaks (acquisition of the tilt board task). This was followed by post-acquisition measurements, where H-reflexes were elicited when performing the tilt board task, the floor task and at rest. M_{max} was re-measured in the three conditions before eliciting H-reflex.

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on the hips and stepped down from an elevated platform after hearing a 'go' signal to execute a one leg stance on the tilt board (board task) or on the floor (floor task). For both tasks, the depth and distance between the starting platform and the spot where the one leg stance had to be executed were the same.

For the board task, subjects were instructed to balance on the tilt board for as long as possible, to a maximum of 20 s, with a one-legged stance. The stance (preferred) leg was determined as the participants' preferred leg to perform a one-legged stance on the floor (i.e. the subject performed a one leg stance on the floor on each leg for a few seconds before deciding which leg is easier to use). For twelve subjects, the preferred leg was the right leg. The tilt board (SENSOBOARD essential; Sensosports GmbH, nsengericht, Germany) consisted of a wooden board covered by a gripping surface (width 80 cm, length 52 cm) positioned over a metal sphere fixed to a support (height 14 cm). The upper board and the support were maintained by elastics (for more details, see Giboin *et al.* 2019*c*). Task performance was defined as the time spent on the tilt board, starting when both feet were outside of the starting platform and finishing with task termination. The task was terminated when one edge of the board touched the ground, or when the subject stepped off the tilt board, or after 20 s. Time was monitored with a stopwatch and given as a feedback to participants after each trial. The floor task consisted of executing a one-legged stance, using the same leg as during the board task, on the floor for ∼2 s.

Electromyography (EMG)

At the beginning of the acquisition and retention sessions, EMG sensors (Trigno wireless system; Delsys, Natick, MA, USA) were taped on the muscle belly of the soleus (Sol), tibialis anterior (TA), vastus lateralis (VL) and biceps femoris (BF) muscles of the preferred leg. All skin sites were shavedand cleaned with sandpaper and alcohol prior to electrode placement. EMG signals were sampled at 4000 Hz (Power1401 and Signal Software 5.08; Cambridge Electronic Design, Cambridge, UK).

Motion capture

Reflective motion capture markers were taped on the preferred leg of the subject at the hallux, fifth metatarsal bone, lateral and medial malleolus, lateral knee joint centre and greater trochanter. Four additional markers were fixed on the lateral side of the tilt board platform to determine its position with respect to the floor. Additionally, for the tilt board task, the starting platform was located over a force plate (BP600400; AMTI, Watertown, MA, USA), which allowed us to calculate the time delay between touchdown of the preferred leg and take-off of the second leg.

Peripheral nerve stimulations

Percutaneous electrical peripheral nerve stimulations were applied to the tibial nerve to elicit H-reflexes and M-waves in the Sol of the preferred leg. The cathode electrode (custom-made, copper, circular, diameter 2 cm, wrapped in a water-soaked sponge) was placed in the popliteal fossa and the anode (custom-made, copper, 7 × 5 cm, wrapped in a soaked sponge) was fixed over the patella. We used a non-compliant strap and additional tape to fix both stimulation electrodes. The electrical stimulation consisted of a 1 ms squared pulse (stimulator DS7A; Digitimer, Welwyn Garden City, UK).

H-reflex and M_{max}

Sol H-reflexes were elicited at rest, with subjects sitting on a chair and with the preferred leg extended at a knee angle of 125°, and also during task execution, with the aim to apply the stimulation just before the subject touched the floor or the tilt board. Because we wanted to compare H-reflex amplitudes when subjects were performing different tasks (rest, floor task and tilt board task) and under different conditions (pre- and post-acquisition and retention), the stimulation intensity was adjusted so that the M-wave amplitude, normalized to the M_{max} obtained for each task and condition, was similar between tasks and conditions (Giboin *et al.* 2019*c*). Therefore, for each task and condition, we ensured a stable and discernible M-wave when being on the ascending part of the H-reflex recruitment curve (Pierrot-Deseilligny & Burke, 2012). When measuring M_{max} during the tilt-board task, subjects were instructed to maintain their balance for only 2 s to minimize the learning effect of these additional trials.

Maximal H-reflex amplitude (H_{max}) and M_{max} at rest

The H_{max} and M_{max} at restwere obtained from 30–50 stimulations, each separated by 4 s. We started the stimulations at submotor threshold level and increased the current progressively until the H-reflex amplitude decreased considerably to one-half of its maximal value (around 15 stimulations). After that, 10–20 more stimulations around the hitherto existing maximal value were delivered to increase the precision of the H_{max} estimation further. Then, we increased the current and reached M_{max} within 5–10 stimulations.

Stimulation during movement

Subjects were first instructed how to perform the floor task and were provided three to five practice trials. Following this, subjects were exposed to the tilt board task three

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times. To limit the learning effect of these three trials, subjects were instructed to stand for no more than 2 s on the tilt board. These three trials were used to control the speed and technique of the subject (with investigator feedback) and determine the correct time delay for eliciting the H-reflex. The aim was to trigger stimulations so they would occur as close as possible but before initial foot contact with the floor or the tilt board. A light barrier (Optogait; Microgate, Bolzano, Italy) was positioned just above the floor or the tilt board such that when the subject's foot crossed the light barrier, the stimulation was triggered. If necessary, a stimulation delay was added. Further more details are provided in Giboin *et al.* (2019c). The simulation intensity to elicit a M-wave with the correct amplitude was adjusted during several trials of the floor task. We used the same stimulation intensity during the tilt board task. Indeed, because we were interested in the modulation of the H-reflex when learning the tilt board task (and especially during the first trials), we had to limit as much as possible the amount of trials that subjects performed (and therefore started learning) on the tilt board task before measuring the baseline H-reflex amplitude.

Analysis

EMG traces from every trial involving a stimulation were examined (2415 trials, with one subject not measured at rest at post-acquisition). M-wave and H-reflex amplitudes were normalized to the M_{max} amplitude obtained for the given condition and task. Trials with a M-wave above or equal to 1% M_{max} and below mean (M-wave) + 2 × SD (M-wave) were included for further analyses (2328 trials remaining). The mean and SD of the M-wave were calculated from the total pool of stimulations. Background EMG of Sol, TA, VL and BF muscles were measured during a time frame of 50 ms before stimulation. The root mean square (rms) of these EMG signals was expressed as % mean rms EMG obtained during rest at the same time level. Nexus, version 1.8.5 (Vicon, Oxford, UK) was used to calculate the kinematic parameters: time delay between the stimulation and foot touchdown on the tilt-board (defined as the first movement of the tilt-board markers), time delay between touchdown and take-off of the stance leg (force level of the force plate underneath the platform returning to baseline), knee angle in the sagittal plane (calculated from the markers on lateral malleolus, lateral knee joint centre and greater trochanter), ankle angle in the sagittal plane (calculated from the markers on fifth metatarsal, lateral malleolus and lateral knee joint centre), velocity of the toe, lateral knee joint centre and greater trochanter markers (discrete derivative of the marker positions over time), and tilt angle of the tilt-board in the anteroposterior and mediolateral axis when the stance leg takes off (deviation of the tilt-board markers from their initial position in the horizontal plane), as well as the distance of the lateral malleolus from the centre of the platform when the stance leg takes off. The tilt angle of the tilt board was normalized to the resting position of the board [i.e. absolute value (tilt angle – 90)].

Statistical analysis

Bayesian linear mixed models: advantages and short description. In neurophysiology research, it is common to measure muscle compound action potentials elicited by nerve or brain stimulation (e.g. H-reflexes or motor evoked potentials). These potentials are known to exhibit between-trial variability (Kiers *et al.* 1993; Funase & Miles, 1999). The source of this variability can be the result of a number of reasons, including the sampling procedure (e.g. the electrode used for nerve stimulation slightly moving as a result of the underlying muscle contraction when measured during movement), physiological mechanisms or noise (e.g. the efficiency of learning appears to be linked to the active modulation of task-specific motor variability) (Wu *et al.* 2014). Therefore, many potentials are elicited and then averaged to obtain an estimate of the true size of the potential in a given subject and a given experimental condition. This averaged value is usually analysed further with 'traditional' tests such as ANOVA. However, averaging potentials from a number of trials can lead to a substancial loss of information that can result in a misinterpretation or a masking of interesting physiological phenomena. To illustrate this problem, we have simulated three different scenarios that could plausibly occur when measuring potentials (e.g. H-reflexes) pre- and post-intervention in one subject (Fig. 2). For each case, we have simulated data (15 data points with a dispersion following the normal distribution) so the pre-intervention mean H-reflex amplitude is equal to 30% M_{max} and the post-intervention mean amplitude equal to 25%. In Fig. 2*A*, the SD was set at 7% M_{max} pre- and post-intervention. In Fig. 2*B*, the SD was set at 0.5% M_{max} pre- and post-intervention. In Fig. 2*C*, the SD was set at 0.5% M_{max} pre-intervention and at 7% M_{max} post-intervention. In Fig. 2*A*, the variability is quite high and it is not clear whether the reduction of the averaged H-reflex is a result of the intervention or a result of variability alone. In Fig. 2*B*, the variability is small and the reduction of the H-reflex following the intervention is clearly apparent. In Fig. 2*C*, it appears that the intervention elicited a change in the variability of the H-reflex. This last result could lead to the discovery of interesting physiological mechanisms responsible for such variability (Wu *et al.* 2014). When using traditional analyses, the three situations are considered as being the same (because only the average value is taken into account) even though they are clearly different. Therefore, it stands to reason

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that statistical procedures should take this within-subject variability into account.

In the present study, we measured 15 H-reflexes per time and condition level for each subject. As expected, the variance of the H-reflex measured differed depending on the task performed, and potentially at the time level (Fig. 3). As explained above, this variance may stem not only from the sampling procedure, but also from physiological mechanisms, and should not be ignored. To take this within-subject variance into account, and provide analyses as close as possible to our data, we used linear mixed models. Roughly, linear mixed models correspond to linear regressions within a linear regression, and allow to cluster non-independent data points together and incorporate the error from these clusters into the total error of the model. The benefits of incorporating linear mixed models rather than traditional statistical approaches include that ability to take into account measurements nested within one subject, to handle missing and unbalanced data, to avoid the loss of information as a result of data averaging, and to have a better estimation of the parameters as a result of the partial pooling strategy (Boisgontier & Cheval, 2016; Nalborczyk *et al.* 2019). For all these reasons, a strong call in favour of transitioning from ANOVA to linear mixed models in neuroscience research has recently been made (Boisgontier & Cheval, 2016).

In addition, we have used Bayesian analyses instead of frequentist statistics because it allowed us to maximize the error structure of the models, in order to limit type I errors (Barr *et al.* 2013). Indeed, this is problematic in a frequentist framework, where the maximized models often do not converge. By contrast, within a Bayesian framework, these models are generally able to converge. Moreover, the Bayesian framework easily deals with multiple comparisons, which is often necessary in training studies with several groups, and it allows to consider whether an effect is credibly different from a null value (Kruschke & Liddell, 2018; Nalborczyk *et al.* 2019). Finally, we find it easier to discuss results in a Bayesian framework,

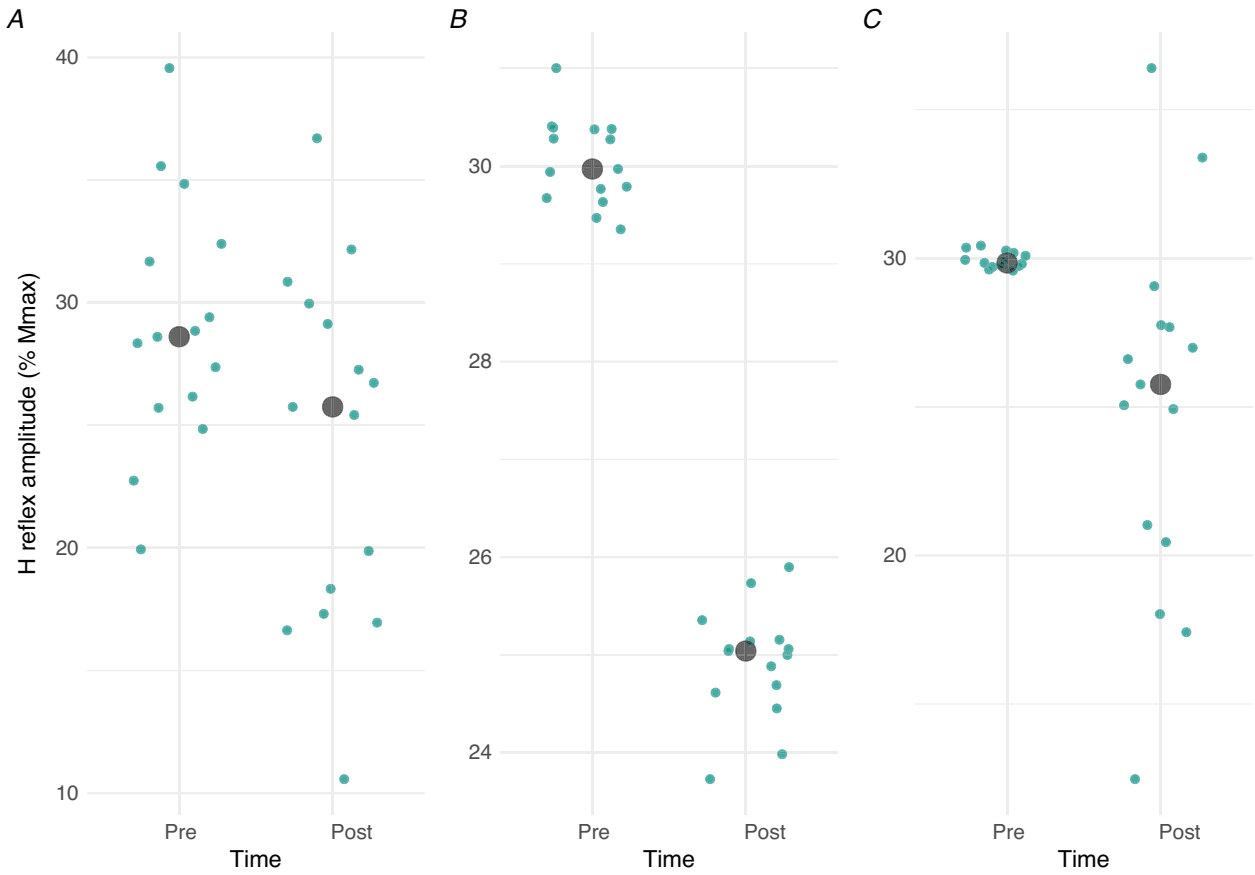

Figure 2. Example of different within-subject error cases
A–C, green points correspond to simulated H-reflexes obtained in one subject pre- and post-intervention and black points correspond to the mean. Data points are simulated so the mean is equal to 30% M_{max} pre-intervention and 25% M_{max} post-intervention (n = 15 per time-level). The distribution of the simulated points follows a normal distribution with SD equal to 7% M_{max} pre-and post-intervention in (*A*), 0.5% M_{max} pre-and post-intervention in (*B*) and 0.5 and % M_{max} pre- and post-intervention respectively in (*C*). [Colour figure can be viewed at wileyonlinelibrary.com]

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where a 95% credible interval indicates that the estimate has 95% of chance of being within the interval boundaries (Kruschke & Liddell, 2018).

Statistical procedures. We used the R package *brms* to perform Bayesian generalized linear mixed models (Bürkner, 2017). Analyses were performed at the trial level clustered within subjects, where, for each model, subject was the group-level effect or random effect depending on the terminology used. Data set distribution was assessed with *Q*–*Q* plots. The details of every model (and priors) used for each test are provided in Table 1. For each model, we maximized the random error structure to limit type I errors (Barr *et al.* 2013). We verified that every model converged correctly and we checked graphically that predicted values from the model were similar to actual data.We used the *brms* function *hypothesis()* to assess whether there was difference between the marginal effects given by the model and calculated an evidence ratio. The *hypothesis()* function allows to test one-sided hypothesis by subtracting the distribution of one estimate by another. For example, to test the hypothesis 'the H-reflex at retention is lower than at pre-acquisition', we subtract the distribution of the H-reflex estimate at pre-acquisition by the distribution of the H-reflex estimate at retention. If the resulting distribution is above zero, we conclude that the H-reflex at pre-acquisition was bigger than at retention. The evidence ratio identifies the ratio between the probability tested and its alternative (i.e. probability of $A > B$ versus probability of $B > A$).

To test whether there was a learning effect, we assessed the effect of trial number on performance at pre-acquisition, post-acquisition and retention. To calculate the intercept correctly, we defined the first trial as trial zero and consequently shifted all trial numbers by one towards zero (trial number = actual trial number − 1). As shown in Fig. 4*A*, the variability of performance was not constant across trials. Therefore, we multiplied performance by 100 to obtain integers and used a generalized linear mixed model with a Poisson family and a log link function (i.e. the function that links the linear predictor to the expected data point) (O'Hara & Kotze, 2010).

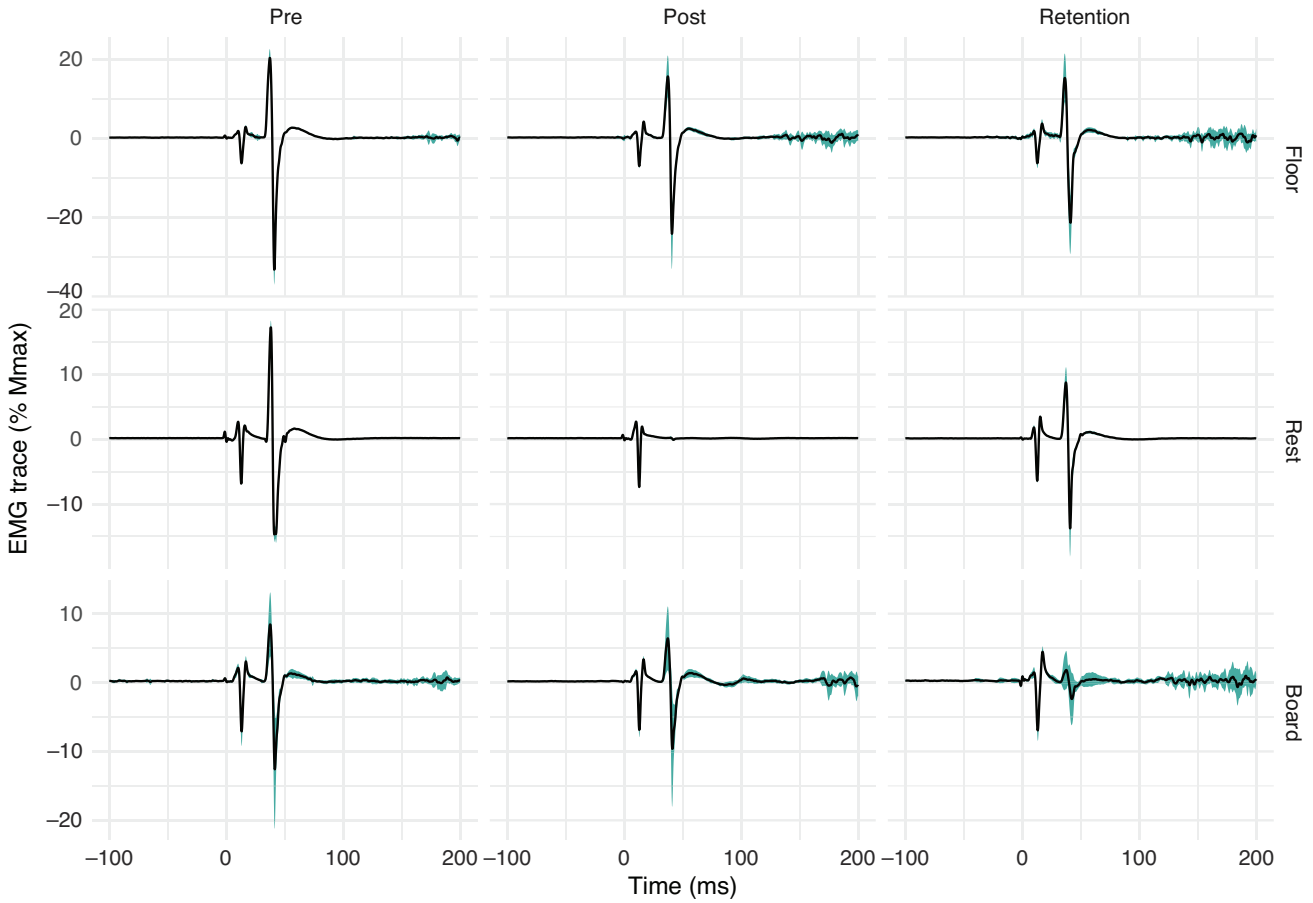

Figure 3. H-reflex and M-wave in one subject
The thick line corresponds to the average of the 15 trials per time and task level. The green area corresponds to the standard deviation. The EMG traces are expressed in percentage of M_{max}. The nerve stimulation is elicited at zero on the *x*-axis. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 1. Statistical models

Model number	Dependent variable	Right side of the model	Family	Link function	Prior	MCMC, iterations
1	Performance × 100	~time × trial+(time × trial|subject)	Poisson	Log	Normal (0,10) for class b cauchy (0,5) for SD	4, 4000 (warm-up of 2000)
2	H-reflex centred and scaled	~task + time +(task + time|subject)	Gaussian	Identity	Normal (0,10) for class b cauchy (0,5) for SD	4, 4000 (warm-up of 2000)
3	H-reflex centred and scaled, M-wave, EMG, joint angles and velocities	~task × time + (task × time|subject)	Gaussian	Identity	Normal (0,10) for class b cauchy (0,5) for SD	4, 4000 (warm-up of 2000)
4	Performance × 100	~tilt mediolateral + tilt anteroposterior + delay stance off + distance foot board + (tilt mediolateral + tilt anteroposterior + delay stance off + distance foot board|subject)	Gaussian	Identity	Normal (0,10) for class b cauchy (0,5) for SD	4, 4000 (warm-up of 2000)

In the present study the log link function exponentiates the linear predictor. We used minimally informative priors for the betas (normal distribution with a mean of 0 and SD of 10) and the group level SD (half-Cauchy distribution with a mean of 0 and SD of 5; Model 1) (Table 1).

For H-reflexes measured at pre-acquisition, post-acquisition and retention, we predicted that H-reflex amplitude would be different between tasks (Koceja *et al.* 1995; Earles *et al.* 2000). Therefore, to better compare the relative changes in H-reflex amplitude between tasks, we centred and scaled the normalized H-reflex amplitude. First, we examined whether there was an interaction between the different tasks and time. For this, we used the function *loo()* (leave-one-out cross-validation) to compare two models. The first model (Model 2) represented main effects of time and task, whereas the second model (Model 3) represented the same main effects but additionally an interaction between time and task (both models had maximized error structures, see Table 1). The smaller the leave-one-out information criterion (LOOIC), the better the model fit to the data (Vehtari *et al.* 2017). As supplementary information, we have calculated the Bayes r^2 for both models, which is the Bayesian homologue of the classical r^2 and corresponds to 'the variance of the predicted values divided by the variance of predicted values plus the expected variance of the errors' (Gelman *et al.* 2019). Then we used the *hypothesis()* function on estimates posteriors from Model 3 to assess our hypothesis. Note that multiple comparisons do not need to be adjusted when using Bayesian mixed models (Gelman *et al.* 2012). We used a Bayesian paired *t* test to assess whether there was a difference in the H_{max} normalized to M_{max} and the *hypothesis()* function to calculate the associated evidence ratio.

To test whether the H-reflex amplitude was sensitive to the number of trial performed across tasks (i.e. comparing the H-reflex slopes across trials between tasks), we used a model with H-reflex amplitude as the dependent variable and an interaction between trial and task with a maximized error structure (model not shown, same priors than Model 3). To assess the association between the H-reflex amplitude and performance, we used Bayesian correlation with Student family because it is more robust to outliers: default priors from the *brms* package, 4 Markov chain Monte Carlo (MCMC), 2000 iterations. Moreover, we used a linear mixed effect model with performance as the dependent variable and main effects and interaction between H-reflex amplitude, trials and time on the right side of the equation (with maximized error structures, model not shown). To pursue further the analysis of performance, we also used a linear mixed model with performance as the dependent variable and, as main effects, the angle of the tilt board in the medio-lateral and antero-posterior axis when the stance leg takes off, the time between the touchdown on the tilt board and the take-off of the stance leg, and the distance between the foot and the centre of the tilt board when the stance leg takes off (with maximized error structures; Model 4). This analysis was conveyed on all trials performed on the tilt board (i.e. trials performed at time level pre- and post-acquisition, during acquisition and during retention). It must be noted that, for the latter two models with performance as the dependent variable, we were unable to use a generalized linear model with Poisson family because it prevented the models from converging. Therefore, we acknowledge

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that, for these two models, the model did not fit the data optimally and care must be taken in their interpretation.

For the control measurements, we were interested in whether there was a difference between tasks or time. Therefore, for M-waves, EMG, joint angles and velocities, we used Model 3. For joint angles and velocities, we did not include data from the rest task. For the delay (i.e. time between the stimulus and the touchdown on the tilt board), we used a model similar to that used for the other control measurements but with time as the only constant effect and a random intercept by subject and a random slope for time by subject. Results are reported in the form of 'estimated mean of the posterior distribution [lower 95% credible interval, upper 95% credible interval]'. The 95% credible interval corresponds to the interval that contains 95% of the most credible values for the mean estimate. This means that the mean estimate has a probability of 0.95 to lie within the credible interval boundaries (Kruschke & Liddell, 2018). When one-sided hypotheses were made (e.g. 'the H-reflex at retention is lower than at pre-acquisition'), the 90% credible interval is used instead of the 95% credible interval because, in that case, only one side of the distribution is relevant. In the present study, when the 95% or 90% credible estimate of one parameter distribution contains zero, we consider that this parameter may not be different from zero (because zero is part of the credible values). In general, the evidence ratio threshold to support one hypothesis is 'set by practical consideration' (Kruschke & Liddell, 2018). It has been proposed that a ratio threshold of 6 should be reached in early lines of research to support evidence (the present case), whereas a threshold of 10 should be reached in

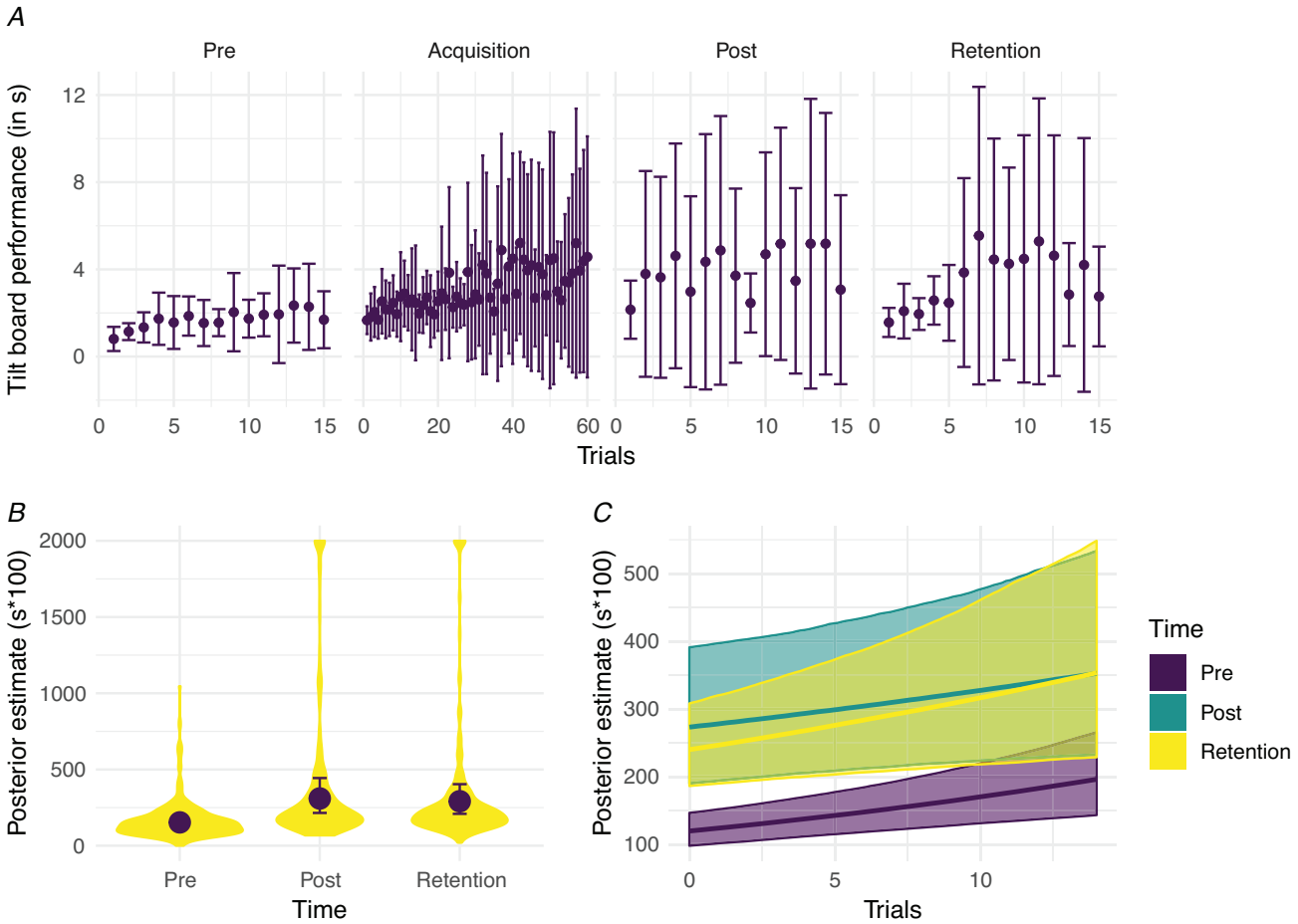

Figure 4. Balance performance
A, balance performance in the tilt-board task across trials at pre-acquisition, during acquisition, post-acquisition and retention. Points represent mean values of the 18 subjects for each trial, and error bars represent one SD. *B*, purple points correspond to the posterior estimate of the mean performance value (performance × 100), and the error bars correspond to the upper and lower bounds of the 95% credible interval. Yellow violin plots correspond to the real data. The *y*-axis value corresponds to performance multiplied by 100 (statistical model with Poisson family). *C*, thick lines represent the posterior estimate of the performance across trials for each time level (interaction between trial and time). The area around the line represents the upper and lower bounds of the 95% credible interval. Note that the first trial is trial number 0. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 2. Posterior estimates of main results

Model	DV	Population effect	Estimate	SD	Lower 95% CI	Upper 95% CI
1	Performance × 100	Intercept	4.78	0.1	4.59	4.99
		Time post	0.83	0.17	0.48	1.17
		Time retention	0.7	0.11	0.47	0.92
		Trial	0.04	0.01	0.02	0.05
		Time post : trial	−0.02	0.01	−0.04	0.01
		Time retention : trial	−0.01	0.01	−0.03	0.01
3	H-reflex	Intercept	0.1	0.23	−0.35	0.56
		Task rest	0.27	0.25	−0.23	0.75
		Task board	0.17	0.1	−0.04	0.37
		Time post	−0.09	0.15	−0.39	0.21
		Time retention	−0.18	0.22	−0.6	0.25
		Task rest : time post	−0.68	0.32	−1.3	−0.05
		Task board : time post	−0.06	0.14	−0.35	0.24
		Task rest : time retention	−0.13	0.27	−0.67	0.4
		Task board : time retention	−0.33	0.18	−0.69	0.03
3	M-wave	Intercept	0.07	0.01	0.05	0.09
		Task rest	0	0.01	−0.02	0.01
		Task board	0	0	0	0.01
		Time post	0	0	0	0.01
		Time retention	0	0.01	−0.01	0.01
		Task rest : time post	0	0.01	−0.01	0.01
		Task board : time post	0	0	−0.01	0.01
		Task rest : time retention	0	0.01	−0.01	0.01
		Task board : time retention	0	0	−0.01	0.01

The intercept corresponds to the mean estimate of baseline factor levels. In Model 1, intercept corresponds to the mean estimate of performance at pre-acquisition and, in Model 3, intercept corresponds to the mean estimate at pre-acquisition for the floor task. Estimates are tested against the Intercept (unless for the interactions with Trial, which are tested against Trial). The colon symbol ':' corresponds to an interaction effect. Estimates of Model 1 are on a log scale (to exponentiate to obtain the estimate on the performance scale) and Model 3 fits the centred and scaled values of the H-reflex amplitude. DV, dependent variable; CI, credible interval.

confirmatory studies (Schönbrodt *et al.* 2017; Kruschke & Liddell, 2018).

Data availability. Data have been uploaded on Figshare.com (https://figshare.com/articles/Motor_learning_induces_time_dependent_plasticity_that_is_observable_at_the_spinal_cord_level/8262248; reviewers can ask directly for the data sets if required).

Results

Performance

Performance results are plotted in Fig. 4 and posterior estimates are shown in Table 2, as well as in Fig. 4*B* and *C*. Balance performance increased from pre-aquisition to post-aquisition and pre-acquisition to retention (Fig. 4*B*). A direct hypothesis testing of retention > post-acquisition gave an estimate of −0.13 [−0.37, 0.11] and an evidence ratio of 0.22. This result indicates that the mean difference of performance between retention and post-acquisition was −0.13 but, because the credible interval contains zero, we cannot support the hypothesis that there was a difference of performance between retention and acquisition. This is also supported by the low evidence ratio, indicating that the probability that performance is higher at retention compared to acquisition is 0.22 higher than the probability that the performance was higher at acquisition compared to retention. Similarly, there was no difference in the slopes of performance across trials between pre- and post-acquisition and pre-acquisition and retention (Fig. 4*C*). The slope at retention was not steeper than the slope at post-acquisition (0.01 [−0.01, 0.03], evidence ratio of 3.87) (Fig. 4*C*).

H-reflex

The M-wave and H-reflex traces of one subject are plotted in Fig. 3. H-reflex amplitudes normalized to M_{max} across time and task are plotted in Fig. 5*A* and the mean and SD are shown in Table 3. The centred and scaled H-reflex amplitudes normalized to M_{max} are plotted in Fig. 5*B*. The LOO showed that the model with the interaction between task and time (Model 3) fitted the centred and scaled data better than the model with only the main effects of task and

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time (Model 2) (LOOIC ± SE, 2825 ± 113 and 4030 ± 86 for Model 3 and Model 2, respectively). The Bayes r^2 was equal to 0.82 [0.81, 0.82] for Model 3 and 0.68 [0.67, 0.69] for Model 2, indicating that Model 3 explained a larger proportion of the variance. This result indicated an interaction effect of task and time on H-reflex amplitude. Direct hypothesis testing (pre-acquisition > post-acquisition and pre-acquisition > retention, estimates given in centred and scaled units) on the posterior estimates from Model 3 showed that the H-reflex amplitude during the board task at pre-acquisition was not higher than at post-acquisition (0.14 [−0.12, 0.41], evidence ratio of 4.72). However, the H-reflex amplitude during the board task at pre-acquisition was higher than at retention (0.51 [0.21, 0.82], evidence ratio of 215). During the floor task, the H-reflex amplitude was not higher at pre-acquisition than at post-acquisition (0.09 [−0.16, 0.33], evidence ratio of 2.7) or at retention (0.18 [−0.17, 0.54], evidence ratio of 4.3). At rest, the H-reflex amplitude was higher at pre-acquisition than at post-acquisition (0.77 [0.27, 1.26], evidence ratio of 122), although not higher compared to the amplitude at retention (0.32 [−0.12, 0.76], evidence ratio of 7.1). It must be noted that, in the present cases where the 95% credible interval framed zero, the rather large credible intervals does not allow us to conclude that there was truly no difference between the marginal effects compared (Kruschke & Liddell, 2018). The H-max normalized to M_{max} measured at rest and at the beginning of each session was not higher pre-acquisition than at retention (60.3 ± 18.3 and 58 ± 23.5% M_{max}, respectively, 2.06 [−3.55, 7.68], evidence ratio of 2.75). Finally, the slope of the H-reflex amplitude across trials was equal to zero during the floor task (−0.0 [−0.01, −0.0]) and there was no difference with the slopes of H-reflex amplitude across trials during the board task (0 [−0.0, 0.01]) or at rest (0 [0. 0.01]).

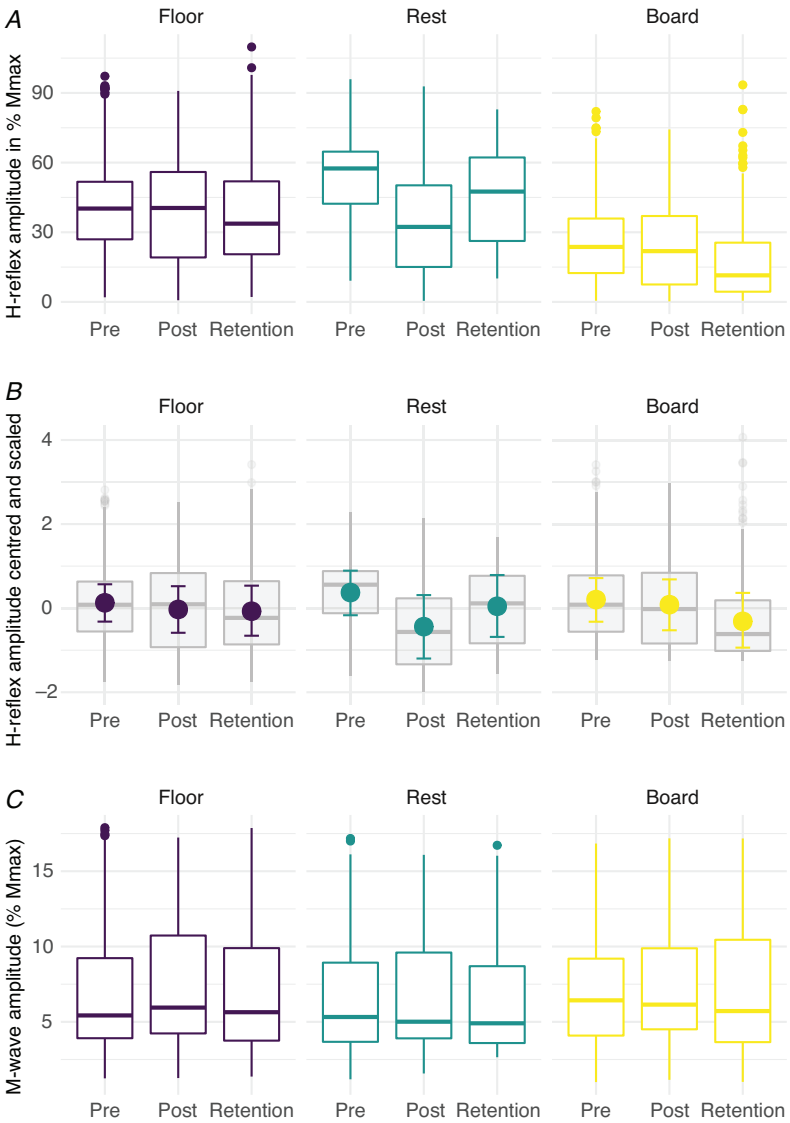

Figure 5. H-reflex and M-wave amplitude
A, H-reflex amplitude normalized to M_{max} across tasks and time. Boxplots represent the median, the first and third quartiles. The whiskers extend to 1.5 × the distance between the first and third quartile. *B*, H-reflex amplitude centred and scaled. The grey boxplots correspond to the data points, the coloured points to the posterior mean and the error bars correspond to the 95% credible interval. *C*, M-wave amplitude normalized to M_{max}. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 3. Mean H-reflex

	Pre-acquisition	Post-acquisition	Retention
Rest	53.1 ± 1.7	35.2 ± 2.5	46.1 ± 2
Floor	39.8 ± 1.9	38.8 ± 2.3	37 ± 2
Board	25.6 ± 1.7	23.4 ± 1.7	17.9 ± 1.7

Mean ± SD of the normalized H-reflex (in % M_{max}) across time and tasks.

H-reflex and performance

As shown in Fig. 6, H-reflex amplitude and task performance were not correlated (correlation coefficient estimates at pre-acquisition, post-acquisition and retention were −0.30 [−0.67, 0.17], 0 [−0.46, 0.44] and −0.19 [−0.59, 0.26], respectively). There was also no correlation between changes in H-reflex amplitude (H-reflex amplitude at retention as a percentage of pre) and changes in performance (time during retention as a percentage of pre; correlation coefficient estimate = −0.11 [−0.57, 0.38]). Additionally, the linear mixed model showed no clear effect of H-reflex amplitude, nor any interaction effects between H-reflex amplitude, trials and time on performance (results not shown). By contrast, performance could be explained by local biomechanical factors. Indeed, Model 4 showed a clear effect of the antero-posterior tilt deviation from the baseline of the tilt board when the stance leg was lifted off the elevated platform (i.e. the subject was only in contact with the tilt board), with a mean estimate of −0.13 [−0.25, −0.01] s. This means that performance decreased by 0.13 s per degree of anteroposterior deviation from baseline. There was also an effect of the distance between the lateral maleollus of the foot that stands on the tilt board and the centre of the board (−0.03 [−0.06, 0]). This means that performance decreased by 0.03 s per mm of distance with the centre of the board. The large 95% credible interval of the time between the touchdown and the stance leg take-off (0.84 [−0.87, 2.76]), as well as the

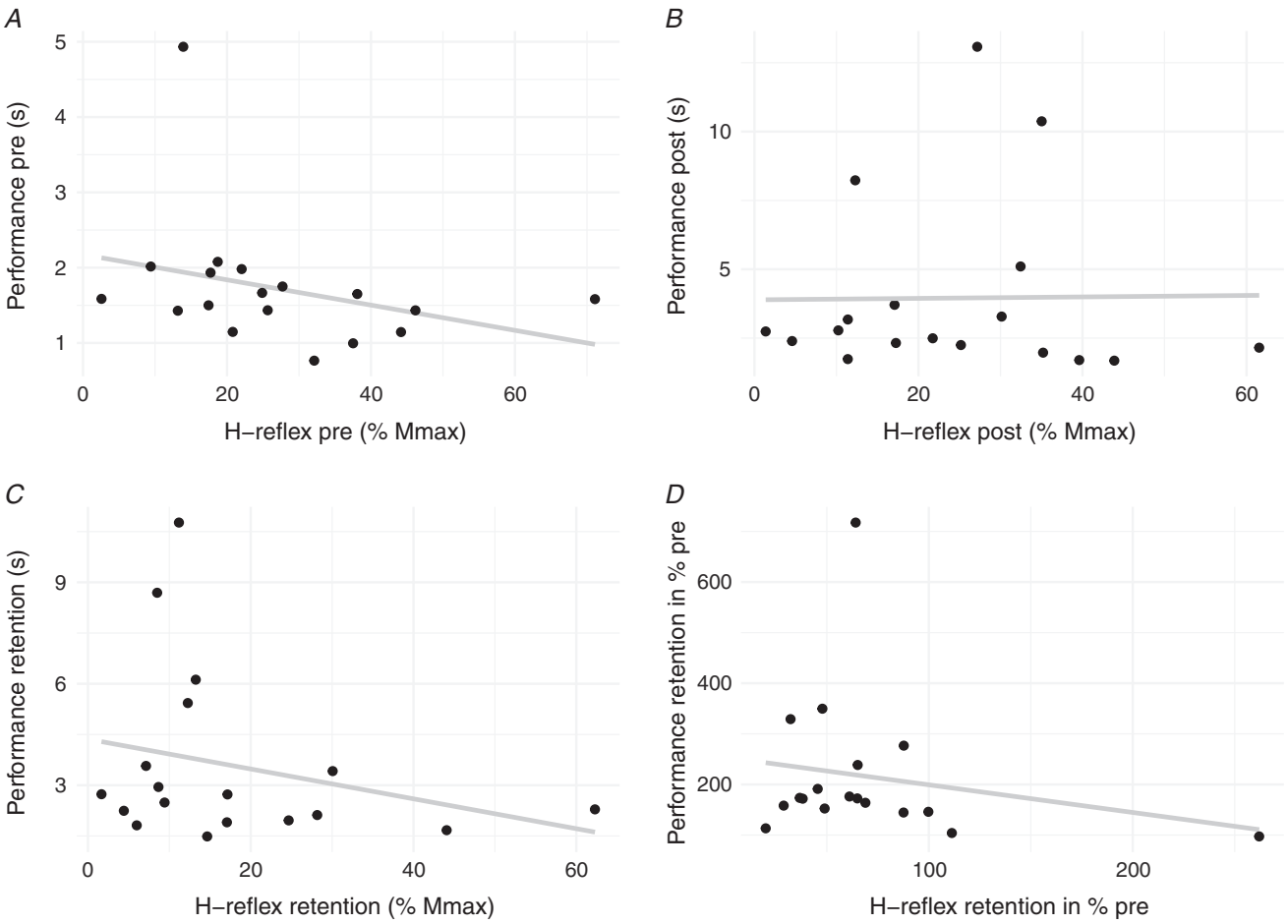

Figure 6. Correlations between H-reflex amplitude and performance
A–C, mean performance plotted against mean H-reflex amplitude (% M_{max}) for each subject at pre-acquisition, post-acquisition and retention, respectively. *D*, mean changes in performance (retention in % pre-acquisition) against mean changes in H-reflex amplitude (retention in % pre-acquisition).

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medio-lateral angle of the tilt board (−0.12 [−0.27, 0.06]), does not allow us to directly infer on their influence on performance.

Control measurements

To ensure that the changes observed in the H-reflex amplitude were probably a result of training-induced neural plasticity rather than changes in proportion of nerve fibre activated or biomechanical constraints (Giboin *et al.* 2019*c*), we tested whether there were any effects of time or task or time × task interaction on the M-wave amplitude; background EMG before stimulation; knee and ankle angle at stimulation; knee, hip and toe velocity at stimulation; and the delay between stimulation and touchdown. For the M-wave, there was no difference between tasks and across time, suggesting that, in regard to H-reflex measurements, the same proportion of afferent fibres were stimulated (data shown in Fig. 5*C*; estimates shown in Table 2).

The background EMG of the Sol, TA, VL and BF muscles are shown in Fig. 7, and the associated model outputs are provided in Table 4. As expected, background EMG activity was greater during the floor and board task than during rest. For the Sol, there was a small interaction between task floor and time post (Table 4). However, direct hypothesis testing showed no difference between task for each time level. For the TA and the VL, there was no difference between tasks and across time. For the BF, direct hypothesis testing showed that background EMG was slightly higher during the board task compared to the floor task at pre (11.47 [0.99, 21.58]%, evidence ratio of 28.3) and retention level (16.4 [2.4, 29.88]%, evidence ratio of 33.19), although not at post (4.41 [−12.4, 21.28]%, evidence ratio of 2.03).

The biomechanical parameters are displayed in Fig. 8 and the model outputs are given in Table 5. For the ankle angle, there was a small difference between tasks (∼3–4°), although this difference was identical across time. For the knee angle, there was a difference between tasks and a slightly reduced angle across time. However, direct hypothesis testing showed that the difference of angles between tasks remained similar across time level (3.41 [2.62, 4.2]°, evidence ratio equal to infinity;

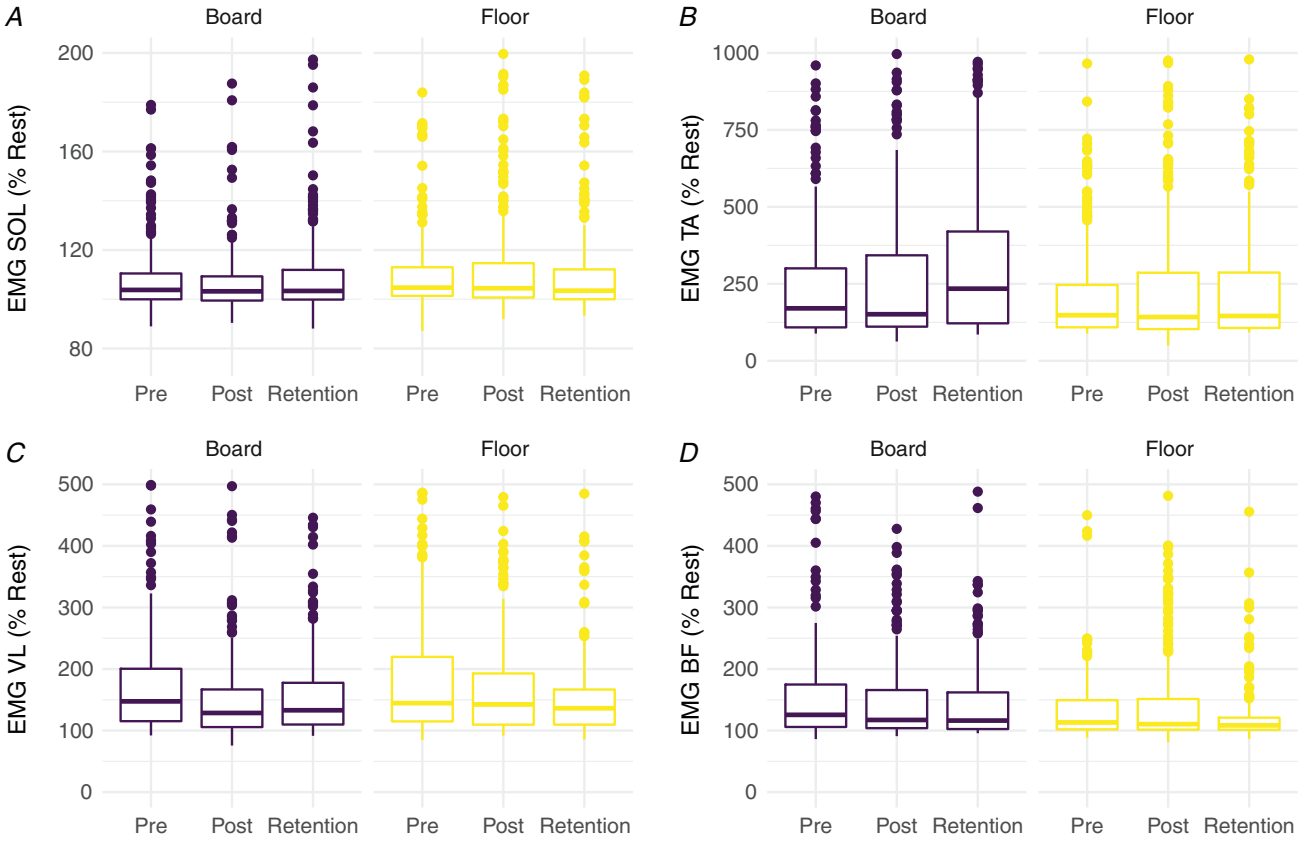

Figure 7. Background EMG
Background EMG during the board and floor tasks normalized to the background EMG obtained during rest at the same time level in the SOL, TA, VL and BF muscles. Note that, for a better overview of the present data, the *y*-axis does not frame all outliers. *A–D*, 66, 65, 85 and 31 data points are located outside of the *y*-axis frame, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 4. Posterior estimates of background EMG

Model	DV	Population effect	Estimate	SD	Lower 95% CI	Upper 95% CI
3	Sol	Intercept	108.61	2.33	103.94	113.11
		Task floor	4.38	3.32	−2.22	10.85
		Time post	2.07	6.89	−11.61	15.54
		Time retention	0.45	2.38	−4.24	5.05
		Task floor : time post	7.48	3.51	0.46	14.28
		Task Floor : time retention	−0.04	4.07	−8.09	8.07
3	TA	Intercept	247.62	47.29	153.97	340.56
		Task floor	−4.74	9.62	−23.59	14.28
		Time post	−3.16	8.34	−19.4	12.73
		Time retention	2.23	9.89	−17.06	21.66
		Task floor : time post	2.34	9.45	−16.29	21.02
		Task floor : time retention	−1.61	9.8	−20.46	17.48
3	VL	Intercept	156.07	17.82	121.07	191.19
		Task floor	2.53	8.77	−14.8	19.41
		Time post	−0.12	9.58	−18.81	18.89
		Time retention	−8.58	9.18	−26.6	9.3
		Task floor : time post	4.89	8.35	−11.94	20.77
		Task floor : time retention	−2.73	7.72	−17.61	12.57
3	BF	Intercept	146.24	13.64	119.27	173.53
		Task floor	−11.47	6.23	−23.5	0.95
		Time post	−3.39	8.13	−19.2	13
		Time retention	−2.68	7.75	−17.88	12.32
		Task floor : time post	7.06	8.69	−9.93	23.99
		Task floor : time retention	−4.93	7.46	−19.52	9.75

The intercept corresponds to the mean estimate of baseline factor levels (e.g. Task board, Pre). Other estimates are tested against the intercept. DV, dependent variable; CI, credible interval.

2.98 [1.94, 4.03]°, evidence ratio equal to infinity; and 3.6 [2.47, 4.72]°, evidence ratio equal to infinity, for pre, post and retention, respectively). An evidence ratio equal to infinity indicates that the ratio tends toward infinity (as a result of the very low probability of the alternative hypothesis). For the hip velocity, there was a difference between tasks, but this difference was consistent across time. For the knee velocity, there was also a difference between tasks, although this difference remained similar across time. There was no difference between time or across tasks for the toe velocity. During the board task, the delay between stimulation and touchdown on the tilt board was ∼8 ms larger at post compared to pre, although there was no difference between pre-acquisition and retention. Importantly, the biomechanical differences observed between tasks or time do not follow the specific time modulation of the H-reflex that was seen during the board task.

Discussion

In the present study, we were interested in the short-term time-course and generalization of spinal neuroplasticity following motor training. To assess the time-course of the spinal neural adaptations, we compared the H-reflex amplitude in the soleus muscle before, immediately after, and 24 h after the learning of a skilled task (i.e. balancing on a tilt board). To assess the generalization of the neural adaptations, we measured the H-reflex at rest, during the skilled and learned task (board task) and during an unskilled and untrained task (floor task). Immediately after the acquisition of the tilt board task, we found that the H-reflex amplitude was decreased at rest but not when performing the board task or the floor task. However, at retention (24 h after), the H-reflex amplitude was decreased when performing the board task but not when performing the floor task or at rest. The main outcome of the present study is a time and task-specific modulation of the H-reflex during the acquisition and retention of a skilled motor task.

Balance performance on the tilt board task was improved at post-acquisition and retention compared to pre-acquisition, demonstrating that the number of trials performed was sufficient to induce behavioural adaptations and the retention of learning. This change in performance indicates that the accompanying changes in the H-reflex can be related to training-induced neural plasticity. Moreover, in the present study, we measured the H-reflex at rest or during the preparatory phase of the balance task. By doing so, we circumvented most of the

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changes in muscle activation or biomechanical constraints that could influence the H-reflex amplitude (Zehr, 2002; Pierrot-Deseilligny & Burke, 2012; Giboin *et al.* 2019*c*). These changes in muscle activation and biomechanical constraints are expected to occur not only when measuring the H-reflex during the balancing task as a result of the stochastic fluctuations of the balance device, but also when measuring H-reflexes during the task before and after a learning session where performance is modified (e.g. increased stability, lesser muscle activation and changes in the muscular synergies used). Furthermore, muscle activity, joint positions and angular velocities were measured during the preparatory phase and were found not to change between tasks and days, or at least not in a confounding manner with the H-reflex. Similarly, we monitored the M-wave amplitude to ensure that the same proportion of afferent fibres was stimulated between conditions (Pierrot-Deseilligny & Burke, 2012). Therefore, it is assumed that changes in H-reflex amplitude observed in the present study are more representative of training-induced neural changes rather than changes in muscle activity or biomechanical constraints.

Similar to previous studies (Perez *et al.* 2005; Mazzocchio *et al.* 2006; Meunier *et al.* 2007; Perez *et al.* 2007), we observed a decrease in the soleus H-reflex amplitude when subjects were tested at rest after the acquisition phase. This result suggests that the acquisition of the balance task was accompanied by general changes at the spinal cord level (i.e. changes that are thought to affect any motor command transiting through the changed networks). This fast occurrence of hypothetic neural plasticity in the spinal cord (i.e., after less than an hour of skill practice) is in line with experiments demonstrating increases in receptive fields of spinal dorsal horn neurons within minutes of cutaneous afferent conditioning (Cook *et al.* 1987), or with high frequency stimulation of Ia afferents enhancing immediately the reflex for several minutes (Lloyd, 1949). It must be noted that this fast change observed in a different context than the trained task (i.e. at rest and not during the board task) could also reflect the presence of 'reactive plasticity'. Reactive plasticity is a term used for plasticity induced by a change of activity in surrounding networks as a result of the 'primary plasticity' or even the compensatory

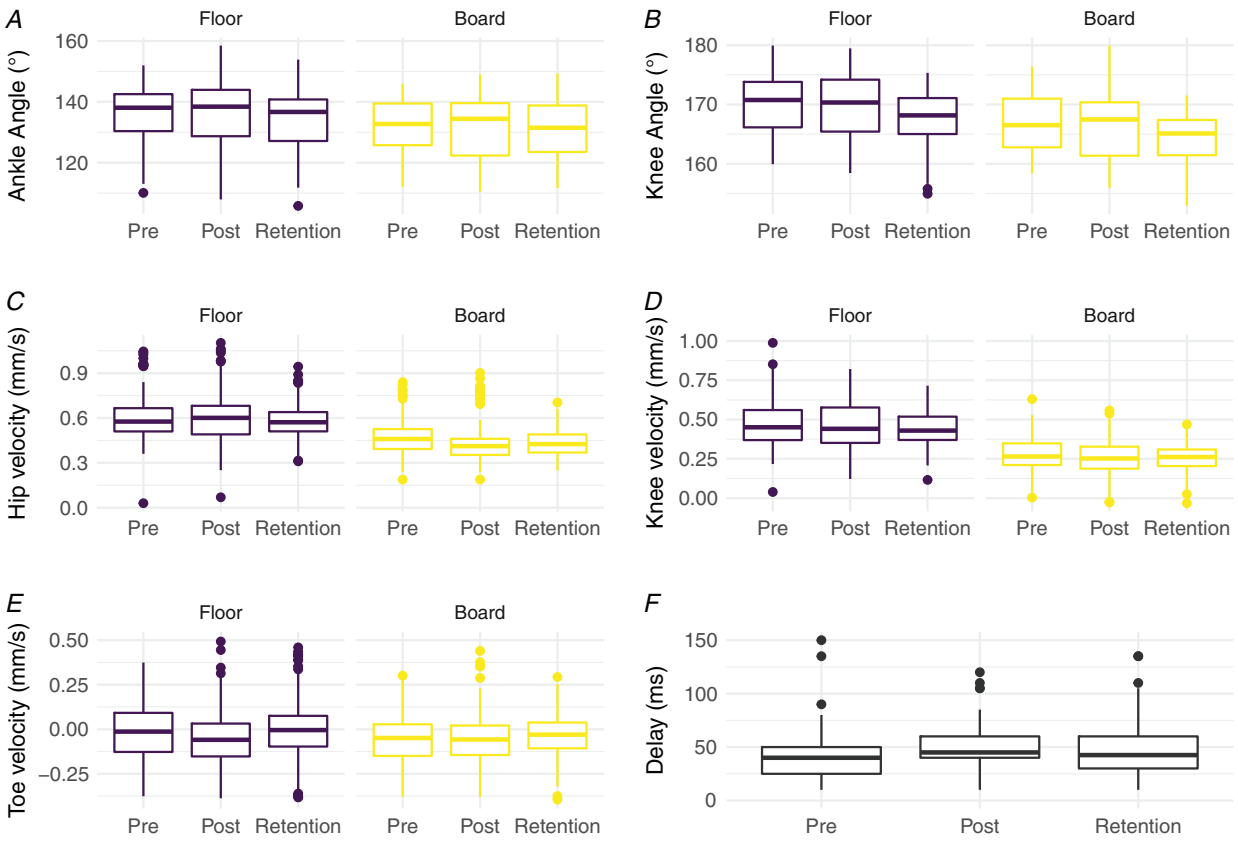

Figure 8. Biomechanical parameters
A and *B*, ankle and knee angle at stimulation. *C–E*, hip, knee and toe velocity at stimulation. *F*, delay between the stimulation and the touchdown during the tilt board task. Note that, for a better overview of the present data, the *y*-axis does not frame all outliers. There are 47, 51, 50, 34, 65 and 35 data points that lie outsides of the *y*-axis boundaries in *A–F*, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 5. Posterior estimates of biomechanical measurements

Model	DV	Population effect	Estimate	SD	Lower 95% CI	Upper 95% CI
3	Ankle angle	Intercept	135.67	2.43	130.88	140.4
		Task board	−3.65	0.81	−5.25	−2.07
		time post	0.2	0.63	−1.05	1.49
		Time retention	−0.77	1.51	−3.78	2.16
		Task board : time post	−0.25	0.9	−2.03	1.5
		Task board : time retention	−0.79	0.86	−2.52	0.89
3	Knee angle	Intercept	170.36	1.3	167.74	172.91
		Task board	−3.41	0.48	−4.35	−2.45
		time post	−0.67	0.34	−1.37	−0.01
		Time retention	−2.47	1.07	−4.57	−0.35
		Task board : time post	0.43	0.59	−0.72	1.62
		Task board : time retention	−0.19	0.52	−1.22	0.84
3	Hip velocity	Intercept	0.62	0.03	0.55	0.68
		Task board	−0.15	0.02	−0.19	−0.1
		Time post	−0.01	0.02	−0.04	0.03
		Time retention	−0.03	0.02	−0.08	0.01
		Task board : time post	−0.04	0.02	−0.08	0.01
		Task board : time retention	0	0.02	−0.05	0.04
3	Knee velocity	Intercept	0.47	0.03	0.4	0.54
		Task board	−0.2	0.02	−0.25	−0.15
		Time post	−0.01	0.02	−0.05	0.02
		Time retention	−0.03	0.02	−0.07	0.02
		Task board : time post	0	0.02	−0.04	0.04
		Task board : time retention	0.02	0.02	−0.02	0.06
3	Toe velocity	Intercept	−0.01	0.03	−0.07	0.05
		Task Board	−0.05	0.04	−0.13	0.02
		Time Post	−0.06	0.03	−0.12	0
		Time retention	0	0.03	−0.06	0.06
		Task board : time post	0.06	0.02	0.02	0.1
		Task board : time retention	0.03	0.04	−0.04	0.1
	Delay	Intercept	41.27	3.25	34.78	47.68
		Time post	7.91	3.54	0.54	14.72
		Time retention	4.44	4.56	−4.7	13.32

The intercept corresponds to the mean estimate of baseline factor levels (e.g. Task floor, Pre). Other estimates are tested against the intercept. DV, dependent variable; CI, credible interval.

plasticity that is itself induced by the primary plasticity. Reactive plasticity is therefore not directly related to the training; for more details, see (Wolpaw, 2010)). However, despite these general changes and in contrast to our hypothesis, there was no decrease in the H-reflex amplitude for the two other tasks (i.e. floor and tilt-board). This lack of observed change in the H-reflex during movement can be explained by training-induced changes in other networks and/or compensatory effects in the physiologically activated networks at the pre- and/or post-synaptic level of the Ia afferent to motoneuron synapse. Interestingly, because there was no effect of trials on the H-reflex amplitude post-acquisition for any tasks, this suggests that compensatory mechanisms were able to take place immediately, much like that described by the negotiated equilibrium model (Wolpaw, 2018). We suggest that the lack of changes observable during the board or floor task after acquisition, or across trials during the board task at retention, can be explained by the adequacy of the adaptive feedback and/or feed-forward mechanisms at play during task execution.

Twenty-four hours after the acquisition phase, the reduction in the H-reflex amplitude was only observed when performing the trained task. This result suggests the presence of a time- (or sleep-) dependent and task-specific functional neural reorganization that was induced by motor skill training. This time- (or sleep-) dependent reorganization and the shift from general to specific adaptations could be explained by the task-specific plasticity of supraspinal systems that regulate reflex gain, such as presynaptic inhibition networks (Rudomin & Schmidt, 1999). In such a scenario, the changes observed in the H-reflex at rest just after the task acquisition should be representative of short-term local spinal plasticity

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that quickly returns to baseline after the end of the acquisition phase (Mazzocchio *et al.* 2006; Lungu *et al.* 2010). By contrast, the task-specific modulation seen 24 h afterward should be driven by the plasticity of supraspinal networks that control presynaptic inhibition of Ia afferents. This hypothesis is particularly intriguing because it has been shown that presynaptic inhibition can adjust the contribution of afferents to the motoneurons according to different balance conditions (Baudry & Duchateau, 2012). However, another possible mechanism that cannot be ruled out by the results of the present study is indeed a task-specific plasticity located at the spinal cord level similar to the task-specific plasticity observed in the mouse motor cortex following skill learning (Xu *et al.* 2009; Yang *et al.* 2014). In this scenario, skill training should immediately induce plasticity on a large scale of spinal neurons and synapses (general plasticity) and that, over time and/or sleep, connections are reorganized such that only the task-specific relevant synapses remain. The immediate compensatory changes from other networks would then return to baseline during the consolidation of spinal changes, limiting the requirement of short-term 'negotiated equilibrium' between the old and new behaviours. With the present data, it remains unclear whether the task-specific adaptations that we were able to observe are indeed directly located at the spinal level or are a result of the modulation of spinal networks by supraspinal projections.

Whatever the mechanisms might be behind the observed task-specific modulation of the H-reflex, these changes might be important because they can determine early consolidation mechanisms (Muellbacher *et al.* 2002), as well as interference and reconsolidations effects (Walker *et al.* 2003). This short-term task specific adaptation may also explain the previously observed task-specific change in balance performance and the lack of performance transfer to an untrained balance task (Giboin *et al.* 2015, 2018; Giboin *et al.* 2019*a, b*) that was accompanied by task-specific H-reflex modulation after 6 weeks of training (Giboin *et al.* 2019*c*). It should be noted that the present results do not preclude the existence of long-term general spinal adaptations, such as that observed in ballet dancers or power-trained athletes (Casabona *et al.* 1990; Nielsen *et al.* 1993). However, it must be noted that these latter studies were cross-sectional and the tested populations were elite in terms of their performance, making it difficult to separate the effect of training and heritability (Georgiades *et al.* 2017). In conclusion, because many different plasticity mechanisms co-exist in the spinal cord (Wang & Brehm, 2017; Bertuzzi *et al.* 2018; Chen *et al.* 2019), short- and long-term general and task-specific adaptations most probably co-exist.

We observed no difference in the H-reflex amplitude over the 15 trials during each task and at each time-level, indicating no requirement of adaptation when switching from one task to another and no influence from the previous task (e.g. from the floor task to the board task and vice versa). This suggests that the H-reflex is appropriately modulated by feedforward mechanisms and that the learned task-specific regulation of the H-reflex amplitude is most probably under the control of supraspinal mechanisms selected even before the task is performed (i.e. before the subject balances over the floor or the tilt board). This hypothesis is in line with previous research showing that different spinal networks, possibly under supraspinal control, could be modulated according to a goal or a specific task to perform (Giboin *et al.* 2012; Weiler *et al.* 2019). It should be noted that feedforward systems also probably exist at the spinal level in humans (i.e. systems able to regulate the motor command of subsequent movement without supraspinal control). These circuits are possibly involved in the regulation of the motor command necessary for stepping and balancing, and are able to adapt to motor training (Barbeau *et al.* 1987; Edgerton *et al.* 2001; Gerasimenko *et al.* 2017). In the present case, we observed slight biomechanical differences (e.g. knee velocity) between tasks. This implies that each task may have a specific proprioceptive signature, which could be sufficient to activate feedforward supraspinal and spinal circuits in a task-specific manner, and also explains the present task-specific modulation of the H-reflex

The modulation of the H-reflex observed after training was not associated with changes in performance and does not allow us to directly infer their influence on performance. We suggest that the down-regulation of the H-reflex represents a shift from a more feedback-based (e.g. reflex and stereotyped motor command) to a more feedforward motor command (Wolpaw & Tennissen, 2001; Seidler *et al.* 2004; Giboin *et al.* 2019*c*). This would be in line with our biomechanical observations, where a better positioned foot over the platform predicted better performance. This very precise foot positioning is most probably dependent of a finely tuned motor command and should suffer from stereotyped muscle activation. Furthermore, our results also fall in line with a theoretical model of learning occurring at the motoneuron or the spinal networks level, where Ia afferents correspond to the instructive input (sensory feedback) and the collaterals to Renshaw cells correspond to the predictive input (forward model). The reduction of the Ia contribution would also indicate a relative increase in the feedforward contribution to the motor command compared to the feedback contribution (Brownstone *et al.* 2015).

In conclusion, we have observed that motor skill learning induces general changes that are observable at the spinal level immediately after task acquisition. These general changes appear to be immediately compensated by other networks during movement. Twenty-four hours after acquisition, spinal changes were observed only during the trained task, suggesting the existence of a time- (or

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sleep-) dependent task-specific functional reorganization at the spinal level. However, it remains unclear whether these task-specific changes are a result of local spinal task-specific plasticity or task-specific modulation of spinal networks by supraspinal pathways. Nevertheless, the present results emphasize the important role of the spinal cord in motor control and motor learning.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

LSG conceived the work. LSG, CT and AK designed the work. LSG, CT, AK and MH collected data. MG provided the resources. LSG analysed and interpreted data, and wrote the draft. AK analysed and interpreted biomechanics data. All authors helped with the data interpretation and revised critically the manuscript. All authors approved the final version of the manuscript submitted for publicationn and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document

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