NeuroImage 85 (2014) 415–422

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Cortical control of normal gait and precision stepping: An fNIRS study

Koen L.M. Koenraadt [a,*], Eefje G.J. Roelofsen [a], Jacques Duysens [a,b], Noël L.W. Keijsers [a]

[a] Sint Maartenskliniek Nijmegen, Department of Research, PO box 9011, 6500 GM Nijmegen, The Netherlands
[b] Katholieke Universiteit Leuven, Faculty of Kinesiology and Rehabilitation Sciences, Department of Biomedical Kinesiology, Tervuursevest 101, BE-3001 Leuven, Belgium

ARTICLE INFO

Article history:
Accepted 19 April 2013
Available online 28 April 2013

Keywords:
Precision stepping
fNIRS
Gait
Motor cortex
Prefrontal cortex
Treadmill

ABSTRACT

Recently, real time imaging of the cortical control of gait became possible with functional near-infrared spectroscopy (fNIRS). So far, little is known about the activations of various cortical areas in more complex forms of gait, such as precision stepping. From previous work on animals and humans one would expect precision stepping to elicit extra activity in the sensorimotor cortices (S1/M1), supplementary motor area (SMA), as well as in prefrontal cortices (PFC). In the current study, hemodynamic changes in the PFC, SMA, M1, and S1 were measured with fNIRS. In contrast to previous fNIRS gait studies, the technique was optimized by the use of reference channels (to correct for superficial hemodynamic interference). Eleven subjects randomly performed ten trials of treadmill walking at 3 km/h (normal walking) and ten trials of 3 km/h treadmill walking on predefined spots for the left and right foot presented on the treadmill (precision stepping). The walking trials of approximately 35 seconds were alternated with rest periods of 25–35 seconds consisting of quiet standing. The PFC revealed profound activation just prior to the onset of both walking tasks. There was also extra activation of the PFC during the first half of the task period for precision stepping. The SMA showed mainly increased activation prior to the start of both tasks. In contrast, the sensorimotor cortex did not show a change in activation during either task as compared to a condition of standing. The SMA, M1, and S1 revealed no significant differences between normal walking and precision stepping. It was concluded that fNIRS is suited to record the planning and initiation of gait. The lack of M1/S1 activation during gait suggests that even in the current precision stepping task the control of ongoing gait depended mostly on subcortical automatisms, while motor cortex contributions did not differ between standing and walking.

Introduction

Most of our knowledge about the control of gait originates from animal studies whereas the cortical control in human gait is still not entirely clear. An attempt of measuring cortical activity in real gait in humans was made by La Fougere et al. (2010). After a walking period of 10 minutes the conversion of intravenously injected [^{18}F]-FDG during this period was reflected by a PET scan. While informative, these data will not show the cortical activity during walking itself. In contrast, studies with functional near-infrared spectroscopy (fNIRS: Kurz et al., 2012; Miyai et al., 2001; Suzuki et al., 2004, 2008) and electro-encephalography (EEG: Gwin et al., 2010, 2011; Presacco et al., 2011; Severens et al., 2012) allow to measure brain activity during gait. In the study of Gwin et al. (2010) for example, EEG recordings were made while subjects walked and even ran on a treadmill. However, EEG has still its limitations, since neck muscles and eye movements unrelated to the instructed task can affect the quality of the recordings. Therefore, it remains a good alternative to use fNIRS.

One of the questions that can be tackled with fNIRS concerns the role of the sensorimotor cortex during different complexities of gait in human. It has long been known from animal studies that the primary motor cortex (M1) is not essential for automated unperturbed gait (Liddell and Phillips, 1944) but it is increasingly important for precision stepping (such as walking on a ladder, Armstrong, 1988). In normal walking sequential activations (i.e. mainly at the end of the swing phase) of subgroups of neurons in M1 were regularly demonstrated (Armstrong, 1986; Beloozerova and Sirota, 1993). In humans the loss of motor cortex affects locomotion more severely, thereby indicating an increasingly important contribution of the cortex in the control of gait (Duysens et al., 2013). However, the recording studies in human show a mixed picture. Some authors found that M1 is not activated in imagined gait (Bakker et al., 2008; la Fougere et al., 2010), whereas in PET studies with real walking M1 did reveal activation (la Fougere et al., 2010; Tashiro et al., 2001). In the studies with fNIRS, an involvement of the motor cortex is controversial. Several studies revealed sensorimotor cortex activity during normal gait (Kurz et al., 2012; Miyai et al., 2001) and backwards walking (Kurz et al., 2012). However, Suzuki et al. (2004) revealed no effect of walking speed on

* Corresponding author. Fax: +31 243659154.
E-mail address: k.koenraadt@maartenskliniek.nl (K.L.M. Koenraadt).

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.04.070

the sensorimotor cortex activation while clear SMA and prefrontal cortex activations changes were demonstrated. In agreement with this, the EEG records from Presacco et al. (2011) showed relatively little activity over the motor cortex. It may be argued that such M1 activations require the use of more complex gait patterns (along the line of the animal studies mentioned above). Hence, fNIRS studies on gait including more complex forms of walking are particularly valuable to throw light on the involvement of M1 in gait.

In the present study the focus is on precision stepping in comparison with normal gait. It is often assumed that cortical activity during a movement implies deliberate conscious control, whereas subcortical and spinal networks are responsible for automatic movements that require little conscious attention. In single-unit recording studies in cats it was shown that undemanding steady state walking involves relatively little cortical activity (Drew et al., 2004). Single-unit recording studies further showed that pyramidal tract neurons in the motor cortex are mostly active when vision is used to adapt gait in conditions of gait challenges (Amos et al., 1990; uneven terrain, obstacle, etc.: Beloozerova and Sirota, 1993; Drew, 1988, 1993; Drew et al., 2002; Drew et al., 1996; Marple-Horvat et al., 1993; Widajewicz et al., 1994). Furthermore, under these conditions, the activity in the motor cortex "contributes primarily to the execution of the gait modifications rather than to their planning" (Drew et al., 2008).

In humans the recording of activity over the motor cortex has only be achieved sparsely. In the fNIRS study of Kurz et al. (2012) the idea of introducing a form of precision stepping was achieved by having the subjects walk forwards and backwards on a treadmill. Gait variability was found to be greater during backward walking compared to forward walking (Hoogkamer et al., 2012; Kurz et al., 2012). The greater gait variability was reflected in higher cortical activity (Kurz et al., 2012). In addition to the primary sensorimotor cortices, the supplementary motor area (SMA) and prefrontal cortices (PFC) are likely to play a role in more complex forms of gait. The PFC is seen to be recruited in fNIRS studies when there is a high attention demand on the gait task (such as with increasing speed: Suzuki et al., 2004). In the fNIRS study of Holtzer et al. (2011), increased activations in the prefrontal cortex (PFC) were seen when walking was combined with talking. In general, the PFC has been reported as being typically active during attention demanding tasks (Wood and Grafman, 2003). The SMA has been shown to play an important role during normal gait in several fNIRS studies, such as those from Suzuki et al. (2004, 2008). The area is known to be involved in the selection, planning, and coordination of voluntary movements. This was supported by the findings of a profound activation of the SMA in the period prior to and around the start of locomotor tasks (Mihara et al., 2008; Sahyoun et al., 2004). More generally, the SMA is also known to be important for interlimb coordination of rhythmic arm and leg movements (Debaere et al., 2001) (Debaere et al., 2001). In fNIRS studies on difficult types of walking (such as backward walking) Kurz et al. (2012) found increased SMA activity (along with other premotor activity) for the more difficult task of backward walking. Hence, the PFC and SMA seem important during gait and particularly during complex gait.

The goal of the present study was to advance our knowledge of precision stepping in comparison with normal gait, in line with the historic importance of this task to evaluate the role of the different motor areas in gait (Drew et al., 2004). fNIRS data were collected from motor related cortical areas (sensorimotor and supplementary motor areas) and prefrontal cortices during rest periods, steady state walking on a treadmill at 3 km/h, and a precision stepping task on the treadmill. Based on the literature described above it is hypothesized that the latter task would require substantial activation in PFC since it is an attention-demanding task. Furthermore, the activation of the primary sensorimotor areas (M1 and S1) is likely to increase with the more complex precision stepping task. Finally, the activation of SMA is expected prior to and around the start of the locomotion tasks and might also play a more prominent role while performing the precision stepping task. An additional benefit of the present study is that the fNIRS technique is currently improved considerably by the use of reference channels to correct for superficial hemodynamic interference. This interference is mainly caused by systemic interference arising from cardiac activity, respiration, and other homeostatic processes (Diamond et al., 2009; Obrig et al., 2000; Toronov et al., 2000), which are very likely to interfere during gait. Several studies have recently suggested to use reference channels (Gagnon et al., 2012; Saager et al., 2011; Zhang et al., 2005), but has never been used in walking studies.

Methods

Subjects and experimental setup

Eleven healthy subjects (3 males, 8 females) with a mean age of 23 years (SD: 4) participated in the present study. The study was conducted according to the Declaration of Helsinki. Written informed consent was obtained prior to the experiment. The subjects performed two different locomotor tasks on a programmable treadmill (Forcelink, Culemborg, The Netherlands). The two conditions consisted of 1) normal walking at 3 km/h and 2) precision stepping at 3 km/h that forced the subjects to step on predefined spots on the treadmill. Each task period was 35 seconds of duration (including instruction and starting and stopping of the treadmill) and was preceded with a baseline period varying between 25 and 35 seconds. During the baseline periods subjects were not allowed to hold on to the safety bars at the treadmill. In total 10 trials of each condition were performed randomly and a short break of approximately 3 minutes was given after 10 trials.

A video projector (Dell 2400MP, The Netherlands) attached on the ceiling above the treadmill was used to present the rectangles for the intended right (red rectangle) and left (green rectangle) steps during the complex task. This procedure was extracted from Bank et al (2011) who also projected rectangles as stepping stones on the treadmill for gait rehabilitation purposes. Variation in step width and step length was induced by presenting the rectangles of each foot at 5 predefined positions in the frontal plane and 5 different positions in the sagittal plane, respectively. The positions in the sagittal plane were based on the step length of each individual that was measured in a 1-minute treadmill walking session and for each step the position of the rectangle in the sagittal plane was randomly adjusted to −30%, −15%, −0%, +15% or +30% of the individual step length. Variation in position of the rectangle in the frontal plane was based on an estimation of preferred step width in humans of 29 cm (Donelan et al., 2001, 2004). For each step, the position of the rectangle in the frontal plane was randomly adjusted with −25 cm, −12.5 cm, 0 cm, +12.5 cm, or +25 cm. Once a rectangle was projected at the predefined spot it moved with the same speed and direction as the treadmill. The video projector was also used for the presentation of the task instructions "Precision stepping" and "Walking" (for 2 seconds) and a fixation cross on the treadmill during the normal walking task and the baseline periods in between.

NIRS imaging

NIRS measurements were conducted with the use of two continuous-wave NIRS instruments (Oxymon, Artinis Medical Systems, Zetten, The Netherlands). The NIRS equipment used two wavelengths, 858 and 764 nm, and the data were sampled at 25 Hz. A soft and in size adjustable headband was placed tightly around the participants head. Subsequently, a six-channel motor cortex unit and a three-channel prefrontal cortex unit were attached to the headband to prevent for displacements.

The six-channel motor cortex unit included four long-separation channels (channel 1–4) with an interoptode distance of 30 mm and

two short separation channels (channel 5–6) with an interoptode distance of 10 mm (Fig. 1). The setup was created using two receivers and five transmitting optodes, which were fixed in 10 mm thick foam with holes for the optodes and Velcro was used to fasten the foam to the headband. The four long-separation channels covered the S1, M1, and supplementary motor areas (SMA and (pre-)SMA) of the left hemisphere with respect to the Cz position of the International 10–20 system (Okamoto et al., 2004).

The prefrontal cortex unit consisted of two long-separation channels with an interoptode distance of 40 mm (lower- and upper channel) and one short separation channel with an interoptode distance of 10 mm (channel 3 right panel of Fig. 1). This setup was created by one receiver and three transmitting optodes, which were placed on the prefrontal cortex with one channel overlapping the Fp1-postion of the international 10–20 system on the forehead. The holes for the optodes of that channel were incorporated into the headband. The remaining optodes were fixed using holes in foam and Velcro was used to fix the foam to the headband.

Physiological and gait parameters

Prior to the start of the experiment subjects were equipped with two tri-axial accelerometers (Analog Devices, ADXL335) on the shoes of both feet closely located above the head of metatarsal II in order to calculate the step length from the 1-minute treadmill walking part and to detect gait variability afterwards. Subsequently, the NIRS setup was attached to the subjects head. A finger cuff was fixed on the middle finger of the left hand to continuously monitor the blood pressure (Finapres Medical Systems BV, Amsterdam, The Netherlands). All data were sampled at 250 Hz.

Data analysis

The Oxymon software preprocessed the NIRS signals by converting the changes in optical density in changes in HbO and HbR using the modified Beer–Lambert law and the age dependent path length factor (Duncan et al., 1996). After the measurements, analysis of HbO and HbR signals was performed using a customized code implemented in MatLab (R2007b). Firstly, a second order low pass Butterworth filter with a cut off frequency of 1.25 Hz was conducted to reduce high frequency noise. In addition, a second order high pass Butterworth filter with a cut off frequency of 0.01 Hz was used to reduce low frequency drift caused by the NIRS system. Subsequently, the short separation channels (channel 3 in prefrontal cortex and channel 5 and 6 in motor cortex, see Fig. 1) were used to remove hemodynamic changes in superficial tissue layers. The short-distance signal was scaled by a factor and subtracted from the nearest long-distance signal. The scaling factor was obtained during the 1 min rest period by matching the short-separation signal with the long-separation channel data (Gagnon et al., 2012; Saager et al., 2011). After the correction for superficial interference, a second order low pass Butterworth filter with a cut off frequency of 1 Hz was conducted. Finally, in order to compare the data between all subjects, the maximal concentration change in HbO and HbR over all trials and channels was determined for each individual. Subsequently, the individual data were normalized by dividing the individual mean hemodynamic response amplitudes of all channels by the corresponding (HbO or HbR) maximum concentration change throughout the whole experiment. This procedure was used to decrease the amplitude differences across subjects.

For the next steps of the data analysis the task period was divided into three phases of 12.5 seconds; 1) a pre-task phase running from 12.5 seconds prior to the task instruction onto the task instruction, 2) an early-task phase running from 6 to 18.5 seconds after the start of the task instruction, and 3) a late-task phase from 18.5 to 31 seconds after the start of the task instruction. There was a 6 seconds period between the pre- and early-task and this consisted of 2 seconds of task instruction succeeded by 4 seconds of treadmill acceleration to reach a constant velocity of 3 km/h (see Fig. 2). From the processed fNIRS signal, the HbO and HbR concentrations were averaged over the pre-task, the early-task, and late-task period for each channel and each trial.

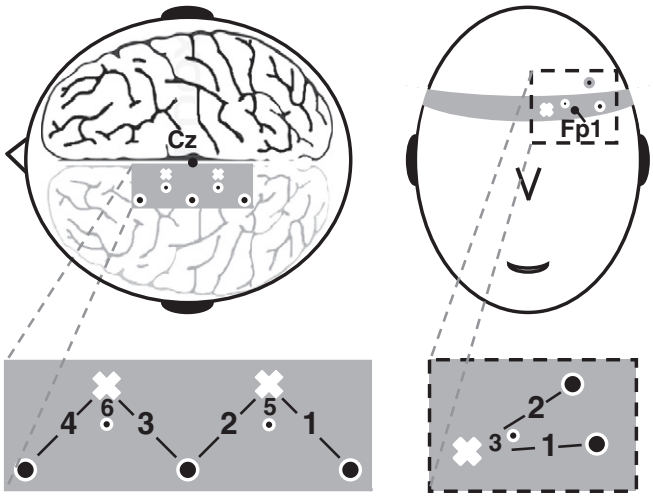

Fig. 1. Optodes configuration. Motor cortex setup (left panels) and prefrontal cortex setup (right panels) of the fNIRS optodes. The upper panels show the optodes with respect to the Cz and Fp1 locations of the International 10–20 system. In the lower panels the channel numbers 1 to 4 represent the S1, M1, SMA and (pre-)SMA channel for the motor cortex setup and 1 to 2 the lower and upper channel on the prefrontal cortex. Channel 5 and 6 for the motor cortex setup and channel 3 for the prefrontal setup represent the reference channels.

To analyze the accelerometer data, a peak detection procedure was written in MatLab (R2007b) to determine all foot contacts. Subsequently, the mean step time for each trial was calculated by averaging the step times during the whole task period (early-task and late-task). Finally, for each trial the gait variability was calculated by taking the standard deviation of the step times.

The increase in mean blood pressure was calculated by subtraction of the pre-task mean blood pressure from the mean blood pressure during the whole task period for each trial. In addition, the heart rate (beats/min) was derived from the blood pressure data and analyzed the same way as the blood pressure.

Statistics

One-way ANOVAs with the different task phases (pre-, early-, and late-task) as repeated measures were used to determine whether a response was seen in the hemodynamic response (HbO or HbR) in each channel. Bonferroni corrections for multiple comparisons were applied during the post hoc analyses. To determine differences in conditions paired *T*-tests were performed between normal walking and precision stepping for the early-task and late-task periods. Differences between the physiological and gait measures for normal walking and precision stepping were tested with paired *T*-tests over the whole task period of 25 seconds (early- and late-task). In addition, paired *T*-tests were performed to indicate differences between the pre-task and whole task period in blood pressure and heart rate.

Results

Fig. 3 shows the average HbO and HbR concentration changes of the motor cortices (S1, M1, SMA, and (pre-) SMA in the left four panels) for the whole study population. The right two panels present the average HbO and HbR concentration changes on the upper and lower channel of the PFC. The two SMA channels demonstrate comparable changes over time, with an increase in HbO and a decrease in

HbR during the pre-task phase. Subsequently, the HbO concentration decreases and the HbR concentration increases during the early-task phase, finally reaching a plateau during the late-task phase. The M1 and S1 channels, on the other hand, show a peak in HbO concentrations right after the treadmill reached its constant speed followed by a small undershoot directly after the peak and a plateau during the late-task phase. In addition, the HbR concentrations in M1 and S1 increase during the early- and late-task phase. The hemodynamic concentration changes in M1, S1, and (pre-)SMA over time are comparable between normal walking and precision stepping. Finally, the PFC channels reveal an increase of HbO and a decrease in HbR during the pre-task, a decrease in HbO and an increase in HbR during early-task and no obvious changes during the late-task phase. For both PFC channels it can be noticed that HbO concentrations for precision stepping rise above HbO of normal walking right after the task instruction until a few seconds after the treadmill reaches constant speed. Moreover, for the upper prefrontal channel the precision stepping task reveals HbR concentrations lower compared to the normal walking task during the early- and late-task.

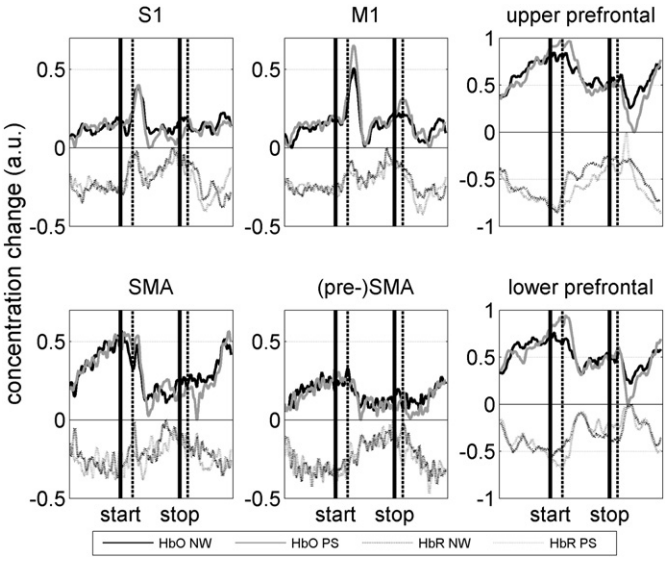

Fig. 3. Mean group hemodynamic responses. HbO (solid) and HbR (dotted) relative concentration changes over time for normal walking (NW, black lines) and precision stepping (PS, gray lines) are presented for the four channels of the motor cortex setup (left four panels) and for the two channels of prefrontal cortex setup (right two panels). Vertical black lines represent in chronological order the start of the instruction (first solid line), treadmill reaching constant speed (first dotted line), slow down of the treadmill (last solid line), and during the last vertical line the treadmill comes to a standstill. Average SDs across the complete time course of HbO ranged from 0.17 to 0.24 for the motor related channels and from 0.27 to 0.35 for the prefrontal channels. For HbR time courses the average SDs ranged from 0.19 to 0.28 for motor and 0.28 to 0.33 for the prefrontal channels. Note that, instead of opposite changes in HbO and HbR, parallel changes seem to occur in the S1 and M1 channels. This has been addressed in many previous studies and might be the result of several underlying mechanisms (Hoshi, 2007; Sato et al., 2005; Yamada et al., 2012; Yamamoto and Kato, 2002).

Normal versus precision stepping

Statistical analyses of the differences between normal and precision stepping revealed a significant larger HbR decrease during the early-task for precision stepping compared to normal walking ($p < 0.05$) in the upper PFC channel (Fig. 4). For the late-task no significant difference was found ($p = 0.26$). In addition, no significant difference was found for the HbR of the lower channel (early-task: $p = 0.49$; late-task: $p = 0.17$) and for the HbO at both channels (p-values ranging from 0.12 to 0.79). In contrast to the PFC, the motor cortex channels revealed no significant difference between the two conditions for the early- and late-task phases (p-values ranging from 0.12 to 0.96).

Prefrontal cortex activation; phase effects

Mean differences in HbO and HbR between the pre-task, and either the early- or late-task are shown in Fig. 4 together with the standard deviations along all subjects. For normal walking, the one-way RM ANOVAs on the HbO data revealed no significant differences between the pre-, early-, and late-task phase on both channels (lower channel: $F(2,10) = 2.9$, $p = 0.08$; upper channel: $F(2,10) = 2.8$, $p = 0.08$). Precision stepping, on the other hand, did reveal a significant phase effect in HbO on the upper channel ($F(2,10) = 4.6$, $p < 0.05$). However, post hoc analyses revealed no significant difference between the different phases (see Fig. 4). For HbR, the upper channel revealed significant phase effects for normal walking ($F(2,10) = 10.3$, $p < 0.001$) and precision stepping ($F(2,10) = 7.8$, $p < 0.005$). Post hoc analyses revealed larger HbR concentrations for early- and late-task compared to pre-task during normal walking, while during precision stepping only the late-task HbR was significantly larger compared to the pre-task phase. Individual analysis revealed one subject that demonstrated no phase effects on both normal and precision stepping.

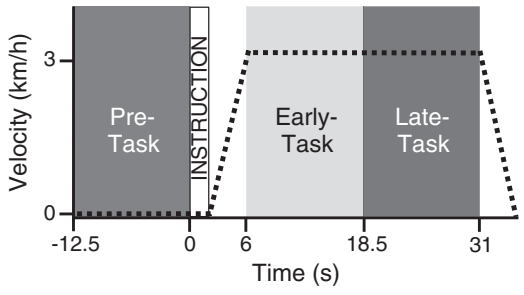

Fig. 2. Timing of the experiment. The gray blocks indicate the three different phases of the task used in the data-analysis. The dotted black line indicates the velocity of the treadmill. After the 2 seconds task instruction, the treadmill reached a constant speed of 3 km/h after 4 seconds.

Motor cortex activation; phase effects

For S1, M1, SMA and (pre-)SMA, the mean differences and standard deviation of the HbO and HbR concentrations between the pre-task and early-task and pre-task and late-task are shown in Fig. 5. Normal walking revealed a significant phase effect of the HbO data for the SMA channel ($F(2,10) = 11.7$, $p < 0.001$). Post hoc analyses revealed significant larger pre-task HbO responses compared to early- and late-task. The same omnibus effect ($F(2,10) = 8.2$, $p < 0.005$) and post hoc results were seen in the precision stepping HbO analyses for the SMA channel. For HbR during normal walking significant omnibus effects were found for the channels S1 ($F(2,10) = 4.4$, $p < 0.05$), M1/SMA ($F(2,10) = 8.2$, $p < 0.005$), and SMA ($F(2,10) = 4.7$, $p < 0.05$). For precision stepping only the (pre-)SMA channel showed significance ($F(2,10) = 6.1$, $p < 0.01$). However, post hoc analyses revealed no significant differences, although several trends were noticed (as shown in Fig. 5). Most remarkably, for the (pre-)SMA in both conditions the pre-task revealed a trend of larger HbR compared to the early- and late-task. Individual analysis revealed only one subject that revealed an absence of phase effects on the motor cortical channels during normal walking. This subject revealed significant differences between the phases for the prefrontal cortical channels. During the precision stepping task all subjects revealed significant phase effects in motor cortical areas.

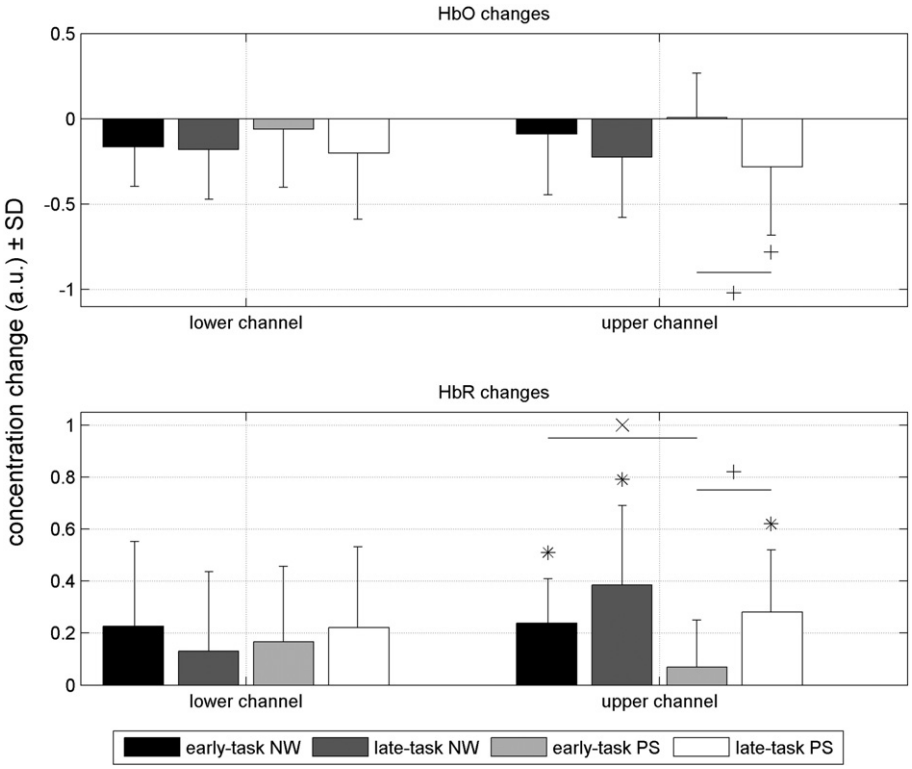

Fig. 4. Average hemodynamic responses for the prefrontal cortex. Mean HbO (upper panel) and HbR (lower panel) responses during the early- and late-task in comparison to the pre-task are shown for the two prefrontal cortex channels. NW = normal walking, PS = precision stepping. For the comparisons within one condition, the asterisk (*) indicates a significant difference with a $p < 0.0167$ and the plus sign (+) indicates a trend with a p-value < 0.05. For the comparisons between the conditions, the "x" indicates a significant difference with $p < 0.05$. One of the two channels was positioned across the Fp1 position of the International 10–20 system (i.e. the lower channel) and the other channel was positioned approximately 1 cm more caudally (the upper channel).

Physiological measures and gait characteristics

The HR increased from 76 (±9) beats/min (during the rest period before normal walking) to 83 (±7) beats/min during the normal walking task ($p < 0.01$) and from 77 (±9) beats/min (during the rest period before precision stepping) to 85 (±8) beats/min during the precision stepping task ($p < 0.01$). No significant difference was found between the increase in HR for the two conditions ($p = 0.12$). The mean BP increased from 89 (±13) mmHg during the rest period before normal walking to 90 (±14) mmHg during the normal walking task ($p < 0.01$) and from 88 (±12) mmHg during the rest period before precision stepping to 90 (±14) mmHg during the precision stepping task ($p < 0.05$). No significant ($p = 0.21$) difference was found between the increase for normal walking and precision stepping.

The accelerometer data revealed mean step times of 0.66 (±0.02) seconds for normal walking and 0.66 (±0.04) seconds for precision stepping. The step time variability of 0.09 seconds (±0.02) for precision stepping was significant larger ($p < 0.001$) compared to a step time variability of 0.04 seconds (±0.01) for the normal walking task.

Discussion

The present fNIRS study examined hemodynamic responses in multiple cortical areas before and during treadmill walking at 3 km/h ("normal walking") and a precision stepping task at 3 km/h ("precision stepping"). Reference optodes were used to correct for superficial hemodynamic interferences. The current study revealed increased activation (increased HbO and/or decreased HbR) in the SMA channels prior to the start of normal walking and precision stepping. The sensorimotor cortex channels (S1 and M1), on the other hand, revealed no differences between the rest periods (i.e. standing) and normal walking or precision stepping. The prefrontal cortex revealed enlarged oxygenation prior to the task compared to the last half of the task phase for normal walking and precision stepping. In addition, for precision stepping the prefrontal cortex showed a prolonged activation during the first half of the task.

With respect to the main purpose of the present study (changes between normal walking and precision stepping), we demonstrated that we introduced successfully more step time variability during the precision stepping task. Considering the hemodynamic changes, the precision stepping task revealed more PFC activation during the first half of the task compared to normal walking. Furthermore, an increase in activity, as indicated by an increase in HbO and a decrease in HbR, occurred mainly before the start of both normal walking andprecision stepping. The prefrontal cortex is known to be activated during attention demanding tasks (Wood and Grafman, 2003; Yogev-Seligmann et al., 2008). This has also been shown with fNIRS. In postural tasks for example, Mihara et al. (2008) showed fNIRS activity in the PFC in conjunction with postural perturbations provided the subjects were warned beforehand. This preparatory activation is most likely related to the "allocation of attention" as typically found in the dorsolateral PFC (Luks et al., 2007; Mihara et al., 2008). This type of activation is also found during task execution with increasing complexity. Recently, Holtzer et al. (2011) found an increased PFC activity during walking while talking in comparison to normal walking. Our findings of a prolonged activation of the PFC for the precision stepping task are in line with these findings and indicate that more attention was needed to perform precision stepping in comparison to normal walking.

In contrast to the PFC, the sensorimotor cortices revealed hardly any significant hemodynamic changes during normal walking and precision stepping, although a peak in HbO concentrations was noticed at the beginning of the task. For S1, only the HbR concentration changes for normal walking indicated somewhat more activity prior to

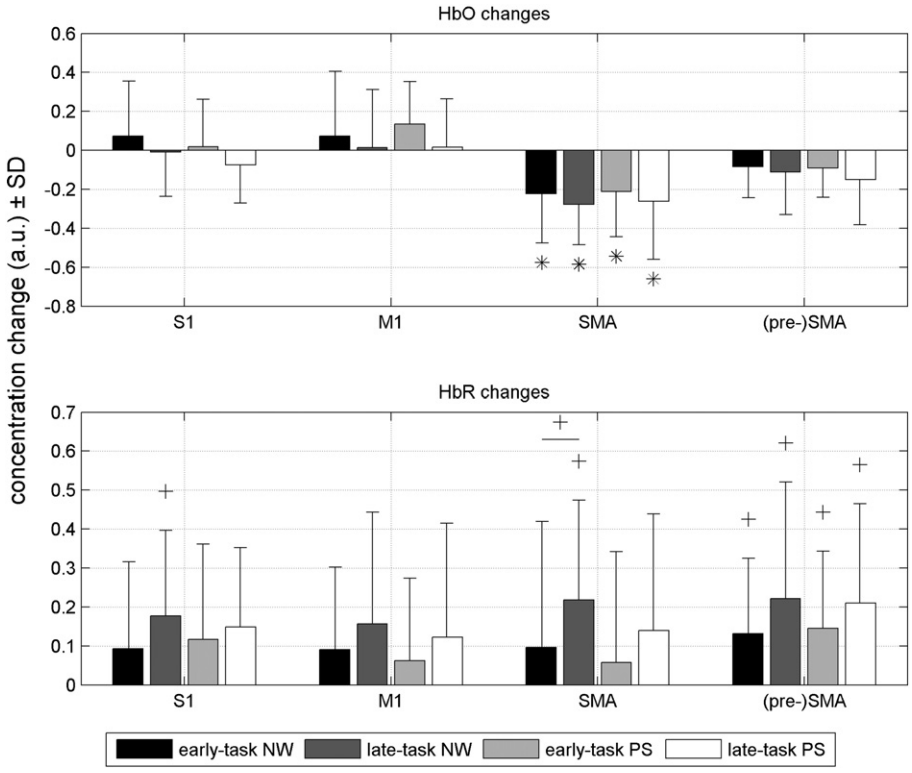

Fig. 5. Average hemodynamic responses for the sensorimotor channels. Average HbO (upper panel) and HbR (lower panel) responses during the early- and late-task in comparison to the pre-task are shown for the four sensorimotor channels. NW = normal walking, PS = precision stepping. * indicates a significant difference with a $p < 0.0167$. + indicates a trend with a $p < 0.05$.

the task compared to the second half of the normal walking period. It should be emphasized that these walking data were compared to a preceding period of standing. This is important since standing by itself may elicit considerable activity. Indeed, in a previous fNIRS study, Mihara et al. (2008) demonstrated an essential role of the sensorimotor cortices in balance control. Since subjects were standing during the rest periods in the present study, it is likely that S1 and M1 were activated during the rest period and this may be part of the reason why no further increment in activity was seen during the walking task (ceiling effect). Previous fMRI, PET, and SPECT studies (Bakker et al., 2008; Dobkin et al., 2004; Fukuyama et al., 1997; la Fougere et al., 2010) used rest periods consisting of supine position and therefore no activation of S1 and M1 was expected during rest in these studies. Nevertheless, some fNIRS studies were able to detect sensorimotor cortex activity related to the legs with experimental gait paradigms comparable to the present study (Kurz et al., 2012; Miyai et al., 2001). However, the slow walking speeds of 1 km/h (Miyai et al., 2001) and 1.6 km/h (Kurz et al., 2012) are remarkable in these studies. A walking speed more comparable with the preferred walking speed is likely to decrease the M1 involvement during gait since then locomotion depends more on subcortical structures (such as CPGs, see den Otter et al., 2004; Duysens and Van de Crommert, 1998; Nielsen, 2003). Furthermore, our negative findings for M1 are in line with those found in an fNIRS study of Suzuki et al. (2004) during 3 km/h and 5 km/h treadmill walking and with those found by the EEG study of Presacco et al. (2011) at a maximum speed of 2.4 km/h. The lack of additional M1 recruitment during precision stepping is also in agreement with previous cat work (Armstrong and Drew, 1984) that demonstrated no changes in neuronal firing rates when the animals increased walking speed or walked uphill.

In contrast to the sensorimotor cortices, the SMA revealed distinct activation primarily before and around the start of the task for both conditions. Since the SMA and (pre-)SMA channels revealed comparable hemodynamic time courses they are discussed as one area. The SMA has previously been reported to be involved in locomotion (Fukuyama et al., 1997; Miyai et al., 2001). In the present study there was also substantial activity of SMA during the rest periods before gait. This might be explained by the balance control necessary during the rest periods. Mihara et al. (2008) identified the SMA as involved in balance control. However, since our precision stepping task requested more balance control and since no differences were seen on the SMA between normal walking and precision stepping this explanation seems somewhat inappropriate for the present findings. Another explanation originates from the role in motor preparation of this specific cortical area. Sahyoun et al. (2004) revealed preparatory activation of the SMA before actual foot flexion and extension in the fMRI. In contrast, after the movement onset the SMA is no longer very active. This was seen in the present experiments but also in earlier findings (la Fougere et al., 2010; Suzuki et al., 2008). For example, the [^{18}F]-FDG PET study of la Fougere et al. (2010) failed to show SMA activity after a 10 min walking period. Nevertheless, an fNIRS study of Kurz et al. (2012) demonstrated an increase in SMA activity during backwards walking compared to normal walking. The absence of additional SMA activity during precision stepping in the present study might be caused by the differences in speed of the treadmill (as mentioned above for M1 discrepancies). The 3 km/h walking speed in the present study for both normal walking and precision stepping is much closer to the preferred walking speed in human compared to the 1.6 km/h used in the study of Kurz et al. (2012). Presumably, the more preferred walking speed in the present study resulted in less dependence on the SMA for planning of the movement. Another explanation for the discrepancy between our findings and those of Kurz et al. (2012) might originate from the fact that they did not use reference channels to correct for superficial interferences. Therefore, factors such as blood pressure changes might have influenced the

hemodynamic responses and thereby the outcome in their study (Gagnon et al., 2012; Saager et al., 2011). Since we did use reference channels in the present study, we concluded the SMA to be mainly active before the start of the task period in walking at 3 km/h, most likely due to a preparatory/initiating function.

One of the major limitations of the present study is the small number of optodes used (as compared to some other gait related studies such as Miyai et al (2001) and Suzuki et al. (2004, 2008)). Therefore, not all the cortical areas involved in gait could be recorded. For example, the parietal cortex is not measured although this area can be expected to be very important in precision stepping (Drew et al., 2004). Instead of increasing the number of cortical areas studied, we chose to sacrifice some optodes to create reference channels in order to correct for superficial hemodynamic interferences (Gagnon et al., 2012; Saager et al., 2011). Since Gagnon et al. (2012) emphasized that systemic interference seems inhomogeneous across the scalp, three short separation reference channels were created for six long distance channels. For the small number of channels used, this seems appropriate but it is recognized that for larger number of channels one could use other approaches. For example, the principal component analysis as described in Zhang et al. (2005) and also used in the fNIRS gait study of Kurz et al. (2012) seems a solid alternative approach to correct for systemic interference when using a large number of channels covering a large cortical area. A second (related) limitation is that it is difficult to be certain about the areas recorded from in view of the small number of optodes. For example, given the position of channel 3 of the motor cortex setup in Fig. 2 it may be argued that this channel was related to M1 and SMA activity rather than purely SMA. In addition, the SMA channels might also cover parts of the dorsal premotor cortex. Future work might therefore focus on better methods of identification, for example one may profit from combining different approaches, such as additional fMRI scans as used by Kleinschmidt et al. (1996). Finally, other continuous wave fNIRS issues like differences in optical path length between subjects, interindividual differences in the type and time course of the hemodynamic response, and the absence of absolute measures of the chromophores might have influenced the results (Strangman et al., 2003). In addition, the slow hemodynamic response following brain activity makes fNIRS unsuitable to study cortical activation changes within the different phases of the gait cycle or initiation of gait. For this purpose EEG seems a more proper approach, as demonstrated by Gwin et al. (2010, 2011). Despite these limitations, it is clear that fNIRS studies definitely have a place in gait research, in particular now that the method can be improved with the addition of reference channels to correct for superficial hemodynamic interferences. This paves the way for applications of fNIRS in future research to provide insight in the neural mechanisms of movement disorders and future applications of fNIRS in gait rehabilitation, for example for use in a brain–computer interface.

Acknowledgments

The authors gratefully acknowledge the support of theBrainGain Smart Mix Programme of The Netherlands Ministry of Economic Affairs and The Netherlands Ministry of Education, Culture and Science. We would also like to thank Jan van Erp from TNO (Zeist, The Netherlands) for the use of an additional fNIRS system.

Conflict of interest

The authors declare no conflict of interest.

References

Amos, A., Armstrong, D.M., Marple-Horvat, D.E., 1990. Changes in the discharge patterns of motor cortical neurones associated with volitional changes in stepping in the cat. Neurosci. Lett. 109, 107–112.
Armstrong, D.M., 1986. Supraspinal contributions to the initiation and control of locomotion in the cat. Prog. Neurobiol. 26, 273–361.
Armstrong, D.M., 1988. The supraspinal control of mammalian locomotion. J. Physiol. 405, 1–37.
Armstrong, D.M., Drew, T., 1984. Discharges of pyramidal tract and other motor cortical neurones during locomotion in the cat. J. Physiol. 346, 471–495.
Bakker, M., De Lange, F.P., Helmich, R.C., Scheeringa, R., Bloem, B.R., Toni, I., 2008. Cerebral correlates of motor imagery of normal and precision gait. Neuroimage 41, 998–1010.
Bank, P.J., Roerdink, M., Peper, C.E., 2011. Comparing the efficacy of metronome beeps and stepping stones to adjust gait: steps to follow! Exp. Brain Res. 209, 159–169.
Beloozerova, I.N., Sirota, M.G., 1993. The role of the motor cortex in the control of accuracy of locomotor movements in the cat. J. Physiol. 461, 1–25.
Debaere, F., Swinnen, S.P., Beatse, E., Sunaert, S., Van, H.P., Duysens, J., 2001. Brain areas involved in interlimb coordination: a distributed network. Neuroimage 14, 947–958.
den Otter, A.R., Geurts, A.C., Mulder, T., Duysens, J., 2004. Speed related changes in muscle activity from normal to very slow walking speeds. Gait Posture 19, 270–278.
Diamond, S.G., Perdue, K.L., Boas, D.A., 2009. A cerebrovascular response model for functional neuroimaging including dynamic cerebral autoregulation. Math. Biosci. 220, 102–117.
Dobkin, B.H., Firestine, A., West, M., Saremi, K., Woods, R., 2004. Ankle dorsiflexion as an fMRI paradigm to assay motor control for walking during rehabilitation. Neuroimage 23, 370–381.
Donelan, J.M., Kram, R., Kuo, A.D., 2001. Mechanical and metabolic determinants of the preferred step width in human walking. Proc. Biol. Sci. 268, 1985–1992.
Donelan, J.M., Shipman, D.W., Kram, R., Kuo, A.D., 2004. Mechanical and metabolic requirements for active lateral stabilization in human walking. J. Biomech. 37, 827–835.
Drew, T., 1988. Motor cortical cell discharge during voluntary gait modification. Brain Res. 457, 181–187.
Drew, T., 1993. Motor cortical activity during voluntary gait modifications in the cat. I. Cells related to the forelimbs. J. Neurophysiol. 70, 179–199.
Drew, T., Jiang, W., Kably, B., Lavoie, S., 1996. Role of the motor cortex in the control of visually triggered gait modifications. Can. J. Physiol. Pharmacol. 74, 426–442.
Drew, T., Jiang, W., Widajewicz, W., 2002. Contributions of the motor cortex to the control of the hindlimbs during locomotion in the cat. Brain Res. Brain Res. Rev. 40, 178–191.
Drew, T., Prentice, S., Schepens, B., 2004. Cortical and brainstem control of locomotion. Prog. Brain Res. 143, 251–261.
Drew, T., Andujar, J.E., Lajoie, K., Yakovenko, S., 2008. Cortical mechanisms involved in visuomotor coordination during precision walking. Brain Res. Rev. 57, 199–211.
Duncan, A., Meek, J.H., Clemence, M., Elwell, C.E., Fallon, P., Tyszczuk, L., Cope, M., Delpy, D.T., 1996. Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. Pediatr. Res. 39, 889–894.
Duysens, J., Van de Crommert, H.W., 1998. Neural control of locomotion; The central pattern generator from cats to humans. Gait Posture 7, 131–141.
Duysens, J., Severens, M., Nienhuis, B., 2013. How can active cycling produce less brain activity than passive cycling? Clin. Neurophysiol. 124 (2), 217–218 (Feb).
Fukuyama, H., Ouchi, Y., Matsuzaki, S., Nagahama, Y., Yamauchi, H., Ogawa, M., Kimura, J., Shibasaki, H., 1997. Brain functional activity during gait in normal subjects: a SPECT study. Neurosci. Lett. 228, 183–186.
Gagnon, L., Cooper, R.J., Yucel, M.A., Perdue, K.L., Greve, D.N., Boas, D.A., 2012. Short separation channel location impacts the performance of short channel regression in NIRS. Neuroimage 59, 2518–2528.
Gwin, J.T., Gramann, K., Makeig, S., Ferris, D.P., 2010. Removal of movement artifact from high-density EEG recorded during walking and running. J. Neurophysiol. 103, 3526–3534.
Gwin, J.T., Gramann, K., Makeig, S., Ferris, D.P., 2011. Electrocortical activity is coupled to gait cycle phase during treadmill walking. Neuroimage 54, 1289–1296.
Holtzer, R., Mahoney, J.R., Izzetoglu, M., Izzetoglu, K., Onaral, B., Verghese, J., 2011. fNIRS study of walking and walking while talking in young and old individuals. J. Gerontol. A Biol. Sci. Med. Sci. 66, 879–887.
Hoogkamer, W., Massaad, F., Jansen, K., Bruijn, S.M., Duysens, J., 2012. Selective bilateral activation of leg muscles after cutaneous nerve stimulation during backward walking. J. Neurophysiol. 108, 1933–1941.
Hoshi, Y., 2007. Functional near-infrared spectroscopy: current status and future prospects. J. Biomed. Opt. 12, 062106.
Kleinschmidt, A., Obrig, H., Requardt, M., Merboldt, K.D., Dirnagl, U., Villringer, A., Frahm, J., 1996. Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy. J. Cereb. Blood Flow Metab. 16, 817–826.
Kurz, M.J., Wilson, T.W., Arpin, D.J., 2012. Stride-time variability and sensorimotor cortical activation during walking. Neuroimage 59, 1602–1607.
la Fougere, C., Zwergal, A., Rominger, A., Forster, S., Fesl, G., Dieterich, M., Brandt, T., Strupp, M., Bartenstein, P., Jahn, K., 2010. Real versus imagined locomotion: a [18F]-FDG PET-fMRI comparison. Neuroimage 50, 1589–1598.
Liddell, E.G., Phillips, C.G., 1944. Pyramidal section in the cat. Brain 67, 1–9.
Luks, T.L., Simpson, G.V., Dale, C.L., Hough, M.G., 2007. Preparatory allocation of attention and adjustments in conflict processing. Neuroimage 35, 949–958.
Marple-Horvat, D.E., Amos, A.J., Armstrong, D.M., Criado, J.M., 1993. Changes in the discharge patterns of cat motor cortex neurones during unexpected perturbations of on-going locomotion. J. Physiol. 462, 87–113.
Mihara, M., Miyai, I., Hatakenaka, M., Kubota, K., Sakoda, S., 2008. Role of the prefrontal cortex in human balance control. Neuroimage 43, 329–336.
Miyai, I., Tanabe, H.C., Sase, I., Eda, H., Oda, I., Konishi, I., Tsunazawa, Y., Suzuki, T., Yanagida, T., Kubota, K., 2001. Cortical mapping of gait in humans: a near-infrared spectroscopic topography study. Neuroimage 14, 1186–1192.

Nielsen, J.B., 2003. How we walk: central control of muscle activity during human walking. Neuroscientist 9, 195–204.
Obrig, H., Neufang, M., Wenzel, R., Kohl, M., Steinbrink, J., Einhaupl, K., Villringer, A., 2000. Spontaneous low frequency oscillations of cerebral hemodynamics and metabolism in human adults. Neuroimage 12, 623–639.
Okamoto, M., Dan, H., Sakamoto, K., Takeo, K., Shimizu, K., Kohno, S., Oda, I., Isobe, S., Suzuki, T., Kohyama, K., Dan, I., 2004. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 21, 99–111.
Presacco, A., Goodman, R., Forrester, L., Contreras-Vidal, J.L., 2011. Neural decoding of treadmill walking from noninvasive electroencephalographic signals. J. Neurophysiol. 106, 1875–1887.
Saager, R.B., Telleri, N.L., Berger, A.J., 2011. Two-detector Corrected Near Infrared Spectroscopy (C-NIRS) detects hemodynamic activation responses more robustly than single-detector NIRS. Neuroimage 55, 1679–1685.
Sahyoun, C., Floyer-Lea, A., Johansen-Berg, H., Matthews, P.M., 2004. Towards an understanding of gait control: brain activation during the anticipation, preparation and execution of foot movements. Neuroimage 21, 568–575.
Sato, H., Fuchino, Y., Kiguchi, M., Katura, T., Maki, A., Yoro, T., Koizumi, H., 2005. Intersubject variability of near-infrared spectroscopy signals during sensorimotor cortex activation. J. Biomed. Opt. 10, 44001.
Severens, M., Nienhuis, B., Desain, P., Duysens, J., 2012. Feasibility of measuring event related desynchronization with electroencephalography during walking. Conf. Proc. IEEE Eng. Med. Biol. Soc. 2764–2767. http://dx.doi.org/10.1109/EMBC.2012.6346537.
Strangman, G., Franceschini, M.A., Boas, D.A., 2003. Factors affecting the accuracy of near-infrared spectroscopy concentration calculations for focal changes in oxygenation parameters. Neuroimage 18, 865–879.
Suzuki, M., Miyai, I., Ono, T., Oda, I., Konishi, I., Kochiyama, T., Kubota, K., 2004. Prefrontal and premotor cortices are involved in adapting walking and running speed on the treadmill: an optical imaging study. Neuroimage 23, 1020–1026.
Suzuki, M., Miyai, I., Ono, T., Kubota, K., 2008. Activities in the frontal cortex and gait performance are modulated by preparation. An fNIRS study. Neuroimage 39, 600–607.
Tashiro, M., Itoh, M., Fujimoto, T., Fujiwara, T., Ota, H., Kubota, K., Higuchi, M., Okamura, N., Ishii, K., Bereczki, D., Sasaki, H., 2001. 18F-FDG PET mapping of regional brain activity in runners. J. Sports Med. Phys. Fitness 41, 11–17.
Toronov, V., Franceschini, M.A., Filiaci, M., Fantini, S., Wolf, M., Michalos, A., Gratton, E., 2000. Near-infrared study of fluctuations in cerebral hemodynamics during rest and motor stimulation: temporal analysis and spatial mapping. Med. Phys. 27, 801–815.
Widajewicz, W., Kably, B., Drew, T., 1994. Motor cortical activity during voluntary gait modifications in the cat. II. Cells related to the hindlimbs. J. Neurophysiol. 72, 2070–2089.
Wood, J.N., Grafman, J., 2003. Human prefrontal cortex: processing and representational perspectives. Nat. Rev. Neurosci. 4, 139–147.
Yamada, T., Umeyama, S., Matsuda, K., 2012. Separation of fNIRS signals into functional and systemic components based on differences in hemodynamic modalities. PLoS One 7, e50271.
Yamamoto, T., Kato, T., 2002. Paradoxical correlation between signal in functional magnetic resonance imaging and deoxygenated haemoglobin content in capillaries: a new theoretical explanation. Phys. Med. Biol. 47, 1121–1141.
Yogev-Seligmann, G., Hausdorff, J.M., Giladi, N., 2008. The role of executive function and attention in gait. Mov. Disord. 23, 329–342.
Zhang, Y., Brooks, D.H., Franceschini, M.A., Boas, D.A., 2005. Eigenvector-based spatial filtering for reduction of physiological interference in diffuse optical imaging. J. Biomed. Opt. 10, 11014.