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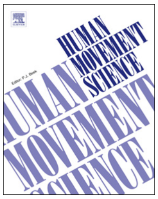

Synergistic influences of sensory and central stimuli on non-voluntary rhythmic arm movements

I.A. Solopova[a,*], D.S. Zhvansky[a], V.A. Selionov[a], Y. Ivanenko[b]

[a] Laboratory of Neurobiology of Motor Control, Institute for Information Transmission Problems, Moscow, Russia
[b] Laboratory of Neuromotor Physiology, IRCCS Fondazione Santa Lucia, Rome, Italy

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ABSTRACT

In recent years, neuromodulation of the cervical spinal circuitry has become an area of interest for investigating rhythmogenesis of the human spinal cord and interaction between cervical and lumbosacral circuitries, given the involvement of rhythmic arm muscle activity in many locomotor tasks. We have previously shown that arm muscle vibrostimulation can elicit non-voluntary upper limb oscillations in unloading body conditions. Here we investigated the excitability of the cervical spinal circuitry by applying different peripheral and central stimuli in healthy humans. The rationale for applying combined stimuli is that the efficiency of only one stimulus is generally limited. We found that low-intensity electrical stimulation of the superficial arm median nerve can evoke rhythmic arm movements. Furthermore, the movements were enhanced by additional peripheral stimuli (e.g., arm muscle vibration, head turns or passive rhythmic leg movements). Finally, low-frequency transcranial magnetic stimulation of the motor cortex significantly facilitated rhythmogenesis. The findings are discussed in the general framework of a brain-spinal interface for developing adaptive central pattern generator-modulating therapies.

1. Introduction

Bipedal locomotion shares common spinal neuronal control mechanisms with the forelimb and hindlimb coordination of quadrupeds (Frigon, 2017; Zehr et al., 2016). Rhythmic arm muscle activity is an inherent part of many locomotor-related tasks in humans (walking, running, swimming, crawling, cycling, skiing, etc.) despite a strong corticospinal control of the upper limbs that allows it to uncouple this connection during voluntary activities. Neural connections between the upper and lower limbs start to be established in very early ontogenesis of locomotion (La Scaleia et al., 2018), later they are manifested during crawling infant behaviour (Patrick, Noah, & Yang, 2012), while an adult-like arm swing emerges within the first few months of independent walking in toddlers (Ledebt, 2000). During adult walking, the most active upper limb muscles are the proximal ones that demonstrate noteworthy electromyographic (EMG) activity synchronized with the step frequency even when the upper limb is immobilized (Kuhtz-Buschbeck & Jing, 2012) or its motion is restricted due to the hand contact with another person (Sylos-Labini, d'Avella, Lacquaniti, & Ivanenko, 2018). Interactions between the upper and lower limbs are also demonstrated by modulation of cross-limb reflexes (Dietz, 2011; Meyns, Bruijn, & Duysens, 2013; Zehr et al., 2016).

In the last decade, many researches put significant effort into assessing and modulating the functional state of the spinal locomotor circuits in humans (Edgerton & Roy, 2012; Gerasimenko et al., 2010; Ivanenko et al., 2017; van den Brand et al., 2012). While

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* Corresponding author at: Laboratory of Neurobiology of Motor Control, Institute for Information Transmission Problems, Russian Academy of Sciences, Bolshoy Karetny 19, Moscow, Russia.

E-mail address: solopova@iitp.ru (I.A. Solopova).

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most efforts have been dedicated to investigating pattern generation of the lower limbs, rhythmogenesis of the cervical spinal cord can also be examined under body weight support conditions. Such studies showed that facilitatory mutual influences exist between the cervical and lumbosacral circuitries at the pattern generation level even though the data in humans are rather limited (Dai, Lv, & He, 2017; Solopova, Selionov, Zhvansky, Gurfinkel, & Ivanenko, 2016; Sylos-Labini et al., 2014). In animal models it has been demonstrated that hindlimb motor function can be improved after a spinal cord lesion by actively engaging functional activity of forelimbs (Shah et al., 2013). Exploiting the neural and mechanical linkages between the arms and legs can increase the locomotor performance in neurologically intact individuals (de Kam et al., 2013) and enhance the recovery of walking in individuals with sensorimotor disorders (Kaupp et al., 2017). Thus, in addition to the physiological relevance of investigating rhythmogenesis of the cervical spinal cord, it has also clinical implications.

The present experiments were designed to provide further insight into excitability of the cervical pattern generation circuitry in humans. We have previously shown that muscle vibration can elicit non-voluntary upper limb oscillations in a gravity neutral position in healthy individuals (Solopova et al., 2016). However, in the context of looking for adaptive therapies for entraining the cervical spinal circuitry, it is of interest to explore the effect of combined stimulations from different sources since the efficiency of one stimulus is normally limited (Selionov, Ivanenko, Solopova, & Gurfinkel, 2009). In addition, a priori one could not exclude that some afferent inputs would result in inhibition rather than activation of rhythmic movements (for instance, many synapses in the spinal cord are inhibitory and contribute to maintain network stability). One might also expect different influences of sensory inputs from the proximal and distal muscles, given a superior involvement of the proximal arm muscle activity during normal walking (Kuhtz-Buschbeck & Jing, 2012; La Scaleia et al., 2014). Finally, it may be a critical combination of afferent signals that is needed to generate a synergistic locomotor pattern (Dietz & Harkema, 2004; Gerasimenko, Sayenko, et al., 2016; Prochazka & Ellaway, 2012) considering also individual differences in the functional state of spinal circuitries. Therefore, here we evaluated the effects of different peripheral and central stimuli (electrical nerve stimulation, muscle vibration, transcranial magnetic stimulation) in eliciting non-voluntary rhythmic upper limb movements.

2. Methods

2.1. Subjects

Twelve healthy volunteers (5 males, 7 females) aged between 26 and 70 yrs (mean 44 ± 20 yrs) participated in the study. The same subjects participated in the previous study (Solopova et al., 2016) and were again preselected based on the fact that, in that study (in ~75% of participants), involuntary rhythmic upper limb movements could be entrained using arm muscle vibration. None of the subjects had any known neurological or motor disorder. The experiments were performed according to the procedures of the Ethics Committee of the Institute for Information Transmission Problems and in conformity with the declaration of Helsinki for experiments on humans. All subjects had given written informed consent.

2.2. Experimental set-up

The experimental setup was similar to that described in our previous studies (Solopova, Selionov, Blinov, Zhvansky, & Ivanenko, 2017; Solopova et al., 2016). To minimize the effects of gravity and external resistance, the subjects lay on their right side on couch with the left arm and leg suspended (Fig. 1A, left panel). The other (right) leg and arm were lying motionlessly and the head was put on a pillow. We applied unilateral sensory and transcranial magnetic stimulation (TMS) stimulation (see below) to evoke or facilitate rhythmic limb movements related to the site of stimulation. While an essential feature of natural locomotor behaviour is alternating movements of two legs, we have previously shown that peripheral sensory stimulation can elicit rhythmic air-stepping movements of both two limbs and only one limb if the other limb is immobilized, thus both limbs might not be critical for the manifestation of rhythmogenesis (Gurfinkel, Levik, Kazennikov, & Selionov, 1998; Selionov et al., 2009). This observation is also consistent with the idea of unit rhythmic pattern generators for each limb, joint, or groups of muscles (Grillner, 1981; Kiehn, 2016), as well as with a preservation or even an increment of ipsilateral arm oscillations if the contralateral upper limb motion is immobilized or limited during stepping (MacLellan, Qaderdan, Koehestanie, Duysens, & McFadyen, 2013; Shapkova, Terekhov, & Latash, 2011).

The trunk was fixed between two rests placed on the breast and back of the trunk to limit its rotation/tilt during limb movements (Fig. 1). This suspension system allowed us to perform a low friction motion of the arm and leg in the horizontal plane. Subjects were instructed to relax and not to intervene with any movement that might be induced by stimulation. Prior to stimulation (initial position), the suspended arm and leg adopted the equilibrium position of joint angles determined by the relative passive stiffness of agonists and antagonists and other soft tissues around the joints. On average, the initial upper limb joint angles were: 165°–185° (across all subjects and trials) for the shoulder (180° refers to the upper arm segment being parallel to the trunk) and 133°–167° for the elbow. For the lower limb, the initial hip joint angle varied between 135° and 160°, the knee joint angle varied between 115° and 155° (180° refers to the hip and knee angles of the extended leg with the thigh and shank segments being parallel to the trunk).

2.3. Stimulation techniques

The following stimulation techniques were used:

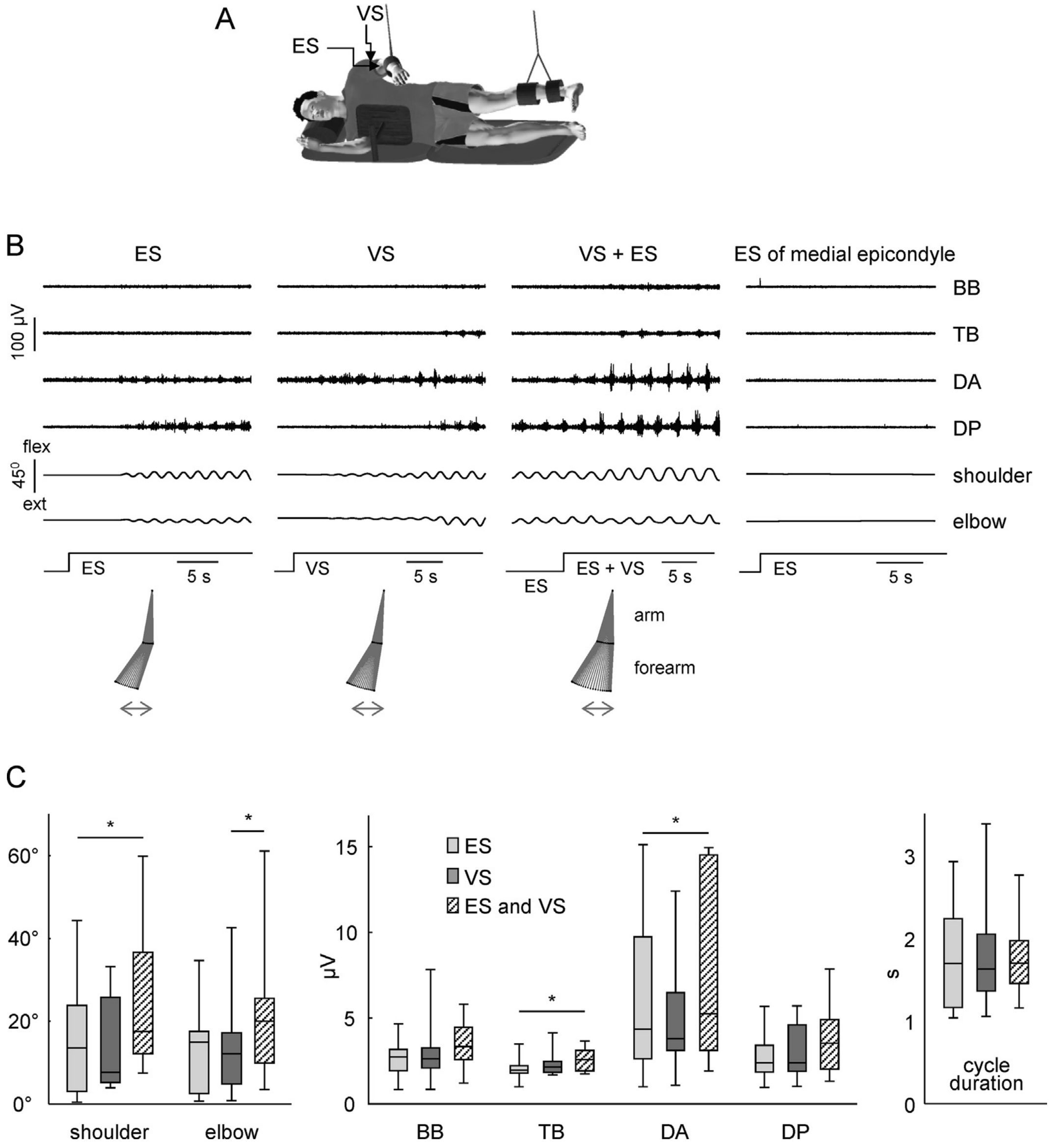

Fig. 1. Combined effects of electrical nerve stimulation and muscle vibration. A – experimental setup for investigating upper limb rhythmic movements in the unloading conditions. The legs were stationary. B – examples of upper limb oscillations elicited by continuous median nerve ES, VS and VS + ES (first three panels), and ES of the medial epicondyle (right panel). Note the absence of limb movements in the latter condition. Lower panels show stick diagrams of upper limb oscillations. C – from left to right: the amplitude of angular motion (median and quartiles), mean amplitude of EMG activity and cycle duration. Adding ES to VS (VS + ES) and VS to ES (ES + VS) significantly potentiated arm movements, this facilitation was similar and the data for VS + ES and ES + VS were pooled together. Asterisks denote significant differences between different conditions of stimulations.

2.3.1. Electrical stimulation (ES) of the median nerve

The rationale for stimulating this nerve innervating hand muscles is that it might have access to the spinal pattern generation circuitry since it plays a part in the cervicothoracic cross-limb reflex modulation during rhythmic movements (Solopova et al., 2017; Zehr et al., 2016). The bipolar stimulation electrodes ($2 \times 2\,\mathrm{cm}^2$ and an inter-electrode distance of 2 cm) were positioned over the median nerve (just below the ulnar fold), firmly pressed with adhesive straps. A custom-made constant-current stimulator provided the desired stimulation train. The non-nociceptive electrical stimulus consisted of repetitive trains of rectangular pulses (0.3-ms duration each, at 60 Hz). Before beginning the recordings, the perception threshold was determined psychophysically by gradually increasing the stimulus amplitude (Duysens, Tax, Murrer, & Dietz, 1996). The stimulus intensity (range 2–3 mA for all subjects) was kept constant at twice the perceptual threshold. Once the intensity value was set, it remained fixed throughout the session. Two modes of ES were used: continuous (60 Hz) and periodic (bursts of 60-Hz pulses with the duration of 1 s applied at a frequency of 0.5 Hz). ES was delivered ~15 s after the onset of each recording and lasted till the end of the probe (1 min).

2.3.2. ES of the medial epicondyle

In the control trial we placed a stimulating electrode on the dorsal part of the elbow (medial epicondyle of the humerus) to test whether the effect of ES could be related to non-specific activation of skin receptors of the upper limb. The parameters of stimulation (intensity and frequency of continuous stimulation) were the same as for the median nerve ES.

2.3.3. Muscle vibration (VS)

Continuous VS (40–60 Hz sinusoid, ~1 mm amplitude) of m. tricepsbrachii (TB) was produced by a small DC motor with an attached eccentric weight. The vibrator was placed in a cylindrical box (3 cm diameter, 7 cm length). Muscle vibration mainly stimulates Ia muscle spindle afferents though other afferent signals may be elicited as well (Roll, Vedel, & Ribot, 1989). The vibrator was fastened with a rubber belt over the belly of the TB muscle.

2.3.4. Head turn

The subject was asked, at the command of the experimenter, to turn his/her head to the left by ~70° (to the side of the suspended leg, Fig. 1 top panel) and keep it in that position for ~20 s, and then to return the head to its original position. Fifteen seconds of non-voluntary arm oscillations before and after head turn were also recorded. The rationale for this test is that neck (and vestibular) inputs affect the tonic state of the spinal circuitry and postural tone, as well as mind-driven motor acts like Jendrassik's manoeuvre might create the conditions for triggering involuntary stepping movements (Selionov et al., 2009).

2.3.5. Passive leg movements

The experimenter imposed passive rhythmic movements of the suspended leg. The frequency and amplitude of imposed movements in the knee and hip joints (~0.6 Hz, 20–40° for hip, 40–70° for knee) were comparable to those during voluntary leg air-stepping. Given the upper and lower limb interactions (MacLellan et al., 2013; Solopova et al., 2016; Sylos-Labini et al., 2014), one might expect a facilitatory effect of these movements.

2.3.6. Transcranial magnetic stimulation

TMS was delivered to the right primary motor cortex corresponding to the arm representation using a figure-8 magnetic coil (diameter of inner wings − 10 cm) attached to the 'Mag-2' stimulator (Schwarzer, Germany, maximum output 2.1 T, 200-ms stimulus duration). The coil was placed tangentially to the scalp, with the handle pointing posterior-laterally at ~30° to the midline with the induced current in the cortex flowing posterior to anterior across the motor strip. The coil was fixed at the optimal position (about 2 cm forward and 2–3 cm to the right of the vertex) for inducing responses in one or several main arm muscles (anterior and posterior deltoid, biceps brachii, triceps brachii and flexor carpi radialis) in the stationary suspended arm. The intensity of stimulation (typically 50–55% of the maximal output of stimulator) was routinely adjusted to elicit motor evoked potentials exceeding at least three times the background (level of noise) EMG activity of muscles (Solopova, Selionov, Kazennikov, & Ivanenko, 2014) in the initial position of the suspended arm. After eliciting and identification of these responses, the coil was fixed and maintained in that position during the experiment. A computer program was used to trigger the TMS-stimuli at a frequency of 0.33 Hz (T = 3 s). Since the inter-stimulus interval (T = 3 s) was different and lower than the cycle duration of non-voluntary arm oscillations (~1.7 s, see Results), the TMS stimuli were applied at different moments of the individual arm movement cycles within the trial. The duration of the TMS stimulation was 40 s. Fifteen seconds of non-voluntary arm oscillations before and after stimulation were also recorded.

2.4. Protocols and experimental conditions

The following experimental conditions were recorded in the random order across participants: 1) Continuous ES; 2) Periodic ES; 3) ES of the medial epicondyle (control experiment); 4) VS (TB vibration); 5) Continuous ES for 20 s on the background of non-voluntary arm oscillations induced by VS (VS + ES); 6) The following stimulations were performed for ~20 s when non-voluntary arm movements induced by continuous ES became stable: a) VS (ES + VS), b) Head turn (ES + head turn), c) Passive left leg movements (ES + leg), d) TMS (ES + TMS) (only 8 subjects participated in this probe). In all conditions, to reduce attention paid to the evoked movements (Selionov et al., 2009; Sylos-Labini et al., 2014), subjects were asked to carry out mental arithmetic (counting down by 7) throughout each trial.

2.5. Data recording and analysis

Recordings of EMG activity were obtained using surface wireless bipolar electrodes (Delsys Trigno EMG system, USA) from 9 muscles on the left side of the body: posterior deltoid (DP), anterior deltoid (DA), biceps brachii (BB), triceps brachii (TB), rectus femoris (RF), biceps femoris (BF, long head), lateral gastrocnemius (LG), tibialis anterior (TA), and levator scapulae (LS). EMG activity was pre-amplified and filtered (bandwidth 20–450 Hz). Angular displacements in the two upper limb joints (shoulder and elbow) and two lower limb joints (hip, knee) were recorded using potentiometers attached laterally to each joint. The kinematic and EMG data were sampled at 1000 Hz and stored for subsequent analyses.

Cycle duration and amplitudes of angular movements in different joints were assessed as the parameters averaged across 8–10 cycles. Raw EMG data were numerically rectified, low-pass-filtered with a zero-lag 4th-order Butterworth filter (10 Hz cut-off), time-interpolated over a time base with 100 points for individual arm movement cycles (max shoulder angle as the onset of the cycle), and the mean amplitude of EMG waveforms was computed for each cycle and then averaged across cycles. In the "ES + TMS" condition, for calculating the amplitude of EMG activity, we selected only the cycles, in which the timing of TMS stimuli did not overlap with the timing of major EMG bursts during evoked arm oscillations. For that reason, since TMS elicits motor evoked potentials that are typically proportional to the background EMG activity (Solopova et al., 2014), their amplitude was minimal in these cycles and it did not affect the calculation of the mean EMG activity (see also *Results*).

2.6. Statistics

The experimental data set did not meet the normal distribution criteria (Shapiro-Wilk's W-test, $p < 0.05$), therefore non-parametric statistics were used for statistical data analysis. Descriptive statistics included medians, quartiles and range of values. For reporting the percentage of participants demonstrating non-voluntary upper limb movements, the presence of oscillations was considered, if the amplitude of cyclic movements in the shoulder or elbow joint exceeded 3°. The following parameters were evaluated: amplitude of joint angular movements, cycle duration, latency of elicited cyclic movements (angular motion $> 3°$) and mean amplitude of EMG activity. For paired comparisons of two related groups, the Wilcoxon matched pair test was used (effect size statistics: $PS_{dep} = n_+/N$, where n_+ is the number of positive difference scores, N – number of pairs). To compare three related groups, we used the Friedman test and Dunn-Bonferroni test for the post hoc analysis (effect size statistics: pairwise Kendall's W – coefficient of concordance). Since most of the considered parameters (EMG magnitudes and joint movement amplitudes) correlated to each other, multiple testing correction was performed according to the Benjamini-Hochberg procedure. The degree of relationship between motion parameters was assessed by Spearman rank-order correlation. The level of statistical significance was set at 0.05.

3. Results

3.1. Rhythmic arm movements induced by peripheral sensory stimulation

Vibration of TB (VS) was effective in eliciting cyclic arm movements in all subjects. Continuous ES of the median nerve was effective for eliciting cyclic arm movements in 90% of participants, while periodic ES was less effective: it induced non-voluntary arm movements in 60% of subjects. Since we used continuous ES for the combined effects of sensory and central stimuli (see Methods), the characteristics of non-voluntary upper limb oscillations reported below (Fig. 1) refer to the effect of continuous ES. It is also worth stressing that the effect of ES was unlikely related to non-specific activation of skin receptors since ES stimulation of the medial epicondyle of the humerus (when we placed a stimulating electrode on the dorsal part of the elbow) failed to elicit non-voluntary upper limb oscillations in all tested subjects.

The latency of the elicited cyclic movements varied significantly (range 0.8–20 s across all trials and subjects, median 3.2 s), nevertheless, rhythmic arm movements with the period 1.0–2.3 s (median 1.7 s) persisted as long as stimulation continued. The amplitudes of angular oscillations in the elbow and shoulder joints, EMG activity and cycle duration are shown in Fig. 1. They were similar in both ES and VS conditions. The mean amplitude of the induced EMG activity was very small (median values well below 5 μV in most cases, Fig. 1C). However, it is also worth noting that during arm swinging in normal (upright) walking the EMG activity of the upper limb muscles is also small (Kuhtz-Buschbeck & Jing, 2012), as well as air-stepping (Fig. 1A) is used as a unique and important model for investigating human rhythmogenesis (Solopova et al., 2015) since its manifestation is largely facilitated by a reduction of external resistance. Angular movements were observed in both shoulder (median amplitude 14°) and elbow (15°) joints, though the two joints were not involved to the same extent in all subjects (see, for instance, Fig. 1A left panel). Movements in the wrist joint were typically absent (as assessed by visual observation though we did not record them). Non-voluntary arm oscillations were accompanied by the bursts of EMG activity (generally more prominent in the proximal muscles, DP and DA) with predominantly reciprocal interaction between flexors and extensors.

3.2. Simultaneous influences of muscle vibration and electrical stimulation

Combined ES and VS stimulation (both VS + ES and ES + VS) was more effective in evoking rhythmic arm movements than each stimulus alone. Fig. 1B illustrates an example of separate and combined (VS + ES) stimulations in one representative subject. Combined stimulation evoked significantly larger movements in the shoulder joint (with a latency of about several seconds, as in the case of single stimulations) accompanied by the prominent activity of shoulder muscles. While there was some inter-individual variability, on the whole, facilitation of rhythmogenesis (with the cycle duration ~1.7 s, Fig. 1C, right panel) was similar in both VS + ES and ES + VS conditions. In particular, the range of motion in the shoulder ($p = 0.03$, post hoc ES vs. VS + ES $p = 0.042$, $W = 0.64$) and elbow ($p = 0.018$, post hoc VS vs. VS + ES $p = 0.025$, $W = 0.45$) joints increased (Fig. 1C) along with the increments in the TB EMG bursts ($p = 0.014$, post hoc ES vs. VS + ES $p = 0.014$, $W = -0.03$ – this effect size may be explained by vibration of TB muscle) and DA EMG bursts ($p = 0.028$, post hoc ES vs. VS + ES $p = 0.04$, $W = 0.61$). The activity of other muscles also tended to increase though non significantly. Thus, incrementing the number of sensory channels can be an essential factor for augmenting non-voluntary rhythmic arm movements.

3.3. Effects of head turn and passive leg movements

To verify whether the movements in other body parts (active or passive) could potentiate non-voluntary arm oscillations, we first evoked non-voluntary arm oscillations by ES of the median nerve and then we asked the participants to turn the head by ~70° and

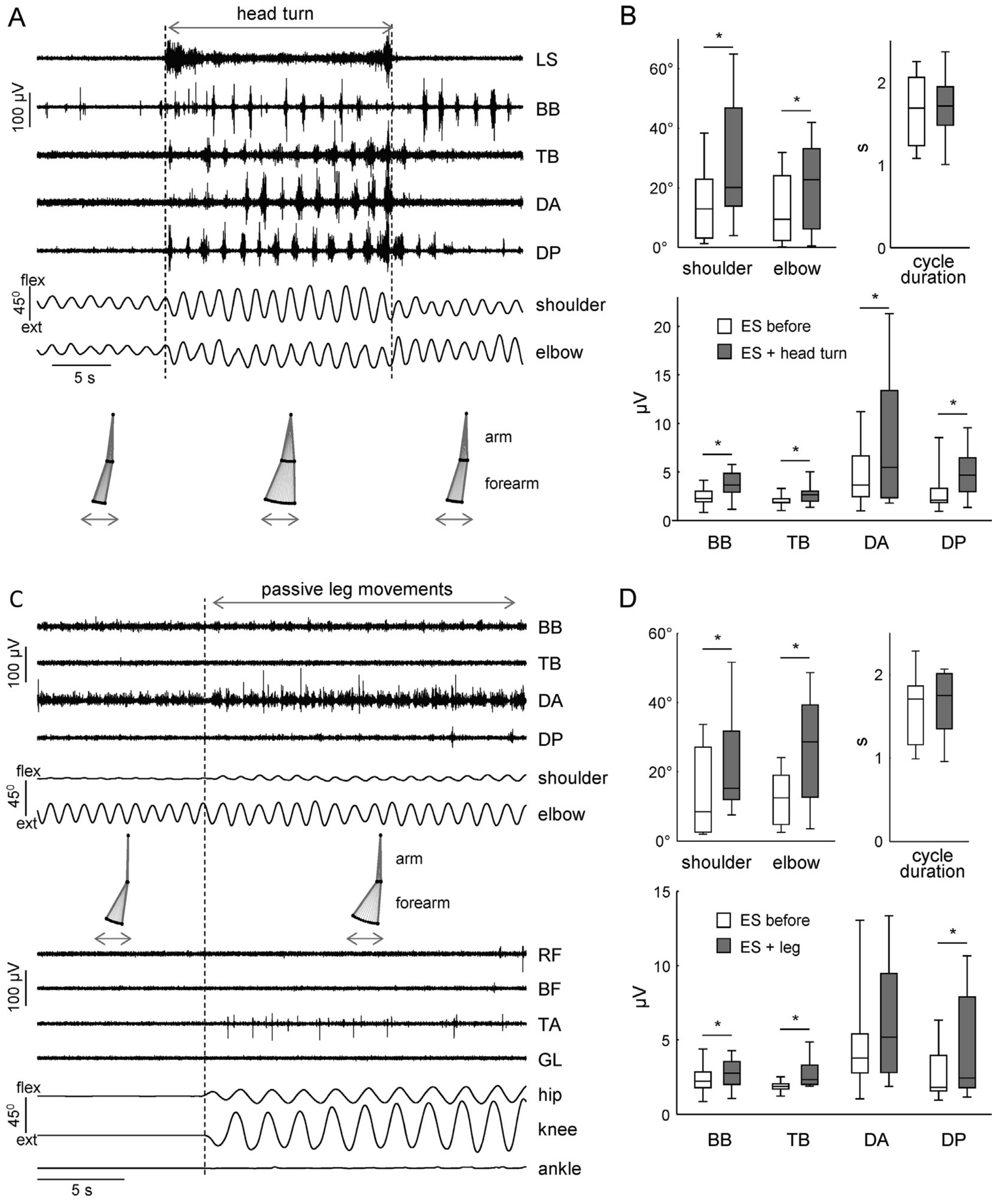

Fig. 2. Facilitatory effects of head turn (A, B) and passive leg movements (C, D) on ES-evoked upper limb oscillations. Similar format as in Fig. 1. Asterisks denote significant differences. Note a significant facilitatory effect of head turn on all kinematic and EMG parameters (B) and a less pronounced effect of passive leg movements (D).

keep it in that position for ~20 s (Fig. 2A) or we imposed rhythmic passive movements of the suspended leg (Fig. 2C). Prior to the head turn, the head was put on a pillow (relaxed). Turning the head (along with activation of neck muscles, Fig. 2A) led to a significant enhancement of the range of motion in the shoulder ($p = 0.014$, PS = 1) and elbow ($p = 0.027$, PS = 0.83) joints, accompanied by an increment in the EMG activity of BB, TB, DP ($p = 0.014$ for all, PS = 0.83, 0.92 and 0.92, respectively) and DA ($p = 0.027$, PS = 0.64) muscles (Fig. 2A and B), though the period of arm oscillations did not change significantly. After the head returned to its original position, the parameters of the induced arm movements returned to the values similar to those prior to the head turn (Fig. 2A). Imposing passive movements of the ipsilateral leg also resulted in a facilitation of arm movements though to a lesser extent (e.g., one subject showed an opposite effect). On average, a significant increase was observed in the range of motion of the shoulder and elbow joints ($p = 0.046$, PS = 0.82 for both), and in the EMG activity of BB ($p = 0.046$, PS = 0.73), TB ($p = 0.021$, PS = 1) and DP ($p = 0.046$, PS = 0.82) (Fig. 2D). Thus, there was a significant facilitatory effect of head turn on all kinematics and

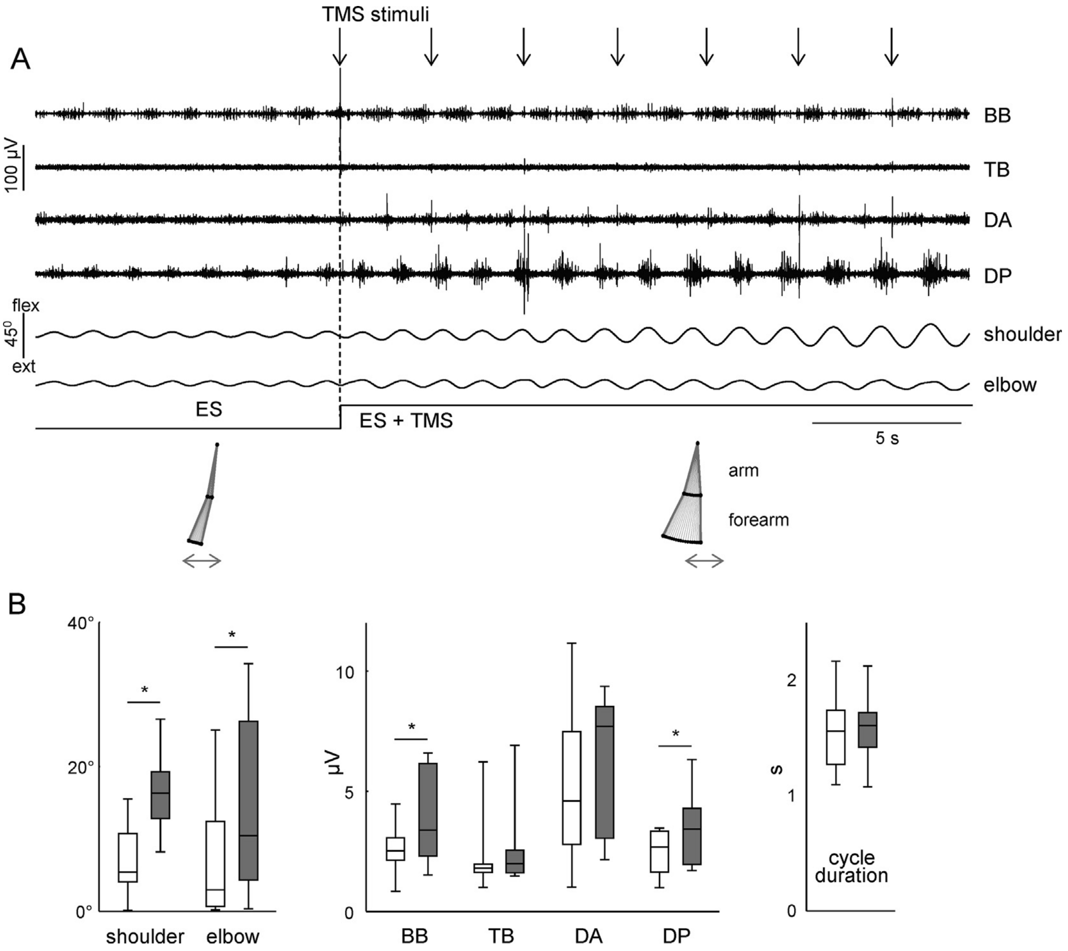

Fig. 3. Effect of low-frequency TMS of the motor cortex during ES-induced non-voluntary upper limb oscillations. The same format as in Fig. 1. Arrows in panel A denote the timing of TMS stimuli.

EMG parameters (Fig. 2B) and a less pronounced effect of passive leg movements (Fig. 2D).

3.4. Effect of TMS

In this test, we aimed at examining whether the low frequency TMS of the primary motor cortex (corresponding to the arm representation) could evoke or interact with non-voluntary rhythmic arm movements. Low-frequency TMS (0.33 Hz) of the motor cortex *per se* did not evoke movements of the suspended arm (not shown). However, it significantly potentiated non-voluntary arm oscillations evoked by ES of the median nerve (Fig. 3A and B). The amplitude of angular motion noticeably increased following the onset of TMS ($p = 0.035$ both for the shoulder ($PS = 1$) and elbow ($PS = 0.9$) joints), though without significant changes in the period of arm movements. As a result, the anterior-posterior amplitudes of wrist oscillations increased and were on average ~12 cm before TMS and ~25 cm during ES + TMS. Increments in the upper limb oscillations were accompanied by augmentations in the muscle activity bursts of BB and DP ($p = 0.04$ for both, $PS = 0.8$ and 0.89, respectively) (Fig. 3). TMS also induced motor evoked potentials is some muscles. However, it is worth noting that, even though motor evoked potentials contributed to the net EMG activity, the reported increments in EMG bursts (Fig. 3B) could not be accounted for by the presence of motor evoked potentials. For calculating the amplitude of EMG activity, we selected only the cycles when the timing of TMS stimuli did not overlap with the timing of major EMG bursts and, thus, the amplitude of motor evoked potentials was minimal in these cycles (and did not affect the calculation of the mean EMG activity). Furthermore, the inter-stimulus interval of TMS ($T = 3$ s) was lower than the cycle duration of non-voluntary arm oscillations (~1.7 s) and the stimuli were applied at different moments of the arm movement cycle, while the enhanced bursts of EMG activity followed the rhythm of arm movements (Fig. 3A and B).

4. Discussion

We have previously argued about an endogenous pattern generator for the upper limbs in humans and showed that nonspecific tonic excitability, such as muscle vibration or the after-effect of muscle contraction (Kohnstamm phenomenon), may elicit cervical CPG activity (Solopova et al., 2017, 2016). Here we extended these findings by demonstrating that low-intensity electrical stimulation of the median nerve can also evoke rhythmic arm movements (Fig. 1) and that combined peripheral and central stimuli are more effective in facilitating upper limb rhythmogenesis (Figs. 1–3).

4.1. *Effects of peripheral sensory stimulation*

In this study, we used diverse peripheral sensory stimulations: TB muscle vibration and ES of the median nerve. Historically, afferent stimulation was the first method for producing rhythmic movements in animal preparations (Roaf & Sherrington, 1910). The afferent signals may increase the excitability of several segments of the spinal cord and facilitate triggering of locomotor-like movements (Gerasimenko et al., 2010; Gurfinkel et al., 1998). Since walking in humans typically involves arm swinging and rhythmic activity in the proximal upper limb muscles (Ivanenko, Poppele, & Lacquaniti, 2006; Kuhtz-Buschbeck & Jing, 2012), the contribution of the sensory input from TB in activating the cervical CPG might be expected. In this respect, the fact that ES of the median nerve also evoked rhythmic upper limb oscillations (Fig. 1) is interesting since it excites sensory fibres not from proximal but from distal upper limb segments. Furthermore, the effect of ES was specific to nerve stimulation, rather than activating a sensory input from skin receptors, since the same stimulus applied to the medial epicondyle of the humerus did not elicit non-voluntary upper limb oscillations (Fig. 1A, right panel).

While TB vibration mainly activates muscle spindles of this muscle (and to a lesser extent other muscle and skin receptors, Roll et al., 1989), ES of the median nerve activates the somatosensory input from the muscles of the hand. The former stimulus is associated with the sensory input from the proximal muscles (triceps brachii), while the latter stimulus is associated with that of the distal muscles. The median nerve is one of the five main nerves of the upper limb originating from the brachial plexus and supplying the muscles of the hand, and it has contributions from ventral roots of C5-C6, C8 and T1 segments. In particular, it supplies the muscles of the front of the forearm and muscles of the thenar eminence, therefore, controlling the coarse movements of the hand. Whereas distal arm muscles are typically not involved in normal walking, the positive effect of median nerve stimulation on the state of the cervical pattern generation circuitry could likely be accounted for by the involvement of these muscles in other locomotor-related tasks (e.g., swimming, skiing, climbing, cycling, crawling). In addition, distal arm muscle activation facilitates leg rhythmogenesis (Solopova et al., 2017) and plays a part in the cervicothoracic cross-limb reflex modulation (Zehr et al., 2016), as well as excitatory and inhibitory relationships between the foot and hand motor cortices were documented (Debaere et al., 2001; Kato et al., 2016). In sum, the neural circuits of the cervical spinal cord may be activated by sensory inputs from both proximal and distal muscles (Fig. 1).

4.2. *Synergistic effects of peripheral and central stimulations*

Another aim of our study was to assess the combined effects of peripheral and central stimulations since the efficiency of one stimulus is typically limited. For instance, the effect of muscle vibrostimulation is higher at intermediate rather than at higher frequencies and is manifested only in a part of subjects (Gurfinkel et al., 1998), while the intensity of electrical nerve or spinal cord stimulation is limited to the acceptable electrical currents. When combined together, however, the resultant non-voluntary arm oscillations were significantly larger (Fig. 1). It is also worth noting that not all stimulations might be effective to the same extent and not all stimulations can evoke rhythmic arm movements. For instance, repetitive TMS of the motor cortex, as well as passive leg movements and head turn, *per se* did not elicit arm oscillations. However, they considerably assisted non-voluntary oscillations evoked by upper limb sensory stimulation (Figs. 2 and 3).

Synergistic effects of stimulation have been previously reported for the lower limbs. For instance, combined spinal cord and plantar stimulation in uninjured subjects under full body weight support stepping movements exhibited a synergistic effect on hip and knee flexion than either modality alone (Gerasimenko, Gad, et al., 2016). The Jendrassik manoeuvre may significantly prolong rhythmic leg movements initiated manually by an experimenter (Selionov et al., 2009). It has also been suggested that there might be a critical combination of afferent signals for generating a synergistic locomotor pattern (Dietz & Harkema, 2004; Gerasimenko, Sayenko, et al., 2016b; Prochazka & Ellaway, 2012). Here, we extended this notion to the upper limbs by demonstrating that the combined effects of peripheral and central stimulation can be beneficial for facilitating rhythmogenesis (Figs. 1–3) and thus may be a general property of pattern generation circuitry providing a promising avenue for enhancing its functional capacity. Considering a quadrupedal nature of human gait (the neural linkages between cervical and lumbosacral pattern generation circuitries, Dietz, 2011; Zehr et al., 2016) and the common principles of synergistic effects of stimulation for lower and upper limb rhythmogenesis, an appropriate involvement of the cervical spinal cord can enhance the recovery of walking in sensorimotor disorders (e.g., Kaupp et al., 2017).

An interesting finding was a facilitatory effect of low-frequency TMS on non-voluntary arm oscillations evoked by sensory stimulation (Fig. 3). This effect could not be simply accounted for by the mechanical consequences of direct muscle stimulation since the enhanced bursts of EMG activity followed the rhythm of arm movements (Fig. 3A and B) and not the TMS cycles, suggesting that TMS potentiated the physiological state and the overall cervical motor pool's excitability.

Even the simplest movement involves numerous brain and spinal cord neuronal structures. Furthermore, motor imagery is able to generate motor commands activating the spinal interneurons without activation of alpha-motoneurons. For instance, enhanced cervico-medullary-evoked and motor-evoked potentials and an interaction between presynaptic conditioning of the H-reflex and task suggest a modulatory effect of motor imagery on the spinal neuronal network (Grosprêtre, Lebon, Papaxanthis, & Martin, 2016).

Given that corticospinal excitation is mediated by polysynaptic pathways and TMS projects not only to the motoneurons but also to the interneurons of the spinal cord (York, 1987) that play a major role in pattern generation, such stimulation might have a powerful functional impact on the physiological state of the network.

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Declarations of interest

None.

References

Dai, C., Lv, R. T. X., & He, J. (2017). Modulation of upper limb locomotor function via transcutaneous electrical stimulation of spinal cord nervous system. In: 2017 IEEE International Conference on Cyborg and Bionic Systems (CBS) (pg. 154–159). https://doi.org/10.1109/CBS.2017.8266088.

de Kam, D., Rijken, H., Manintveld, T., Nienhuis, B., Dietz, V., & Duysens, J. (2013). Arm movements can increase leg muscle activity during submaximal recumbent stepping in neurologically intact individuals. *Journal of Applied Physiology, 115*(1), 34–42.

Debaere, F., Swinnen, S. P., Béatse, E., Sunaert, S., Van Hecke, P., & Duysens, J. (2001). Brain areas involved in interlimb coordination: A distributed network. *NeuroImage, 14*(5), 947–958. https://doi.org/10.1006/nimg.2001.0892.

Dietz, V. (2011). Quadrupedal coordination of bipedal gait: Implications for movement disorders. *Journal of Neurology, 258*(8), 1406–1412.

Dietz, V., & Harkema, S. J. (2004). Locomotor activity in spinal cord-injured persons. *Journal of Applied Physiology (Bethesda, Md.: 1985), 96*(5), 1954–1960. https://doi.org/10.1152/japplphysiol.00942.2003.

Duysens, J., Tax, A. A., Murrer, L., & Dietz, V. (1996). Backward and forward walking use different patterns of phase-dependent modulation of cutaneous reflexes in humans. *Journal of Neurophysiology, 76*(1), 301–310. https://doi.org/10.1152/jn.1996.76.1.301.

Edgerton, V. R., & Roy, R. R. (2012). A new age for rehabilitation. *European Journal of Physical and Rehabilitation Medicine, 48*(1), 99–109.

Frigon, A. (2017). The neural control of interlimb coordination during mammalian locomotion. *Journal of Neurophysiology, 117*(6), 2224–2241. https://doi.org/10.1152/jn.00978.2016.

Gerasimenko, Y., Gad, P., Sayenko, D., McKinney, Z., Gorodnichev, R., Puhov, A., ... Edgerton, V. R. (2016a). Integration of sensory, spinal, and volitional descending inputs in regulation of human locomotion. *Journal of Neurophysiology, 116*(1), 98–105. https://doi.org/10.1152/jn.00146.2016.

Gerasimenko, Y., Gorodnichev, R., Machueva, E., Pivovarova, E., Semyenov, D., Savochin, A., ... Edgerton, V. R. (2010). Novel and direct access to the human locomotor spinal circuitry. *The Journal of Neuroscience, 30*(10), 3700–3708.

Gerasimenko, Y., Sayenko, D., Gad, P., Liu, C.-T., Tillakaratne, N. J. K., Roy, R. R., ... Edgerton, V. R. (2016b). Feed-forwardness of spinal networks in posture and locomotion. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*. https://doi.org/10.1177/1073858416683681.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods and fish. In V. B. Brooks, J. M. Brookhart, & V. B. Mountcastle (Eds.). *Handbook of physiology: Section 1: The nervous system, volume II, Part. 1 motor control* (pp. 1179–1236). Bethesda, MD: American Physiological Society.

Grosprêtre, S., Lebon, F., Papaxanthis, C., & Martin, A. (2016). New evidence of corticospinal network modulation induced by motor imagery. *Journal of Neurophysiology, 115*(3), 1279–1288. https://doi.org/10.1152/jn.00952.2015.

Gurfinkel, V. S., Levik, Y. S., Kazennikov, O. V., & Selionov, V. A. (1998). Locomotor-like movements evoked by leg muscle vibration in humans. *The European journal of neuroscience, 10*(5), 1608–1612.

Ivanenko, Y. P., Gurfinkel, V. S., Selionov, V. A., Solopova, I. A., Sylos-Labini, F., Guertin, P. A., & Lacquaniti, F. (2017). Tonic and rhythmic spinal activity underlying locomotion. *Current Pharmaceutical Design, 23*(12), 1753–1763. https://doi.org/10.2174/1381612823666170125152246.

Ivanenko, Y. P., Poppele, R. E., & Lacquaniti, F. (2006). Spinal cord maps of spatiotemporal alpha-motoneuron activation in humans walking at different speeds. *Journal of Neurophysiology, 95*(2), 602–618.

Kato, K., Muraoka, T., Mizuguchi, N., Nakagawa, K., Nakata, H., & Kanosue, K. (2016). Muscle relaxation of the foot reduces corticospinal excitability of hand muscles and enhances intracortical inhibition. *Frontiers in Human Neuroscience, 10*, 218. https://doi.org/10.3389/fnhum.2016.00218.

Kaupp, C., Pearcey, G. E., Klarner, T., Sun, Y., Barss, T. S., Cullen, H., & Zehr, E. P. (2017). Rhythmic arm cycling training improves walking and neurophysiological integrity in chronic stroke-the arms can give legs a helping hand in rehabilitation. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.00570.2017.

Kiehn, O. (2016). Decoding the organization of spinal circuits that control locomotion. *Nature Reviews Neuroscience, 17*(4), 224–238. https://doi.org/10.1038/nrn.2016.9.

Kuhtz-Buschbeck, J. P., & Jing, B. (2012). Activity of upper limb muscles during human walking. *Journal of Electromyography and Kinesiology, 22*(2), 199–206.

La Scaleia, V., Ivanenko, Y., Fabiano, A., Sylos-Labini, F., Cappellini, G., Picone, S., ... Lacquaniti, F. (2018). Early manifestation of arm-leg coordination during stepping on a surface in human neonates. *Experimental Brain Research, 236*(4), 1105–1115. https://doi.org/10.1007/s00221-018-5201-y.

La Scaleia, V., Sylos-Labini, F., Hoellinger, T., Wang, L., Cheron, G., Lacquaniti, F., & Ivanenko, Y. P. (2014). Control of leg movements driven by EMG activity of shoulder muscles. *Frontiers in Human Neuroscience, 8*, 838. https://doi.org/10.3389/fnhum.2014.00838.

Ledebt, A. (2000). Changes in arm posture during the early acquisition of walking. *Infant Behavior and Development, 23*(1), 79–89. https://doi.org/10.1016/S0163-6383(00)00027-8.

MacLellan, M. J., Qaderdan, K., Koehestanie, P., Duysens, J., & McFadyen, B. J. (2013). Arm movements during split-belt walking reveal predominant patterns of interlimb coupling. *Human Movement Science, 32*(1), 79–90.

Meyns, P., Bruijn, S. M., & Duysens, J. (2013). The how and why of arm swing during human walking. *Gait & Posture, 38*(4), 555–562.

Patrick, S. K., Noah, J. A., & Yang, J. F. (2012). Developmental constraints of quadrupedal coordination across crawling styles in human infants. *Journal of Neurophysiology, 107*(11), 3050–3061.

Prochazka, A., & Ellaway, P. (2012). Sensory systems in the control of movement. *Comprehensive Physiology, 2*(4), 2615–2627. https://doi.org/10.1002/cphy.c100086.

Roaf, H. E., & Sherrington, C. S. (1910). Further remarks on the mammalian spinal preparation. *Quarterly Journal of Experimental Physiology, 3*(2), 209–211. https://doi.org/10.1113/expphysiol.1910.sp000064.

Roll, J. P., Vedel, J. P., & Ribot, E. (1989). Alteration of proprioceptive messages induced by tendon vibration in man: A microneurographic study. *Experimental Brain Research, 76*(1), 213–222.

Selionov, V. A., Ivanenko, Y. P., Solopova, I. A., & Gurfinkel, V. S. (2009). Tonic central and sensory stimuli facilitate involuntary air-stepping in humans. *Journal of*

Neurophysiology, 101(6), 2847–2858.

Shah, P. K., Garcia-Alias, G., Choe, J., Gad, P., Gerasimenko, Y., Tillakaratne, N., ... Edgerton, V. R. (2013). Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain: A Journal of Neurology, 136*(Pt 11), 3362–3377. https://doi.org/10.1093/brain/awt265.

Shapkova, E. Y., Terekhov, A. V., & Latash, M. L. (2011). Arm motion coupling during locomotion-like actions: an experimental study and a dynamic model. *Motor control, 15*(2), 206–220.

Solopova, I. A., Selionov, V. A., Blinov, E. O., Zhvansky, D. S., & Ivanenko, Y. P. (2017). Rhythmic wrist movements facilitate the soleus H-reflex and non-voluntary air-stepping in humans. *Neuroscience Letters, 638*, 39–45. https://doi.org/10.1016/j.neulet.2016.12.007.

Solopova, I. A., Selionov, V. A., Kazennikov, O. V., & Ivanenko, Y. P. (2014). Effects of transcranial magnetic stimulation during voluntary and non-voluntary stepping movements in humans. *Neuroscience Letters, 579*, 64–69.

Solopova, I. A., Selionov, V. A., Sylos-Labini, F., Gurfinkel, V. S., Lacquaniti, F., & Ivanenko, Y. P. (2015). Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Frontiers in Systems Neuroscience, 9*, 14. https://doi.org/10.3389/fnsys.2015.00014.

Solopova, I. A., Selionov, V. A., Zhvansky, D. S., Gurfinkel, V. S., & Ivanenko, Y. (2016). Human cervical spinal cord circuitry activated by tonic input can generate rhythmic arm movements. *Journal of Neurophysiology, 115*(2), 1018–1030. https://doi.org/10.1152/jn.00897.2015.

Sylos-Labini, F., d'Avella, A., Lacquaniti, F., & Ivanenko, Y. (2018). Human-human interaction forces and interlimb coordination during side-by-side walking with hand contact. *Frontiers in Physiology, 9*, 179. https://doi.org/10.3389/fphys.2018.00179.

Sylos-Labini, F., Ivanenko, Y. P., Maclellan, M. J., Cappellini, G., Poppele, R. E., & Lacquaniti, F. (2014). Locomotor-like leg movements evoked by rhythmic arm movements in humans. *PLoS One, 9*(3), e90775.

van den Brand, R., Heutschi, J., Barraud, Q., DiGiovanna, J., Bartholdi, K., Huerlimann, M., ... Courtine, G. (2012). Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science, 336*(6085), 1182–1185.

York, D. H. (1987). Review of descending motor pathways involved with transcranial stimulation. *Neurosurgery, 20*(1), 70–73.

Zehr, E. P., Barss, T. S., Dragert, K., Frigon, A., Vasudevan, E. V., Haridas, C., ... Sun, Y. (2016). Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. *Experimental Brain Research, 234*(11), 3059–3081. https://doi.org/10.1007/s00221-016-4715-4.