Human cervical spinal cord circuitry activated by tonic input can generate rhythmic arm movements

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Running title: Rhythmogenesis of human cervical spinal cord circuitry

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Abstract

The coordination between arms and legs during human locomotion shares many features with that in quadrupeds, yet, there is limited evidence for the central pattern generator for the upper limbs in humans. Here we investigated whether different types of tonic stimulation, previously used for eliciting stepping-like leg movements, may evoke non-voluntary rhythmic arm movements. Twenty healthy subjects participated in this study. The subject was lying on the side, the trunk was fixed and all four limbs were suspended in a gravity neutral position, allowing unrestricted low-friction limb movements in the horizontal plane. The results showed that peripheral sensory stimulation (continuous muscle vibration) and central tonic activation (post-contraction state of neuronal networks following a long-lasting isometric voluntary effort, Kohnstamm phenomenon) could evoke non-voluntary rhythmic arm movements in most subjects. In about 40% of subjects, tonic stimulation elicited non-voluntary rhythmic arm movements together with rhythmic movements of suspended legs. The fact that not all participants exhibited non-voluntary limb oscillations may reflect inter-individual differences in responsiveness of spinal pattern generation circuitry to its activation. The occurrence and the characteristics of induced movements highlight the rhythmogenesis capacity of cervical neuronal circuitries, complementing the growing body of work on the quadrupedal nature of human gait.
Introduction

In animals, the rhythmic activity of forelimb and hindlimb muscles during locomotion is coordinated by central pattern generators (CPG) in the cervical and lumbosacral spinal cord, respectively, and influenced by sensory and supraspinal inputs (Grillner, 1981). Both descending and ascending connections between cervical and lumbosacral CPGs, as well as an intrinsic rhythmogenesis capacity of the thoracic spinal network, have been described in quadrupedal animals (Beliez et al., 2015). The lumbar locomotor network plays an essential role in the generation of locomotor outputs (Falgairolle et al., 2006). Nevertheless, the cervical neurons also have the capacity to produce reciprocal activation of agonist and antagonist forelimb muscles, as it has been shown, for instance, during fictive forelimb locomotion in the immobilized decerebrate cats with the lower thoracic cord transected under stimulation of the mesencephalic locomotor region (Amemiya and Yamaguchi, 1984; Yamaguchi, 1992). Furthermore, forelimb movements may facilitate or even trigger hindlimb stepping in both normal and decerebrate animals (Shik and Orlovsky, 1965; Miller et al., 1975; Gerasimenko et al., 2009), as well as cervicolumbar CPG coordination is reduced after a thoracic midsagittal section or local synaptic transmission blockade in isolated spinal cord preparations (Juvin et al., 2012), suggesting facilitatory neural coupling between limb neural controllers. The neuronal organization of the central pattern generator for forelimb locomotion in the cat (Saltiel and Rossignol, 2004; Yamaguchi, 2004) and its interaction with brain areas (Zelenin et al., 2011; Drew and Marigold, 2015) have also been studied extensively.

Substantial evidence indicates that humans use quadrupedal coordination during locomotion (Zehr et al., 2009; Dietz, 2011), even though the coupling between upper and lower limbs movements is likely weaker in humans in relation to the evolution of bipedal locomotion and the need to free the upper limbs for manipulative tasks. Both mechanical and neuronal coupling contribute to arm-leg coordination (Murray et al., 1967; Collins et al., 2009; Barthelemy and Nielsen, 2010; Bruijn et al., 2010; Meyns et al., 2013). The inter-limb coupling
during human locomotion shares many features with that in quadrupeds (Wannier et al., 2001; Falgairolle et al., 2006; Patrick et al., 2009), including the reliance on propriospinal connections (Nathan et al., 1996; Dietz, 2011) and cross modulation of reflexes between the limbs (Haridas and Zehr, 2003; Mezzarane et al., 2011; Massaad et al., 2014). Furthermore, voluntary rhythmic arm movements increase leg muscle recruitment during sub-maximal recumbent stepping (de Kam et al., 2013), modulate leg muscle activity during standing (Danna-Dos-Santos et al., 2009), and can even evoke stepping-like leg movements (Sylos-Labini et al., 2014).

Despite evidence for neural and mechanical coupling between arms and legs during human locomotion, the capacity of the cervical spinal circuitry for rhythmogenesis has not been directly demonstrated. Indeed, the presence of arm muscle activity during walking (Ballesteros et al., 1965; Hogue, 1969; Ivanenko et al., 2006a; Barthelemy and Nielsen, 2010; Kuhtz-Buschbeck and Jing, 2012) and phase-specific inter-limb reflex modulation (Zehr et al., 2009; Dietz, 2011) might not necessarily imply that, in the absence of lumbosacral CPG activity, the cervical spinal cord has the capacity of rhythmogenesis. While it is difficult to investigate the hypothetical human cervical CPG in isolation, one way to study it, is to find the conditions for producing non-voluntary reciprocal arm muscle activation.

We hypothesized that different types of tonic non-invasive stimulation, previously used for eliciting stepping-like leg movements (Selionov et al., 2009), may evoke non-voluntary rhythmic arm movements. For instance, tonic sensory inputs have access to the functional state of CPG and may initiate a stepping-like output (Sherrington, 1910). In addition to peripheral sensory stimulation, an intriguing approach related to the role of central tonic influences is the Kohnstamm phenomenon, which consists in the appearance of involuntary muscle activity and a particular sensation of “lightness” after the cessation of a long-lasting (30–40 s) strong isometric contraction (Kohnstamm, 1915). The involuntary activity is typically observed on the previously contracted muscles, however, appearance of motor post-contractions in other muscles (not involved in the previous voluntary activity) has also been documented (Craske and Craske, 1986;
Gurfinkel et al., 1989; Duclos et al., 2004). The exact physiological mechanisms underlying this phenomenon have not yet been clearly identified, even though extensive literature exists (for a review see Craske and Craske, 1986; Ghosh et al., 2014). What is important for this report is that long-lasting central motor outputs undoubtedly drive the Kohnstamm movement (Ghosh et al., 2014) and this has been used for changing the state of the locomotor circuitry and for eliciting rhythmic leg movements (Gurfinkel et al., 1998; Selionov et al., 2009). We used simulated weightlessness conditions as a model for investigating human rhythmogenesis since its manifestation is largely facilitated by a reduction of external resistance (Selionov et al., 2009; Gerasimenko et al., 2010; Sylos-Labini et al., 2014; Solopova et al., 2015). Since the strength of the neural coupling between the arms during cyclic movements appears to be weaker than between the legs (Zehr et al., 2003) we compared the effects of bilateral and unilateral tonic stimulations for arm and leg movements.

Methods

Subjects

Twenty healthy volunteers (11 males, 9 females) aged between 26 and 70 yrs (mean 49±17 yrs) participated to the study. Five of these subjects participated in the earlier work and were preselected again based on the fact that, in the previous study, leg air-stepping could be entrained using sensory stimulation (Selionov et al., 2009), and we expected that they also might be responsive to tonic stimulation for producing cyclic arm movements. 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.
Experimental set-up

The previously used experimental set-up for leg air-stepping (Selionov et al., 2009) was modified by adding the block for suspension of the upper extremities (Fig. 1). The subjects lay on a canvas hammock on their right side and their right arm hung down through a hole (~24 cm diameter) in the hammock. All extremities were suspended using long ropes attached to the ceiling (4 m height) providing low friction angular motion of all limb segments without noticeable vertical motion component. The shank segment of the right lower leg was placed on a 2.5-m long horizontal wood board that was suspended to the ceiling using 2 vertical ropes of the 4-m length. The right elbow was suspended analogously. The length of the horizontal board was long enough in order for each extremity to fulfill unrestricted forth and back movements. A bearing junction was introduced between the shank (or elbow) and the board allowing free rotation of the shank (or elbow) relative to the board around the vertical axis. The left limbs were suspended directly to the ceiling using a long rope attached to the shank or elbow segments. The foot and hand segments remained unrestrained in the air so that there was no resistance in the ankle and radiocarpal joints motion. The weight of the upper and lower leg suspension systems (0.9 and 1.5 kg, respectively) and the weight of the upper and lower arm suspension systems (0.2 and 1.1 kg, respectively) were much less than that of any limb so that it had minimal impact on the inertia of the system. The trunk was fixed between the 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 to perform a low friction motion of both legs and arms 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 limbs 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 hip joint angle varied between 135° and 160° (across all subjects and trials), 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). The upper limb joint angles were: 165°-185° for the shoulder (180° refers to the upper arm segment being parallel to the trunk) and 133°-167° for the elbow.

**Stimulation techniques**

The following stimulation techniques were used:

1) **Continuous muscle vibration.** Muscle vibration (30-60 Hz sinusoid, ~1 mm amplitude) 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 afferent though other afferent signals may be elicited as well (Roll et al 1989). For the arms, the vibrator was fastened with a rubber belt over the belly of the applicable muscle: deltoid (posterior head) (DP), biceps brachii (BB), triceps brachii (TB) and flexor carpi radialis (FCR). In the latter case, vibration was applied to other neighbor flexor wrist muscles (since the size of the vibrator was 3x7 cm) but for simplicity we will use the abbreviation of the muscle, where the vibrator was fixed (FCR). In each probe, only one muscle of the upper limb was vibrated (see *Protocols and experimental conditions*). For the legs, the vibrator was fastened with a rubber belt over the quadriceps muscle (Q), about 5 cm over the patella. The vibration to each muscle was delivered \( \sim 5 \) s after the onset of each recording and lasted till the end of the probe (1.5 min).

2) **Kohnstamm aftereffect** (aftereffect of a strong long-lasting isometric muscle contraction of one or both limbs). In static conditions, the cessation of the voluntary effort may result in involuntary contraction of the preconditioned muscles (Kohnstamm, 1915). In healthy humans, the post-contraction aftereffects emerge most clearly in proximal rather than in distal muscles (Gurfinkel et al., 1989). They are commonly thought to result from long-lasting changes (tens of seconds) in the excitatory state of the neuromuscular system that may include supraspinal structures, spinal interneurons, motoneurons and enhanced activity of peripheral sensory inputs.
(Gurfinkel et al., 1989; Duclos et al., 2004; Ivanenko et al., 2006b; Ghosh et al., 2014). Subjects were positioned on their right sides (Fig. 1) with both legs relaxed, but the extended arms performed a strong (about half-maximal) isometric contraction of one arm against another in the horizontal plane for about 30 s: one arm in flexion (forward direction) and the other in extension (backward direction) against resistance provided by a research assistant. This procedure was analogous to the classic Kohnstamm maneuver (Kohnstamm, 1915), except for anterior-posterior rather than medio-lateral force exertion. The research assistant stabilized a distal part of the forearm segments of both arms (or one arm in the case of a unilateral Kohnstamm effect) during a voluntary effort. The effort was mainly performed by the shoulder (and also elbow) flexor and extensor muscles of both arms. Immediately after the cessation of the voluntary contraction, the research assistant released both arms of the subject thus allowing a manifestation of any unconstrained arm motion in the horizontal plane. The Kohnstamm maneuver of the legs was performed analogously (Selionov et al., 2009).

Protocols and experimental conditions

The following experimental conditions were recorded in random order across the subjects:

1. Vibration of the same muscles of both arms in random order (DP and BB in all subjects, TB in 16 subjects and FCR in 15 subjects). 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 out aloud by 7’s) throughout each trial.

2. When vibration was effective in eliciting non-voluntary arm swinging, we tested the effect of a transient block of right or left arm. To this end, an experimenter blocked manually the subject’s forearm in the neutral (the middle of its excursion) position for several seconds and then released the arm. In 6 subjects, we additionally blocked the arm in the maximally forward and backward positions.
3. Vibration of only one muscle (DP, BB, TB, FCR) of the arm. We vibrated both left and right limb muscles separately.

4. Kohnstamm maneuver of two arms (one trial) and of only one arm (another trial).

5. Vibration of the Q muscle of both legs (one trial) and only one leg (another trial).

6. Kohnstamm maneuver of two legs (one trial) and of only one leg (another trial).

Prior to data recording, 1-2 sensory stimulation (muscle vibration) probes were delivered so that the subjects could familiarize with the stimulus. Each experimental condition was performed 1-2 times and ~1-3 min periods of rest were taken between testing probes. The duration of each probe was 1.5 min. The total duration of the experiment was about 2-2.5 hours.

**Data recording**

Recordings of EMG activity were obtained using surface wireless bipolar electrodes (Delsys Trigno EMG system, USA) from 8 muscles on each side of the body (16 muscles total): rectus femoris (RF), biceps femoris (BF, long head), lateral gastrocnemius (LG) and tibialis anterior (TA), posterior deltoid (DP), anterior deltoid (DA), biceps brachii (BB), triceps brachii (TB). EMG activity was pre-amplified and filtered (bandwidth 20 Hz - 1 kHz). Angular movements in the three joints (hip, knee and ankle) of both legs and two joints (shoulder and elbow) of both arms were recorded using potentiometers attached laterally to each joint of all limbs. The kinematic and EMG data were sampled at 1000 Hz and stored for subsequent analyses.

**Data analysis**

Cycle duration and amplitudes of angular joint movements were assessed as the movement parameters averaged across 10 cycles. Raw EMG data were numerically rectified, low-pass-filtered with a zero-lag Butterworth filter (10 Hz cutoff), time-interpolated over a time base with 100 points for individual step cycles (max shoulder angle as the onset of the cycle),
and averaged. We also calculated phase shifts between right and left shoulders, shoulder and elbow joint angles of each arm and shoulder and ipsilateral hip joint angles for the cases, when arm and/or leg movements were observed. The phase shift was defined as the interval between maxima of correspondent angles divided by the cycle duration. The onset of motion was determined by visual inspection as the onset of a prominent angular deviation (by ~3° relative to the initial baseline) in the elbow, shoulder, hip and knee joint angles prior to the first rhythmic cycle.

Statistics

Descriptive statistics included means and standard deviation (SD) of the mean. Paired t-tests and Repeated Measures (RM) ANOVA were used to compare means between different conditions. Post-hoc tests and multiple comparisons analysis were performed by means of Tukey HSD (Honestly Significant Difference) test. The level of statistical significance was set at 0.05.

Results

Rhythmic arm swinging produced by peripheral sensory stimulation

An example of non-voluntary rhythmic arm movements is shown in Fig. 2A. The results demonstrated that most participants (70-80%, Table 1) were responsive to peripheral sensory stimulation and produced cyclic arm movements. The remaining subjects did not show arm movements on any sensory stimuli. The latency of the elicited cyclic movements varied significantly (on average, 10.6±7.3 s across all trials and subjects, range 1-26 s). Generally, cyclic movements increased monotonically for 2-7 cycles until they reached relatively constant amplitude of angular oscillations (coefficient of variation in the shoulder and elbow joints across 10 successive cycles was 10±5% across all trials and subjects, without any visible wrist
movements) and persisted as long as stimulation continued (i.e., for \(-1.5\) min). It was often difficult to determine the direction of the first angular deviation since cyclic movements increased monotonically and the first few oscillations were very small (<3°, ‘undefined’ in Fig. 1D). Nevertheless, it is interesting to note that, when the first deviation was prominent (>3°, e.g., Fig. 1A), it predominantly corresponded to flexion rather than extension in the shoulder joint, independent of the muscle being vibrated (Fig. 1D, left). On average, rhythmic arm movements started with the flexion (forward arm movement) in 33% of trails vs. only 7% of trials with the extension (Fig. 1D, right).

We analyzed arm oscillations for 10 cycles in a steady state stable response (typically, during the 15-40 s period after the onset of muscle vibration). Non-voluntary arm oscillations were accompanied by bursts of EMG activity mainly in the proximal (DP, DA) muscles with predominantly reciprocal interaction between right and left arms (Fig. 2A). The characteristics (cycle duration, angular amplitudes) of non-voluntary arm swinging are presented in Fig. 2B. On average, the cycle duration was 1.7±0.2 s and did not depend significantly on the muscle being vibrated (RM-ANOVA, \(F_{3,18}=0.92, p=0.45\)). The amplitude of elbow and shoulder angular oscillations was almost identical in both arms and therefore it was averaged across the right and left sides (Fig. 2B). The angular motion was not significantly different across different (stimulated muscles) conditions (\(F_{3,18}=0.98, p=0.42\) for shoulder and \(F_{3,18}=0.35, p=0.84\) for elbow, RM-ANOVA). Movements of both arms were predominantly alternating between the left and right arm (~50%-cycle phase shift, Fig. 2C left panel), although in a few cases we could observe almost synchronous arm oscillations (~0% phase shift). The phase shift between elbow and shoulder angular movements varied, though it was positive in most trials (Fig. 2C).

It should be noted that we used mental arithmetic to minimize attention of the subjects to arm movements. According to the literature (Passingham, 1996; Wu et al., 2004), evidence that a task is automatic and carried out with minimal attention is provided by the fact that a secondary task is performed with little interference. In 10 subjects we compared non-voluntary arm
swinging with and without the concurrent mental task. The joint angular excursion (peak-to-peak) was larger during mental arithmetic (RM-ANOVA, F_{1,18}=35.08, p<0.001).

In sum, most participants (Table 1) demonstrated alternating arm movements and EMG activity (~1.5-2 s cycle duration) in response to continuous sensory stimulation. Rhythmic movements persisted as long as stimulation continued (up to 1.5 min).

**Effect of transient block of one arm**

To further investigate the nature of evoked arm movements, we blocked one arm movement manually. Fig. 3A illustrates examples of the evoked responses in 2 subjects: dashed lines delimit the period of a transient arm block (interruption and resumption of arm displacements). In some cases we could observe tonic or phasic EMG responses in the blocked arm muscles. Table 2 contains the percentage of trials with tonic and phasic responses. In the remaining trials, there was no evident rhythmic or tonic arm muscle EMG activity. It is important to note that all subjects in all trials resumed automatic arm movements upon release of the other arm (Fig. 3A).

Interestingly, in the non-blocked arm, a significant increase in the EMG activity level was observed in all recorded muscles (one way RM-ANOVA; for DA - in 1.5 times, F_{1,80}=19.8, p<0.001; for DP- in 1.3 times, F_{1,80}=10.2, p<0.001; for BB - in 1.2 times, F_{1,80}=6.56, p<0.01; for TB- in 1.2 times, F_{1,80}=4.46, p<0.03) and in the amplitude of the shoulder and elbow joint angular motion (p<0.001, paired t-tests) (Fig. 3A,B). In additional trials in 6 subjects, we compared the block of the arm in three different positions: neutral, forward and backward. The response of the contralateral (non-blocked) arm depended on this position (RM-ANOVA, F_{2,26}=4.24, p<0.03). Post-hoc analysis (Turkey test) showed that the amplitude of angular motion in the non-blocked arm increased when the other was blocked in the backward position relative to the forward one (p<0.02 for shoulder and p<0.04 for elbow) (Fig. 3C).
Fig. 4A illustrates an example of the observed aftereffect of the long-lasting (30s) isometric effort (Kohnstamm maneuver) in one representative subject. After the termination of the voluntary effort, 75% of subjects (15/20, Fig 4B) showed non-voluntary alternating movements of both arms with appropriate reciprocal activation of proximal arm muscles, rather than a simple tonic arm ‘lift’ as it occurs in the classic Kohnstamm maneuver. Even though the release could provide a small impulse to the arm, it could not account for the observed long lasting arm swinging (Fig. 4A). In addition, the angular changes for the 2-s period following the cessation of the voluntary effort were very small in many cases (<10°) and thus likely could not trigger the response. Typically, the amplitude of arm oscillations first increased, reached a steady state level and then decreased (Fig. 4C). The frequency of arm oscillations was approximately constant from the beginning to the end of movements (Fig. 4A) and similar to that of vibration-induced arm movements (Fig. 2). Even though the effects were bilateral (both arms were involved in arm swinging), muscles performing the isometric contraction showed larger EMG bursts during non-voluntary arm movements (e.g., DP-l vs. DP-r and DA-r vs. DA-l in Fig. 4A). On average, the amplitude of EMG bursts was ~20-30% larger if the muscle was previously involved in the isometric contraction (Fig. 4D). The duration of non-voluntary rhythmic movements varied across subjects from 8 to 60 s (~5-35 cycles), corresponding to a typical time course of involuntary muscle contraction for the Kohnstamm phenomenon (Craske and Craske, 1986). Thus, not only continuous sensory stimulation (Fig. 2, Table 1) but also the Kohnstamm maneuver is able to generate upper limb rhythmicity (Fig. 4).

Involvement of legs in air-stepping

Interestingly, in some subjects, non-voluntary rhythmic arm movements were accompanied by non-voluntary leg movements, when we used either the Kohnstamm (6/15 subjects) or sensory stimulation (8/17 subjects) techniques (Fig. 5). On average, the cycle
duration of arm and leg oscillations was similar (1.8±0.2 s during vibration and 2.0±0.2 s during Kohnstamm effect, Fig. 5B). The phase shift between shoulder and hip joint angular motion varied significantly across subjects. Both diagonal and lateral coordination of the lower and upper limbs could be observed (though in 1 subject the phase shift was not constant since the frequency of leg and arm movements differed). The amplitudes of angular joint motion were similar during vibration and during the Kohnstamm phenomenon (Fig. 5C). The delay of leg involvement with respect to the onset of arm movements varied. During the Kohnstamm maneuver it was small (0-3 s), while during muscle vibration in half of trials it was 0-20 s and in the other half the onset of leg oscillations preceded arm movements by 2-25 s.

Comparison of single arm and leg stimulation

To test the hypothesis that the strength of the contralateral influences for the cervical pattern generator may be weaker (Zehr et al., 2003), we compared the effects of unilateral tonic stimulations for the upper and lower limbs (Fig. 6). All subjects who demonstrated non-voluntary one- or two-legged air-stepping (when tonic stimulation was applied to the leg, Fig. 6) also demonstrated arm rhythmic movements when tonic stimulation was bilaterally applied to the upper limbs (Table 1), suggesting similar responsiveness of cervical and lumbosacral pattern generators. Nevertheless, unilateral tonic stimulation of the lower limb was more effective for the involvement of the contralateral limb (75-93%, Fig 6 right panels) than stimulation of the upper limb (25-30%, Fig. 6 left panels). Rhythmic movements in the contralateral lower or upper limb appeared with some delay (0-15 s in the case of muscle vibration and ~0 s in the case of Kohnstamm effect) and the amplitude of angular movements in the contralateral and stimulated limbs were similar. For the lower limb stimulation, rhythmic movements of both limbs were typically alternating. For the upper limb stimulation, in ~75% of trials they were alternating and in the other 25% they were rather unstable (no specific phase shift). In sum, tonic peripheral and
central stimulation may activate both lumbosacral and cervical circuitries though the strength of the contralateral influences seems to be weaker for the cervical pattern generator (Fig. 6).

Discussion

Even though human walking is primarily bipedal, rhythmic arm muscle activity is often present during natural locomotor-like movements – walking, running, crawling, swimming, and in more recently acquired rowing, cycling, etc. Therefore, it is reasonable to suggest that the cervical spinal cord may possess an ability of sustaining rhythmic activity. The results (Fig. 2-6) confirmed our hypothesis that peripheral and central tonic input is able to activate cervical and lumbosacral circuitries in humans, as it does in animals, and we can thus propose the existence of arm CPG that can be activated alone or with that of the legs (Fig. 5).

The idea that tonic input may activate the spinal CPG circuitry and initiate stepping is not new. The findings corroborate earlier pioneering observations in decerebrated cats that stepping can be induced using a simple tonic stimulation pattern applied to the mesencephalic locomotor region (Shik et al., 1966), but they also show that this type of control can be initiated at the spinal cord levels (Selionov et al., 2009; Gerasimenko et al., 2010). Indeed, in addition to the control of the timing of major phase transitions and muscle activity production during locomotion (Nielsen and Sinkjaer, 2002; Pearson, 2004), sensory activity has access to the functional state of CPG and may initiate a stepping-like output. For instance, electrical stimulation of the dorsal roots may evoke stepping in the decerebrate cat (Sherrington, 1910). The afferent signals due to stimulation of peripheral nerves may increase the excitability of several segments of the spinal cord, which may facilitate triggering of locomotor-like leg movements in humans (Gurfinkel et al., 1998; Selionov et al., 2009; Gerasimenko et al., 2010). Also, due to an asymmetric organization of pattern-generating circuits and sensory feedback,
with flexor circuits having a dominant role (Duysens, 2006; Zhong et al., 2012), one might expect that the first burst to appear after the onset of sensory stimulation would be a flexor burst. Indeed, the first angular deviation occurred more frequently in the forward (shoulder flexion) rather than backward (extension) direction, independent of the muscle being vibrated (Fig. 1D). These results may be consistent with the evolutionary primacy of flexor pattern generation (Duysens et al., 2013; Machado et al., 2015). A particularly interesting result of our study is that stimulation via tonic sensory inputs may not only evoke rhythmic leg movements (when it is applied to the legs) but also upper limb movements (when it is applied to the upper limbs), either in isolation (Fig. 2,3) or along with lower limbs (Fig. 5).

In addition to peripheral sensory stimulation, central tonic facilitatory influences may be used for eliciting limb oscillations. The Kohnstamm manoeuvre provides an interesting experimental model for investigating an excitatory state of neuronal circuits after a sustained isometric voluntary effort, that can last for more than half a minute (Gurfinkel et al., 1989). The *Kohnstamm generator* is thought to be a ‘tonogenic’ central network triggered by either persistent peripheral or central signals. The neurophysiological substrates underlying such behavior may include supraspinal structures, spinal interneurons, motoneurons and enhanced activity of peripheral sensory inputs. For instance, one of the possible neural mediators of prolonged excitation of spinal motor circuits may be serotonin, which has a critical role in activating locomotor rhythms and shaping the pattern of motor output through a long-lasting modulation of plateau properties at the cellular level (Schmidt and Jordan, 2000). A current view on the mechanism of the Kohnstamm movement tends to consider a central contribution, although the involvement of ‘residual’ sensory inputs following the cessation of voluntary contraction has also been demonstrated (Gilhodes et al., 1992; Duclos et al., 2004). For instance, sustained voluntary contraction in one muscle may trigger involuntary motor after-effects in other muscles (Gurfinkel et al., 1989; Duclos et al., 2004), and imagining the effort necessary for the voluntary contraction may induce a long-lasting involuntary arm lift (Craske and Craske,
1986). These studies favor the central origin of the Kohnstamm phenomenon. Indeed, it is unlikely that purely peripheral factors such as thixotropic properties of muscle spindles can account for the observed phenomenon (Gilhodes et al., 1992; Hagbarth and Nordin, 1998) since the aftereffect during air-stepping (Fig. 4-6) would be expected to disappear after 1-2 steps (as muscle thixotropy decreases considerably following muscle shortening/lengthening, Proske et al., 1993). Based on the silent period in EMG activity in response to transcranial magnetic stimulation of the motor cortex during involuntary contraction, it has even been speculated that the Kohnstamm command, like voluntary motor command, may arise upstream of the motor cortex (Ghosh et al., 2014). Whether the Kohnstamm generator is peripherally or centrally driven remains to be clarified; what is important for our study is that a long-lasting tonic input may change the state of the locomotor circuitry and trigger upper limb oscillations (Fig. 4,6B).

The main evidence for the neural coupling between arm and leg movements comes from the presence of phasic arm muscle activity (Ballesteros et al., 1965; Hogue, 1969; Ivanenko et al., 2006a; Barthelemy and Nielsen, 2010; Kuhtz-Buschbeck and Jing, 2012) and modulation of cross-limb reflexes during human walking (Zehr et al., 2009; Dietz, 2011). These reflexes indirectly imply the existence of the cervical CPG in humans, though their properties are in accordance with some features of the upper limb rhythm generation observed in our study. For instance, the strength of the neural coupling (reflex modulation) between the arms during cyclic movements appears to be weaker than between the legs (Zehr et al., 2003), consistent with the weaker effect of unilateral stimulation for arm swinging as opposed to leg air-stepping (Fig. 6). The rhythmogenesis capacity of the cervical spinal circuitry in humans has not been previously reported, to our knowledge. We showed that both peripheral (Fig. 2) and central (Fig. 4) tonic excitability may elicit arm oscillations. Furthermore, the non-voluntary arm cycling rebounded in all participants after the transient block of one arm (Fig. 3). This paradigm is reminiscent of ‘reduced gait’: to reduce the impact of one limb movements while still evoking a locomotor-like rhythmic muscle activation (Van de Crommert et al., 1996; Faist et al., 1999; Ferris et al., 2004).
For instance, with this paradigm, it has been shown that the rhythmic modulation pattern of the biceps femoris tendon jerk reflex observed during normal gait is present also in the rigid held leg despite the reduction in limb movements (Van de Crommert et al., 1996; Faist et al., 1999). In the cat, it was observed that blocking the rhythms is especially effective when the limb is held in a backward position (similar to the present findings, Fig. 3C), and the contralateral rhythm could be enhanced as well (Fig. 3B) (Duysens and Pearson, 1980). Furthermore, during the transient block of one arm, some subjects exhibited a phasic or tonic response in arm muscles (Fig. 3, Table 2), suggesting that the generator continued to operate in the background despite the limb is held stationary, such examples were documented in the literature (e.g., Grillner and Rossignol, 1978; Duysens and Pearson, 1980; Pang and Yang, 2000; Ferris et al., 2004; Sylos-Labini et al., 2014).

Initiation of non-voluntary arm movements could be accompanied by leg movements (Fig. 5). Both diagonal and lateral coordination of the lower and upper limbs could be observed, supporting the hypothesis that the biomechanical factors, such as leg propulsion, trunk torsion, etc., may contribute to the emergence of a specific arm-leg coordination phase during walking (Murray et al., 1967; Collins et al., 2009; Bruijn et al., 2010). Nevertheless, this finding (Fig. 5) favors quadrupedal coordination of human gait (Dietz, 2011) and is consistent with a facilitatory effect of voluntary rhythmic arm movements on leg muscle recruitment during sub-maximal recumbent stepping (de Kam et al., 2013) or leg air-stepping (Sylos-Labini et al., 2014). The results further reinforce the idea that there exists a functional coupling between arm and leg CPGs.

Overall, the findings allow us to speculate about an endogenous pattern generator for the upper limbs in humans and show that nonspecific tonic excitability may elicit or facilitate cervical CPG activity. Nevertheless, caution is required in applying the same logic for non-voluntarily- and voluntarily-initiated stepping movements. For instance, in a recent study we showed that they are dissociable: the H-reflex and motor evoked potentials in response to
transcranial magnetic stimulation of the motor cortex are significantly larger during voluntary than during non-voluntary air-stepping movements at matched levels of EMG activity and angular movements (Solopova et al., 2014). Novel pharmacological strategies (Roy et al., 2012; Borton et al., 2014; Guertin, 2014) and electromagnetic stimulation techniques (Shapkova and Schomburg, 2001; Minassian et al., 2007; Gerasimenko et al., 2008; Selionov et al., 2009, 2013; Angeli et al., 2014) are being developed aimed at modulating spinal activity and restoring the locomotor function. Motor centers in the brain, and the motor cortex in particular, play an essential role in human walking (Capaday, 2002; Yang and Gorassini, 2006; Petersen et al., 2012; Beloozerova et al., 2013), and greater responsiveness to central/sensory inputs during voluntary stepping (Solopova et al., 2014) supports the idea of active engagement of supraspinal motor areas in developing central pattern generator-modulating therapies (van den Brand et al., 2012). Another interesting issue is how specific the arm CPG activation is. Indeed, each individual interneuron, as a rule, receives a broad convergence of various supraspinal and sensory inputs (Hultborn, 2001; Poppele and Bosco, 2003) and therefore may contribute to a vast repertoire of motor actions. It should also be noted that not all participants exhibited non-voluntary limb oscillations (Table 1). As it was emphasized by Sten Grillner (2006), “if CPGs invariably produced rigidly fixed action patterns, animals would behave like automata - stereotypic robots or soldiers in a parade”. In reality, CPG activity depends on a variety of modulatory influences. We do not know why not all participants exhibited rhythmic activity. Non-voluntary CPG activity and coupling between arms and leg locomotor movements is not easily demonstrated in fully conscious humans, thereby explaining why some of the evidence was not seen for all subjects (Table 1, Fig. 5). Nevertheless, large inter-individual differences in humans in the delay and responsiveness of spinal pattern generation circuitry to its activation (Gurfinkel et al., 1998; Selionov et al., 2009; Gerasimenko et al., 2010) and in the motor response to muscle vibration (Eklund and Hagbarth, 1966) have also been reported in previous studies. Thus, the extent to which tonic stimulation or pharmacological interventions imitate the
actual ‘locomotor’ state of the spinal circuitry represents an interesting avenue for future research.

Concluding remarks

In this study we examined whether in healthy humans a CPG network for the arms can be demonstrated. The ability to tap into the cervical neuronal circuits to produce rhythmic outputs may be facilitated by a reduction of external resistance; to this end the limbs in our experiments were suspended reducing any gravity effects or friction. Most participants showed alternating arm movements accompanied with EMG activity upon vibration of different arm muscles and the Kohnstamm (post-contraction) phenomenon. In the former case, rhythmic movements persisted as long as sensory stimulation continued, while in the latter case the duration of rhythmic movements was limited (Fig. 4), in line with a limited duration of the Kohnstamm phenomenon (Craske and Craske, 1986). The rhythmogenesis capacity of the cervical spinal cord (Amemiya and Yamaguchi, 1984), involvement or facilitation of hindlimb stepping (Shik and Orlovsky, 1965; Miller et al., 1975; Gerasimenko et al., 2009) and motor responses to the block of one limb (Duysens and Pearson, 1980) in animals show similarities with the results obtained in our study (Fig. 2-5).

Our findings can motivate new studies to investigate the properties of the cervical pattern generator in humans. In addition to the physiological relevance, they may have clinical implications. For instance, arm movements may increase muscle activity in both paretic and non-paretic legs in stroke patients supporting the inclusion of arm movements in gait rehabilitation protocols (Stephenson et al., 2010). A normal reciprocating arm swing may facilitate lower limb locomotor output in individuals with incomplete spinal cord injury, in whom neural connections in the spinal cord between regions controlling upper and lower limbs were at least partially preserved (Visintin and Barbeau, 1994; Behrman and Harkema, 2000; Kawashima et al., 2008).
Furthermore, the design of human-machine interfaces and neuroprosthetic technologies, such as those of assistive lower limb exoskeletons, may take advantage of the high involvement of shoulder muscles in most locomotor-related movements (La Scaleia et al., 2014). A better understanding of the role that the rhythmogenesis capacity of the spinal cord plays in injured populations might bear important implications for locomotor rehabilitation in neurological injuries which disrupt interlimb coordination (Debaere et al., 2001; Crenna et al., 2008; Zehr et al., 2009; Dietz, 2011; Meyns et al., 2012).

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References


Zelenin PV, Deliagina TG, Orlovsky GN, Karayannidou A, Dasgupta NM, Sirota MG, Beloozerova IN.

Legends to figures

Fig. 1. Experimental setup (general view and three projections) for investigating upper and lower limb rhythmic movements in the unloading condition (A) and schematic definitions for joint angles (B, positive during flexion). A pendulum-like support (3-m ropes) for both arms and legs in a high-ceilinged room was used. The subject laid on her/his right side with the limbs suspended, such that movements were possible only in the horizontal plane. The upper body of the subject was secured through a chest and back fixation, while the head rested on a pillow. The shank and forearm segments of the right upper and lower limbs, respectively, were placed on a long aluminum stick (suspended to the ceiling using two vertical ropes) that permitted motion in the horizontal plane with minimal resistance (bearing was introduced between the limb and the stick). The left limbs were suspended directly to the ceiling by using a long rope.

Fig. 2. Upper limb rhythmic movements evoked by arm muscle vibration. A – an example of arm swing evoked by continuous BB vibration. The arrow indicates the onset of a prominent angular deviation (flexion) in the shoulder joint. B – mean (+SD) cycle duration and amplitude of joint angles across all subjects and conditions (vibration of different arm muscles). C – histograms of the phase shift between left and right shoulders (left) and between elbow and shoulder angles (positive when shoulder leads elbow) across all subjects and probes. Note predominantly alternating left and right arm movements (phase shift ~50%), though in a few cases there were synchronous arm movements (phase shift ~0%). D – direction of the first angular deviation in the shoulder joint (percentage of trials: flexion, extension or ‘undefined’) after the onset of bilateral muscle vibration: left – for each muscle, right – the results for all muscles were pooled together. Note predominantly forward (flexion) rather than backward initial movement, independent of the muscle being vibrated.
Fig. 3. Effect of transient arm block during non-voluntary arm movements evoked by muscle vibration. A – upper limb kinematics and EMG activity in two subjects during the transient block of the arms performed manually by an experimenter. Dashed lines delimit the period of transient arm block. One subject (s1) exhibited a tonic response in the left BB muscle and a phasic response in the DA muscle during the block, while the other (s2) showed a phasic response. All subjects restored non-voluntary air stepping movements after the release of the arm. B – mean amplitude of joint angle oscillations of the contralateral (not-blocked) arm across all subjects. In upper scatter plots each point illustrates the mean joint amplitude (peak-to-peak) before and during the block for each single trial in all participants. Linear regression lines with corresponding r values are reported (dashed lines indicate 1:1 ratios). Bars in the lower panel represent mean (+SD) amplitudes over all subjects. C – mean (+SD) amplitude of contralateral arm oscillations during the block of the arm performed in different (backward, neutral and forward) positions (n=6 subjects). Asterisks denote significant differences.

Fig. 4. Kohnstamm-evoked arm rhythmic movements. A – an example of non-voluntary alternating arm movements after long-lasting (30-s) isometric voluntary contraction of arm muscles (left arm exerted the force in the backward direction and the right arm forward, see schematic drawing on the left). A research assistant stabilized a distal part of the forearm segments of both arms during a voluntary effort. Non-voluntary alternating arm movements were observed for the period of about 20 s after the termination of the voluntary effort. B – pie chart showing the percentage of subjects showing no response and non-voluntary arm oscillations. C – time course of the post-contraction effect on the amplitude and period of non-voluntary rhythmic arm movements in 13 subjects (the data for the left and right arms were averaged). Note a monotonic increment and subsequent decrement of the amplitude of arm motion. D – mean
amplitude of EMG bursts during non-voluntary arm rhythmic movements evoked by the Kohnstamm maneuver across different trials, depending on whether the muscle was involved in the isometric contraction in this trial or not (asterisks denote significant differences).

Fig. 5. Involvement of legs in air-stepping following long-lasting voluntary contraction of arm muscles (Kohnstamm effect) and during arm muscle vibration. A – an example of non-voluntary alternating arm and leg movements after long-lasting (30-s) voluntary contraction of arm muscles (Kohnstamm effect). In this subject, non-voluntary alternating arm movements were observed for the period of about 1 min after the termination of the voluntary effort. B – pie charts showing the percentage of subjects with no leg responses and non-voluntary leg oscillations during Kohnstamm- (left panels) and vibration-evoked (right panels) arm movements. Bars in the lower panels represent mean (+SD) cycle durations and amplitudes of angular movements when both upper and lower limbs were involved in rhythmic movements. Note similar cycle durations for arms and legs.

Fig. 6. Involvement of the contralateral limb during unilateral tonic stimulation of arms (left panels) and legs (right panels). A – vibration induced rhythmic movements of arms (left) and legs (right). B – Kohnstamm-induced rhythmic movements. Pie charts show the percentage of subjects showing involvement of the contralateral limb in rhythmic movements. Note a lower percentage of the contralateral arm involvement, consistent with the weaker neural coupling between the arms than between the legs.
Table 1. Number of subjects that demonstrated non-voluntary upper limb rhythmic movements in response to bilateral muscle (BB, TB, DP and FCR) vibration.

<table>
<thead>
<tr>
<th></th>
<th>BB</th>
<th>TB</th>
<th>DP</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects tested</td>
<td>20</td>
<td>16</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Number of subjects who demonstrated arm swinging</td>
<td>14</td>
<td>12</td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2. The percentage of trials with tonic and phasic responses in BB, TB, DA and DP muscles during the transient block of the arm.

<table>
<thead>
<tr>
<th></th>
<th>BB</th>
<th>TB</th>
<th>DA</th>
<th>DP</th>
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</thead>
<tbody>
<tr>
<td>tonic response</td>
<td>18%</td>
<td>5%</td>
<td>9%</td>
<td>5%</td>
</tr>
<tr>
<td>phasic response</td>
<td>5%</td>
<td>5%</td>
<td>7%</td>
<td>8%</td>
</tr>
</tbody>
</table>
Fig. 2
Fig. 3

A. Subject 1 (TB vibration) and Subject 2 (BB vibration) showing tonic and phasic responses during arm block.

B. Graphs showing shoulder and elbow oscillations during the block of the arm in different positions. Regression lines with equations $y = 1.30x$ and $r = 0.68$ for shoulder oscillations; $y = 1.14x$ and $r = 0.80$ for elbow oscillations.

C. Bar graphs comparing amplitude of contralateral arm oscillations before and during arm block.
voluntary arm muscle contraction

Kohnstamm effect

BB-I
BB-r
TB-I
TB-r
DA-I
DA-r
DP-I
DP-r
shoulder-l
shoulder-r
elbow-l
elbow-r

30 s voluntary arm muscle contraction

10 s

Fig. 4

% of subjects

no response
non-voluntary rhythmic movements

shoulder

elbow

mean amplitude of EMG bursts

DA DP BB TB

involved in Kohnstamm
non involved

mean amplitude of EMG bursts

μV

0 5 10 15 20

0 1 2 3

0 5 10 15 20

0 1 2 3

cycle number

cycle duration, s

amplitude

amplitude
Fig. 5

Kohnstamm effect vibration

no leg movements
non-voluntary rhythmic leg movements

cycle duration, s
0
0.5
1.0
1.5
2.0
2.5

arms
legs

amplitude
0
10
20
30
40
50
60

hip
knee

leg involvement
involvement of the contralateral limb during unilateral tonic stimulation

**A**
left shoulder DP vibration
- BB-l
- TB-l
- DA-l
- DP-l
- BB-r
- TB-r
- DA-r
- DP-r

right leg RF vibration
- RF-l
- BF-l
- TA-l
- GL-l
- RF-r
- BF-r
- TA-r
- GL-r

flex
- shoulder-l
- elbow-l
- shoulder-r
- elbow-r

vibration
10 s

![Diagram A]

no contralateral arm movements
involvement of contralateral arm

**B**
left arm Kohnstamm
- BB-I
- TB-I
- DA-I
- DP-I
- BB-r
- TB-r
- DA-r
- DP-r

left leg Kohnstamm
- RF-I
- BF-I
- TA-I
- GL-I
- RF-r
- BF-r
- TA-r
- GL-r

flex
- shoulder-l
- elbow-l
- shoulder-r
- elbow-r

vibration
10 s

![Diagram B]

no contralateral arm movements
involvement of contralateral arm

voluntary arm muscle contraction
10 s

30 s

Fig. 6