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TITLE: Modulation of motoneurone excitability during rhythmic motor outputs

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Running Head: Corticospinal excitability during rhythmic motor output

Invited review for the special issue on “Nervous System and Exercise”

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Abstract

In quadrupeds, special circuitry located within the spinal cord, referred to as central pattern generators (CPGs), is capable of producing complex patterns of activity such as locomotion in the absence of descending input. During these motor outputs, the electrical properties of spinal motoneurons are modulated such that the motoneuron is more easily activated. Indirect evidence suggests that like quadrupeds, humans also have spinally located CPGs capable of producing locomotor outputs, albeit descending input is considered to be of greater importance. Whether motoneuron properties are reconfigured in a similar manner to those of quadrupeds is unclear. The purpose of this review is to summarize our current state of knowledge regarding the modulation of motoneuron excitability during CPG-mediated motor outputs using animal models. This will be followed by more recent work *initially* aimed at understanding changes in motoneuron excitability during CPG-mediated motor outputs in humans, which quickly expanded to also include supraspinal excitability.

Keywords: corticospinal, motoneuron, central pattern generator, MEP, CMEP

Introduction

Humans move about their environment quite easily, performing seemingly simple motor tasks, such as walking or cycling, without much thought. Despite their deceptive simplicity, however, the neural control of these rhythmic motor outputs is actually quite complex. Consider the number of muscles, bones, and joints throughout the body that must work together in a well-coordinated manner to produce these goal-oriented tasks; tasks that are elegantly controlled by the central nervous system. Indirect evidence exists that spinal circuitries known as central pattern generators (CPGs; (Grillner 1981)) are capable of inducing stepping-like movements in humans (Calancie et al. 1994; Dimitrijevic et al. 1998; Solopova et al. 2014; Solopova et al. 2016), much as they are in quadrupeds. In essence, supraspinal structures activate motor circuitry within the brainstem and spinal cord to ultimately activate spinal motoneurons; neurons that in turn project to and activate skeletal muscle. The motoneuron is the ‘final common path’ of the motor system (Sherrington 1906) and as such developing a fundamental understanding of the factors regulating motoneuron excitability is essential if we are to facilitate one’s ability to move voluntarily.

The majority of research focusing on motoneuron excitability during CPG-mediated motor outputs has been conducted using animal preparations (adult cat, adult rat, neonatal rat) (Krawitz et al. 2001; Power et al. 2010; MacDonell et al. 2015). While providing useful information on the basic mechanisms associated with changes in motoneuron excitability, one of the main goals of animal research is to eventually assist in our understanding of human physiology. This led us to ask the question, “Do motoneurons in humans behave similarly to those in animal experimentations during CPG-mediated motor output?” This is an interesting question that we first asked almost a decade ago and decided to try and address experimentally.

This is not an easy question to address in humans, mainly given the technical difficulties in assessing motoneurone excitability during a rhythmic motor output. This is further complicated by the fact that descending input arising from supraspinal structures likely plays a larger role in the production of CPG-mediated motor output in humans than our quadrupedal counterparts (Barthelemy et al. 2011; Field-Fote et al. 2017).

The final common path

It is the sole path which all impulses, no matter whence they come, must travel if they are to act on the muscle fibres to which it leads. --- Sir Charles S. Sherrington, "The Integrative Action of the Nervous System," (1906)

Alpha motoneurons located within the spinal cord (hereafter referred to as motoneurons) provide an essential link between the central nervous system and motor behaviour. They were referred to as the "final common path" by Sir Charles Sherrington (1906) to emphasize the point that the nervous system must act directly or indirectly on motoneurons to produce movement, independent of its level of complexity. Once seen as having a passive role in motor output, it is now accepted that a complex process of neuronal integration, taking into account not only the passive properties of the motoneurone, but also its active (intrinsic) properties, converts the synaptic drive to motoneurons arising from descending systems, sensory fibres, and the CPG into action potentials during motor behaviour. Changes in motoneurone properties that alter their responses to any or all of these inputs may thus have substantial effects on motoneurone recruitment, firing, and subsequent motor output.

Reconfiguration of motoneurone properties during CPG-mediated motor outputs in quadrupeds

The onset of a rhythmic motor output, such as locomotion, is initiated via descending commands that increase the excitability of spinal interneurons, causing the spinally-located network of neurones, collectively referred to as the central pattern generator (CPG) to oscillate and recruit motoneurons for motor output. Locomotion is a complex motor behaviour characterized by a functional reorganization of intrinsic spinal cord excitability at its onset, creating, in essence, a new functional locomotor “state” within the spinal cord when compared to rest. We now know that the biophysical properties of motoneurons undergo transient, state- and task-dependent changes *prior to and throughout* motor output that would result in enhanced excitability (Krawitz et al. 2001; Power et al. 2010; MacDonell et al. 2015). These changes in motoneurone properties have collectively been referred to as “state-dependent,” in that during a CPG-mediated motor output the motor system is placed in a “state” of enhanced excitability compared to rest.

The following sections provide a brief description of the motoneurone properties that have demonstrated state-dependence, which include reductions in (1) the voltage threshold (V_{th}) for action potential initiation (i.e. V_{th} hyperpolarization) and (2) the afterhyperpolarization (AHP) amplitude, as well as (3) the emergence of voltage-dependent excitation due to activation of persistent inward currents (PICs). These intraspinally-mediated changes have been demonstrated using several experimental models and appear to be conserved across species. Regardless of the experimental paradigm utilized, the aforementioned changes in motoneurone properties would collectively act to reduce the magnitude of excitatory input required to recruit and maintain motoneurone firing and thus, muscle contraction.

Voltage threshold hyperpolarization

Motoneurone V_{th} represents the point at which the inward Na^+ current overcomes the outward K^+ current and an action potential is initiated. Modulation of the V_{th} will have direct effects on the excitability of the motoneurone, either facilitating (i.e. V_{th} hyperpolarized) or inhibiting (i.e. V_{th} depolarized) motoneurone recruitment and firing. Krawitz et al. (2001) provided the first description of a state-dependent hyperpolarization of the V_{th} during brainstem-evoked fictive locomotion in the adult decerebrate cat. Subsequent work has confirmed that the V_{th} hyperpolarizes during CPG-mediated motor outputs in different experimental paradigms including the *in vitro* neonatal rat preparation (Gilmore and Fedirchuk 2004), fictive scratch in the adult decerebrate cat (Power et al. 2010) and fictive locomotion in the adult decerebrate rat (MacDonell et al. 2015). Some prominent features of V_{th} hyperpolarization have been identified using the aforementioned experimental paradigms. First and foremost, V_{th} hyperpolarization appears to be independent of whether the innervated muscle fibres are flexors, extensors or bifunctionals (i.e. can contribute to both flexion and extension) (Krawitz et al. 2001; Power et al. 2010) or whether they are fast or slow (Krawitz et al. 2001; Power et al. 2010; MacDonell et al. 2015), suggesting that all motoneurons, at least in the lumbar spinal cord, are capable of demonstrating V_{th} hyperpolarization.

There is also mounting evidence that the existence and/or degree of V_{th} hyperpolarization does not depend on the *intensity* of the CPG-mediated motor output. Previous work has shown that the amount of V_{th} hyperpolarization is not correlated with the amplitudes of the locomotor (Krawitz et al. 2001) or scratch drive potentials (Power et al. 2010) which provide a general index of the drive to the motoneurone pool (Jordan 1983). In fact, we recently demonstrated that during fictive locomotion in the adult decerebrate rat, V_{th} hyperpolarization is not limited to motoneurons actively engaged in locomotion, but also occurs in motoneurons that were ‘idle’

(i.e. no locomotor drive potentials). Importantly, we also showed that V_{th} hyperpolarization also occurred during the inhibitory components of rhythmic activity. This includes during the approach (flexion) phase of scratch when the membrane potential of extensor motoneurons are tonically hyperpolarized (Perreault 2002; Power et al. 2010) and also during the hyperpolarized phase of rhythmic scratch (i.e. when the flexors are active) when reciprocal inhibition is present (Geertsen et al. 2011). Regardless, V_{th} hyperpolarization still occurred, albeit a larger depolarizing current injection was required, likely to overcome the background inhibition. Thus, not only does V_{th} hyperpolarization occur independent of motoneuron pool activity, but it also occurs in the presence of inhibitory input. This strongly suggests that V_{th} hyperpolarization is an integral part of CPG-mediated motor outputs that may be regulated by a type of priming effect, perhaps by metabotropic sources (Gilmore and Fedirchuk 2004). A detailed discussion of the mechanisms responsible for V_{th} hyperpolarization or the other changes in motoneuron properties discussed are beyond the scope of this review. Two important factors are worth mentioning, however: 1) the neuromodulators serotonin and noradrenaline (Fedirchuk and Dai 2004) have been shown to induce V_{th} hyperpolarization and 2) V_{th} hyperpolarization does not rely on enhanced persistent inward currents (described below) (Power et al. 2012).

Afterhyperpolarization amplitude reduction and the frequency current relationship

Action potentials in motoneurons are followed by a prolonged hyperpolarization, referred to as the afterhyperpolarization (AHP). In the quiescent state, a reduction in AHP amplitude results in an increase in firing frequency or slope of the frequency/current (F/I) relationship. Brownstone et al. (1992) investigated repetitive firing produced by intracellular current injection (control/rest) and compared it with repetitive firing produced during fictive locomotion in the decerebrate cat. They demonstrated that the AHPs evoked at rest were

significantly larger than those produced at similar membrane potentials during fictive locomotion. Similar results were demonstrated during pharmacologically evoked locomotor-like activity using the *in vitro* neonatal rat spinal cord, indicating that AHP amplitude reduction can be mediated via an intraspinal mechanism (Schmidt, 1994). More recent work in adult cat and rat experiments have confirmed that the AHP amplitude is reduced; an effect that persists during fictive scratch following spinal transection (Power et al. 2010), confirming that this effect is intraspinally mediated. Additionally, Brownstone et al. (1992) reported that the F/I relationship was abolished during fictive locomotion and suggested that the AHP does not control rhythmic firing. MacDonell and colleagues (2015) recently demonstrated a reduction and leftward shift in the F/I slope when compared to rest and tonic contraction. Taken together, a reduced AHP amplitude and decreased F/I slope supports the notion that the AHP does little, if anything, to control rhythmic firing during locomotor output in quadrupeds.

Persistent inward currents

A persistent inward current (PIC) is a voltage-gated inward current, carried by Ca^{++} and Na^{+} , that is activated at subthreshold membrane potentials and enhances cell excitability (Hounsgaard & Kiehn, 1985; Hounsgaard & Kiehn, 1989) (Lee & Heckman, 1998) (Harvey *et al.*, 2006). PICs can be visualized using intracellular voltage-clamp recordings and are described as a region of “negative slope conductance” in the current-voltage relationship (Schwindt and Crill, 1977). The term “negative slope conductance” arises from recordings whereby during a linearly rising voltage ramp, there is a strong downward deflection in the net current. If the PIC is large relative to the leak conductance, it induces a region of negative slope conductance in the net current-voltage relationship. An important characteristic of PICs is that the deactivation of PIC on the descending limb of a voltage command occurs at a more hyperpolarized membrane

potential than does PIC activation on the ascending limb, a process known as hysteresis (Heckman *et al.*, 2008). Once activated, PICs exemplify several important characteristics. They act to amplify synaptic input, thus enhancing motoneurone firing rates and can maintain firing upon removal of an excitatory stimulus, a process referred to as self-sustained firing. PICs are more prevalent in extensor than flexor motoneurons (Cotel *et al.* 2009; Wilson *et al.* 2015) a characteristic typically attributed to extensors being anti-gravity muscles associated with posture. The general idea is that PIC activation reduces the need for descending or sensory input to recruit motoneurons and/or maintain their firing.

Brownstone *et al.* (1994) showed that excitatory post synaptic potentials in motoneurons activated via the locomotor CPG demonstrated non-linear changes in amplitude such that their amplitude increased with depolarization. In the same study, motoneurons were studied during fictive scratch to see whether the same effect was present. Depolarizing current pulses did not elicit plateau potentials during control, but did demonstrate voltage-dependent excitation during the active phase of the fictive scratch cycle, work we subsequently confirmed (Power *et al.* 2010).

State-dependent changes in motoneurone excitability are task-dependent

An important feature of the state-dependent changes in motoneurone properties discussed above is that they are seemingly restricted to CPG-mediated motor outputs. The best example of this comes from work done on the cat using fictive scratch and weight support as motor outputs for comparison. During rhythmic scratching of the ipsilateral hindlimb, activity in the contralateral limb is characterized by tonic activity in the extensors (Perreault 2002). We examined the excitability of the same motoneurone during the two different motor outputs and demonstrated that when a motoneurone was engaged in rhythmic scratching the V_{th}

hyperpolarized and the AHP was reduced. When the same motoneurone was active during stance the V_{th} depolarized and the AHP amplitude was unchanged (see Figure 1). It is also important to remember that these state-dependent changes occur prior to the rhythmic activity. Given that state-dependent changes are also task-dependent and occur in both the cat and rat, it appears that increasing the excitability of motoneurons via reconfiguration of their electrical properties is an integral component of CPG-mediated *rhythmic* motor outputs.

-----Insert figure 1 approximately here-----

Modulation of supraspinal and motoneurone excitability during CPG-mediated motor outputs in humans

State- and task-dependent changes in motoneurone properties during quadrupedal CPG-mediated motor outputs is conclusive. Based on those findings, the question we posed that formed the basis of our research program was, “do similar state- and task-dependent changes in motoneurone properties occur in humans during CPG-mediated motor outputs?” In setting out to address this question experimentally we thus had to solve two main methodological dilemmas: 1) what model of a human CPG-mediated motor output could we use to represent fictive locomotion and scratch that have been previously used in reduced preparations (?) and 2) how could we assess motoneurone excitability during rhythmic CPG-mediated motor output(?). The following sections provide a brief overview of the methods we currently employ to address these issues.

Arm cycling as a human model of a CPG-mediated motor output:

Substantial indirect evidence suggests that like quadrupeds, humans also possess spinally located CPGs capable of producing rhythmic motor outputs, such as locomotion and leg or arm cycling (Calancie et al. 1994; Dimitrijevic et al. 1998; Zehr 2005; Solopova et al. 2014; Solopova et al. 2016). Calancie and colleagues (1994) for example, observed involuntary stepping in an individual unable to do so voluntarily following an incomplete spinal cord injury while Dimitrijevic and colleagues (1998) used electrical stimulation of the spinal cord to produce rhythmic activity in the leg muscles of patients with complete spinal cord injury. Over almost two decades, Zehr and colleagues have extensively examined reflex modulation during arm cycling and have quite convincingly shown that arm cycling is at least partially mediated by spinal CPGs, the details of which are beyond the scope of this review (Zehr et al. 2016). When choosing a human-based CPG model of rhythmic motor output to assess corticospinal excitability, arm cycling is an ideal motor output given the head stability and ease with which one can use stimulation techniques to assess corticospinal excitability, the reduced influence of balance related influences and the fact that much of the methodological work completed on the spinal stimulation technique that we use was based on findings in the upper-limb, namely the biceps brachii (see below) (Petersen et al. 2002; Taylor et al. 2002; Taylor 2006). Thus, arm cycling was the motor output we chose to represent the CPG-mediated motor outputs previously used in quadrupeds to examine state- and task-dependent changes in motoneurone excitability.

Because reflex and corticospinal modulation during locomotor outputs are phase-dependent, we also divide the arm cycling motion into flexion and extension phases. Measurements can be taken at any point during the motion, but we typically obtain measurements from the biceps brachii, and more recently the triceps brachii, at two different locations: 6 and 12 o'clock made relative to a clock face. These positions are chosen to represent

periods of high (6 o'clock or mid-elbow flexion) and low (12 o'clock or mid-elbow extension) activity for the biceps brachii with the opposite being true for the triceps brachii. We define flexion as the movement from 3 o'clock (maximal elbow extension) and 9 o'clock (maximal elbow flexion) and extension from 9 o'clock to 3 o'clock.

Assessing corticospinal excitability: transcranial magnetic stimulation

In humans, it is generally accepted that supraspinal pathways, including the corticospinal tract, provide larger contributions to motoneurone activation during CPG-mediated motor outputs than they do in quadrupeds (see (Barthelemy et al. 2011) (Field-Fote et al. 2017)). Thus, as opposed to examining only motoneurone excitability during locomotor outputs in humans, it is also prudent to assess the excitability of descending pathways, the most importance of which is arguably the corticospinal tract. To do so, transcranial magnetic stimulation (TMS) of the motor cortex is used.

TMS-evoked responses are normally recorded from a target muscle as compound muscle action potentials in the surface EMG trace, and are referred to as motor evoked potentials (MEPs) (Rossini et al. 2015). While a variety of MEP properties can be assessed to examine changes in corticospinal excitability across different experimental conditions, typically, changes in the peak-to-peak amplitude of the MEP are used to infer changes in corticospinal excitability (Rossini et al. 2015). Using this measurement, a corresponding increase or decrease in MEP amplitude represents an increase or a decrease in corticospinal excitability, respectively. Since the MEP pathway involves synapses at the cortical and spinal level, as well as at the neuromuscular junction, the size of the MEP depends on the excitability of cortical/spinal neurones and the muscle fibres involved. Therefore, changes in MEPs cannot fully disclose where along the motor pathway the change in excitability is occurring (Rossini et al. 2015). As a

result, an independent measure of spinal excitability is often used in conjunction with TMS to help differentiate whether the change in MEP may be due to supraspinal or spinal factors, assuming peripheral excitability is considered (i.e. responses made relative to $M_{\max}$ to account for changes in peripheral excitability).

Assessing motoneurone excitability: transmastoid electrical stimulation

Also referred to as brainstem or cervicomedullary junction stimulation (Ugawa et al. 1991), TMES involves an electrical current being passed through surface electrodes placed over or near the mastoid processes (Taylor 2006). This electrical stimulation activates the axons of the corticospinal tract that decussate near the cervicomedullary junction. In contrast to TMS of the motor cortex, TMES produces a single descending volley that travels along the corticospinal tract and synapses onto spinal motoneurons; a response that is subsequently recorded from the target muscle via surface EMG. The observed response is known as a cervicomedullary motor evoked potential (CMEP), and changes in the peak-to-peak amplitude of these responses (expressed as a percentage of the maximal compound muscle action potential) is considered the best means currently available to assess motoneurone excitability, particularly at rest and during dynamic motor outputs (McNeil et al. 2013).

TMES-evoked CMEPs reflect a predominantly monosynaptic response of the motoneurons to electrical stimulation of large diameter corticospinal tract axons, and can be performed in awake human participants at rest, during, and/or following voluntary motor outputs (Taylor et al. 2002) (Petersen et al. 2002; McNeil et al. 2013). These are important features in that TMES has allowed us to examine motoneurone excitability *at rest prior to* arm cycling and also *during* arm cycling. In addition, TMES-evoked CMEPs are not influenced by conventional presynaptic inhibition as descending corticospinal axons are not prone to this type of inhibition

(Nielsen and Petersen 1994) and unlike TMS-evoked MEPs, CMEPs are not influenced by changes in cortical excitability (McNeil et al. 2013). CMEPs have been suggested to be the most direct means available to examine a motoneurone's response to synaptic input (Taylor and Gandevia 2004; McNeil et al. 2013).

TMES is thus the technique that we have chosen to assess motoneurone excitability during arm cycling in humans. As previously mentioned, our main muscle of interest is the biceps brachii, which is important because the majority of methodological studies conducted to determine the appropriateness of using TMES as a measure of motoneurone excitability were completed using the biceps brachii (Taylor et al. 2002) (Petersen et al. 2002; McNeil et al. 2013). While our goal is to determine whether similar changes in motoneurons occur in humans as they do in quadrupeds, it is important to note that although TMES-evoked CMEPs provide us with an overall output of the motoneurone pool, they *do not* provide specific information related to V_{th} , AHP amplitudes or PICs. We thus hypothesize, based on the experimental question, whether changes in these motoneurone properties may *contribute* to findings we observe using CMEPs as a surrogate measurement, though concrete conclusions are not possible.

Comparing the neural control between locomotor outputs and tonic contractions

As previously mentioned, the basic, rhythmic patterns of locomotor outputs are thought to be controlled, at least partially, by specialized networks of cells within the spinal cord. In contrast, the activation of these networks is reduced, if not absent, during non-locomotor outputs such as tonic or isometric contractions. A common method to examine the neural control of rhythmic motor outputs is thus to compare a locomotor output to a tonic contraction (Capaday et al. 1999; Pyndt and Nielsen 2003b; Carroll et al. 2006; Forman et al. 2014). In order for this method to be effective in providing information on the neural control of different tasks, however,

the overall output from the motoneurone pool between the tasks must be the same. If the output from the motoneurone pool differs, then the comparison between the two tasks cannot be made since potential changes in corticospinal excitability may now be due to differences in the amount of central drive to the motoneurone pool. Thus, motoneurone pool output between tasks is matched based on the EMG produced in the muscle of interest, given that EMG reflects the output of the motoneurone pool (Carroll et al. 2006; Forman et al. 2014) . If the EMG is matched correctly, then any differences in the excitability measurements between the two tasks can more confidently be attributed to differences in the neural control of the tasks.

Modulation of corticospinal excitability prior to and during the initiation of locomotor outputs in humans

As previously stated, motoneurone excitability in the cat and rat models is modulated *before* CPG-mediated motor output begins (no visible nerve activity). Thus, the first question we set out to examine was whether premovement changes in motoneurone excitability occur in humans. While premovement changes in the excitability of various neural structures had been previously demonstrated – including the cerebellum, basal ganglia, supplementary motor area, and premotor and primary motor cortices – none had combined locomotor output and motoneurone assessment simultaneously. To address this issue, we employed TMS and TMES using a time-preparation protocol in two separate experiments (Power and Copithorne 2013; Copithorne et al. 2015). The protocol consisted of auditory signals used to indicate a warning signal (get ready) which began a 1s preparatory period, followed by a response signal. Both TMS and TMES were administered following the response signal with participants not intending to move (control) or ~50ms prior to EMG onset (pre-cycling). We hypothesized that motoneurone excitability would be enhanced prior to arm cycling, as was the case prior to locomotion and

scratch in the cat. This did not occur (no change in CMEPs), though supraspinal excitability was enhanced as shown via an enhanced MEP amplitude and also via a shortened latency (time from stimulus artefact to MEP onset). Thus, supraspinal but not motoneurone excitability was enhanced prior to arm cycling.

These findings were followed by another premovement study using the same time-preparation protocol, with two important differences. First, work by Geertsen and colleagues (Geertsen et al. 2010) showed that soleus motoneurone excitability was enhanced prior to a tonic contraction when assessed 25 ms prior to EMG onset. We thus reasoned that perhaps we needed to assess spinal motoneurone excitability closer to EMG onset (motor output). Second, in addition to our control (rest) and arm cycling conditions, we added an intensity matched tonic contraction to the experimental condition to allow a direct comparison to arm cycling. Following the cycling trials, participants were required to produce a position and intensity-matched (same EMG between motor tasks) tonic contraction following the auditory cue. As with cycling, TMS and TMES were placed approximately 25 ms prior to the onset of EMG produced by the tonic contraction. Again however, the results showed that motoneurone excitability was not enhanced prior to either motor output (no change in CMEP amplitude or latency). In fact, we also showed, much to our surprise, that although supraspinal excitability was enhanced prior to motor output, it was not different from the intensity-matched tonic contraction (i.e. not task-dependent).

In comparison to quadrupeds, we thus concluded that motoneurone excitability in humans is not enhanced prior to a CPG-mediated motor output, at least not as assessed using TMES. We also concluded that a common neural drive is likely involved in the initiation of both motor outputs, which was especially interesting given that we had recently shown that supraspinal excitability was higher *during* arm cycling than an intensity-matched tonic contraction (Forman

et al. 2014) (see subsequent section). This led to an obvious question - when do task-dependent changes in corticospinal excitability emerge between arm cycling and tonic contraction as an individual transitions from rest to steady-state arm cycling?

To address this question, we examined corticospinal excitability of the biceps brachii from the *onset* of arm cycling to a point where a constant cadence was reached in comparison to an intensity-matched tonic contraction. Corticospinal and spinal excitability were assessed using TMS-evoked MEPs and TMES-evoked CMEPs, respectively at three points throughout the motor outputs: 1) during the initiation of arm cycling, 2) after the completion of the 4th revolution, and 3) after the completion of the 9th revolution (at which time, participants had reached a constant cycling cadence; steady-state). We showed that corticospinal excitability to the biceps brachii was similarly modulated between arm cycling and a tonic contraction at the initiation/onset of the motor outputs and that task-dependent differences (higher corticospinal excitability during arm cycling) became evident after only the 4th revolution. It remains unclear when the transition from common neural drive becomes task-dependent for these motor outputs.

Modulation of corticospinal excitability during locomotor outputs in humans

Recall that motoneurone excitability was higher *throughout* rhythmic motor output (i.e. not phase-dependent) than during a tonic contraction in quadrupeds. Thus, the second question we set out to examine was whether motoneurone excitability was higher *during* CPG-mediated motor output compared to tonic contraction in humans. Prior to a discussion on findings related to motoneurone excitability, however, a brief review of previous work aimed at examining corticospinal excitability during locomotor output is warranted, particularly given the importance of the corticospinal tract in the production of locomotor output in humans (Barthelemy et al. 2011).

Schubert and colleagues (1997) were the first to use TMS to investigate the corticospinal control during a locomotor output. Examining MEPs from the tibialis anterior and the gastrocnemius during walking, the authors reported phase-dependent increases in corticospinal excitability to both muscles. MEPs from the tibialis anterior were largest during the swing phase of walking, while MEPs from the gastrocnemius were largest during the stance phase. The authors suggested that this modulation in MEPs was likely due to increases in EMG from each muscle during the corresponding phase of walking; the tibialis anterior EMG was largest during swing, while the gastrocnemius EMG was largest during stance. However, they noted that facilitation of the MEP was larger in the tibialis anterior (i.e. ankle flexor) than the gastrocnemius (i.e. ankle extensor).

In addition, the authors reported a task-dependent change in corticospinal excitability as MEPs were larger during the corresponding phases of walking than when compared to an intensity-matched tonic dorsiflexion contraction. The authors suggested that the facilitation of the MEPs between tasks could not simply reflect changes in motoneurone activity since EMG was matched, and therefore may be due to supraspinal and/or spinal mechanisms. Similar phase-dependent modulation of corticospinal excitability to the tibialis anterior has been noted by Christensen et al. (2001), who found that MEPs were larger during the swing phase of walking (i.e. when the tibialis anterior is most active) than the stance phase (i.e. when the tibialis anterior is less active). This provides further evidence that corticospinal excitability is modulated in task- and phase-dependent manners during locomotor outputs.

Capaday et al. (1999) performed a similar study in which they examined the corticospinal contribution to the tibialis anterior and soleus muscles during the stance phase of walking and a tonic plantar flexion task. In opposition to the findings from Schubert et al. (1997), the authors

reported a suppression of MEPs from the soleus (i.e. an ankle extensor) during the stance phase of walking, while MEPs from the tibialis anterior were enhanced when both were compared to an intensity-matched tonic plantar flexion contraction. It was suggested that during stance, corticospinal excitability to the tibialis anterior was enhanced due to increased cortical activity, which consequently would bring the tibialis anterior motoneurons closer to threshold. As for the reduction in corticospinal excitability to the soleus, the opposite was proposed. They suggested that during stance, motor cortical activity to the ankle extensor (i.e. soleus) was reduced, which would account for the suppression of the MEP.

Although not discussed in the paper, a potential reason for the observed discrepancy between the two studies could represent a muscle-dependent modulation in corticospinal excitability. The notion of a muscle-dependent modulation of corticospinal excitability during locomotor output is becoming a central theme in our research program, the details of which are discussed in an upcoming section.

During leg cycling, Pyndt and Nielsen (2003) investigated the transmission in the corticospinal and Ia afferent pathways to soleus and tibialis anterior motoneurons throughout the full cycle. They reported that soleus H-reflexes and MEPs were modulated similarly throughout the crank cycle, whereby responses were largest during down-stroke and smallest during up-stroke. In contrast, tibialis anterior MEPs were large during up-stroke and much smaller during down-stroke. Thus, the responses were directly related to the EMG from each muscle during each phase, since during down-stroke the soleus is most active and during up-stroke the tibialis anterior is most active. Additionally, at matching ankle angles and levels of background EMG, the authors found that soleus MEPs were larger during early down-stroke during bicycling compared to a tonic plantarflexion contraction, while soleus H-reflexes were

depressed during late down-stroke compared to tonic contraction. These findings are similar to the modulation observed during walking (Schubert et al. 1997; Capaday et al. 1999), which suggests that a common neural control may exist across locomotor tasks. The authors suggested that task-dependent facilitation in soleus MEPs during early down-stroke may be due to changes in the transmission of the corticospinal pathway, while the suppression of the H-reflex during late down-stroke of leg cycling was likely due to changes in the Ia afferent pathway.

Modulation of corticospinal excitability has also been shown during arm cycling (Carroll et al. 2006; Forman et al. 2014). Carroll and colleagues (2006) used TMS and H-reflexes to record responses from the flexor carpi radialis (FCR) at four different positions during arm cycling (i.e. 3, 6, 9, and 12 o'clock, relative to a clock face). Stimulations were also delivered during position- and intensity-matched tonic contractions. During the mid-flexion phase of arm cycling (6 o'clock position), MEP and H-reflex amplitudes were significantly smaller than when compared to position- and intensity-matched tonic contractions. At the onset of flexion (3 o'clock position), MEPs remained unchanged, while H-reflexes were significantly larger during tonic contraction than during cycling. In addition, TMS facilitated the H-reflex during tonic contractions but not cycling. Thus, the authors concluded that their findings reflected a decrease in the contribution of the motor cortex to the generation of arm cycling when compared to tonic contractions, likely due to the contribution of the spinal CPG during rhythmic arm cycling.

Based on the work discussed herein, it is quite clear that corticospinal excitability during locomotor tasks is modulated in a task-, phase-, and muscle-dependent manner with similar results having been reported using cycling as a model of locomotion (Pyndt et al. 2003b; Carroll et al. 2006; Sidhu et al. 2012; Forman et al. 2014). It was unclear whether the observed changes

484 in TMS-evoked MEPs and/or H-reflexes in those studies could be explained by changes in
485 motoneurone excitability.

486 Sidhu et al. (2012) were the first study to assess motoneurone excitability during
487 locomotor output using a leg-cycling experimental-paradigm. Several, notable findings emerged
488 from that work. First, they showed that subthreshold TMS led to the suppression of ongoing
489 EMG during cycling, indicating that the inhibitory interneurons in the cortex were active during
490 cycling. Second, they showed that TMS-evoked MEPs and TMES-evoked CMEPs were
491 modulated in a similar manner throughout the phase of a full cycle, suggesting that changes in
492 corticospinal excitability were driven by changes at the spinal level (motoneurone). A
493 comparison to a tonic contraction was not performed, however, thus it was unclear whether the
494 changes in corticospinal, and importantly motoneurone excitability were specific to leg cycling;
495 a CPG-mediated motor output.

496 We recently sought to determine whether motoneurone excitability, as assessed via
497 CMEPs, was higher during a CPG-mediated motor output compared to a tonic contraction
498 (Forman et al. 2014), as has been demonstrated in quadrupedal studies. We showed that
499 corticospinal excitability to the biceps brachii was higher during arm cycling than a tonic
500 contraction at the initiation (3 o'clock position) and mid-point (6 o'clock position) of elbow
501 flexion. There was no difference in motoneurone excitability between cycling and tonic
502 contraction at mid-flexion, suggesting higher supraspinal excitability during cycling. At the
503 initiation of flexion, however, motoneurone excitability was higher during cycling. These
504 findings were somewhat surprising. First, given the work of Carroll and colleagues we did not
505 expect corticospinal excitability to be higher during cycling than tonic contraction; a finding that
506 reinforces the concept that the control of CPG-mediated motor outputs is muscle-dependent. It is

possible that the motor control strategies for the FCR – primarily a wrist stabilizer during arm cycling – are different than the motor control strategies for the biceps brachii – a prime mover during arm cycling. Second, unlike quadrupeds, enhanced motoneurone excitability was phase-dependent (i.e. only higher at the initiation of flexion). Recall that during fictive motor outputs in adult cats and rats, motoneurone excitability is enhanced *throughout* the motor output. That is, motoneurone excitability was enhanced during the inhibitory phase of the cycle (when the membrane potential is hyperpolarized and receiving inhibitory synaptic input) and when the motoneurone was inactive or ‘idle.’ Based on this data from the cat, we expected motoneurone excitability to be higher during the elbow extension phase of arm cycling when the biceps brachii was inactive when compared to rest at a similar joint angle. This was not the case, possibility due to reciprocal inhibition arising from the active triceps brachii (Katz et al. 1991) keeping in mind that even in the cat, state-dependent changes in motoneurone properties during the inhibitory phase of scratch and locomotion were evident, but required higher stimulation intensities likely to overcome the background inhibition. Based on this data and evidence that higher leg cycling cadence was shown to reduce reciprocal inhibition (Pyndt et al. 2003a), we assessed motoneurone excitability during the extension phase of arm cycling using different cycling cadences and stimulation intensities. We hypothesized that this combination of high cadence and high stimulation intensities would result in large increases in CMEP amplitudes, resulting from state-dependent changes in motoneurone excitability. The finding was surprising in that the opposite occurred, motoneurone excitability was decreased. The influence of intensity on spinal and corticospinal excitability are further discussed in a later section.

Ultimately, we suggested that the enhanced supraspinal excitability during cycling was due to enhanced motor cortical excitation, potentially in an attempt to ensure adequate

motoneurone recruitment and firing during the propulsive phase of cycling in the biceps brachii. While there are many potential explanations for the observed enhancement of motoneurone excitability at the initiation of flexion, such as changes in synaptic input to the motor pool, it is also possible that motoneurone properties (V_{th} , AHP, PICs) are modulated, as is the case in quadrupeds. A limitation of assessing motoneurone excitability using CMEPs is that they do not provide insight in to these detailed mechanisms but is instead a gross measurement of motoneurone pool excitability in response to synaptic input.

Intensity-dependent modulation of corticospinal excitability during cycling

An increase in motor output intensity will enhance motoneurone recruitment and firing rates, however evidence from animal studies suggests that *reconfiguration* (i.e. state-dependent changes) of motoneurone properties do not depend on the *intensity* of the CPG-mediated motor output, just that the motor output has been initiated (Krawitz et al. 2001; Power et al. 2010; MacDonell et al. 2015). To date, there are only three studies that have examined the modulation of corticospinal excitability as the intensity of a cycling task is altered (Forman et al. 2015; Weavil et al. 2015; Spence et al. 2016). Weavil and colleagues (2015) used TMS and TMES to investigate the effects of increased leg cycling workload on the modulation of corticospinal excitability. Recording from vastus lateralis, participants performed brief constant-load bouts on a cycle ergometer at a constant cadence of 80 rpm, and at various power outputs (100, 200, 300, and 400 W). Stimulations were delivered in a randomized order throughout the cycling bouts at 45 degrees after top-dead-centre, which was chosen because it represented the peak EMG in the vastus lateralis during cycling. Tonic contractions were also performed at matching levels of background EMG. The authors found that increases in workload, via increases in power output, resulted in an increase in corticospinal excitability during cycling, with both MEPs and CMEPs

following a similar pattern. MEPs and CMEPs increased with increasing power output until a plateau was reached, in which further increases in power output did not cause additional changes in MEP and CMEP amplitude. The similar pattern of modulation between MEPs and CMEPs suggests that the observed increase in corticospinal excitability during leg cycling with increasing power output was mainly driven by spinal mechanisms, although the exact mechanism remains unknown. Additionally, MEPs and CMEPs from the rectus femoris increased with increased power output but did not demonstrate a plateauing effect. Thus, there may also be muscle-dependent modulation in corticospinal excitability as intensity increases.

Work from our lab examined corticospinal excitability projecting to the biceps brachii during both the flexion (6 o'clock) and extension (12 o'clock) phases of arm cycling as the cadence was increased (Forman et al., 2015). We reported cadence-dependent changes in overall corticospinal excitability, as both MEPs and CMEPs increased in a similar manner as the cadence increased from 30 to 60 to 90 rpm at the 6 o'clock position. Thus, it was concluded that enhanced spinal excitability during the flexion phase of arm cycling must be partially responsible for the increase in overall corticospinal excitability. However, at the 12 o'clock position, overall corticospinal excitability (MEPs) increased with increased cadence, but spinal excitability (CMEPs) decreased in a manner that was exactly opposite to MEPs. This modulation suggests that at the 12 o'clock position, supraspinal mechanisms must account for the increase in overall corticospinal excitability as the cadence of the motor output is increased. The decrease in spinal excitability, we argued, is likely due to a combination of factors such as reduced afferent excitation resulting from increased presynaptic inhibition (disfacilitation) and/or enhanced reciprocal inhibition arising from triceps brachii activation (inhibition).

576 A recent study from our laboratory examined the effect of arm cycling at different
577 relative power outputs on corticospinal excitability projecting to muscles of the upper limb
578 (biceps and triceps brachii) during arm cycling (Spence et al., 2016). MEPs and CMEPs were
579 recorded from the biceps and triceps brachii of the dominant arm during cycling trials at 5 and
580 15% of each participant's peak power output (PPO), which was calculated from a maximal arm
581 cycling sprint test performed on a cycle ergometer. While cycling at a constant cadence of 60
582 rpm, participants received stimulations at the 6 and 12 o'clock position in a randomized order at
583 each of the two power outputs (i.e. 5 and 15% PPO). We reported both phase- and workload-
584 dependent changes in corticospinal excitability projecting to the biceps brachii, as both MEPs
585 and CMEPs increased in amplitude with power output, with MEPs and CMEPs being larger at
586 the 6 o'clock position than the 12 o'clock. Corticospinal excitability to the triceps brachii was
587 workload- (i.e. higher at the highest power output), but surprisingly did not show a phase-
588 dependent change, while perhaps the most interesting finding was that motoneurone excitability
589 projecting to the triceps brachii was *higher* at the 6 o'clock position during arm cycling than at
590 the 12 o'clock position. This was unexpected since the triceps brachii is most active during the
591 extension phase of arm cycling, as demonstrated by the higher EMG. The lack of phase-
592 dependent modulation in corticospinal excitability combined with enhanced motoneurone
593 excitability suggests that supraspinal excitability is reduced the triceps motoneurone pool during
594 the flexion phase of cycling. Several putative mechanisms are discussed in that manuscript,
595 including the idea that enhanced motoneurone excitability may be due to a stretch induced
596 activation of PICs that would act to amplify synaptic input to the motoneurone pool (Wilson et
597 al. 2015), thus reducing the need for supraspinal input. The findings from this study suggest that
598 corticospinal excitability projecting to antagonistic muscles during arm cycling is modulated

differently as the intensity, via an increase in power output, is altered. This is further evidence that the manner in which corticospinal excitability is modulated during arm cycling depends on the muscle being examined.

Muscle-dependent modulation of corticospinal excitability during locomotor output

A recurring theme when assessing corticospinal excitability differences between locomotor output and tonic contraction is muscle-dependence. Our initial insight was based on our findings compared to Carroll et al. (2006). Whereas Carroll and colleagues reported decreased corticospinal excitability during arm cycling compared to tonic contraction, we showed the opposite when assessed in the biceps brachii. We suggested that the discrepancy between the results was likely due to the role that the target muscle from each experiment plays throughout arm cycling. The biceps brachii is a prime mover and shows distinct active and inactive phases during cycling. In contrast, the flexor carpi radialis acts primarily as a stabilizer of the wrist and is thus more active throughout arm cycling. Our subsequent finding of no phase-dependence in corticospinal excitability to the triceps brachii and the very surprising higher motoneurone excitability during elbow flexion, was again opposite to that in the biceps brachii (strong phase dependence with spinal excitability higher during flexion). Based on these results it appears that cortical input may be of greater importance in the control of flexor motoneurone pools. This theory is indirectly supported by findings from previous work during locomotion. Schubert and colleagues (1997) reported phase-dependent increases in corticospinal excitability to the tibialis anterior and gastrocnemius during locomotion but noted that facilitation of the MEP was *larger* in the tibialis anterior, suggesting greater cortical involvement to the flexors. A similar conclusion was reached by Capaday and colleagues (1999) who showed that MEPs in the

soleus were smaller during the stance phase of locomotion compared to an intensity matched tonic contraction, whereas the tibialis anterior MEPs were larger, even when it was inactive.

Indeed, the corticospinal pathway appears to preferentially influence the flexor portion of the spinal CPG during locomotion in the cat (Dietz, 1992). This seems logical given the higher amount of cortico-motoneuronal monosynaptic connections for flexor motoneurone pools compared to extensors (Brouwer and Ashby 1990) and also the higher amount of PICs in extensors (Wilson et al. 2015). This suggests that during CPG-mediated motor outputs there is greater cortical control of flexors motoneurone pools whereas extensor motoneurone pools do not require the same degree of cortical activation given that they are capable of amplifying synaptic input and self-sustained firing (see Figure 2).

-----Insert figure 2 approximately here-----

Perspective

We originally set out in our research program to examine motoneurone excitability in humans, a line of questioning based on prior work in quadrupeds. To do so, we used, and still use, arm cycling as our model of a CPG-mediated motor output to represent the locomotor outputs examined in quadrupeds. Though our research program is still heavily based on work in quadrupeds, we have begun to include questions that have arisen from our human experimentation that once addressed will enhance our understanding of the neural control of locomotor outputs.

In addition to this basic science approach, we are also aware that arm cycling is frequently used as a rehabilitation tool following injuries and/or disease and is becoming increasingly more popular as an exercise tool in healthy, able-bodied individuals. As with any

rehabilitation and/or exercise paradigm, it is important to know how the movement is generated by the nervous system in order to capitalize on our ability to facilitate neuroplasticity and in some cases, functional recovery. Determining the contributions of supraspinal and spinal activity to the production of a ‘spinally-mediated’ rhythmic motor output (i.e. arm cycling) and how these interactions may be influenced by factors such as cadence, power output, the phase of the motor output and even the muscle of interest, thus becomes crucial. These findings may ultimately prove useful in elucidating mechanisms of impaired motor function and contribute to novel rehabilitation/exercise strategies in a time when ‘personalized medicine’ seems to be gaining traction in our health care system.

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Conflict of Interest

The authors have no conflicts of interest to report.

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Figure Legends

Figure 1. V_{th} is depolarized during fictive weight support and hyperpolarized during ipsilateral scratching. **A** shows continuous ENG records, first during ipsilateral rhythmic scratching evoked by rubbing the ipsilateral ear (8-15s in the run), and then during scratch on the contralateral side evoked by rubbing the other ear (48-58s) in a decerebrate cat without spinal transection. During the approach phase of contralateral scratch (i.e. during tonic activity in the contralateral flexor, coTA) the ipsilateral extensor ipSmAB displays tonic “fictive weight support” activity (48-52s). The intracellular record from a SmAB motoneurone is shown below the ENGs, and representative values of the V_{th} measured throughout the run are shown below. Expanded records of the regions indicated by the grey vertical bars are shown in **B** and **C**. Note that depolarizing pulses of current were delivered to evoke action potentials throughout the run, so evoked firing is present in addition to the spontaneous firing associated with the bouts of fictive scratch. The control V_{th} of -45.6 mV on the left becomes hyperpolarized by -1.8 mV (to -47.4 mV) during the rhythmic phase of ipsilateral scratch (see **B**) and recovers a few seconds after the end of ipsilateral scratch activity. During the fictive weight support that begins at about 48s, V_{th} depolarized by +6.4 mV from the control value immediately before contralateral scratch began (i.e. from -43.3 to -36.9 mV; see **C** for values from individual spikes). Figure reproduced from Journal of Physiology with permission (Power et al. 2010).

Figure 2. Schematic showing several factors influencing the neural control of fictive scratch in the cat and arm cycling in humans. **Top panels:** Fictive scratch in the adult cat (**A**) and arm cycling in humans (**B**). **Bottom panels for A and B:** The amount of input is represented by line

861 thickness and colour in descending order, thick black, thick grey, thin black. The dashed boxes
862 represent spinally located networks and neurons. Abbreviations: CPG, central pattern generator;
863 CST, corticospinal tract; Ext, extensor muscle; Ext MN, extensor motoneurone; Flex MN, flexor
864 motoneurone; Flex, flexor muscle.

Draft

Figure 1

Figure 2

A **B**