Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion

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Sidhu SK, Cresswell AG, Carroll TJ. Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. J Appl Physiol 113: 401–409, 2012. First published June 7, 2012; doi:10.1152/japplphysiol.00486.2012.—The excitability of the motor cortex increases as fatigue develops during sustained single-joint contractions, but there are no previous reports on how corticospinal excitability is affected by sustained locomotor exercise. Here we addressed this issue by measuring spinal and cortical excitability changes during sustained cycling exercise. Vastus lateralis (VL) and rectus femoris (RF) muscle responses to transcranial magnetic stimulation of the motor cortex (motor evoked potentials, MEPs) and electrical stimulation of the descending tracts (cervicomedullary evoked potentials, CMEPs) were recorded every 3 min from nine subjects during 30 min of cycling at 75% of maximum workload (Wmax), and every minute during subsequent exercise at 105% of Wmax until subjective task failure. Responses were also measured during nonfatiguing control bouts at 80% and 110% of Wmax prior to sustained exercise. There were no significant changes in MEPs or CMEPs (P > 0.05) during the sustained cycling exercise. These results suggest that, in contrast to sustained single-joint contractions, sustained cycling exercise does not increase the excitability of motor cortical neurons. The contrasting corticospinal responses to the two modes of exercise may be due to differences in their associated systemic physiological consequences.

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LOCOMOTOR EXERCISES involving multiple limb muscles can compromise both the ability of muscles to generate force and the central nervous system to fully recruit muscles (17, 29, 36, 38, 40). Although there is ongoing debate regarding the mechanisms of central fatigue during tasks that require rhythmic activation of large muscle groups (2, 21, 31), there is evidence to suggest that the output from the motor cortex is insufficient for complete muscle activation as a consequence of fatiguing exercise of this kind (36, 38, 40). The data indicate that sustained locomotor exercise reduces the ability of the motor cortex to drive 1) remote upper limb muscles during exercise (36), and 2) the exercised leg muscles in the recovery period after exercise (38, 40). However, little is known about how the responsiveness of brain and spinal cord projections to exercising lower limb muscles is modulated during sustained locomotor tasks such as cycling.

Noninvasive stimulation of the brain via transcranial magnetic stimulation (TMS) has been widely used to probe the central nervous system and provide direct evidence of changes in responsiveness of the human corticospinal tract during sustained maximal and submaximal single-joint contractions, but not yet during locomotor exercise. Because of the inability to dissociate cortical vs. spinal contributions to any changes in TMS evoked responses, stimulation of the descending tracts at the cervicomedullary junction (CMS) can be used to account for changes in excitability of spinal motoneurons (48). Since the responses to these two methods of stimulation appear to involve many of the same descending axons (23, 49), their comparison allows dissociation of effects at cortical vs. spinal levels of the corticospinal tract. During sustained maximal contractions and submaximal contractions at a constant torque, the responses to CMS [i.e., motor evoked potentials (MEPs)] increase in size over the course of the contraction (28, 42, 45, 46). There is also a similar increase in size of MEPs during repetitive, dynamic single-joint contractions (20), which suggests that increases in corticospinal responsiveness are independent of the type of muscle contraction. In contrast, during the last quarter of a 2-min sustained maximal voluntary contraction (MVC), cervicomedullary evoked potential (CMEP) amplitude decreases (7, 24) or returns to control level (28, 45). Because responses to cortical stimulation increase whereas responses to descending tract stimulation do not, the data imply an increased responsiveness of the motor cortex during sustained maximal contraction. CMEP amplitude also decreases when a constant EMG output is maintained during a sustained submaximal contraction (27), and although there is a progressive increase in size of CMEPs over the course of submaximal single-joint contractions at constant torque (15, 18), this is probably due to a concurrent increase in EMG (19, 27). Furthermore, CMEPs elicited at task failure of a sustained submaximal contraction are smaller than those elicited in brief, prefatigue MVCs, whereas MEPs are of comparable size to those elicited during prefatigue MVCs (15). The results of these studies suggest that cortical responsiveness is enhanced as a consequence of sustained maximal and submaximal single-joint contractions, whereas, for a given level of voluntary EMG, motoneuronal responsiveness is reduced.

Given that the amount of muscle work and associated oxygen demands are typically greater during locomotor than single-joint exercise, there is the potential for considerable differences in systemic responses to single-joint vs. locomotor exercises. Thus the consequences of perturbations to whole body homeostasis such as hyperthermia, respiratory muscle loading, arterial hypoxemia, decreases in cerebral oxygenation and brain catecholamines (4, 8, 33, 37, 43, 44) may have a greater capacity to influence central nervous system drive during exhaustive locomotor exercise. In the present study, we investigated cortical and spinal modulations of corticospinal responsiveness during sustained cycling exercise. This was done via comparisons of responses evoked by TMS and CMS in the locomotor knee extensor muscles at 3-min intervals during a 30 min bout of cycling, and every minute thereafter at a higher intensity until volitional exhaustion. We tested the hypothesis that cortical responsiveness would increase during sustained cycling exercise as occurs during single-
joint contractions, despite likely differences in systemic vascular effects.

**METHODS**

**Subjects.** Nine healthy subjects (7 men, 2 women), with a mean age of 28.7 ± 1.5 yr, body mass of 72.2 ± 1.2 kg, and height of 177.6 ± 0.9 cm were recruited for the study. Each subject gave informed consent prior to the study and completed a health-screening questionnaire for participation in studies involving TMS and endurance exercise. The experimental procedures were approved by the local university ethics committee.

**Electromyogram and electrocardiogram recordings.** Electromyogram (EMG) recordings were taken from the right knee extensors [rectus femoris (RF), vastus lateralis (VL)]. Electrocardiogram (ECG) was used to record electrical activity of the heart to monitor and record the heart rate throughout the study. Areas under the electrodes were shaved, abraded, and cleansed with alcohol swabs. EMG was recorded via bipolar configurations (Ag-AgCl, 10-mm diameter, 2-cm interelectrode distance) positioned over the muscle bellies. ECG was also recorded via bipolar configuration with the anode and cathode positioned ~10 cm apart across the chest. The leads connected to the electrodes were secured at the greater trochanter using medical tape to ensure that there was minimal movement of the leads and to minimize movement artifacts (see Fig. 1). The signals were amplified (200–1,000 times; Digitimer, Neurolog Systems), band-pass filtered (50–2,000 Hz; NL844, Digitimer) and analog to digitally converted at a sampling rate of 2,000 Hz using a 16-bit Micro 1401 mk-II and Spike 2 data collection software via custom-written scripts (Cambridge Electronic Design). Data collected were stored on PC for further analysis.

**Cycle ergometer set-up.** The subjects were positioned on the cycle ergometer with the seat height adjusted and their feet fastened securely into the pedals. They rested their arms on a custom-made adjustable frame securely attached to the front of the ergometer. A chin rest attached to the frame was used to ensure that the upper body and head were kept relatively stable in space during stimulations, which aided consistent application of magnetic stimulation (see Fig. 1A). The crank angle was monitored continuously via a calibrated linear potentiometer coupled to the pedal end of the crank (see Fig. 1B).

**Subjective responses.** Subjects indicated their perceived exertion value every 3 min during both sessions according to Borg scale of 6–20 (6: no exertion; 20: maximal exertion) (6) and were allowed to drink water throughout exercise. Subjects were also required to keep their cycling cadence constant at 80 revolutions per min (rpm) via self-monitoring on a cadence meter in front of the ergometer (mean group cadence during both sessions: 82.2 ± 0.04 rpm).

**Protocol.** Each subject participated in two sessions, an incremental session for determination of maximum workload and a sustained cycling session to perform a fatigue protocol, separated by at least 48 h, during a 1-wk period.

**Incremental session.** The cycling exercise began with a standard warm-up at 1 W/kg body wt for 5 min. The subjects were then given 3 min of rest, after which they were required to perform an incremental cycle exercise test to exhaustion on a mechanically braked cycle ergometer (Lode Rehcor, Lode BV) for determination of maximum workload (Wmax) to set target power output for subsequent test session. The incremental exercise began with 100 W for 3 min, after which power output was increased by 20 W (women) and 30 W (men) every 3 min until volitional exhaustion. In the latter stage of this test, subjects were encouraged to exert themselves maximally. Wmax was taken as the highest 3-min average of power output (mean group workload: 236.7 ± 6.8 W).

**Sustained cycling session.** The session began with warm-up cycling for 5 min at 1 W/kg body wt. The EMG obtained during warm-up cycling was rectified before it was averaged with respect to a “position” signal from the top dead center (i.e., 0°) on the crank cycle during a 20-s segment midway through the bout of cycling. This allowed the determination of the relationship between crank angle and quadriceps EMG activity (see Fig. 1B). A point on the cycle where EMG activity in the quadriceps was close to maximum was chosen for consistently eliciting stimulations at the same position during cycling (mean position: 36.6 ± 2.1° relative to top dead center).

**Motor nerve stimulations.** While subjects remained seated on the cycle ergometer, with their leg positioned at the point on the crank cycle where pulses were elicited for the rest of the experiment, the optimal position at the femoral nerve (located midway between the greater trochanter and iliac crest on the right leg) for eliciting M-waves was ascertained by applying low-intensity single-pulse electrical current (pulse width 500 μs; 50 mA) using a constant-voltage stimulator (model DS7AH, Digitimer). The chosen stimulation position was one that consistently produced the largest M-wave (via direct orthodromic activation of the axon). The optimal stimulation intensity was determined while subjects cycled at a low workload (1 W/kg body wt). The stimulation intensity was increased in 50-mA increments until the size of the M-wave demonstrated no further increase (i.e., maximal M-wave; Mmax). The stimulation intensity was then increased by a further 50% (mean intensity: 227.7 ± 18.7 mA). This was done to ensure a maximal activation of the muscles and was kept constant throughout the session.

**Corticospinal tract stimulations.** Subjects were initially seated on a chair and asked to perform two MVCs of the knee extensors (with 3 min rest in between) against a wall to establish maximum VL EMG and also to set submaximal EMG levels (i.e., 20% of EMG during MVC) for subjects to match to establish the optimal stimulation intensities for TMS and cervicomedullary stimulation (CMS). An electrical percutaneous stimulator was used to activate the cervicomedullary junction at the back of the neck to evoke responses in VL. This was done by passing a high-voltage pulse (duration 100 μs, D-185 mark IIa, Digitimer) between a set of self-adhesive electrodes attached to the skin in the groove between the mastoid process and the occiput (1–2 cm posterior and superior to the tip of the mastoid processes with the cathode on the left, contralateral to the right limb muscles). The intensity of the stimulator was set to produce ~10% of Mmax size (mean intensity: 450 ± 12.4 V) during a submaximal contraction (20% of maximum EMG measured during MVC). Following this, a transcranial magnetic stimulator (model 200T, The Magstim) with a concave double-cone coil (130-mm diameter) was used to elicit MEPs in the right knee extensors during contractions of the same strength (i.e., 20% of maximum EMG measured during MVC). The junction of the figure-eight was aligned tangentially with the sagittal plane, with the center of the coil 1–2 cm to the left of the vertex. The optimal coil position (with posterior-to-anterior induced current flow within the cortex) to elicit responses in the VL was determined prior to the experiment. The position was marked directly on the scalp for accurate placement throughout the session. The intensity of the stimulator (mean intensity: 41.4 ± 0.9%) was set to produce a MEP of similar size to CMEP (i.e., ~10% of Mmax).

**Control and sustained cycling.** Subjects performed four control bouts: two bouts each at 80% and 110% of Wmax (i.e., each workload was performed in alternate order and each bout lasted 1 min with at least 3 min rest in between to avoid the effects of sustained cycling on control measurements). One set of stimulations (see below) was elicited in each bout. After the completion of control bouts, subjects were required to cycle at 75% of Wmax for 30 min. Before the end of each 3-min phase (~40–50 prior), one stimulation set (see below) was elicited. At the end of 30 min of cycling, the workload was increased to 105% of Wmax and subjects were asked to sustain this workload until task failure. One set of pulses was elicited every minute during this phase of cycling (see Fig. 1C).

**Stimulation set.** One set of stimulations encompassed five TMS pulses, one CMS, and one motor nerve stimulation (MNS) during cycling at the selected point of stimulation (see Fig. 1C). The five TMS pulses were randomized with CMS, while MNS was always
Fig. 1. Experimental set-up and schematic diagram of control and fatigue cycling during the sustained cycling session. A: diagram of experimental set-up. Throughout the study, a transcranial magnetic stimulation (TMS) coil was held stable at optimal position on the motor cortex by an experimenter. Self-adhesive electrodes attached to the groove between the mastoid process and the occiput were used to activate the descending tracts at the cervicomedullary junction (i.e., cervicomedullary stimulation; CMS). Motor nerve stimulations (MNS) were elicited at the femoral nerve (position is represented by circle at the femoral triangle). Subjects were required to rest their chin on a chin rest during all stimulations. Responses were measured from vastus lateralis (VL) and rectus femoris (RF). B: crank angle (in degrees after top dead center) and EMG from VL and RF during cycling in a single subject. Stimulations were consistently elicited at a fixed position (represented by dotted line) on the crank angle (which was determined via a calibrated potentiometer attached to the pedal) where EMG was close to maximum in the quadriceps. C: experimental protocol for sustained cycling. Subjects were required to cycle at 75% of maximum workload (W\textsubscript{max}) for 30 min. At the end of 30 min of cycling, the workload was increased to 105% of W\textsubscript{max} and subjects were asked to sustain this workload until task failure. One set of stimulations (represented by an arrow) was elicited before the end of every 3 min during sustained cycling. Each set of stimulations encompassed eliciting 5 TMS pulses, 1 CMS, and 1 MNS during cycling at the selected point of stimulation. The 5 TMS pulses were randomized with CMS, while MNS was always elicited at the end of each set. Each of these pulses was elicited at a random cycle separated by at least 5–6 s. At the end of each stimulation set, subjects were asked to provide a rating of perceived exertion (RPE) according to the Borg Scale. Measurements made every 9 min (3 × 3 min = 9 min in each phase) were pooled and averaged into 3 phases. Responses elicited in the last sustained phase (irrespective of the number of additional bouts the subject could sustain beyond the 30 min exercise) were averaged as a single time point named “task failure.”
elicted at the end of each set. We chose to only elicit one CMS in every set because of the high levels of stimulation intensity essential to activate the corticospinal axons that are imbedded quite deep beneath the skin and the scalp and the consequent discomfort associated with it (48). MNS was always elicited at the end of each set because electrical stimulation of the nerve tended to cause considerable disruption to the normal activation pattern during cycling. Thus, to prevent any confounding effects on the corticospinal tract stimulations, it was always elicited at the end. The cycle during which each pulse was elicited was randomized and separated by at least five full cycles. Each set of stimulations lasted ~30 s.

Data analysis. Data were analyzed off-line. The peak-to-peak amplitude of the evoked responses (i.e., MEPs, CMEPs, and Mmax) and peak-to-peak duration of the Mmax response was measured using custom-made Spike 2 scripts. The cycling EMG signal was rectified and waveform average analysis was performed on a 10-s segment toward the end of each 1 min (control bouts) or 3-min phase (sustained cycling bouts) just before the stimulation set was elicited. The reference point for overlaying and averaging was taken as the same point on the crank angle that was selected for eliciting stimulations during cycling (in the test session). Average EMG was measured for a 100-ms time window (50 ms prior to and 50 ms after the selected point) from the waveform average. Average EMG was also measured during the MVCs from a rectified EMG segment 1 s in duration (at the point of peak EMG).

For control cycling, responses from the two bouts at each of the workloads (i.e., 80% and 110% Wmax, C-80 and C-110) were pooled and averaged. During the 30-min sustained cycling, measurements made every 9 min (3 × 3 min = 9 min in each phase) were pooled and averaged into three phases (see Fig. 1C). Responses elicited in the last sustained phase, irrespective of the number of additional bouts the subject could sustain beyond the 30 min exercise, were averaged as a single time point named “task failure.” All data are reported as means ± standard error of the mean (SE).

Statistical analysis. Paired t-tests were used to test for differences between 1) MEPs and CMEPs during control bouts and 2) maximum heart rate between the two sessions. One-way ANOVAs, with repeated measures on the factor “exercise bout” (i.e., C-80, C-110, phases 1–3, and task failure), were conducted to examine the effect of the sustained cycling exercise on the following measurements: heart rate, rating of perceived exertion (RPE), cycling EMG, and Mmax. Two-way ANOVA, with repeated measures on the factors “exercise bout” (i.e., C-110, phases 1–3 and task failure) and “stimulation type” (TMS, CMS), were conducted for 1) MEPs and CMEPs normalized to Mmax to account for activity-dependent changes in the muscles and 2) MEPs and CMEPs normalized to cycling EMG. Since EMG during the three phases of sustained cycling was not significantly different from control bouts at C-110, only C-110 was included in the two-way ANOVA. Paired t-tests were used to test for changes between the control bout and each fatigue bout when indicated by significant main and interaction effects (phases 1–3 and task failure). Statistical significance was set at P ≤ 0.05.

RESULTS

Cycling duration. All subjects successfully completed the prescribed steady-state exercise (75% Wmax for 30 min; 177.5 ± 5.1 W). On average, the subjects sustained the cycling exercise for 31.3 ± 0.2 min, including the additional cycling at 105% Wmax until task failure.

Psychophysiological responses. One-way ANOVA revealed that both heart rate and RPE modulated significantly across sustained cycling bouts (F_{5,40} > 6.7; P < 0.05; Table 1). Heart rate throughout the sustained exercise bouts (i.e., from phase 1 to task failure) was significantly greater than that recorded during C-110 (t > 7.9; P < 0.05). At task failure, heart rate was at 97.6 ± 1.3% of maximum recorded in the last bout of the incremental session with no significant difference between the two sessions (t > 1.4; P = 0.2). Compared with C-110, RPE was significantly less during the first phase of the 30-min cycling exercise (t > 2.5; P < 0.05) but similar in the second phase (t > 1.2; P = 0.26). At phase 3 and task failure, RPE was significantly greater than that during C-110 (t > 3.0; P < 0.05). At task failure, perceived exertion was 19.9 ± 0.7, indicating that all subjects had reached a high level of subjective exertion.

Average rectified electromyogram. One-way ANOVA revealed that average cycling EMG amplitude modulated during exercise in both VL and RF (F_{5,40} > 8.5; P < 0.05). EMG during the three sustained exercise phases (i.e., up to 27 min of exercise at 75% Wmax) was significantly greater than C-80 in both VL and RF (t > 3.5; P < 0.05) but similar in magnitude to C-110 (t > 1.7; P > 0.05). At task failure, average cycling EMG in both VL and RF was significantly greater than C-110 (t > 2.6; P < 0.05; Fig. 2, A and B).

Peripheral changes. Figure 3 shows the raw traces of EMG responses in VL for one representative subject during phase 1 of the sustained exercise and at task failure and demonstrates a decrease in the Mmax response. One-way ANOVA revealed that the amplitude of the Mmax response modulated across the exercise bouts in VL (F_{5,40} = 6.5; P < 0.05; Fig. 2C) but not in RF (F_{5,40} = 0.82; P = 0.53; Fig. 2D). Specifically, responses in VL were significantly reduced during phase 3 and at task failure compared with responses during C-110 (t > 3.0; P < 0.05; Fig. 2C), indicating peripheral changes. The duration of the Mmax response did not change significantly across bouts in either VL (F_{5,40} = 2.1; P > 0.08; Fig. 2E) or RF (F_{5,40} = 1.0; P = 0.43; Fig. 2F).

Changes in MEP and CMEP (normalized to Mmax). The amplitude of the MEPs and CMEPs evoked in both muscle groups was large relative to the maximal M-wave (~50% Mmax), indicating that the corticospinal tract stimulations (i.e., TMS and CMS) activated a high proportion of knee extensor motor units during cycling (Fig. 4, A and B). Averaged across subjects and muscles, the mean amplitude of MEPs in phase 1 of the fatigue task was ~87% of the amplitude during the control cycling bouts (C-110), and ~100% of C-110 at task failure. In contrast, CMEP amplitude was ~100% of C-110 in phase 1 of the fatigue task, and ~120% of C-110 at task failure. There were no significant differences between MEP and CMEP amplitudes at C-80 or C-110 in either muscle group (t < 0.7; P > 0.18). During sustained exercise, there was no significant interaction between stimulation type and bout (F_{4,32} < 2.3; P > 0.05; Fig. 4, A and B).

### Table 1. Group mean data of heart rate and rating of perceived exertion

<table>
<thead>
<tr>
<th>Bouts</th>
<th>HR</th>
<th>RPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-80</td>
<td>122.7 ± 1.5</td>
<td>12.6 ± 0.2</td>
</tr>
<tr>
<td>C-110</td>
<td>131.1 ± 1.9</td>
<td>15.6 ± 0.3</td>
</tr>
<tr>
<td>Fatigue phase 1</td>
<td>155.7 ± 1.6*</td>
<td>14.1 ± 0.2*</td>
</tr>
<tr>
<td>Fatigue phase 2</td>
<td>163.0 ± 1.4*</td>
<td>16.3 ± 0.2</td>
</tr>
<tr>
<td>Fatigue phase 3</td>
<td>166.0 ± 1.3*</td>
<td>17.7 ± 0.2*</td>
</tr>
<tr>
<td>Task failure</td>
<td>177.2 ± 1.9*</td>
<td>19.9 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. C-80 and C-110 represent control bouts at 80% and 110% of maximum workload (Wmax), respectively. HR, rate; RPE, rating of perceived exertion. *Significant difference in values during the exercise phases from control bout values at C-110: P ≤ 0.05.
and no significant main effect of bout ($F_{4,32} < 1.6; P > 0.05$; Fig. 4, A and B) or stimulation type ($F_{1,8} < 1.5; P > 0.05$; Fig. 4, A and B) for responses elicited in both VL and RF.

Changes in MEP and CMEP (normalized to EMG). Since centrally evoked responses could be influenced both by fatigue and changes in background muscle activity, MEPs and CMEPs were normalized to background EMG for further analysis. MEPs decreased from $\sim 83\%$ of C-110 at phase 1 to $66\%$ of C-110 at task failure. In contrast, CMEPs decreased from $\sim 97\%$ of C-110 at phase 1 to $86\%$ of C-110 at task failure. There were no significant differences between normalized MEPs and CMEPs during C-80 and C-110 bouts in either muscle group ($t_{8} < 0.4; P > 0.46$). There was no significant interaction between stimulation type and bout ($F_{4,32} < 0.9; P > 0.05$; Fig. 4, C and D) and no significant main effect of stimulation type ($F_{1,8} < 1.6; P > 0.05$; Fig. 4, C and D). There was however a significant main effect of bout on responses elicited in both RF and VL ($F_{4,32} > 4.5; P < 0.05$; Fig. 4, C and D). Although not strictly justified due to the lack of a significant interaction (30), compared with responses at C-110, MEP responses from phase 2 to task failure were significantly lower in both muscle groups ($t_{8} > 2.2; P < 0.05$; Fig. 4, C and D). In contrast, CMEPs were not significantly different from responses at C-110 throughout exercise in both muscle groups ($t_{8} < 1.8; P > 0.05$; Fig. 4, C and D).
DISCUSSION

Main findings. The aim of the present study was to investigate the changes in cortical and spinal responsiveness caused by sustained cycling exercise in relation to the locomotor muscles. The data provide evidence that during the 30-min steady-state sustained cycling exercise, the responsiveness of the motor cortex and motoneurons was similar to baseline. This pattern of change in cortical and spinal responsiveness differs markedly from that observed during sustained single-joint contraction, where corticospinal excitability increases substantially (15, 26, 28, 45). The results suggest differences in cortical and spinal responses to sustained single-joint vs. locomotor exercises.

Cycling EMG and muscle excitability. Cycling muscle activity (EMG) during the steady-state fatiguing exercise at 75%
of $W_{\text{max}}$ was not different from control bouts at 110% of $W_{\text{max}}$. This indicates that additional motor units were recruited in the first few minutes of exercise via an increase in motoneuronal drive (5) to sustain the exercise at a relatively lower workload (i.e., 75% of $W_{\text{max}}$). EMG amplitude remained relatively constant during the 30-min cycling bout, however, indicating that even though exercise was becoming progressively more difficult (shown via increases in psychophysiological responses), additional motor units were not recruited. At task failure, an additional increase in EMG activity indicated an increase in the number of units recruited and/or their firing rates to counteract fatigue of the exercising muscles (5, 12) and the increased prescribed workload.

To provide evidence for peripheral changes within the muscle, the amplitude and duration of the responses to motor nerve stimulation were monitored during the course of the cycling exercise. A reduction in the amplitude of $M_{\text{max}}$ in VL in the last two phases of exercise suggests that sarcolemmal excitability was reduced as a consequence of fatigue. Previous studies have shown reduction in M-wave amplitude in the last 2 h of long-duration (i.e., 5 h) cycling and running exercises (34) and at the end of 1.5 to 2 h exercise (16, 35). The present study provides new evidence of reduction in M-wave amplitude during a 30-min high-intensity cycling exercise whereby M-wave responses were measured at the same crank position during the course of cycling with no disruption to the task. Consequently, changes in M-wave properties demonstrated within the present study have greater relevance to the task and instantaneous biochemical state of the muscle. Since a similar reduction was absent in RF, it is also evident that there were intermuscle differences in the extent of peripheral changes.

Central responsiveness. The lack of change in MEPs and CMEPs suggests that the responsiveness of the corticospinal pathway was not modulated during the steady-state sustained cycling exercise. This pattern of change is in contrast to that observed during sustained single-joint contraction, where corticospinal excitability increases substantially (15, 26, 28, 45). In contrast to responses elicited during constant-torque contractions (15, 18), recent evidence showed that when a constant-EMG contraction was held, CMEPs were reduced in size while MEPs remained unchanged during the course of a contraction (26). In addition to confirming previous evidence that cortical excitability is enhanced relative to spinal changes during single-joint contractions, the results indicated that the evoked responses are partly sensitive to progressive increases in EMG levels during a constant-torque contraction (25). Interestingly, when we normalized MEP and CMEP amplitudes to cycling EMG as a crude control for motoneuron output in the present study, we found that CMEPs were not different throughout exercise while MEPs were significantly reduced from phase 2 to task failure of the exercise. While post hoc tests of differences in modulation of MEPs vs. CMEPs are not strictly justified due to the lack of an interaction effect, the pattern suggests that, if anything, there was a tendency toward reduced cortical excitability, both during the steady-state phase of a sustained cycling bout and at task failure. This pattern of changes contrasts markedly with results for single-joint exercise.

The differences in corticospinal responsiveness observed during sustained locomotor exercise vs. during sustained single-joint exercise (15, 28, 45) indicate that the responsiveness of human corticospinal tract neurons to fatiguing exercise is task specific (47). During the sustained 30-min exercise in the present study, there was progressive increase in psychophysiological demands, as evidenced by both a significant increase in heart rate and RPE. At task failure, both of these variables were close to maximum, indicating that the subjects had reached high levels of psychophysiological stress. Given that cardiorespiratory demands are greater during exhaustive locomotor exercise compared with that of single-joint exercise (1, 4, 11), there is greater potential for factors such as temperature, blood glucose, catecholamines, and cerebral oxygenation to regulate homeostasis during locomotor endurance exercises (10, 13, 32, 36, 39). These factors may differentially influence the responsiveness of motor cortical cells to fatigue induced by locomotor vs. single-joint exercise.

It is also interesting that a pattern of reduced MEP size and increased CMEP size has been reported when pain was induced artificially by injecting hypertonic saline to cause increased activity in group III and IV afferents during single-joint contractions (25). Thus a lack of an increase in cortical responsiveness in the present study might be related to exaggerated activation of group III and IV afferents during exhaustive locomotor exercise. A possible mechanism for a reduction in the excitability of cortical neurons in the presence of increased input from group III and IV afferents is an increase in intracortical inhibition. For example, sustained single-joint contractions typically result in a lengthening of cortically evoked silent periods, which reflects an increase in intracortical inhibitory activity. However, silent period lengthening is attenuated when feedback from muscle afferents is blocked via lumbar anesthesia after single-joint fatiguing contractions (14), which suggests that fatigue-sensitive afferents may influence cortical responsiveness via modulating intracortical inhibition. There is also evidence via the twitch interpolation technique that when a muscle is held ischemic at the end of a fatiguing single-joint contraction, the output from the motor cortex remains insufficient to activate the muscle maximally, possibly via increased input from fatigue-sensitive afferents acting at the supraspinal level (9). A more recent study also reported a reduction in cortical activation of the remote elbow flexors during leg cycling exercise in hypoxia (36). Although the capacity of the motor cortex to drive the muscles does not always parallel changes in the responsiveness of the corticospinal neurons (11, 40–42, 44), it is possible that the mechanisms related to oxygen availability either originating centrally (4) or from within the exercising muscles (3) may have contributed to the lack of an increase in excitability of cortical cells observed in the present study. Alternatively, the lack of an increase in excitability of the cortical cells may also be related to intrinsic brain regulation mechanisms associated with an increased internal sense of effort (21) and/or mental stress and fatigue (22).

Conclusion. In conclusion, the present results show that the sustained cycling exercise induced considerable psychophysiological stress and significant effects on the responsiveness of both the corticospinal pathway and the exercised muscles.
After accounting for changes within the muscle, responses evoked by stimulation at the motor cortex and spinal cord failed to increase. This suggests a lack of an increase in the responsiveness of motor cortical cells during sustained locomotor exercise, possibly via increased effectiveness of intracortical inhibitory mechanisms.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.K.S., A.G.C., and T.J.C. conception and design of research; S.K.S. performed experiments; S.K.S. collected and analyzed data; S.K.S. and T.J.C. interpreted results of experiments; S.K.S., prepared figures and tables; S.K.S. drafted manuscript; S.K.S., A.G.C., and T.J.C. edited and revised manuscript; S.K.S., A.G.C., and T.J.C. approved final version of manuscript.

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