Muscle fatigue is characterized as a reduction in the maximal force exerted by a muscle or a muscle group (21). The force-generating capacity of muscles becomes progressively impaired during exercise and gradually recovers once exercise is terminated. The degree of fatigue and the duration of recovery depend on the intensity and type of exercise performed. Fatigue can be caused both by peripheral factors within the muscle and central factors that alter the capacity of the central nervous system to drive the motor neurons (e.g., Refs. 3, 9). Peripheral fatigue can be established if the amplitude of a resting twitch response to supramaximal stimulation of the motor nerve is reduced. The mechanisms may include the accumulation of intramuscular metabolites, muscle damage, depletion of energy substrates, and disturbances in excitation-contraction coupling occurring at or distal to the neuromuscular junction (1, 19, 20, 49). Central fatigue is indicated when electrical stimulation of the peripheral motor nerves, or magnetic activation of corticospinal output to motoneurons, during a maximal voluntary contraction (MVC) produces a larger twitch than was elicited before exercise (22). An increase in this “superimposed” twitch demonstrates that a larger proportion of motor units received insufficient drive to produce tetanus. Thus the voluntary activation of the muscle is reduced.

Deficits in voluntary activation identified with the conventional method of electrical nerve stimulation could be mediated at any site proximal to the motoneuron axons (i.e., including reflex, spinal, brainstem, or supraspinal circuits). Thus the exact site of central fatigue cannot be deciphered with motor nerve stimulation. More specific information regarding the site of central fatigue can be obtained by using transcranial magnetic stimulation (TMS), rather than (or in addition to) conventional motor nerve stimulation (21). An increase in superimposed twitch amplitude implies a failure of drive brought about by factors located proximal to the point of stimulation. Thus if extra force is evoked by TMS (i.e., motor cortical stimulation), corticospinal cell output must be inadequate to maximally activate motoneurons. This indicates that central fatigue has a supraspinal component. Supraspinal fatigue has been demonstrated after maximal and submaximal isometric contractions (53, 54, 64, 65), hyperthermia (63), and lengthening contractions (42). However, the site(s) of impairment in central drive during a maximal muscle contraction after locomotor cycling exercise is not known (e.g., Refs. 30, 31).

The relative importance of central and peripheral factors in the development and recovery of fatigue is influenced by the type of exercise performed (for review, see Ref. 61). Compared with the effects of sustained maximal contractions of a relatively short duration (22, 58), supraspinal fatigue is more dominant and takes longer to recover after prolonged isometric contractions at submaximal intensities (53, 54). In particular, prolonged isometric contractions at very low forces induce supraspinal fatigue of a longer duration (i.e., 5% MVC, at least 29 min; Ref. 53) than prolonged isometric contractions at higher forces (i.e., 15% MVC, ~3 min; Ref. 54). In contrast, maximal contractions induce more peripheral fatigue for a similar reduction in MVC (e.g., Refs. 22, 65). The differences in fatigue properties could be due to variations in the extent of motor unit recruitment, blood perfusion, and afferent feedback (for review, see Ref. 61).

Central fatigue and peripheral fatigue are also induced by locomotor running or cycling exercises of relatively long (i.e., 90 min to 4 h; Refs. 30, 36, 41, 45) and short durations (i.e., 5

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to 30 min; Refs. 6, 31, 44, 46). Although reductions in the force-generating capacity of muscle fibres contribute to locomotor fatigue directly, some of the mechanisms of fatigue in “whole body” locomotor exercise may differ from those associated with isolated muscle contractions. The factors that limit central drive during locomotor exercise are controversial (3, 32); however, there is a greater potential for factors related to the regulation of whole body homeostasis (e.g., temperature and blood glucose control) to influence central drive during and after locomotor exercise. For example, it has been suggested that decreases in cerebral oxygenation (8, 48, 51, 57) and brain catecholamines (25) might contribute to central fatigue.

The aim of the current study was to determine whether a failure of drive from the motor cortex contributes substantially to the reduction in the force-generating capacity of the human knee extensor muscles after locomotor exercise. Voluntary activation was assessed using motor nerve and motor cortex stimulation during brief (2 s) and sustained MVCs (30 s) before and after a series of eight, 5-min bouts of cycling. We tested the hypothesis that locomotor exercise would induce supraspinal fatigue of a considerably longer duration than that which typically occurs after isolated muscle contractions of high or moderate force.

METHODS

Subjects. Ten moderately active, healthy subjects (5 males; 5 females), with a mean age of 22.6 ± 1.4 yr, body mass of 62.2 ± 2.8 kg, and maximum aerobic capacity (\(V\dot{O}_2\) max) of 45.8 ± 1.8 ml·kg\(^{-1}\)·min\(^{-1}\), were recruited for the study. The experimental procedures were explained to the subjects both verbally and in an information document. Each subject gave informed consent before the study and completed a health-screening questionnaire for participation in studies involving TMS and locomotor cycling exercise. The University of New South Wales Human Research Ethics Committee approved the experiment procedure, which conformed to the Declaration of Helsinki.

Experimental protocol. Each subject participated in three sessions (familiarization session, control session, and exercise session) separated by at least 48 h during a 2-wk period. The control and the exercise sessions were performed in a random order under normal environmental conditions (temperature: 22.6 ± 0°C; relative humidity: 46.9 ± 1.5%) at the same time of the day. In the exercise session, subjects performed a series of isometric knee extension contractions before and after locomotor cycling exercise. In the control session, subjects performed the same series of isometric knee extensions but rested for a period equivalent to the duration of the exercise. The protocol is described schematically in Fig. 1. Note that postexercise testing times are expressed relative to the first brief contraction set after exercise (0 min). This set was performed as soon as possible after the completion of the final cycling bout (mean time = 2.6 ± 0.2 min).

Familiarization session. An incremental cycle exercise test to exhaustion was performed on a mechanically braked cycle ergometer (Danpri; Melbourne, Australia) equipped with SRM training system (Germany) for determination of the maximum workload (Wmax) and \(V\dot{O}_2\) max (ml·kg\(^{-1}\)·min\(^{-1}\)). The reliability of the SRM training system for measurement of power output in cycling has been reported (24). The test began with a warm-up at 1 W/kg body wt for 3 min, after which the power output was increased by 20 W (female) or 30 W (male) every 3 min until volitional exhaustion. \(V\dot{O}_2\) max was taken as the highest 30-s average of oxygen uptake, and Wmax was taken as the highest 3-min average of power output. The optimal nerve and TMS stimulus intensities were determined, and the subjects were familiarized.
ized with the two types of contractions (brief and sustained sets) that were performed during the subsequent sessions.

**Motor nerve stimulation.** Single electrical stimuli (200-μs duration) were delivered to the femoral nerve via self-adhesive electrodes using a Digitimer D57AH stimulator (Welwyn Garden City, Hertfordshire, UK). The cathode was positioned over the nerve in the femoral triangle, and the anode was placed midway between the greater trochanter and the iliac crest. The stimulus intensity required to evoke a maximal compound muscle action potential (Mmax) was determined at rest and during submaximal isometric knee contractions (50–60% MVC) before the experiment on each day. Since the current required to elicit Mmax was higher during contractions than at rest, the stimulus intensity was set to 120% of that required to elicit Mmax during the submaximal contraction and kept constant throughout the protocol. An experimenter applied pressure to the anode to reduce the stimulus intensity required to produce Mmax (mean current = 341 ± 14 mA). The amplitude of each Mmax was carefully checked during every trial (both online and during postprocessing) to ensure consistency in the effective stimulus strength. No trials were detected in which the Mmax amplitude substantially differed from that of trials executed immediately before or after. The reliability of this protocol in our laboratory has been established (52).

**TMS.** A Magstim 200² transcranial magnetic stimulator (Magstim Limited) with a concave double cone coil (130 mm diameter) was used to elicit motor-evoked potentials (MEPs) in the right knee extensors. The junction of the figure-8 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) was determined before the experiment in each session (mean position: 1 cm lateral to vertex). The position was marked directly on the scalp. The stimulator output (30–60% of maximum) was set to produce the largest possible MEP in the rectus femoris (on average, MEP area was ~90% of Mmax during 50% MVC contractions), while causing a small MEP in the biceps femoris (<10% of the raw quadriceps MEP amplitude). The same stimulator intensity was used throughout the protocol.

**Neuromuscular function test.** For the neuromuscular function test, subjects were seated comfortably on a custom-built chair such that the hip and knee were at 110° of flexion (note: 0° of knee extension refers to a horizontal leg-thigh). The chair had a long, rigid back-rest to provide full back and head support. A cuff attached to a strain gauge was placed 2 cm above the lateral malleolus of the right leg using a Velcro strap. Subjects grasped the handles at the side of the chair for support during contractions. Leg position information was recorded to ensure identical positioning for each test occasion.

**Force and electromyographic (EMG) parameters.** Force and electromyographic (EMG) parameters were assessed before and for 50 min after ~47 min of locomotor exercise or rest (Fig. 1A). Each set of brief contractions involved four contractions. TMS was delivered during contractions at 100, 50, and 75% MVC, and motor nerve stimulation was delivered during 100% MVC. An additional motor nerve stimulus was delivered at rest, 2 s after the superimposed motor nerve stimulus, to determine the resting, potentiated twitch amplitude (Fig. 1B). The contractions were separated by 6 s of rest, and the submaximal target force levels were calculated from the first contraction (i.e., MVC) in each set. The reliability of the TMS protocol for the determination of voluntary activation and resting twitch parameters (see Data analysis) has been established (52). Each set of sustained contractions consisted of a MVC that was maintained for 30 s, followed by a voluntary reduction in force output to match 75 and then 50% of the final (i.e., fatigued) MVC force. Stimulation was applied to the femoral nerve and motor cortex, 5 s apart, just before the end of the sustained MVC. The order of motor cortical and motor nerve stimulation varied randomly across subjects but was held constant for each person. TMS was also delivered during each of the 75 and 50% contractions and motor nerve stimulation was delivered at rest, 2 s after the final 50% contraction, to determine the resting, potentiated, and twitch amplitude (Fig. 1B). Subjects were provided with visual feedback of the target force to meet in each contraction.

**EMG and force recordings.** After careful preparation of the skin surface (cleaning with alcohol swabs and light abrasion), self-adhesive bipolar electrodes (Ag-AgCl, 10-mm diameter, 5-cm interelectrode distance) were positioned over the muscle belly of the rectus femoris and the lateral head of the biceps femoris. Please note that although MEPs and Mmax area were recorded from electrodes placed above rectus femoris, compound muscle action potentials would have been evoked in all muscles innervated by the femoral nerve (in the case of Mmax) and all muscles strongly activated during knee extension (in the case of MEPs). Thus our measures of voluntary activation reflect the contribution of all major muscles that contribute to knee extension. Although differences in the timing, duration, and amplitude of muscle activity between various knee extensors have been suggested during cycling, the rectus femoris is a substantial contributor to leg cycling exercise. We therefore assumed that compound muscle action potentials recorded from electrodes positioned above rectus femoris should reflect any fatigue-related changes in MEP or Mmax responses that occur within the knee extensor group as a whole. Voluntary and evoked forces were measured using a linear strain gauge (Precision Transducers; AST-100 tension load cell) coupled to the chair and the leg with a rigid, noncompliant device. Surface EMG signals were amplified, band-pass filtered (30–1,000 Hz; Grass PS511), and sampled (5,000 Hz) for later analysis with a 12-bit National Instruments (Austin, TX) analog-to-digital (A/D) board interfaced with microcomputer running custom written software (Labview, Austin, TX).

**Locomotor exercise bout.** The endurance exercise bout consisted of a warm-up for 5 min at 60% of Wmax, followed by eight, 5-min bouts of cycling at 80% of Wmax, with a 60-s rest between each bout. This type of exercise is typical of current training practices used by elite and recreational athletes to improve cardiorespiratory fitness and performance (26, 29). Subjects indicated their perceived exertion value according to the Borg scale of 6–20 (6: no exertion; 20: maximal exertion) and were allowed to drink water at the end of each 5-min bout.

**Blood sampling.** Blood lactate was measured from arteriovenous blood samples obtained via small punctures made at the fingertip using sterile techniques at the following time points: before baseline brief sets, immediately after the locomotor exercise or rest period, and immediately after the final neuromuscular test contractions (Fig. 1A). Blood lactate concentration (mM/l) was measured using a portable lactate analyzer (i-lactate Pro, LT-1710), the reliability of which has been established (43).

**Data analysis.** The force at the onset of each twitch was determined and twitch amplitude was taken as peak force minus the onset force (see Fig. 2 for examples of raw twitch data). The amplitude of the resting twitch evoked by motor cortex stimulation was estimated rather than measured directly as motor cortex and spinal cord excitability increase with activity (16, 67), and thus at weak contraction strengths interpolated Twitches are smaller than the “expected” size (52). According to the method developed by Todd et al. (65, 66), the “estimated” resting twitch (ERT) was determined as the y-intercept of the linear regression between the amplitude of superimposed Twitches evoked by TMS and voluntary torque recorded during 50, 75, and 100% MVC. The validity and reliability of this method for the human knee extensors have been established (52). This study demonstrated that the reliability and size of cortical measures of voluntary activation were comparable with those derived from motor nerve stimulation when the resting Twitches were estimated on the basis of as few as three TMS trials. Voluntary activation was quantified according to the conventional formula: voluntary activation (%) = [(1 − superimposed twitch/resting twitch (or ERT)) × 100 (22, 27).

During contractions in which either motor nerve stimuli or TMS were delivered, the areas of MEPs and Mmax were measured between cursors placed to encompass all phases of the evoked potentials from
electrodes placed over the rectus femoris and biceps femoris muscles. The area of each MEP was normalized to that of M_max elicited during MVC in the same set (23, 60). Voluntary EMG amplitude was taken as the average rectified EMG during MVCs in the 95 to 5 ms before stimulation and normalized to the M_max amplitude elicited at MVC. The duration of the silent period was determined as the interval from stimulus to return of continuous EMG by visual inspection.

Statistical analysis. All data are reported as means ± SE. Pearson’s correlation coefficients were calculated to determine the relationship between the following: the percent reduction in brief MVC force from baseline vs. the blood lactate concentration immediately postexercise; the percent reduction in brief MVC force vs. the rating of perceived exertion immediately after the last exercise bout; and the percent change in both resting twitch amplitude and ERT amplitude vs. blood lactate concentration postexercise. For all inferential analyses of evoked and voluntary force and EMG measures, data from the four sets conducted at baseline were pooled (i.e., before exercise/rest), as were data from the first four sets after exercise or rest (0 –15 min) and from the final four sets conducted after exercise or rest (30 – 45 min). Two-way ANOVAs with repeated measures on condition (control or exercise session) and time (baseline, 0 –15 min, and 30 – 45 min) factors were conducted. When ANOVA revealed a significant main effect or interaction, planned contrasts were used to test for differences between conditions at baseline and for changes from baseline across time within each session. Paired t-tests were also conducted to directly compare the relative (percent) changes from baseline between the exercise and control sessions. For the sustained trials, two-way ANOVAs with repeated measures on condition (control or exercise session) and time (brief set at 15 min, sustained set at 20 min, brief set at 45 min, and sustained set at 50 min) factors were conducted. We used values from the brief sets at 15 and 45 min as the baseline for the sustained MVCs to assess the “additional” effects of the 30-s MVC. Planned contrasts were used to test the effect of the sustained contractions within conditions, and paired t-tests were used to compare relative (percent) changes from baseline (i.e., the previous brief MVC). The data from two people were excluded from the analysis of TMS measures in the sustained contractions due to large head movements, which led to incorrect positioning of the TMS coil. Statistical significance was set at P ≤ 0.05.

RESULTS

Lactate, perceived exertion, and W_max. The percent increase in blood lactate concentration from baseline was significantly higher immediately after the locomotor exercise than at the corresponding time point in the control session \((t_0 = 4.1; P < 0.001)\) and remained significantly higher 50 min later \((t_0 = 2.2; P = 0.05; \text{Table 1})\). At the termination of the final exercise bout, the perceived exertion was 18.2 ± 0.4, indicating that the subjects had reached a high level of subjective exertion. Subjects successfully completed the required work for the locomotor exercise protocol (80.2 ± 1.3% of W_max).

MVC force. Maximal voluntary force was reduced to 76.9 ± 6.3% of baseline immediately after (i.e., 2–3 min) locomotor exercise \((t_0 = 3.1; P < 0.05)\) and partially recovered to 87.7 ± 2.8% of baseline by the final brief MVC trial, 45 min later \((t_0 = 3.9; P < 0.01)\). The percent reduction in MVC force from baseline for brief contractions was significantly greater in the
exercise session than in the control session at both time points (at 0–15 min, $t_9 = 3.2, P < 0.05; 30–45 \text{ min}, t_9 = 3.9, P < 0.01; \text{Fig. 3A}$), indicating that the locomotor exercise induced fatigue that persisted throughout the experiment. In addition, the percent reduction in MVC force during brief contractions from 0 to 15 min postexercise was related to the absolute blood lactate concentration taken immediately postexercise ($r = -0.63; P < 0.05$) and to the perceived exertion recorded immediately after the last cycling bout ($r = -0.80; P < 0.01$). In both the control and exercise sessions, the absolute MVC force recorded at the conclusion of the 30-s sustained contractions was significantly lower ($F_{1,9} > 6.8; P < 0.05$) than that recorded during the previous brief MVC (i.e., 15 and 45 min) at both time points (i.e., at 20 and 50 min). Thus the sustained MVCs induced additional fatigue in both sessions. However, the percent reduction in MVC force was significantly greater in the exercise session than in the control session at both time points ($t_9 > 2.5; P < 0.05; \text{Fig. 3B}$). This indicates that knee extensor muscles are fatigued to a greater extent during a sustained MVC after locomotor exercise than when the muscles are in a rested state.

**Twitch contractile properties at rest.** Figure 2 shows raw traces of EMG and force responses from one representative subject at baseline and in the first brief set conducted (within 2–3 min) after the locomotor exercise bouts, and demonstrates a decrease in the amplitude of a resting twitch evoked by motor nerve stimulation and a reduced cortically evoked twitch amplitude vs. voluntary force relation (i.e., resulting in a smaller ERT; see METHODS). There was a significant interaction between session and time for resting twitch amplitudes evoked by both motor cortical and motor nerve stimulation in brief trials ($F_{2,18} > 10.2; P < 0.01$). Resting twitches evoked by both forms of stimulation were reduced significantly more after locomotor exercise than in the control session at both time points.

Table 1. *Blood lactate concentration during exercise and at rest*

<table>
<thead>
<tr>
<th>Blood Lactate Concentration, mM</th>
<th>Baseline</th>
<th>After EX/RE</th>
<th>End Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.66±0.1</td>
<td>2.04±0.2</td>
<td>3.80±0.4†</td>
</tr>
<tr>
<td>Exercise</td>
<td>1.53±0.1</td>
<td>8.44±1.2*†</td>
<td>4.62±0.8*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. EX, exercise; RE, rest. *Significant difference in percentage increase from baseline between control and exercise session at the time point ($P \leq 0.05$). †Significant difference from baseline values in each session ($P \leq 0.05$; within condition analyses).
points \( (t_9 > 3.0; P < 0.05; \text{Fig. 3C}) \). Lactate concentration postexercise was strongly correlated with the initial reduction in resting twitches derived from both motor cortical and motor nerve stimulation (i.e., the percent change from baseline to 0–15 min; ERT: \( r = -0.85, P < 0.01; \) resting twitch: \( r = -0.83, P < 0.01 \)). The 30-s sustained MVCs significantly reduced the amplitude of resting twitches evoked by motor nerve stimulation at both time points in the control session \( (F_{1,9} > 25.0; P < 0.001; \text{Fig. 3D}) \) and at 50 min postexercise in the locomotor exercise session \( (F_{1,9} = 15.5; P < 0.01; \text{Fig. 3D}) \). In contrast, at 20-min after locomotor exercise, the sustained MVC failed to significantly reduce the resting twitch amplitude \( (F_{1,9} = 2.2; P = 0.18) \). The percent reduction in resting twitch amplitude was greater in the control session than in the exercise session at both the 20-min measurement \( (t_9 = 2.8; P < 0.05; \text{Fig. 3D}) \) and the 50-min measurement \( (t_9 = 2.3; P = 0.05; \text{Fig. 3D}) \). This suggests that a 30-s MVC induces a less peripheral fatigue after locomotor exercise than when the muscles are in a rested state, despite a greater overall exercise-induced reduction in MVC. There were no significant changes in ERT amplitude during sustained contractions in either session (interaction between session and time; \( F_{1,7} < 0.9; P > 0.30 \)).

**Cortical voluntary activation**. Voluntary activation measured with motor cortex stimulation was high at baseline in both sessions \( (~90\%; \text{Fig. 3E}) \), as has previously been reported for the knee extensors \( (52) \). Locomotor endurance exercise significantly reduced cortical voluntary activation during brief MVCs at both time points \( (~89\% \text{ of baseline at } 0–15 \text{ min}; \text{to } ~89\% \text{ of baseline at } 30–45 \text{ min; } F_{1,9} > 10.7; P < 0.01) \), whereas there was no significant change in cortical voluntary activation in the control session \( (F_{1,9} < 2.6; P > 0.14; \text{see Fig. 3 for absolute voluntary activation levels}) \). There was a significant ANOVA interaction between session and time \( (F_{2,18} = 5.9; P < 0.05) \), and the percent reduction in cortical voluntary activation from baseline was significantly greater after locomotor exercise than in the control session at both time points \( (t_9 > 2.7; P < 0.05) \). Thus the cycling bouts induced central fatigue with a supraspinal component that persisted for at least 30–45 min after exercise. For the sustained contractions, there was a significant reduction in cortical voluntary activation at 20 min \( (23\% \text{ reduction, from } ~86 \text{ to } ~66\%; F_{1,7} = 7.9; P < 0.05) \) and 50 min \( (12\% \text{ reduction, from } ~83 \text{ to } ~71\%; F_{1,7} = 6.9; P < 0.05) \) relative to the previous brief MVC, after locomotor exercise, but not in the control session \( (20 \text{ min: } F_{1,7} = 0.8, P = 0.40; 50 \text{ min: } F_{1,7} = 3.9, P = 0.09; \text{Fig. 3F}) \). The percent reduction in voluntary activation \( (\text{i.e., from the previous brief MVC}) \) was significantly greater in the exercise session, but only at the 20-min measurement \( (t_7 = 2.8; P < 0.05) \).

**Voluntary activation measured with motor nerve stimulation**. Motor nerve estimates of voluntary activation were comparable with those derived from motor cortex stimulation at baseline in both sessions \( (~90\%; \text{Fig. 3E}) \). Voluntary activation measured with motor nerve stimulation was significantly reduced from baseline for brief MVCs performed 0–15 and 30–45 min after locomotor exercise \( (F_{1,9} > 11.4; P < 0.01) \). There were no statistically significant changes in voluntary activation across time in the control session \( (F_{1,9} < 0.1; P > 0.70) \). There was a significant ANOVA interaction between session and time \( (F_{2,18} = 6.7; P < 0.01) \), and the percent reduction in voluntary activation from baseline was significantly greater in the exercise session than in the control session at both measurement times \( (t_9 > 3.0; P < 0.05) \). There was a trend towards a reduction in voluntary activation at the completion of the 30-s MVC that was performed 20 min after locomotor exercise \( (\text{relative to the brief MVC executed at } 15 \text{ min, from } 87 \text{ to } 74\%; F_{1,9} = 4.9; P = 0.06; \text{Fig. 3F}) \) and a trend toward a greater percent reduction in voluntary activation \( (\text{relative to the brief MVC executed at } 15 \text{ min}) \) in the exercise than the control session \( (t_9 = 2.1; P = 0.07; \text{Fig. 3F}) \). The sustained MVC performed at 50 min produced no additional reductions \( (F_{1,9} = 0.0; P = 0.90) \) in motor nerve measures of voluntary activation in either session.

**MEPs and silent period**. The area of MEPs evoked in the knee extensors was large relative to the maximal M wave \( (\text{Fig. 4A}) \), indicating that the motor cortex stimulation activated a high proportion of the knee extensor motor units. The average area, pooled across brief and sustained contractions from both sessions, of MEPs obtained during contractions at 50% MVC was 89% of \( M_{\text{max}} \). During contractions at 75% MVC was 84% of \( M_{\text{max}} \), and during contractions at 100% MVC was 70% of \( M_{\text{max}} \). There were no main effects or interactions between session and time for MEP areas recorded at any contraction strength during brief \( (F_{2,18} < 1.9; P > 0.10) \) or sustained contractions \( (F_{1,7} < 2.4; P > 0.10; \text{Fig. 4A}) \), indicating that the effective stimulus intensity was similar across all conditions. The size of MEPS recorded from the antagonist knee flexors was small compared with MEPS recorded from the knee extensors \( (\text{knee flexor MEP was } 10.2 \pm 2.0\% \text{ of knee extensor MEP;} t_9 = 6.7; P < 0.001) \). There were also no significant main effects or interactions between session and time for the area of MEPS elicited in the knee flexors \( (\text{Fig. 4B}) \).

The duration of the period of EMG silence elicited by TMS during brief contractions did not change across time in either the exercise or the control session \( (F_{1,9} < 2.9; P > 0.10; \text{Fig. 4C}) \). However, the silent period duration was significantly greater at the end of the 30-s sustained contractions \( (\text{relative to that recorded during the previous brief MVC}) \) at both measurement times and in both sessions \( (~145 \text{ vs. } ~92 \text{ ms;} F_{1,7} > 47.0; P < 0.001) \). The percent increase from baseline \( (~60\%) \) was similar in both sessions and measurement times \( (t_7 < 1.3; P > 0.20) \).

**Average rectified EMG**. There were no significant changes across time in the amplitude of voluntary EMG recorded during brief or sustained MVCs in either session \( (\text{expressed as a percent of } M_{\text{max}} \text{ amplitude recorded during the same contraction; } F_{1,9} < 2.5; P > 0.10) \). There was, however, a significant interaction for voluntary EMG amplitude between session and time \( (F_{1,9} = 8.5; P < 0.05) \) during the sustained MVC at 20 min \( (\text{control session: } ~6 \text{ vs. } 7\%; \text{exercise session: } 6 \text{ vs. } 5\% \text{ at } 15 \text{ and } 20 \text{ min, respectively}) \).

**DISCUSSION**

The aim of this study was to examine the site of central fatigue induced by locomotor exercise involving the knee extensor muscles. Voluntary isometric force was significantly reduced for at least 45 min after exercise. This loss in force was associated with a decline in the amplitude of resting twitches evoked by motor nerve and motor cortex stimulation \( (\text{i.e., ERT}) \).
and resting twitch), indicating the presence of peripheral fatigue. However, central fatigue also contributed to the reduction in force, since voluntary activation measured both by motor nerve and motor cortex stimulation was impaired. The cortical voluntary activation data provide evidence that supraspinal factors play an important role in the reduction in voluntary force production after locomotor exercise. Locomotor nerve and motor cortex stimulation was impaired. The supraspinal fatigue persistence of peripheral fatigue throughout the recovery period studied (i.e., 45 min). There was also a strong relationship between the accumulation of blood lactate during the exercise bout and the decrease in the amplitude of twitches evoked by both motor cortical and motor nerve stimulation. A similar relationship has been demonstrated previously for twitches elicited by motor nerve stimulation (55, 56). The implication is that subjects who experienced greater metabolic fatigue during the exercise bout also displayed more peripheral fatigue in the knee extensor muscles during recovery, although a causal relationship between the blood and/or intramuscular lactate concentration and subsequent peripheral muscle fatigue is unlikely (3, 17, 28, 40). The most likely explanation for the reduction in resting twitch amplitude is an impairment in the excitation-contraction coupling process. Reductions in Ca²⁺ release from the sarcoplasmic reticulum (68) and a reduced capacity to form strong cross-bridge bonds between the contractile proteins (18, 19) due to metabolite accumulation (e.g., H⁺ and inorganic phosphate; Ref. 35) may play a role in this decrement.

Central fatigue. The reduction in voluntary activation measured via motor nerve stimulation after locomotor exercise indicates suboptimal output from the motoneurons and shows that the exercise bout induced central fatigue. The reduction in cortical voluntary activation demonstrates that locomotor fatigue reduces the capacity of the motor cortex to drive the motoneurons during recovery. The supraspinal fatigue persisted for at least 30–45 min, which is considerably longer than has been reported to occur after maximal and submaximal isolated muscle contractions (e.g., Refs. 22, 53, 54, 63), unless the contraction duration is long and the force is very low (i.e., ~70 min at 5% MVC; Ref. 53). In the current study, cortical voluntary activation fell to ~89% and voluntary activation measured via motor nerve stimulation fell to ~94% of preintervention levels during brief contractions. The reductions in voluntary activation during the sustained contractions were also greater for TMS measures of voluntary activation than for motor nerve measures of voluntary activation. Although direct, quantitative comparison of voluntary activation measured via motor cortex and motor nerve stimulation is problematic for many reasons (65), including differences in the shape of the
force vs. superimposed twitch relationship, the data indicate that a large proportion of the failure in voluntary drive after locomotor exercise occurs because of suboptimal output from the motor cortex.

The question of whether there is a supraspinal contribution to central fatigue after prolonged locomotor exercise has been considered previously, but no studies to date provide conclusive evidence on the issue. Studies in which strength was measured in remote muscles after locomotor exercise (i.e., handgrip; Refs. 36, 41) failed to provide evidence of supraspinal fatigue, and the results of a recent study (47) that used TMS to estimate voluntary activation in four subjects after marathon running are difficult to interpret. Because neither the amplitude of MEPs elicited during voluntary activation measurements nor the size of the ERTs were reported, it was unclear whether the effective stimulus strength was constant or whether the responses to motor cortical stimulation were maximal.

As the relationship between force and voluntary activation measured via motor cortex stimulation is linear (52, 64), it is possible to estimate the contribution of supraspinal fatigue to the total force loss. The MVC force decreased to \(-83\%\) of baseline after locomotor exercise (average value during 0–15 min postexercise), whereas voluntary activation measured via motor cortex stimulation decreased from \(-91\%\) to \(-81\%.\) This \(10\%\) decrease in voluntary activation should have reduced MVC by \(10\%\) in the absence of peripheral fatigue (i.e., to \(90\%\) of baseline). Thus \(59\%\) of the total force decline (i.e., to \(83\%\) of initial MVC) was due to supraspinal fatigue. The proportion of the (additional) reduction in force, caused by sustained maximal contraction, that could be attributed to supraspinal factors 20 min after locomotor exercise was much higher (\(\sim 52\%)\) than in the control session (\(\sim 4\%).\) Thus locomotor muscle fatigue exacerbates the supraspinal component of central fatigue induced subsequently by sustained maximal contractions and reduces the development of additional peripheral fatigue.

**Causes of supraspinal fatigue.** The results of recent studies (3–5, 32) suggest that the degree of central motor drive during exhaustive locomotion is inversely related to the level of locomotor muscle fatigue. According to one interpretation of this relationship, the central nervous system regulates the extent of voluntary motoneuron activation to prevent the development of excessive peripheral fatigue beyond a tolerance limit associated with tissue damage and pain (3). There is evidence that the firing of group III and IV afferents, which are sensitive to metabolites of fatigue, may act at a supraspinal level to impair voluntary activation during prolonged MVC (11, 22). Afferent supraspinal projections are known to involve multiple ascending pathways to subcortical and cortical structures, including the thalamus, limbic system, and prefrontal cortex (2), and may affect processes such as motivation (50) and motor processing (13). It is not known how long group III and IV afferents continue to fire after fatiguing locomotor exercise of the type performed in the current study, but the persistence of circulating lactate suggests that the exercise induced a relatively long-lasting disturbance in metabolic homeostasis. According to an alternative viewpoint, reductions in the intrinsic force-generating capacity of the locomotor muscles are related to reductions in central drive via the increased sense of effort required to maintain a given workload under fatigued conditions (33). While our data do not allow conclusions on the causes of task failure during locomotor exercise, they do suggest that locomotor muscle fatigue can reduce central drive independently from the influence of an increased sense of effort. This is because effort is, by definition, maximal during MVC. Our current data show that cortical voluntary activation is impaired after locomotor exercise, indicating that the capacity to drive the motor cortical output cells is impaired in the presence of locomotor muscle fatigue, despite maximal effort.

Supraspinal fatigue during prolonged exercises at a relatively low intensity has also been linked to the influence of serotonin as an agent to reduce corticospinal drive (14, 37). An increase in serotonin can result from an increase in lipolysis due to glycogen depletion, which increases plasma and brain free tryptophan (10, 12, 15). Increases in core and/or brain temperature (4, 38, 63) and inadequate cerebral oxygen delivery during endurance exercise have also been proposed as mechanisms of central fatigue during locomotor exercise (8, 39, 48, 57). Many of these factors might persist for some time during recovery and might contribute to the long-lasting supraspinal fatigue observed in the current study.

**Central excitability.** The size of MEPs depends on the balance of all excitatory and inhibitory influences acting on corticospinal neurons, on the response of the motor neuron pool to the descending volleys, and on the muscle fibre action potentials (which can be compensated for by normalization to \(M_{max}\); Ref. 21). After the MEP, there is a silent period in the ongoing voluntary EMG, the latter part of which is thought to be dependant on the inhibition of the voluntary descending output from the motor cortex through the actions of the intracortical inhibitory interneurons (62). MEPs increase in size during prolonged MVC (34, 59), demonstrating an increase in cortical excitability to provide additional cortical drive to motoneurones (16). In addition, the duration of the silent period typically lengthens during fatiguing exercise involving maximal sustained contractions (58, 63). However, both MEP amplitude and silent period duration measured during brief MVCs recover quickly upon cessation of the fatiguing contractions (11, 58, 59) and consequently can be dissociated from impairments in voluntary activation (7, 22, 64). Neither silent period nor MEP amplitude was altered during brief MVCs as a consequence of locomotor exercise in the current study. This suggests that fatiguing exercise did not impair the responsiveness of neurons in the pathway from the motor cortex to the muscle output. Despite this, output from the motor cortex remained insufficient for complete muscle activation for up to 45 min after exercise, which implies that the physiological drivers of central fatigue act upstream of the motor cortical outputs (note: the failure could occur within the motor cortex, but presynaptic to the motor cortex output cells).

In conclusion, the present study shows that locomotor exercise induces fatigue in the knee extensor muscles of both central and peripheral origin. In addition to peripheral fatigue, which persists for at least 45 min postexercise, \(-60\%\) of the initial loss in force could be attributed to a failure of the motor cortex to optimally drive the knee extensor motoneurons. Based on the persistent reduction in resting twitch amplitude, the presence of metabolites in circulation, and the lack of change in corticospinal excitability and inhibition, we conclude that much of the failure in voluntary drive to produce maximum force after locomotor exercise is mediated by systemic or...
intramuscular fatigue signals that reduce the cortical drive “upstream” of the motor cortical output cells.

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