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Title: Effects of ipsilateral and contralateral fatigue and muscle blood flow occlusion on the complexity of knee extensor torque output in humans

Authors: Jamie Pethick
Samantha Lee Winter
Mark Burnley

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Running Title: Fatigue-induced loss of torque complexity

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exponent (DFA α). Neuromuscular fatigue was consistently associated with a loss of torque complexity in all conditions (e.g., IPS bout 1 ApEn from [mean {plus minus} SD]: 0.46 {plus minus} 0.14 to 0.12 {plus minus} 0.06 [P < 0.001]). In IPS-OCC, occlusion abolished the recovery from fatigue and torque complexity remained at the values observed at task failure in the preceding bout (IPS-OCC bout 2, first minute: 0.14 {plus minus} 0.03, P < 0.001). Prior contralateral contractions, with or without blood flow occlusion, had no effect on torque complexity.

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Effects of ipsilateral and contralateral fatigue and muscle blood flow occlusion on the complexity of knee extensor torque output in humans.

Jamie Pethick, Samantha L. Winter and Mark Burnley

Endurance Research Group, School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, Kent, UK.

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Corresponding author:
Dr Mark Burnley
Endurance Research Group
School of Sport and Exercise Sciences
University of Kent
The Medway Building
ME4 4AG
UK
m.burnley@kent.ac.uk

New findings

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Abstract

Neuromuscular fatigue reduces the temporal structure, or complexity, of torque output during muscular contractions. To determine whether the fatigue-induced loss of torque complexity could be accounted for by central or peripheral factors, nine healthy participants performed four experimental trials involving intermittent isometric contractions of the knee extensors at 50% of the maximal voluntary contraction (MVC) torque. These trials involved: 1) two bouts of contractions to failure using the right leg separated by 3 min recovery (IPS); 2) the same protocol but with cuff occlusion during the 3-min recovery (IPS-OCC); 3) contractions of the left leg to failure, followed 1 min later by contractions of the right leg to failure (CONT); and 4) the same protocol but with cuff occlusion applied to the left leg throughout both the recovery period and right leg contractions (CONT-OCC). Supramaximal electrical stimulation during MVCs was used to determine the degree of central and peripheral fatigue, whilst complexity was determined using Approximate Entropy (ApEn) and Detrended Fluctuation Analysis α exponent (DFA α). Neuromuscular fatigue was consistently associated with a loss of torque complexity in all conditions (e.g., IPS bout 1 ApEn from [mean ± SD]: 0.46 ± 0.14 to 0.12 ± 0.06 [$P < 0.001$]). In IPS-OCC, occlusion abolished the recovery from fatigue and torque complexity remained at the values observed at task failure in the preceding bout (IPS-OCC bout 2, first minute: 0.14 ± 0.03, $P < 0.001$). Prior contralateral contractions, with or without blood flow occlusion, had no effect on torque complexity.

Introduction

Physiological systems produce outputs that inherently fluctuate over time (Goldberger *et al*., 2002). Such fluctuations are typically quantified according to their *amplitude*, using the standard deviation (SD) or coefficient of variation (CV), or their *frequency* content, using the fast Fourier transform. It is now recognised that these fluctuations can also be quantified according to their temporal *structure* or "complexity". Complex outputs are characterised by temporal irregularity, time irreversibility and long-range (fractal) correlations (Lipsitz and Goldberger, 1992; (Pincus, 1994; Goldberger *et al*., 2002), properties which amplitude and frequency metrics cannot quantify. Measures of complexity can be divided into those that quantify the regularity of the output (e.g. Approximate Entropy [ApEn]; Pincus, 1991) and those that quantify temporal fractal scaling and noise colour (e.g. Detrended Fluctuation Analysis [DFA]; Peng *et al*., 1994). The presence of complex outputs in physiological systems are thought to be a signature of good health (Peng *et al*., 2009). Consequently, a loss of complexity is indicative of system dysfunction, as frequently observed in ageing (as seen, *inter alia*, in heart rate dynamics, gait and muscle torque output; Goldberger *et al*., 2002; Manor and Lipsitz, 2012).

We have extended the loss of complexity observed in ageing to neuromuscular fatigue, demonstrating a reduction in torque complexity during intermittent isometric contractions of the knee extensors (Pethick *et al*., 2015). These experiments have demonstrated that as fatigue develops during high-intensity contractions (at 40-50% of the maximal voluntary contraction [MVC]), ApEn declines and the DFA α scaling exponent increases to values approximating 'Brownian' noise (DFA α = 1.50), indicating a torque output that has become more regular and in which its previously fractal nature has broken down. However, because both central and peripheral fatigue developed during these contractions (i.e., mechanisms of force loss residing in the central nervous system or the muscle itself, respectively), the precise mechanistic origin of the fatigue-induced loss of torque complexity is not clear. As a first step towards resolving the mechanistic basis of the fatigue-induced loss of torque complexity, we have designed a series of experiments intended to accentuate either central or peripheral fatigue.

The fatigue-induced loss of torque complexity has been observed only during contractions performed above the critical torque (Pethick *et al*., 2016), a threshold above which metabolite-mediated peripheral fatigue (assessed using the potentiated doublet torque) appears to be the dominant mechanism of force/torque loss (Burnley, 2009; Burnley *et al*., 2012). This suggests that metabolite-mediated peripheral fatigue could be a major contributor to the loss of torque complexity during high-intensity contractions (Pethick *et al*., 2016). If so, we would expect to observe no recovery of torque complexity when a fatigued muscle is subject to blood flow occlusion, which arrests arterial inflow and prevents recovery from peripheral fatigue (Bigland-Ritchie *et al*., 1986; Quistorff *et al*., 1993; Gandevia *et al*., 1996; Lanza *et al*., 2006).

We have recently observed that caffeine ingestion attenuates the development of central fatigue (assessed using the twitch interpolation technique) and the fatigue-induced loss of torque complexity, independently of the development of peripheral fatigue (Pethick *et al*., 2018). This suggests the central processes make a small, but significant, contribution to the fatigue-induced loss of torque. If the loss of torque complexity is mechanistically linked to the myriad of central nervous system adjustments responsible for central fatigue, we would expect that increased central fatigue at the start of an exercise bout, induced by prior exercise of the homologous muscles of the contralateral limb (Zijdewind *et al*,. 1998; Todd *et al*., 2003; Rattey *et al*., 2006), would result in reduced torque complexity.

It has also been proposed that central and peripheral fatigue mechanisms interact (Amann and Dempsey, 2008); with metabosensitive group III and IV muscle afferents within working muscle detecting exercise-induced metabolic perturbations associated with peripheral fatigue (Kaufman *et al*., 2002). This results in a feedback loop, proposed to limit voluntary drive (i.e. increases central fatigue) and restrict the development of further peripheral fatigue (Amann *et al*., 2006; Amann *et al*., 2013). If such a feedback loop is involved in the fatigue-induced loss of torque complexity, we would expect contractions performed whilst fatigued contralateral muscle blood flow is occluded would result in a reduction in torque complexity.

The purpose of the present study was, therefore, to attempt to separate the effects of central fatigue, peripheral fatigue and afferent feedback. The experimental hypotheses tested were: 1) that pre-existing peripheral fatigue, induced by circulatory occlusion, would decrease time to task failure and reduce torque complexity; 2) that pre-existing central fatigue, induced by

prior exercise of the contralateral limb, would decrease time to task failure and reduce torque complexity at the start of an exercise bout; and 3) that enhanced afferent feedback, induced by prior exercise and occlusion of the contralateral limb, would decrease time to task failure and reduce torque complexity at the start of an exercise bout.

Materials and Methods

Ethical approval

Nine healthy participants (5 male, 4 female; mean ± SD: age 23.9 ± 5.7 years; height 1.74 ± 0.09 m; body mass 66.0 ± 12.4 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent (Prop_54_2014_2015), and which adhered to the Declaration of Helsinki, except for registration in a database. Participants were instructed to arrive at the laboratory rested (having performed no heavy exercise in the preceding 24 hours) and not to have consumed any food or caffeinated beverages in the three hours before arrival. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on five occasions, with a minimum of 48 hours between visits. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings for the dynamometer and stimulator were recorded. During visits two to five, participants performed a series of intermittent isometric contractions to task failure, during which we attempted to manipulate the type and degree of neuromuscular fatigue that the participants experienced ("*Experimental trials*"; see below). These trials were presented in a randomised order.

Dynamometry

Participants sat in the chair of a Cybex isokinetic dynamometer (HUMAC Norm; CSMi, Stoughton, Massachusetts, USA), initialised and calibrated according to the manufacturer's instructions. Participants sat with relative hip and knee angles of 85° and 90°, respectively with full extension being 0°. The leg to be tested was attached to the lever arm of the dynamometer, with the seating position adjusted to ensure that the lateral epicondyle of the femur was in line with the axis of rotation of the lever arm. The lower leg was securely attached to the lever arm above the malleoli with a padded Velcro strap. Straps secured firmly

across the waist and shoulder prevented any extraneous movement and the use of the hip extensors during the isometric contractions. The seating position was recorded during the familiarisation and replicated during each subsequent visit.

Femoral nerve stimulation

The anode, a carbon rubber electrode with adhesive gel (100 mm x 50 mm; Phoenix Healthcare Products Ltd., Nottingham, UK), was placed lateral to the ischial tuberosity, on the posterior aspect of the leg. The position of the cathode was located using a motor point pen (Compex; DJO Global, Guildford, UK), and an Ag/AgCl electrode (32 x 32 mm; Nessler Medizintechnick, Innsbruck, Austria) was placed over that point. Establishment of the appropriate stimulator current was performed as described by Pethick *et al*. (2015), using a constant-current, variable voltage stimulator (Digitimer. DS7AH, Welwyn Garden City, UK). Briefly, current was incrementally increased until knee extensor twitch torque and the compound motor unit action potential (M-wave) response to single twitches had plateaued and was verified with stimulation delivered during a contraction at 50% MVC to ensure a maximal M-wave was also evident during an isometric contraction. The stimulator current was then increased to 130% of the current producing a maximal M-wave. In all trials, doublet stimulation (two 200 μs pulses with 10 ms interpulse interval) was then used.

Electromyography

On arrival at the laboratory participants had the leg(s) to be tested shaved and cleaned using an alcohol swab over the belly of the *vastus lateralis* and on the medial aspect of the tibia at the level of the tibial tuberosity. Two Ag/AgCl electrodes (32 x 32 mm; Nessler Medizintechnik, Innsbruck, Austria) were placed on the *vastus lateralis* in line with the muscle fibres and a single electrode placed on the tibial tuberosity for EMG acquisition.

Protocol

All visits followed a similar pattern of data acquisition, beginning with the instrumentation of the participants and the (re-)establishment of the correct dynamometer seating position and supramaximal stimulation response. Participants then performed a series of brief (3 s) MVCs to establish their maximum torque. These contractions were separated by 60 s rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the

subsequent EMG signals were normalised ("*Data analysis*"; see below). The second and third MVCs were performed with peripheral nerve stimulation. In all instances, where MVCs were performed with stimuli, the stimuli were manually delivered ~1.5 s into the contraction to coincide with maximal torque, in order to test the maximality of the contraction and provide the voluntary activation; and 2 s after the contraction, to provide a resting potentiated doublet.

In the visits involving contractions performed on both legs, after ten minutes rest participants repeated this process for the left leg. Following the establishment of maximal torque, participants rested for a further ten minutes and then performed one of the experimental trials (see below).

Experimental trials

The four experimental trials were termed: 1) Ipsilateral trial (IPS); 2) Contralateral trial (CONT; 3) Ipsilateral trial with occlusion (IPS-OCC); and 4) Contralateral trial with occlusion (CONT-OCC). All four trials consisted of two bouts of exercise. IPS involved exercising the right leg to task failure, followed by three minutes rest, and then exercising the right leg to task failure again. CONT involved exercising the left leg to task failure, then switching to the right leg and exercising to task failure. The switch from the left to right leg in the CONT and CONT-OCC conditions took approximately 50 seconds, and the second exercise bout was commenced 60 seconds after completion of the first bout. IPS-OCC involved exercising the right leg to task failure, then resting for three minutes with the blood flow to the right leg occluded, and then exercising the right leg to task failure again (with the occlusion released). CONT-OCC involved exercising the left leg to task failure, then occluding blood flow to the left leg and immediately switching to the right leg and exercising to task failure. During this trial, the occlusion of the left leg was released after six minutes of contractions or at task failure, whichever occurred sooner. Blood flow occlusion in the IPS-OCC and CONT-OCC trials was accomplished using a standard, double-bladder, adult thigh cuff, rapidly inflated to a pressure of 200 mmHg using compressed air (AG101, D.E. Hokanson Inc., Washington, USA). The trials are presented schematically in Figure 1.

During visit two (the first of the experimental trials), the highest instantaneous pre-test measure of voluntary torque was recorded as the peak MVC torque, and 50% of this value was used as the target torque for the subsequent trials. As in our previous work (Pethick *et al*., 2015; Pethick *et al*., 2016), the submaximal contractions were performed using a duty

cycle of 0.6; with contractions held for 6 s, followed by 4 s rest. Participants were instructed to match their instantaneous torque with a target bar superimposed on the display in front of them and were required to continue matching this torque for as much of the 6 s contraction as possible. At the end of each minute (i.e. every sixth contraction), participants performed an MVC, accompanied by peripheral nerve stimulation. Each exercise bout was conducted until task failure, the point at which the participant failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the test, but were informed of each "missed" contraction. After the third consecutive missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by peripheral nerve stimulation.

Following the MVC at the end of the first exercise bout, participants rested for three minutes and exercised the same leg again (IPS and IPS -OCC) or switched (over the course of 60 s, see above) to exercising their other leg (CONT and CONT-OCC). Immediately prior to the commencement of the second exercise bout, participants performed an MVC of the leg to be exercised in the second bout, accompanied by peripheral nerve stimulation. The second exercise bout was then performed in an identical manner to the first.

Data acquisition and participant interface

Data acquisition was performed in the same manner as described in Pethick *et al*. (2015). Briefly, all peripheral devices were connected via BNC cables to a Biopac MP150 (Biopac Systems Inc., California, USA) and a CED Micro 1401-3 (Cambridge Electronic Design, Cambridge, UK) interfaced with a personal computer. All signals were sampled at 1 kHz. The data were collected in Spike2 (Version 7; Cambridge Electronic Design, Cambridge, UK). A chart containing the instantaneous torque was projected onto a screen placed ~1 m in front of the participant. A scale consisting of a thin line (1 mm thick) was superimposed on the torque chart and acted as a target, so that participants were able to match their instantaneous torque output to the target torque during each test.

Data analysis

All data were processed and analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA). The data analysis focused on three specific areas: 1) measures of torque and EMG; 2) measures of central and peripheral fatigue; and 3) measures of the variability and complexity of torque output.

Torque and EMG. The mean and peak torque for every contraction in each exercise bout conducted on the right leg were determined. The mean torque was calculated based on the steadiest five seconds of each contraction. Task failure was determined as in Pethick *et al*. (2015). The mean contraction torque produced during the first five contractions was calculated, and task failure was deemed to have occurred when participants' mean torque output failed to achieve that of the first five contractions by more than 5 N·m for three consecutive contractions, with the first of these contractions being the point of task failure.

The EMG output from the vastus lateralis was filtered (10-500 Hz) and full-wave rectified with a gain of 1000. The average rectified EMG (arEMG) for each contraction was then calculated and normalised by expressing the arEMG as a fraction of the arEMG obtained during an MVC from the fresh muscle performed at the beginning of each trial.

Central and peripheral fatigue. Measures of central and peripheral fatigue were calculated based on the stimuli delivered during and after the MVCs performed pre-test, during the exercise bouts and at task failure. Peripheral fatigue was assessed by a fall in the peak potentiated doublet torque; and central fatigue was assessed by the decline in voluntary activation, quantified using the twitch interpolation technique (Behm *et al*., 1996):

$$Voluntary\ activation\ (\%) = (1 - superimposed\ doublet/resting\ doublet) \times 100 \quad [1]$$

where the superimposed doublet was measured during the contraction of interest and the potentiated doublet was measured at rest 2 seconds after the contraction.

Variability and complexity. All measures of variability and complexity were calculated using the steadiest five seconds of each contraction; that is, the five seconds containing the lowest standard deviation (SD; Forrest *et al*., 2014). The amount of variability in the torque output of each contraction was measured using the SD, which provides a measure of the absolute amount of variability in a time-series, and the coefficient of variation (CV), which provides a measure of the amount of variability in a time-series normalised to the mean of the time-series.

The temporal structure, or complexity, of torque output was examined using multiple time domain analyses. As in our previous work (Pethick *et al*., 2015; Pethick *et al*., 2016), the complexity of the torque output was determined using Approximate Entropy (ApEn; Pincus, 1991), and temporal fractal scaling was estimated using Detrended Fluctuation Analysis (DFA; Peng *et al*., 1994). Sample Entropy was also calculated (Richman and Moorman, 2000), though as shown in Pethick *et al*. (2015) this measure did not differ from ApEn and was not included in the present analysis. As detailed in Pethick *et al*. (2015), ApEn was calculated with the template length, *m*, set at 2, and the tolerance, *r*, set at 10% of the standard deviation of torque output; and DFA was calculated across time scales (57 boxes ranging from 1250 to 4 data points).

Statistics

All data are presented as means ± SD. The first exercise bout of the IPS trial (IPS1) acted as a control, against which the second exercise bouts of the experimental trials (IPS2, CONT2, IPS-OCC2 and CONT-OCC2) were compared. The first exercise bouts of the CONT, IPS-OCC and CONT-OCC trials were used to induce pre-existing fatigue in the right leg and were not considered for analysis. Two-way ANOVAs with repeated measures were used to test for differences between conditions and time points, and for a condition x time interaction for MVC torque, arEMG, potentiated doublet torque, voluntary activation, variability and complexity. The variability and complexity measures were analysed using means from the first minute and final minute before task failure. The rates of change in all parameters were analysed using one-way ANOVAs with repeated measures. Main effects were considered significant when $P < 0.05$. When main effects were observed, Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences.

Results

Time to task failure and MVC torque

Time to task failure in IPS1 (the control trial, with no pre-existing fatigue) was 4.7 ± 2.7 min. There was a significant effect of condition on time to task failure ($F = 17.52$, $P < 0.001$). Time to task failure was significantly shorter in IPS2 and IPS-OCC2 compared to IPS1 (paired samples confidence intervals (CIs): IPS1 vs. IPS2, –5.0, –0.4 mins; IPS vs. IPS-OCC2, –6.8, –1.4 mins; Table 1). Time to task failure was not significantly different in

CONT2 or CONT-OCC2 compared to IPS1 (CIs: IPS1 vs. CONT2, –1.1, 2.8 mins; IPS1 vs. CONT-OCC2, –0.4, 2.0 mins; Table 1).

Task failure occurred when participants were no longer able to achieve the target torque (106.6 ± 31.6 N·m), despite a maximal effort. All trials resulted in significant decreases in MVC torque (F = 25.66, P = 0.001), except for IPS-OCC2 (CIs: –26.6, 28.6 N·m), in which neither the pre- nor post-test MVC torques were significantly different from the target torque. At task failure neither the peak, nor the mean, MVC torques in any trial were significantly different from the torque produced during the submaximal contractions (Table 1). MVC torque was significantly lower at the start of the second exercise bout compared to IPS1 for all conditions (F = 21.99, P < 0.001), except for CONT2 (CIs: –8.3, 65.3 N·m). Significant recovery of the right leg was observed at the start of IPS2 (CIs: 8.0, 75.5 N·m), but not IPS-OCC2 (CIs: –8.4, 38.4 N·m).

Peripheral and central fatigue

There was a condition x time interaction for potentiated doublet torque (F = 8.92, P = 0.004), and all trials resulted in significant reductions in potentiated doublet torque (F = 47.22, P < 0.001; Table 1), indicating the presence of peripheral fatigue. Potentiated doublet torque was significantly lower at the start of the second bout of exercise compared to IPS1 for all conditions, except for CONT-OCC2 (CIs: –12.3, 26.7 N·m; Table 1). The values attained at task failure were not significantly different between the trials (Table 1). Significant recovery was observed at the start of IPS2 (CIs: 9.1, 39.9 N·m), but not IPS-OCC2 (CIs: –30.9, 5.1 N·m).

Voluntary activation demonstrated a condition x time interaction (F = 4.45, P = 0.022), with VA declining across IPS2 (CIs: –2.4, –22.2%) and CONT2 (CIs: –35.0, –9.2%), indicating the presence of central fatigue. Voluntary activation was significantly lower at the start of the second bout of exercise compared to IPS1 for IPS2 (CIs: –23.0, –2.9%) and IPS-OCC2 (CIs: –28.4, –8.5%). The values attained at task failure were not significantly different between the conditions (Table 1). No recovery was observed at the start of either IPS2 (CIs: –20.4, 6.7%) or IPS-OCC2 (CIs: –23.0, 20.2%).

Variability and complexity

The variability and complexity data are presented in Table 2. There were significant condition x time interactions for both the SD (F = 5.62, P = 0.002) and CV (F = 7.74, P = 0.004). The SD significantly increased over time in IPS1 (CIs: 2.8, 8.3 N·m) and CONT-OCC2 (CIs: 1.6, 3.9 N·m). The CV significantly increased in all conditions, except for IPS-OCC2 (CIs: –0.02, 0.01 %). The amount of variability was significantly greater at the start of IPS-OCC2 compared to IPS1 (CIs: SD, 0.3, 5.3 N·m; CV, 0.007, 0.06 %). The values attained at task failure were not significantly different for either the SD or CV (Table 2).

The torque profiles of contractions in a representative participant in all conditions is shown in Figure 2. The mean time course of complexity in the ipsilateral and contralateral conditions is shown in Figures 3 and 4, respectively. Complexity, as measured by ApEn, demonstrated a condition x time interaction (F = 14.97, P < 0.001). The ApEn decreased as a function of time in all conditions except for IPS-OCC2 (CIs: –0.02, 0.05). The ApEn was significantly lower at the start of IPS-OCC2 compared to IPS1 (CIs: –0.5, –0.2). There were no significant differences between conditions at task failure (Table 2). Significant recovery was observed at the start of IPS2 (CIs: 0.04, 0.4), but not IPS-OCC2 (CIs: –0.1, 0.07).

There was a significant condition x time interaction for the DFA α exponent (F = 18.45, P < 0.001). The DFA α exponent increased with time in all conditions, except for IPS-OCC2 (CIs: –0.03, 0.03). DFA α was significantly greater at the start of IPS-OCC2 compared to IPS1 (CIs: 0.03, 0.3). There were no significant differences between the values attained at task failure between the different conditions (Table 2). Significant recovery was observed at the start of IPS2 (CIs: 0.03, 0.2), but not IPS-OCC2 (CIs: –0.08, 0.2).

Discussion

The major novel findings of the present study were as follows: 1) that fatigue in the ipsilateral limb, followed by 3 minutes of passive recovery (the IPS trial), resulted in the recovery of torque output complexity to values close to that in fresh muscle at the onset of subsequent isometric contractions. 2) The recovery from fatigue, and of torque complexity, was abolished when muscle blood flow was occluded (the IPS-OCC trial), and participants were unable to complete a full minute of contractions. 3) Contractions of the contralateral limb performed to task failure, followed by contractions of the unexercised limb (the CONT trial)

resulted in no crossover of central fatigue and no significant effect on torque output complexity. 4) Performing contractions of the contralateral limb and occluding blood flow at task failure in order to accentuate afferent feedback (the CONT-OCC trial) did not result in increased central fatigue or significant reductions in torque output complexity. These findings suggest that the fatigue-induced loss of torque complexity can be attributed primarily to events occurring in the periphery. Ultimately, however, the torque output (and its complexity) represents the integration of central and peripheral processes, as reflected in the lack of recovery of central fatigue in the IPS-OCC condition.

Complexity and neuromuscular fatigue in pre-fatigued muscle

At the start of the second bout of the IPS trial (IPS2), which was designed to provide incomplete recovery from neuromuscular fatigue, significant decrements in MVC torque, potentiated doublet torque and voluntary activation were evident compared with fresh muscle (Table 1: IPS1). These observations indicate that neuromuscular function remained compromised for the subsequent exercise bout, a fact confirmed by the significantly shorter time to task failure in IPS2 (Table 1). Nevertheless, the complexity values at the start of IPS2 were not significantly different from fresh muscle, though were, nonetheless, blunted (Table 2; Figure 2). That there was evidence of neuromuscular fatigue at the onset of exercise suggests that the recovery kinetics of neuromuscular complexity is somewhat faster than that of neuromuscular fatigue. One interesting observation is that in IPS2 the initial EMG amplitude was higher as a fraction of the normalised maximum than IPS1 (~70% vs. ~55%) suggesting that a larger recruitment and/or firing frequency was required throughout IPS2. The complexity of torque output in this bout rapidly declined to values similar at task failure to IPS1. We have previously demonstrated that torque complexity can be systematically reduced by both increasing the absolute demand of a task (i.e. by increasing torque requirements) or by increasing the relative demand of a task (i.e. by fatiguing the muscle; Pethick *et al*., 2015; Pethick *et al*., 2016). In this case, it appears that the carry-over effects of fatigue in IPS2 more rapidly increased the relative demand of the task, resulting in a precipitous fall in complexity alongside the mechanical measures of central and peripheral fatigue.

The IPS-OCC condition was designed to prevent the recovery from peripheral fatigue by occluding the leg for 3 min after contractions performed to task failure. The results showed that occlusion completely abolished the recovery from fatigue of all types (Table 1; Figure 2),

a finding consistent with previous research (Bigland-Ritchie *et al*., 1986; Woods *et al*., 1987; Quistorff *et al*., 1993). As a result, the time to task failure during subsequent contractions was significantly shorter than when fresh, with participants unable to complete a full minute of exercise (Table 1). Knee extensor torque complexity at the start of IPS-OCC2 was also no different than at task failure in IPS1 (Table 2), indicating that circulatory occlusion prevented its recovery. Given that circulatory occlusion holds the muscle ischaemic, preventing the recovery of the muscle metabolic milieu (Yoshida and Watari, 1997; Lanza *et al*., 2006), it is likely that the failure of ApEn and DFA α to demonstrate any recovery was mediated, at least partially, by this maintained peripheral fatigue. However, the loss of torque complexity does not simply appear to be caused by a peripheral fatigue-induced failure to transduce central drive into mechanical output, since a depression in voluntary activation was also present at the onset of contractions. The mechanism of this maintained central fatigue following occlusion is not as obvious as its peripheral counterpart, but the previous observation of the rapid recovery in the EMG response to motor cortex stimulation during cuff occlusion of the arm suggests the effects occur upstream of the motor cortex (Gandevia et al., 1996). Specifically, the perturbed muscle metabolic milieu may have been detected by group III and IV afferents, resulting in inhibitory feedback acting to limit motor cortical drive (Gandevia et al., 1996; Amann and Dempsey, 2008; Amann *et al*., 2011). Such a response would seem to suggest that peripheral and central fatigue are inextricably linked under these experimental conditions, with changes in torque output complexity reflecting the integrated response to neuromuscular fatigue.

We performed two trials which initially exercised the left knee extensors to task failure followed by 1 min rest and then contractions of the right knee extensors to failure (CONT), or the same protocol with cuff occlusion from task failure of the left knee extensors maintained until task failure of the right knee extensors occurred (CONT-OCC). Both were performed in an attempt to isolate the effects of central fatigue on subsequent exercise. CONT-OCC was itself performed in an attempt to further diminish central drive consequent to afferent feedback. In contrast to our hypotheses, neither condition influenced the extent or progression of central fatigue nor the loss of knee extensor torque complexity (Table 1, Table 2, Figure 3 and Figure 4). Therefore, fatiguing contralateral exercise, with or without cuff occlusion, did not reduce voluntary activation. Although the potentiated doublet was significantly reduced following the CONT trial, this reduction was relatively small and does not appear to have had any functional impact, since surface EMG, as well as measures of

variability and complexity did not change compared to IPS1. These results suggest that contralateral exercise had no meaningful effect on central or peripheral function in the unexercised leg, and thus no effect on torque complexity. Our failure to disentangle the effects of central and peripheral fatigue experimentally is most likely an indication that the fatigue-induced loss of torque complexity has both central and peripheral components which cannot be effectively separated.

Physiological basis for changes in neuromuscular system behaviour

The sustained loss of torque complexity only following ipsilateral exercise and femoral occlusion adds weight to our previous suggestion that peripheral fatigue is a major contributor to the loss of neuromuscular complexity (Pethick et al., 2016). This loss of torque complexity was associated with both peripheral and central fatigue at the onset of contractions in IPS-OCC2. The reduced mechanical output of the motor units, reflected in the decreased potentiated doublet torque, cannot on its own explain the reduced torque complexity, since this would only serve to reduce the amplitude of torque fluctuations. Such a reduction would have no effect on the complexity metrics used in the present study, and in any case the amplitude of torque fluctuations actually increased with muscle fatigue (SD and CV data in Table 2). To alter torque complexity, the pattern of motor unit firing must also have changed in some way. It may be, therefore, that metabolite-mediated peripheral fatigue is simply a pre-requisite for central adjustments which act on the motor unit pool and are themselves responsible for the loss of torque complexity (see below).

We have previously speculated that increased common synaptic input to motoneurons could be responsible for the fatigue-induced loss of torque complexity (Pethick *et al*., 2016). It has recently been demonstrated that common synaptic input to motoneurons is increased with the development of neuromuscular fatigue (Castronovo *et al*., 2015). As common synaptic input has been proposed as the main determinant of torque variability (Diderkisen *et al*., 2012; Farina *et al*., 2014), any increase in this common synaptic input could be reflected in a change in torque complexity. Motor unit synchronisation, the correlated discharge of action potentials (Semmler, 2002), is a necessary consequence of common synaptic input and should, therefore, also increase as common synaptic input increases. Increased motor unit synchronisation has been associated with reduced force steadiness during simulated contractions (Yao *et al*., 2000), decreased complexity of postural tremor with ageing (Sturman *et al*., 2005), and increased regularity in the surface EMG as fatigue develops

(Mesin *et al*., 2009; Beretta-Piccoli *et al*., 2015). Fatigue at the start of the IPS-OCC2 bout may, therefore, have been accompanied by increased common synaptic input and motor unit synchronisation, with this being responsible for the reduced complexity observed. However, direct measurements of motor unit behaviour will be required to confirm this.

As observed previously (Pethick et al., 2016), the values of each of ApEn, the DFA α exponent, and the potentiated doublet torque reached consistently low values at task failure across experimental conditions, despite each commencing with different levels of fatigue and complexity. It has been suggested that consistent levels of peripheral fatigue at the termination of exercise might reflect the achievement of a 'sensory tolerance limit' (see Hureau *et al*., 2016 for review). The sensory tolerance limit proposes that metabolic perturbations (i.e. those contributing to peripheral fatigue) are detected by group III and IV afferents, which provide inhibitory feedback to the central nervous system at various levels. This, in turn, reduces central motor drive in order to restrict the development of peripheral fatigue beyond a certain limit (Amann *et al*., 2006). Evidence is accumulating to suggest an important role for the aforementioned afferents in CNS adjustments during various types of physical exercise (Blain et al., 2016). It is therefore tempting to link the consistency of torque complexity to the sensory tolerance limit hypothesis. However, present data show only that torque complexity is one of a number of parameters which reach similarly low (or high) values at task failure. The functional significance of these findings is unclear, but consistently low complexity at task failure could be viewed in the following way: neuromuscular fatigue results in maximal or near maximal effort being required to attain the desired target torque, and maximal efforts are associated with low torque complexity (Pethick et al., 2015). In addition, the increased variability in torque output results in targeting error which, by virtue of low physiological complexity, the neuromuscular system can no longer correct with sufficient haste (i.e., the system has lost its adaptability; Pethick *et al*., 2016). Task failure, from this perspective, is a fatigue-induced loss of motor control and adaptability. To what extent the processes purported to account for the sensory tolerance limit might contribute to the loss of motor control at this point (by limiting further increases in central drive) requires further experimentation.

Limitations

In the present experiments, it was not possible to perform the switch between measures of the left (contralateral) leg and the right leg instantaneously, due to the design of the dynamometer. The delay between measurements (1 minute) may, therefore, have reduced the degree of central fatigue measured in CONT and CONT-OCC. Voluntary activation has been shown to recover within 2 min following fatiguing contractions (for review, see Carroll *et al*., 2016). However, as occlusion has been shown to prevent the recovery of voluntary activation (Gandevia *et al*., 1996) any reduction would have been preserved in the CONT-OCC condition. This was not the case, and we therefore concluded that central fatigue did not influence either of the CONT conditions. We were also unable to measure muscle metabolite concentration or muscle oxygenation to establish the effect of occlusion on these parameters. However, we are confident that occlusion was effective due to the lack of recovery in both fatigue and complexity in the IPS-OCC trial. Finally, direct measurements of motor unit behaviour during fatiguing contractions will be necessary in future work to establish the precise neurophysiological basis for the loss in torque output complexity.

Conclusion

In summary, this study has demonstrated that pre-existing fatigue influences the complexity of knee extensor torque at the start of an exercise bout. Specifically, when recovery from fatigue was prevented by occluding the previously exercised leg for 3 minutes, the recovery of complexity was also abolished, in contrast to the same protocol performed without occlusion. Contralateral contractions performed to failure, with or without subsequent occlusion, did not significantly diminish torque complexity during subsequent contractions of the opposite leg. These results support the notion that peripheral fatigue is a primary contributor to the loss of torque complexity. However, since torque output complexity is ultimately the expression of both central and peripheral processes, the loss of torque complexity is most likely to be an integrated response of both to this peripheral fatigue.

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Author contributions:

This work was completed at the University of Kent. Pethick, Winter and Burnley were each involved in the conception and design of the study and contributed to the writing and critical revisions of the manuscript. Pethick collected the data; Winter wrote the MATLAB code to process the data. All authors were involved in the analysis and interpretation of the data. All authors approved the final version of the manuscript.

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Table 1. Voluntary torque, potentiated doublet, voluntary activation, and EMG responses during contractions in the first exercise bout of IPS and the second exercise bouts of IPS, CONT, IPS-OCC and CONT-OCC.

Parameter	IPS1	IPS2	CONT2	IPS-OCC2	CONT-OCC2
Mean test torque, N·m	106.6 ± 31.6				
Time to task failure, min	4.7 ± 2.7	2.0 ± 0.9[b]	3.8 ± 2.3	0.6 ± 0.4[b]	3.9 ± 1.4
Global fatigue					
Pre-exercise MVC, N·m	218.9 ± 72.0	157.2 ± 37.3[b,c]	190.4 ± 68.8	100.4 ± 15.0[b]	180.6 ± 57.5[b]
Peak MVC at task failure, N·m	115.4 ± 19.1[a]	112.4 ± 31.6[a]	108.1 ± 14.4[a]	99.4 ± 24.5	119.6 ± 16.7[a]
Mean MVC at task failure, N·m	97.9 ± 23.2[a]	93.2 ± 25.2[a]	97.3 ± 19.1[a]	85.1 ± 23.1	100.3 ± 13.1[a]
Peripheral fatigue					
Pre-exercise doublet, N·m	95.7 ± 23.6	77.3 ± 17.7[b,c]	86.4 ± 22.0[b]	65.7 ± 15.5[b]	88.5 ± 29.0
Doublet at task failure, N·m	52.8 ± 9.7[a]	53.7 ± 13.4[a,b]	55.7 ± 12.6[a,b]	55.9 ± 15.0[a,b]	58.8 ± 14.0[a,b]
% Change at task failure	43.4 ± 11.7	30.7 ± 7.2	34.6 ± 11.4	15.2 ± 12.3	31.3 ± 15.6
Central fatigue					
Pre-exercise VA, %	92.5 ± 2.6	79.5 ± 9.8[b]	88.9 ± 8.9	74.1 ± 6.7[b]	89.6 ± 5.5
VA at task failure, %	72.7 ± 18.0	67.2 ± 14.7[a,b]	66.8 ± 9.2 [a,b]	73.9 ± 7.3[b]	75.2 ± 13.4[b]
% Change at task failure	21.6 ± 20.1	16.2 ± 12.9	24.5 ± 10.8	0.2 ± 10.5	15.5 ± 18.0
Surface EMG					
arEMG at task beginning, % MVC	55.6 ± 6.9	70.1 ± 14.9	56.3 ± 10.3	68.7 ± 13.8	61.0 ± 9.1
arEMG at task failure, % MVC	75.7 ± 20.5[a]	74.1 ± 16.7	71.5 ± 26.5	69.1 ± 14.1	77.2 ± 23.8

Values are means ± SD, n = 9. IPS1 is the first exercise bout in the global fatigue condition; IPS2, CONT2, IPS-OCC2 and CONT-OCC2 are the second exercise bouts in these respective conditions; MVC, maximal voluntary contraction; VA, voluntary activation; arEMG, average rectified EMG of the vastus lateralis. Letters indicate a statistically significant difference compared to the following: [a]pre-exercise/task beginning value, [b]IPS1, [c]IPS1 at task failure.

Table 2. Variability, complexity and fractal scaling responses during contractions in the first exercise bout of IPS and the second exercise bouts of IPS, CONT, IPS-OCC and CONT-OCC.

Parameter	IPS1	IPS2	CONT2	IPS-OCC2	CONT-OCC2
SD					
SD at task beginning, N·m	3.1 ± 1.0	3.6 ± 1.6	3.6 ± 1.6	5.9 ± 3.0[b]	4.4 ± 2.7
SD at task failure, N·m	8.6 ± 1.1[a]	7.6 ± 1.6	8.4 ± 2.0	6.3 ± 0.9	7.2 ± 1.0[a]
CV					
CV at task beginning, %	2.9 ± 0.4	3.4 ± 1.1	3.6 ± 1.3	6.1 ± 2.1[b]	4.1 ± 2.0
CV at task failure, %	8.8 ± 1.6[a]	7.7 ± 3.1[a]	8.4 ± 3.6[a]	6.8 ± 2.4	7.3 ± 2.4[a]
ApEn					
ApEn at task beginning	0.46 ± 0.14	0.35 ± 0.17[c]	0.38 ± 0.16	0.14 ± 0.08[b]	0.34 ± 0.16
ApEn at task failure	0.12 ± 0.06[a]	0.15 ± 0.06[a]	0.14 ± 0.09[a]	0.12 ± 0.07	0.14 ± 0.05[a]
DFA α					
DFA α at task beginning	1.39 ± 0.10	1.49 ± 0.08[c]	1.44 ± 0.10	1.56 ± 0.10[b]	1.48 ± 0.11
DFA α at task failure	1.60 ± 0.05[a]	1.58 ± 0.05[a]	1.62 ± 0.06[a]	1.56 ± 0.10	1.62 ± 0.06[a]

Values are means ± SD, n = 9. IPS1 is the first exercise bout in the neuromuscular fatigue condition; IPS2, CONT2, IPS-OCC2 and CONT-OCC2 are the second exercise bouts in these respective conditions; SD, standard deviation; CV, coefficient of variation; ApEn, approximate entropy; DFA α, detrended fluctuation analysis. Letters indicate a statistically significant different compared to the following: [a]pre-exercise value/value at task beginning, [b]IPS1, [c]IPS1 at task failure.

Figure legends

Figure 1: Schematic of the experimental trials

IPS, ipsilateral trial; IPS-OCC, ipsilateral occlusion trial; CONT, contralateral trial; CONT-OCC, contralateral occlusion trial. Black bars represent intermittent contractions of the leg in question, grey bars represent periods of occlusion.

Figure 2: Raw torque output during contractions in a representative participant

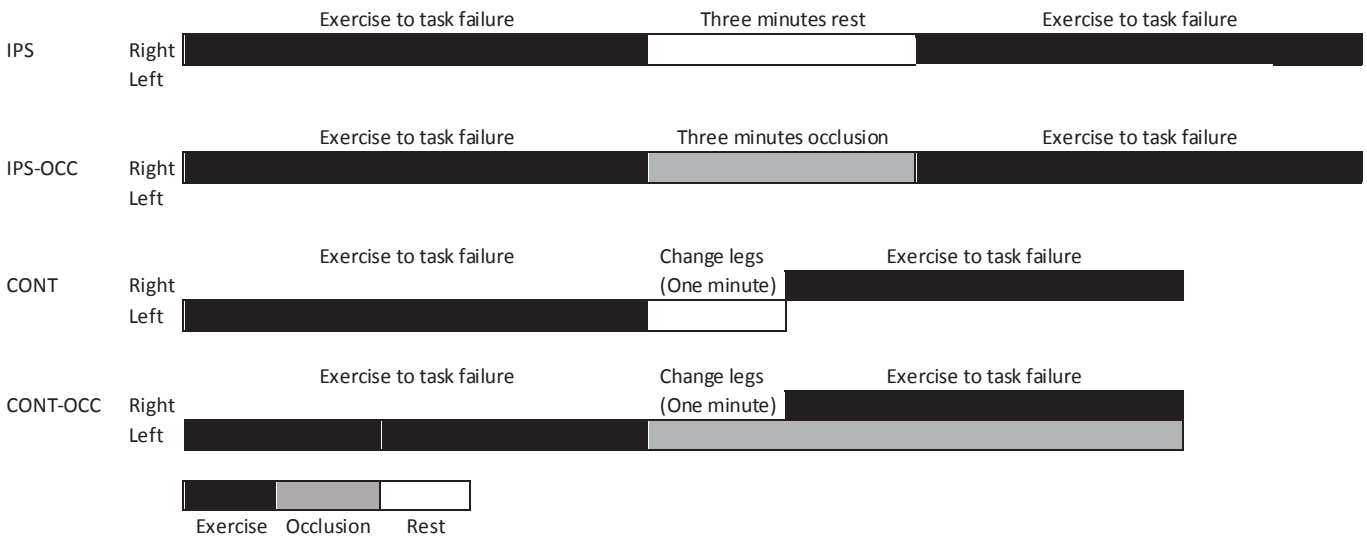
Contractions are all drawn from the first minute of exercise to illustrate the effect of each trial on torque complexity. The ipsilateral trial's first bout is presented as the 'fresh muscle' condition (panel A). Note that complexity is substantially reduced in the first minute of the Ipsilateral Occlusion trial only (panel C).

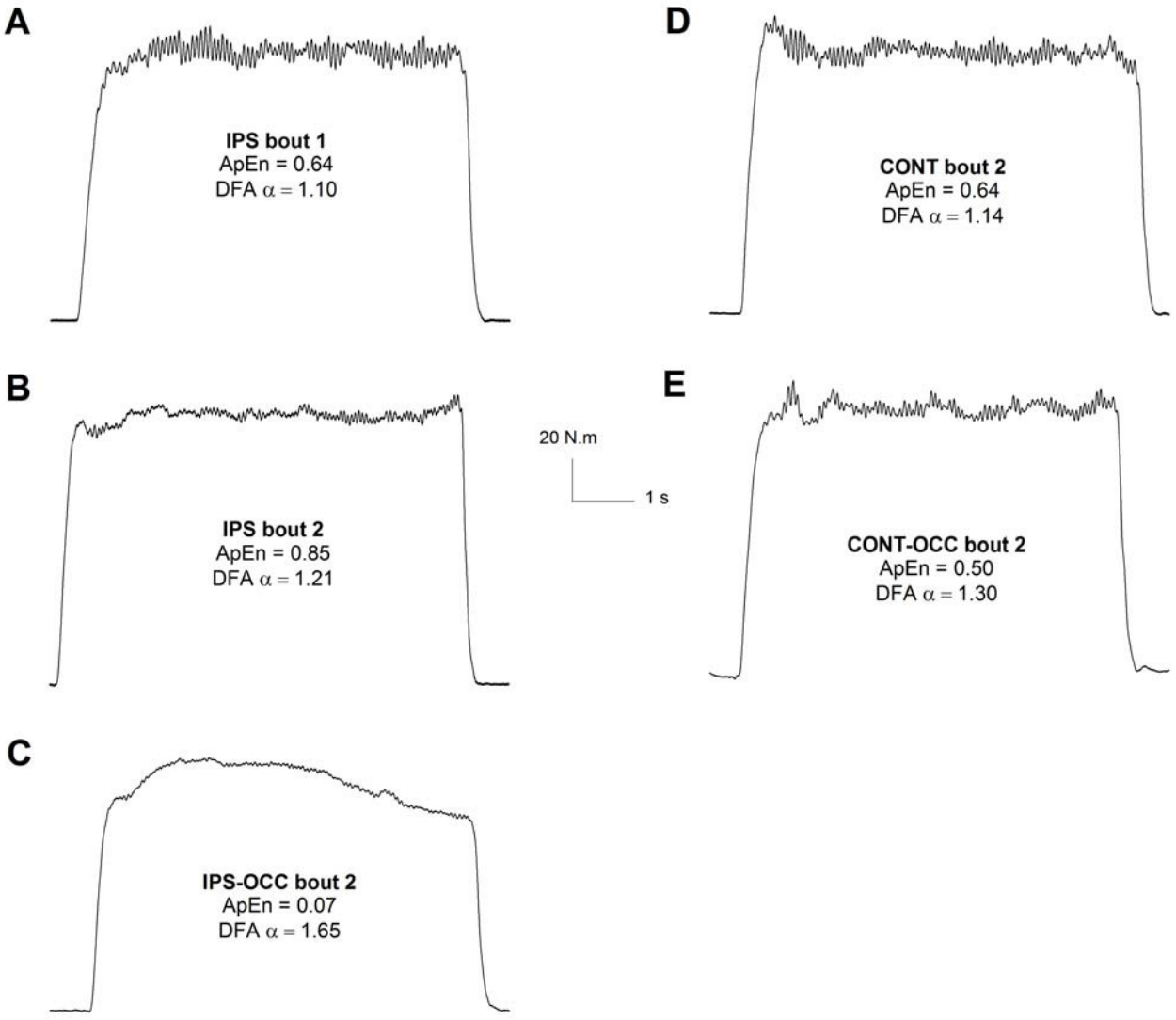
Figure 3 Torque output complexity during the ipsilateral trials (IPS, IPS-OCC)

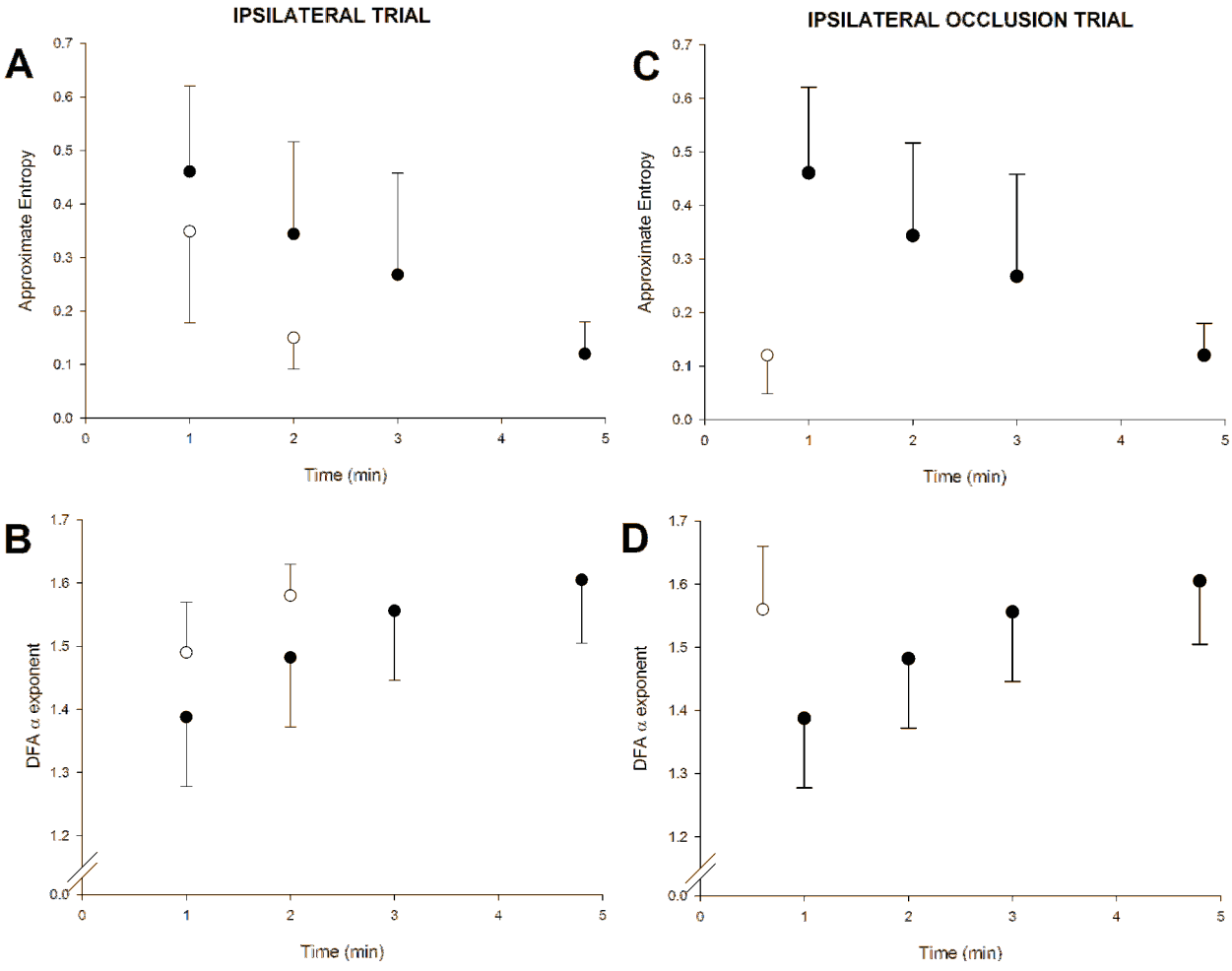
The top panels (A and C) show the Approximate Entropy (ApEn) values during each trial. Black symbols represent the first bout of contractions in the ISP condition, whilst the white symbols represent the second bout of IPS (Panels A and B) and IPS-OCC (Panels C and D). Bottom panels (B and D) show the detrended fluctuation analysis α exponent (DFA α). Note the reduction in ApEn and increase in DFA α as the contractions progress, as well as the lack of recovery at the start of the second Ipsilateral Occlusion trial. Values are mean ± SD, n = 9.

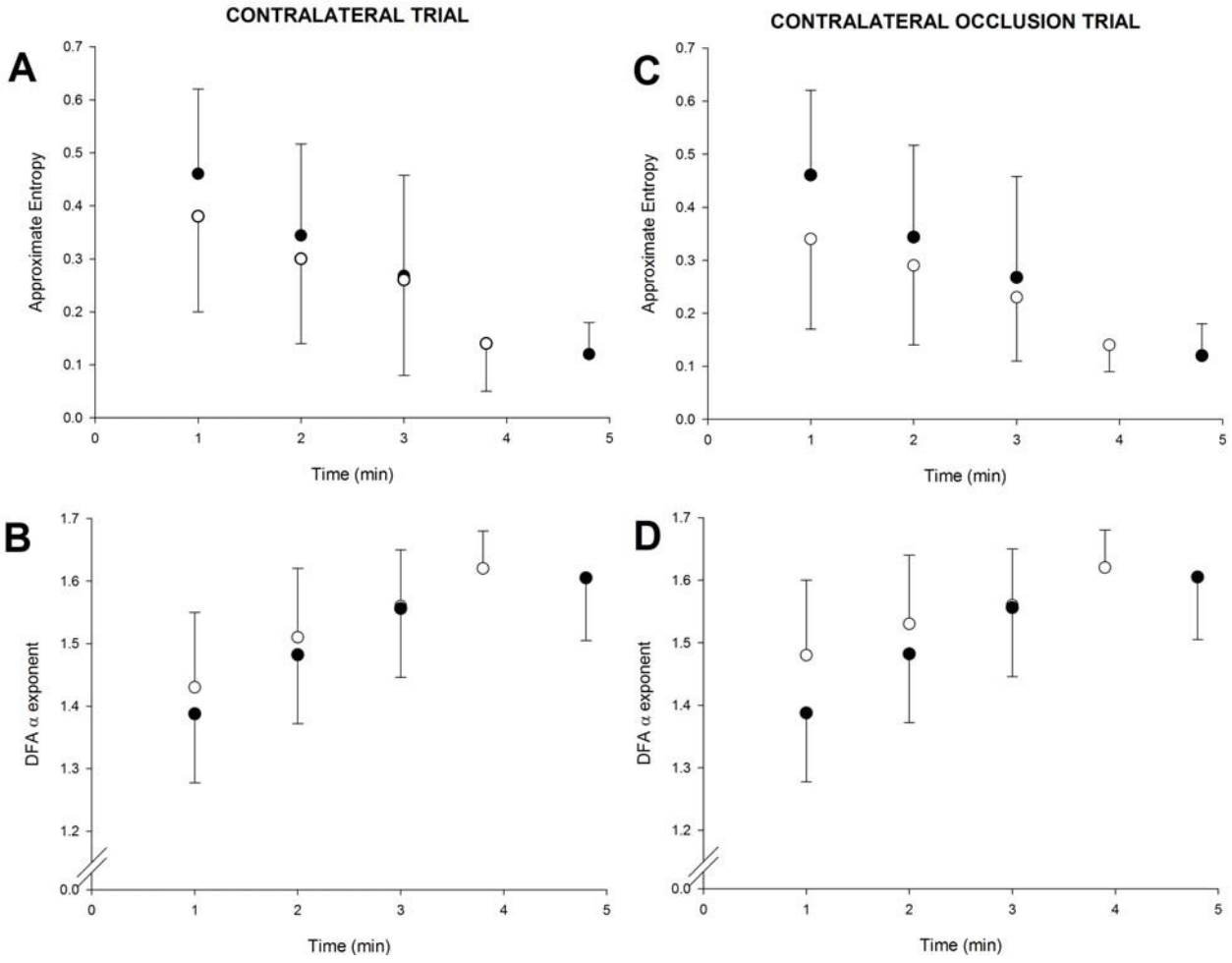
Figure 4: Torque output complexity during the contralateral trials (CONT, CONT-OCC)

The top panels (A and C) show the Approximate Entropy (ApEn) values during each trial. Black symbols represent the first bout of contractions in the ISP condition, whilst the white symbols represent the second bouts in CONT (Panels A and B) and CONT-OCC (Panels C and D). Bottom panels (B and D) show the detrended fluctuation analysis α exponent (DFA α). Note the lack of significant alterations in ApEn and in DFA α during the second contraction buts in each condition. Values are mean ± SD, n = 9.


Exercise to task failure
Three minutes rest
Exercise to task failure
IPS
Right
Left
Exercise to task failure
Three minutes occlusion
Exercise to task failure
IPS-OCC
Right
Left
Exercise to task failure
Change legs
(One minute)
Exercise to task failure
CONT
Right
Left
Exercise to task failure
Change legs
(One minute)
Exercise to task failure
CONT-OCC
Right
Left
Exercise
Occlusion
Rest


A
IPS bout 1
ApEn = 0.64
DFA α = 1.10
B
IPS bout 2
ApEn = 0.85
DFA α = 1.21
C
IPS-OCC bout 2
ApEn = 0.07
DFA α = 1.65
20 N.m
1 s
D
CONT bout 2
ApEn = 0.64
DFA α = 1.14
E
CONT-OCC bout 2
ApEn = 0.50
DFA α = 1.30


IPSILATERAL TRIAL
IPSILATERAL OCCLUSION TRIAL
A
B
C
D
Approximate Entropy
DFA α exponent
Time (min)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1.2
1.3
1.4
1.5
1.6
1.7
0
1
2
3
4
5


CONTRALATERAL TRIAL
CONTRALATERAL OCCLUSION TRIAL
A
B
C
D
Approximate Entropy
DFA α exponent
Time (min)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1.2
1.3
1.4
1.5
1.6
1.7
0
1
2
3
4
5