DYNAMICS OF NEUROPHYSIOLOGICAL ADJUSTMENTS DURING FATIGUING EXERCISE WITH CONCOMITANT WORKING MEMORY CHALLENGES

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Abstract

**Purpose:** This study aimed to determine the neurophysiological mechanisms associated with reduced endurance performance during cognitive-motor dual-task (CMDT) compared to a motor task alone.

**Methods:** Eighteen healthy men performed isometric quadriceps contractions at 15% of maximal voluntary contraction (block of 170 s interspaced by neuromuscular evaluations) until exhaustion. This task was performed on three separate days: i) in the absence of concomitant cognitive task; ii) with concomitant 1-back task and iii) with concomitant 2-back task. Autonomic nervous system activity (i.e. heart rate variability and pupil diameter), perceptions of muscle and mental efforts, and cognitive performance were continuously monitored. Peripheral and central determinants of neuromuscular function were assessed at rest, between each block, and at task failure using electrical femoral nerve stimulation.

**Results:** Endurance time was shorter during 2-back (1273±723 s; p<0.001) and 1-back (1470±791 s; p=0.04) conditions, compared with control (1704±1157 s). Central voluntary activation exhibited a larger decrease in 1-back (-6.7%; p=0.04) and 2-back (-8.7%; p<0.001) conditions, compared to control (-3.7%). Sympathetic activity showed a greater increase in 2-back condition compared to control. Perceived muscle effort was higher during 2-back than during control. Cognitive performance decreased similarly with time during both CMDT but was always lower during 2-back condition.

**Conclusions:** Motor performance is reduced when adding a concomitant demanding memory task to a prolonged isometric exercise. This can be explained by the interaction of various neurophysiological factors, including i) higher level of central fatigue; ii) greater perturbations of autonomic nervous system activity and iii) higher perception of effort.

**Keywords:** Dual task; Neuromuscular fatigue; Mental fatigue; N-back task; Cognitive control; Working memory

**Abbreviations:**
ACC: Anterior cingulate cortex
ANS: Autonomic nervous system
CMDT: Cognitive-motor dual-task
HR: Heart rate
HRV: Heart rate variability
ln HF: Natural logarithm of high frequency
ln RMSSD: Natural logarithm of the root-mean-square difference of successive RR intervals
MVC: Maximal voluntary contraction
NA: Non-answer
NASA-TLX: NASA-Task Load Index
PFC: Prefrontal cortex
RPE: Ratings of perceived exertion
RR interval: Time interval between consecutive heart beats
RT: Reaction time
TF: Task failure
Tw_p: Potentiated twitch peak
Tw_s: Superimposed twitch
VAL: Voluntary activation level

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Author Contribution Statement: CC conducted the experiments, performed data and statistical analysis and wrote the manuscript. RR conceived and designed research and contributed to data analysis and interpretation. FV, TR and JMV conducted the experiments and contributed to data interpretation. TB conceived and designed research, conducted the experiments and contributed to data interpretation. MG conceived and designed research, conducted the experiments, contributed to data interpretation and wrote the manuscript. All authors read and approved the manuscript.
Introduction

Activities of daily-living, sports, military operations and activities in the industrial field all require the ability to perform multiple tasks simultaneously. Cognitive-motor dual-task situations (CMDT) require the simultaneous accomplishment of a cognitive task and a motor task. The effect of adding a cognitive task to a motor task such as walking has been widely explored. Previous research have shown a decrease in performance “quality” (e.g. stride or gait velocity) when two tasks are performed simultaneously compared to a separate execution, in both healthy and patients’ populations (Beauchet et al. 2005; Beurskens and Bock 2012; Heraud et al. 2018). Beyond this effect on task execution, the ability to maintain a physical effort can also be anticipated in case of prolonged dual-task performance. Through the prolongation of a physical exercise, neuromuscular fatigue, which originates from central (i.e. spinal or supraspinal) and peripheral (e.g. excitation-contraction coupling) sites, occurs and eventually leads to the inability to sustain the task. Similarly, several studies have shown that the completion of a prolonged cognitive task could induce a state of mental fatigue, defined as “a psychobiological state characterized by subjective feelings of tiredness and lack of energy” (Boksem and Tops 2008). Mental fatigue may also have a detrimental effect on the accomplishment of a subsequent physical exercise [e.g. for review, Van Cutsem et al. (2017)]. Although many studies have considered these two types of fatigue in isolation, the model of prolonged CMDT, which may induce i) neuromuscular fatigue caused by the achievement of the motor task and ii) mental fatigue caused by the accomplishment of the cognitive task, is less explored.

Contemporary research showed that the addition of a cognitive task to a motor task impaired endurance time (Yoon et al. 2009; Mehta and Agnew 2012; Keller-Ross et al. 2014; Pereira et al. 2015). For instance, Yoon et al. (2009) reported a reduced endurance time during submaximal contraction of the elbow flexors (i.e. 20% of maximal voluntary contraction; MVC) in the presence of a cognitive task (i.e. subtraction from a 4-digit number by 13 or 7 every 3 s). However, the neurophysiological mechanisms underlying changes in motor performance in these studies remain unclear. A better understanding of why performance is affected in CMDT is of importance in both clinical and sports settings, notably to develop specific countermeasures.

Findings from Yoon et al. (2009) suggested that the reduction of endurance time during CMDT could be related to changes in the autonomic nervous system (ANS) activity with larger increase in sympathetic drive, altering skeletal muscle blood flow and contractility. Otherwise, it has been shown that the achievement of a working memory task (i.e. n-back task), regardless of the level of complexity (i.e. 1-back, 2-back, 3-back) or the type of stimulus presented (i.e. auditory, visual), induced predominant brain activity in the frontal and parietal areas (Owen et al. 2005). In addition, the prefrontal cortex (PFC) plays a key role in motor skills since it is closely linked to movement planning by projecting on different motor areas. Changes in PFC oxygenation are associated with the ability to generate force (Rasmussen et al. 2007; Alexandre et al. 2014). Mehta & Parasuraman (2014) found a higher PFC oxygenation during the initial stages of CMDT (i.e. submaximal handgrip and concomitant mental-math task) compared to control condition, indicating a greater mobilization of brain resources to accomplish both tasks simultaneously. However, PFC oxygenation was lower at exhaustion in CMDT compared to control condition. Changes in PFC activation during CMDT may in turn impact the activity of downstream motor areas (e.g., the supplementary motor area or the primary motor cortex), leading into neuromuscular impairments (Tanaka and Watanabe 2012).

Keller-Ross et al. (2014) evaluated neuromuscular fatigue before and after submaximal contraction of the elbow flexors (i.e. 20% MVC until exhaustion), with or without a cognitive task (i.e. subtraction by 13 from a 4-digit number every 3 s), using transcranial magnetic stimulation. These authors found similar reductions in cortical voluntary activation at exhaustion in CMDT and control conditions, despite a briefer endurance time in CMDT. It has been suggested that the addition of a cognitive task might induce specific adjustments from supraspinal sites (e.g. motor cortex and upstream) causing a faster decrease in voluntary muscle activation. Although such an assumption is plausible, this latter remains hypothetical since fatigue measurements were carried out only before and after the fatiguing task, impeding to firmly conclude to an early appearance of central fatigue during CMDT. From a methodological point of view, measuring fatigue only at exhaustion may also present some limits, as discussed elsewhere (Twomey et al. 2017). Endurance time in such isometric tasks is notably dependent on subjects’ cooperation and confounding factors, such as pain and motivation, which may
largely influence the fatigue level at exhaustion. The use of intermittent protocols allowing repeated fatigue measurements throughout the protocol is thus essential i) to obtain fatigue measurements at isotime (i.e. with similar amount of work), allowing optimal comparisons between different conditions (e.g. CMDT vs control), and ii) to provide new insights about the kinetics of both peripheral and central fatigue.

It is currently well documented that the addition of a mental-math task has a negative impact on upper limbs endurance performance (Yoon et al. 2009; Keller-Ross et al. 2014; Pereira et al. 2015; Shortz and Mehta 2017). However, very few studies have yet examined the impact of CMDT involving lower limb muscles that are engaged in many sports and daily-living activities. The corticospinal structures involved in the control of skeletal muscles may differ according to the investigated muscle group (Martin et al. 2006; Gruet et al. 2014), potentially leading to different fatigue kinetics between upper and lower limb muscles. In addition, the nature of the cognitive task added to the motor task may also influence the motor performance, as different cognitive tasks may not involve the exact same brain areas (Yi-Rong et al. 2011). To our knowledge, no study has yet investigated the addition of a cognitive task during prolonged contraction of the quadriceps. A working memory task (n-back task) was chosen because it is an executive task that predominantly relies on prefrontal structures (Owen et al., 2005). In addition, working memory is particularly relevant in sports and exercise contexts as many sport activities require memorizing trajectories (e.g. motor sports, sailing, or orienteering) or action plans (e.g. a choreography in artistic sports or a strategy in collective sports). The n-back task has the advantage to be easily adjustable to create different versions of cognitive load. While a 1-back task leads to small cognitive demands and changes in PFC activity, a 2-back implies much more cognitive effort and PFC activation (Owen et al., 2005). In contrast to previous CMDT studies where no information was available in cognitive performance, the use of such a well-controlled task also makes possible to obtain reliable indices of performance throughout the task (i.e. serial n-back tasks, (Tempest et al. 2017)) to carefully link cognitive activity to neuromuscular fatigue.

Accordingly, the aim of this study was to evaluate the effects of adding working memory demands (i.e., either a 1-back or a 2-back task) to prolonged contractions of the quadriceps on: i) the development of neuromuscular fatigue; ii) the adjustments in ANS activity and iii) the cognitive performance. We hypothesized that endurance time would be further impaired by adding cognitive demands (2-back vs 1-back and control), notably via an earlier appearance of central fatigue.

Methods

Participants

Eighteen active men (age: 23±5 years; height: 177±6 cm; weight: 71±8 kg) participated in this study. All subjects were healthy with no cardiovascular, musculoskeletal, auditory or cognitive disorders. Written informed consent was obtained for all participants before their involvement in the study, which was approved by the local ethics committee and conducted in accordance with the declaration of Helsinki. All subjects were kept naive to the hypotheses of the study until the study completion.

Experimental design

Each subject visited the laboratory on four occasions over a 2-4 weeks period. The first visit was a familiarization session with the equipment and experimental procedures which was followed by three experimental sessions. Each session was conducted on different days, around the same time of the day for a given subject (i.e. ± 60 min interval), and was separated by at least 48h in order to avoid residual muscle fatigue effects. Subjects were refrained from both caffeine and meals at least two hours prior to each experimental session. Subjects were also asked not to be involved in strenuous physical activities the day preceding each session.

The three experimental sessions were as follows: i) control condition, during which subjects performed the motor task alone; ii) 1-back condition, during which the motor task was performed simultaneously with a 1-back cognitive task; iii) 2-back condition, during which the motor task was performed simultaneously with a 2-back cognitive task. The order of the three experimental sessions was randomized and counterbalanced across subjects.

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**Experimental setup**

Subjects sat upright in an adjustable custom-built chair with both hips and knees at 90° of flexion (Fig. 1). The lower right leg was securely attached to the force sensor (F2712 200 daN, Celiens MEIRI, France) ~ 2 cm above the malleoli of the ankle joint with an inextensible padded velcro strap. Subjects were firmly secured to the chair using non-compliant racing car seat belts across the waist in order to minimize any extraneous body movements during the motor task.

Peripheral nerve stimulation

Single electrical stimuli (1-ms duration) were delivered via a constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to the right femoral nerve using a cathode of 15-mm diameter (Controle Graphique, Brie-Comte-Robert, France) located high in the femoral triangle and 50x90-mm rectangular anode (Durastick Plus, DJO Global, Vista, CA, USA) placed in the right gluteal fold. Single stimuli were elicited in an incremental fashion until reaching a plateau in the resting twitch amplitude and concomitant M-wave. The optimal stimulus intensity was then set at 30% above the level required to obtain maximal resting twitch and M-wave amplitudes. This intensity was used throughout the protocol for a given experimental session but recalculated for each experimental session.

Muscle activity

Electromyography activity from the vastus lateralis of the right leg was continuously recorded using bipolar surface electrodes (Controle Graphique, Brie-Comte-Robert, France, Ag-AgCl, 15-mm diameter; 25-mm center-to-center inter-electrode distance). For each session, low electrodes–skin impedance was achieved by shaving, softly abrading the skin with abrasive sponge and cleaning it with isopropyl alcohol. The reference electrode was placed on the right patella. Signals were amplified, filtered (band-pass 10-500 Hz) and digitized on-line at a sampling rate of 2 kHz and analyzed offline using commercially available software (Acqknowledge 4.1, MP150BIOPAC Systems, Inc., Santa Barbara, CA, USA).

ANS activity

Heart rate (HR) and heart rate variability (HRV) were continuously recorded via an automated beat-by-beat monitor (RS800CX, POLAR Electro, Inc., Finland). Pupillary responses were continuously assessed using an eye-tracker (REDn Scientific, SensoMotoric Instrument, Germany) at a sampling rate of 60 Hz. The distance subject-camera was ~ 70 cm (Fig. 1). The experiments were conducted in a room isolated from daylight, so that the luminance level did not vary within and between the sessions.

**Questionnaires**

The Brunel Mood State (Terry et al. 2003) was used to assess mood before and after each experimental session. The NASA-Task Load Index (TLX) questionnaire (Hart and Staveland 1988) was used to assess subjective workload after each experimental session. Success and intrinsic motivation related to the task (motor task alone or motor task + concomitant cognitive task) were evaluated before each experimental session (Matthews et al. 2001).

**Cognitive tasks**

During both n-back tasks, items were sequentially presented using an auditory procedure. Subjects had to evaluate whether the present item was similar to the one presented n positions back in the sequence. Seven letters with clearly distinct sound in the French language (i.e. H, R, O, I, E, P and U) were presented using E-prime software (E-prime 2.0, Psychology Software Tools, Sharpsburg, MD, USA) and audio speakers. Both 1-back and 2-back cognitive tasks were designed in blocks of 165 s. Each block was divided itself in two sub-blocks of 75-s duration, separated by 15 s with no cognitive task (Fig. 2a). Each sub-block comprised 37 letters presented every 2000 ms in a semi-randomized way in order to have 7±1 matches per sub-block. For the 1-back task, subjects had to compare the current letter to the previous one. If the current letter presented was similar to the previous one (i.e. match trial), the subjects had to press the right computer mouse. In the other case (i.e. non-match trial), the subjects
had to press the left computer mouse. For the 2-back task, subjects had to compare the current letter to the one presented two trials before. Similarly to the 1-back task, they were told to press the right computer mouse for a match trial and the left for a non-match trial. Subjects were instructed to provide their answers for each trial as accurately and quickly as possible. No feedback was given between each trial or after each block. During the control condition (i.e. motor task without concomitant cognitive task), subjects were asked to hold the computer mouse with their hands to replicate the same posture as during both other sessions.

Motor task
The motor task consisted in intermittent submaximal isometric contractions of the right knee extensors performed at 15% MVC until task failure (TF). The MVC obtained during the first experimental session was used to calculate the target force level and was kept for the two next sessions. Thus, the motor task for the three experimental sessions was performed at the same absolute force level. The motor task was divided in the same blocks and sub-blocks as the cognitive task (Fig. 2b). However, the motor task was prolonged by 5-s more after the end of the cognitive task (i.e. 75-s duration, see above) for each sub-block to ask the subjects to estimate their ratings of perceived muscular exertion (leg RPE) and ratings of perceived mental exertion (mental RPE) with a 10-grade Borg scale (Borg 1982). Then, each block of motor task was composed by 80 s at 15% MVC, 10 s of rest and then 80 s at 15% MVC once again (Fig. 2a).

Neuromuscular evaluation
A neuromuscular evaluation was performed at baseline, immediately at the end of each block, at TF, and during recovery (see below). The timing of neuromuscular evaluation was standardized and each evaluation was performed in 40 s (Fig. 2c). Each neuromuscular evaluation included two brief MVCs of ~ 4-s duration. During each MVC, one superimposed stimulation was manually delivered on the force plateau, then, followed after 2 s by 2 stimulations delivered in the relaxed muscle (Fig. 2c).

Experimental protocol
At the arrival to the laboratory, subjects completed the Brunel Mood State and Motivation questionnaires. Then, if the experimental condition was a CMDT (i.e. 1-back or 2-back condition), participants performed the cognitive task during 165 s. The subjects had to repeat this “mental warm-up” if their overall accuracy was below 90%. Then, subjects performed a standardized warm-up of the knee extensors (i.e. 360 s intermittent contractions at increasing force levels). After a 5-min recovery period, resting measurements were obtained during 345 s to collect baseline ANS data (i.e. pupil diameters, HR, HRV). The first 180 s corresponded to a “total rest period” (i.e. neither cognitive task nor motor task). When the task of day was a dual-task condition, subjects had to perform one block of the cognitive task of the day (i.e. 1-back or 2-back during 165 s) alone, corresponding to a “cognitive period”. At the end of the 345 s, subjects were asked to estimate leg and mental RPE. Then, a baseline neuromuscular evaluation was performed (see above) just before beginning the fatiguing task.

During the fatiguing task, blocks of dual-task (or motor task alone for control condition) and neuromuscular evaluations were repeated until TF (Fig. 2b). TF was defined as the inability to maintain the target force level for 4 consecutive seconds. Verbal encouragements were given only during neuromuscular evaluations (but not during submaximal fatiguing contractions as they may interfere with the cognitive auditory procedure). Participants were asked not to give priority to a task during CMDT conditions and to perform both tasks as well as possible. ANS activity parameters and cognitive performance were recorded continuously during the fatiguing task.

Neuromuscular evaluations were performed 150 s and 300 s after TF (recovery period). ANS activity parameters were also continuously recorded during the 300-s recovery period. Immediately after recovery measurements, the subjects were asked to complete the NASA-TLX questionnaire and the Brunel Mood State.

Data analysis
Neuromuscular data
Endurance time was calculated as the time between the beginning of the first block and TF,
excluding the periods of neuromuscular evaluations. M-wave and potentiated peak twitch (Tw\(_p\)) amplitudes were obtained from evoked responses in the relaxed muscle. The voluntary activation level (VAL) was quantified by measurement of superimposed twitch (Tw\(_s\)) during MVC and calculated as follows: VAL = (1 – Tw\(_s\)/ Tw\(_p\) \(\times\) 100 with Strojnik & Komi correction (Strojnik and Komi 1998). MVC, Tw\(_p\), VAL and M-wave were averaged for each neuromuscular evaluation in order to obtain only one value for each neuromuscular parameter.

**Cognitive performance**
A d’ was computed for each block using the following formula: d’ = ZHit – ZFA (Macmillan and Creelman 1990), where Hit represents the proportion of correct matches (hits/(hits + misses)), and FA represents the proportion of match responses on non-match trials (false alarms/(false alarms + correct negative)). The use of d’ is recommended to provide a cognitive performance index that is not biased by individual responses tendencies (Haatveit et al. 2010). The mean reaction time (RT) was also computed for each block. Values below 100 ms, corresponding to an anticipation were excluded. The number of non-answer (NA) was also computed for each block.

**HR and HRV data**
All data were analyzed with a freeware HRV analysis software (Kubios HRV Analysis, Standard version 3.0.1, Finland). Data sets were first visually inspected to exclude artifacts by eliminating the time intervals between consecutive heart beats (RR intervals) which differed from the previous and the subsequent RR intervals. Removed RR intervals were replaced by spline interpolation and then data sets were smoothed using the smoothness prior method of the software (Luque-Casado et al. 2016). The natural logarithm of the root-mean-square difference of successive normal RR intervals (ln RMSSD) and the natural logarithm of high frequency (ln HF; HF: 0.15 – 0.40 Hz) were used as indices of vagal control (Laborde et al. 2017).

**Resting measures.** Data were first averaged across 120 s windows during the “total rest period”, excluding the first and last 30 s. During the “cognitive period”, data were averaged across the last 30 s of each sub-block of cognitive task.

**Measures during the fatiguing task.** Using the same method than during “cognitive period”, data during the fatiguing task were averaged across the last 30 s of each sub-block of cognitive task, excluding the last 5 s during which the muscle contraction was prolonged alone (Fig. 2a).

**Recovery measures.** HR and HRV data were averaged across 30 s windows centered at 30 s, 60 s, 90 s, and 120 s after neuromuscular evaluation performed at TF.

**Pupillary data**
The pupillary raw data were collected using the BeGaze software (version 3.0, SensoMotoric Instrument, Germany). Identified blinks and short periods of signal loss were removed from the data and a 10-points moving average was used to smooth signals. Pupillary data were averaged across the same temporal windows than HR and HRV data (Fig. 2a).

For HR, HRV parameters, pupil diameter, leg RPE and mental RPE, one value was obtained for each sub-block. These values were then averaged in order to obtain only one value per completed block (i.e. both sub-blocks completed).

**Analyses at isotime**
Neuromuscular data for the three experimental sessions were compared at six time points: i) before the fatiguing task; ii) at 50% of the duration of the shortest test for a given subject (from the three experimental sessions); iii) at 100% of the duration of the shortest test for a given subject (from the three experimental sessions); iv) at TF; v) at 150 s post-TF and vi) at 300 s post-TF. If no neuromuscular evaluation corresponded to exactly 50% or 100% of the duration of the shortest test for a given subject, the nearest neuromuscular evaluation was considered.

Leg RPE, mental RPE and cognitive performance were compared at five time points: i) at “cognitive period”; ii) at first block of dual task; iii) at block corresponding to 50% of the duration of the shortest test for a given subject; iv) at block corresponding to 100% of the duration of the shortest test for a given subject; v) at TF (i.e. last block entirely completed).
Pupillary, HR and HRV data were compared at ten time points: i) at “total rest period”; ii) at “cognitive period”; iii) at first block of dual task; iv) at block corresponding to 50% of the duration of the shortest test for a given subject; v) at block corresponding to 100% of the duration of the shortest test for a given subject; vi) at TF (i.e. last block entirely completed); vii) at 30 s post-TF; viii) at 60 s post-TF; ix) at 90 s post-TF; x) at 120 s post-TF. These analyses at isotime are necessary to interpret the dynamics of cognitive and neurophysiological parameters at the exact same amount of work, as previously suggested (Rupp et al. 2015).

Statistical analysis
All statistical procedures were performed on Statistica version 7 (Statsoft, Tulsa, OK, USA). Normality of distribution and homogeneity of variances of the main variables were confirmed using Shapiro-Wilk normality test and Levene’s test, respectively. One-way ANOVAs for repeated-measures were used to test the effect of condition (i.e. 1-back vs 2-back vs control) on endurance time, motivation scores, NASA-TLX scores, all neuromuscular variables at TF, all ANS activity variables at TF and leg RPE at TF. Paired t-tests were used to assess the effect of condition (i.e. 1-back vs 2-back) on mental RPE at TF and all cognitive variables at TF. Two separated two-way ANOVAs with repeated-measures were used to test the effect of condition and time on: i) all dependent variables at isotime and ii) all dependent variables during recovery. Post hoc Fisher’s LSD test was applied to determine the difference between two mean values if ANOVA revealed a significant main effect or interaction effect. Significance was set at 0.05 for all analyses. All data are presented as mean ± SD in the text and as mean ± SEM in the figures.

Results

Exercise performance
Significant main effect of condition (P=0.002) was found for endurance time (Fig. 3a). Endurance time was significantly longer in control condition (1306±836 s) compared to 1-back (1128±592 s; P=0.04) and 2-back condition (982±545 s; P<0.001) but no significant difference (P=0.10) was found between 1-back and 2-back conditions despite a numerical difference suggesting a longer endurance time in 1-back than in 2-back condition.

Neuromuscular fatigue
Fatiguing tasks. Tw (Fig. 3b) and MVC (Fig. 3c) decreased significantly during the fatiguing tasks (P<0.005), with no significant differences between conditions at isotime and TF. There was no significant main effect of time or condition on M-wave amplitude. VAL (Fig. 3d) decreased significantly during fatiguing tests (P<0.001). VAL at 100% of the shortest test duration was significantly lower in 1-back (P=0.04) and 2-back (P<0.001), compared to control condition. However, at TF, VAL did not differ between the three experimental conditions.

Recovery. Independently of the condition, Tw (Fig. 3b) and MVC (Fig. 3c) were significantly higher after 150 s post-TF compared to TF (P<0.001), but no significant differences were found between 150 s and 300 s post-TF. Independently of the condition, VAL (Fig. 3d) was significantly lower at TF than at 300 s post-TF (P=0.03) with no differences between 150 s and 300 s post-TF.

ANS activity
Fatiguing tasks. Significant main effect of time (P<0.001) and condition-time interaction (P<0.05) were found for HR (Fig. 4a). HR was significantly higher at 0%, 50% and 100% of the shortest test duration in 2-back than in control condition (P<0.01). HR was significantly higher at 50% and 100% of the shortest test duration in 2-back than in 1-back condition (P<0.05). However, at TF, HR did not differ between the three conditions. Significant main effect of time (P<0.001) and condition-time interaction (P<0.01) were found for pupil diameter (Fig. 4b). Pupil diameter was significantly higher in 2-back than in both control and 1-back conditions at 0%, 50% and 100% of the shortest test duration (P<0.01). However, at TF, pupil diameter did not differ between the three conditions. Significant main effect of time (P<0.001) and condition-time interaction (P<0.01) were found for ln HF values (Fig. 4c).
In 1-back condition, ln HF was significantly higher at 0% and at 50% of the shortest test duration compared to 2-back condition (P<0.01). Similarly, in HF was significantly higher in control condition at 0% and at 100% of the shortest test duration than in 2-back condition. ln RMSSD (Fig. 4d) decreased significantly during the fatiguing tasks (P<0.001), with no significant differences between conditions at isotime and TF.

Rest and Recovery. HR decreased similarly during recovery (P<0.001) but remained higher than pre-fatigue (P<0.001), independent of the condition (Fig. 4a). Pupil diameter decreased significantly during recovery (P<0.001) but was significantly higher at 30 s, 60 s, 90 s and 120 s post-TF than pre-fatigue (P<0.001), regardless of the condition (Fig. 4b). Pupil diameter was significantly higher in 2-back than in 1-back and control conditions during period of “cognitive task alone” (P<0.001) and at 90-s recovery (P<0.05). ln HF (Fig. 4c) and ln RMSSD values (Fig. 4d) remained stable, whatever the condition, during recovery but was systematically lower than pre-fatigue (P<0.05).

Perception of muscular and mental effort

Leg RPE (Fig. 5a) increased significantly during the fatiguing tasks (P<0.001) and was significantly higher in 2-back condition (P<0.005) compared to control, without interaction effect (P=0.11). No difference was found between control and 1-back conditions (P=0.07) and between 1-back and 2-back conditions (P=0.25). At TF, leg RPE did not differ between the three conditions. Significant main effect of time (P<0.001) and condition-time interaction (P=0.01) were found for mental RPE (Fig. 5b). Mental RPE was significantly higher in 2-back compared to 1-back condition for all isotime points (P<0.02) and at TF (P=0.03).

Cognitive performance

The d’ score (Fig. 6a) decreased significantly during the fatiguing tasks (P<0.001) and was significantly higher in 1-back (P<0.001) compared to 2-back condition, without condition-time interaction effect (P=0.20). At TF, d’ was significantly lower in 2-back compared to 1-back condition (P<0.03). RT (Fig. 6b) decreased significantly during the fatiguing tasks (P=0.02) and was significantly slower in 2-back (P<0.001) compared to 1-back condition, without condition-time interaction effect (P=0.19). At TF, no difference was found for RT between 1-back and 2-back conditions (P=0.13). The number of NA (Fig. 6c) increased significantly during the fatiguing tasks (P<0.001) without main condition (P=0.38) or condition-time interaction (P=0.99) effects. At TF, no difference was found between 1-back and 2-back conditions on the number of NA (P=0.95).

Questionnaires

Motivation. There were no significant differences between conditions for intrinsic (1-back 7.2±2.8; 2-back 7.3±3.0; control 7.3±2.5; P=0.89) and success (1-back 15.3±4.1; 2-back 15.5±4.0; control 15.1±4.2; P=0.77) motivation.

Mood. The mood questionnaire revealed a significant increase in fatigue (P<0.001) and in anger subscales (P=0.04) with no significant differences between conditions (Table 1). No main effect of time or condition was found for confusion (P>0.38) and depression (P>0.14) subscales. The mood questionnaire revealed a significant decrease in tension (P=0.03) and in vigor subscales (P<0.01) with no significant differences between conditions.

Subjective workload. The overall score was significantly higher in 1-back and 2-back conditions compared to control (P=0.02) and in 2-back compared to 1-back condition (P=0.01) (Fig. 7a). Subjects reported a higher mental demand in 1-back and 2-back conditions compared to control (P<0.001) and a higher mental demand in 2-back compared to 1-back condition (P<0.001) (Fig. 7b). No main effect of condition was found for physical demand (P=0.85), temporal demand (P=0.19), performance (P=0.43), effort (P=0.14) and frustration (P=0.37).

Discussion

The current study investigated the impact of two working memory tasks (i.e. 1-back and 2-back) repeatedly induced during prolonged exercise of the knee extensors on motor performance (i.e.
endurance time) and the associated modulation in neuromuscular function, ANS activity and cognitive performance. We found reduced lower endurance time in CMDT compared to control condition. The endurance time was further reduced when increasing the complexity of the cognitive task. The nature of the experimental design, using intermittent protocol with comprehensive repeated multimodal measurements sheds new light on the dynamics of neurophysiological and cognitive adjustments that take place during prolonged CMDT. Reduced endurance during prolonged CMDT may be explained by the combination of various neurophysiological mechanisms including greater decrease in voluntary central drive, larger changes in ANS activity and higher perceived exertion.

**Endurance changes during CMDT**

The magnitude of endurance impairment reported in the current study was -13% and -25% in 1-back and 2-back respectively, compared to control condition. These findings are consistent with previous studies reporting a similar range of decline in endurance time during CMDT (i.e. ~ -10 to -25%) using concomitant complex mental-math tasks (Yoon et al. 2009; Keller-Ross et al. 2014; Pereira et al. 2015). However, in these three studies endurance was unchanged compared to a simple mental-math task condition. All together these results suggest that the reduction in endurance during CMDT is related on the difficulty of the cognitive task. Moreover, the aforementioned studies found a negative impact of CMDT on upper-limbs endurance. Our results extend this finding to the lower-limbs and suggest that the decline in performance during CMDT is not related to the investigated muscle. This latter hypothesis will have to be confirmed in a study comparing the impact of CMDT in various muscle groups using a similar CMDT protocol.

**Neurophysiological mechanisms underlying reduced endurance during CMDT**

Our main hypothesis to explain reduced endurance during CMDT was related to potential higher neuromuscular perturbations in this condition compared to control. We found a similar amount of neuromuscular fatigue at TF (i.e. identical decrease in MVC between conditions), despite significantly lower endurance time in both CMDT compared to control condition. With the aim to clarify the origin of the neuromuscular impairments, we investigated the kinetics of peripheral and central mechanisms of fatigue throughout two CMDT protocols and a control condition (i.e. task motor alone). At 100% of the shortest test duration, we found a greater loss of VAL in 2-back (-8.3%) compared to 1-back (-6.4%) and control (-3.5%). This pattern of VAL decrease is consistent with the pattern of endurance time, with performance during 2-back < 1-back < control. Thus, we suggest that reduced endurance time during CMDT may be explained, at least in part, by central alterations causing reduced VAL. We propose several arguments to support this assumption.

Firstly, this larger reduction in VAL can be related in part to changes in subjects’ psychological state throughout CMDT. Enoka & Duchateau (2016) recently proposed a fatigue model involving an interdependence between perceptual factors (i.e. homeostasis and psychological state) and physiological factors (i.e. muscle activation and contractile function). It is possible that changes in some modulating factors of subjects’ psychological state (e.g. motivation, mood, performance feedback, expectations) may have a negative impact on voluntary activation patterns. Mental RPE findings showed that the subjects perceived i) an increased difficulty to accomplish the cognitive tasks throughout both CMDT and ii) a more important difficulty during the 2-back task compared to the 1-back task. Thus, a greater alteration in the factors modulating subject’s psychological state (e.g. motivation, expectations and performance feedback) is expected during 2-back vs 1-back and 1-back vs control. These factors can, alone or in combination, contribute to earlier disengagement from the motor task when adding a complex cognitive task and precipitate reduction in VAL. This is also consistent with the motivational intensity theory (Brehm and Self 1989) and those developed in the psychobiological model of exercise tolerance (Marcora and Staiano 2010), suggesting that the subject may reduce its voluntary effort once the demands of the task overstep the upper limit of what he/she is disposed to do.

A second argument that can explain the reduced VAL could be the appearance of a mental fatigue state during prolonged CMDT. Many studies have shown a negative impact of mental fatigue on subsequent motor performance (Marcora et al. 2009; Pageaux et al. 2013; Pageaux and Lepers 2016). In these studies, the performance impairment is mainly explained by the fact that mentally fatigue subjects reached their maximum level of perceived exertion faster, leading to earlier task disengagement. Our results on leg RPE, showing that subjects reached their maximum level of perceived exertion earlier
in 2-back than in control condition, indicate that similar mechanisms may take place during CMDT. The appearance of mental fatigue is also supported by the gradual decrease in cognitive performance (i.e. $d'$ reduction) during both CMDT, as previously shown during the completion of prolonged cognitive tasks alone (Lorist et al. 2005; Van der Linden and Eling 2006). In addition, several studies highlighted that the mental demand could be evaluated by changes in ANS activity (Kahneman and Beatty 1966; Callister et al. 1992; Kennedy and Scholey 2000; Causse et al. 2017). The mental demand generated by the cognitive task would cause an increase in sympathetic drive causing, for example, an increase in HR (Callister et al. 1992; Kennedy and Scholey 2000) or pupil diameter (Kahneman and Beatty 1966; Causse et al. 2017). Our data on pupil diameter and HR support a greater sympathetic activity in 2-back compared to the two other conditions, confirming with objective markers the higher mental demand in this condition. Although the 1-back did not induce more sympathetic drive compared to control, it does not preclude the occurrence of mental fatigue during 1-back. The muscle contraction also causes an increase in ANS activity. This can concealed the increase in sympathetic drive caused by the accomplishment of the concurrent cognitive task. Moreover, results from the NASA-TLX questionnaire provide subjective indications that 1-back generated a higher mental demand than control condition.

Some authors argued that mental fatigue would cause an impairment in dopamine transmission, a neurotransmitter that allows communication within the central nervous system (Chaudhuri and Behan 2000; Boksem et al. 2006). This impairment would mainly affect the anterior cingulate cortex (ACC) (Lorist et al. 2005; Boksem et al. 2006). It has been shown that the ACC is involved in working memory (Habeck et al. 2005), in motor output (Tanaka and Watanabe 2012) and in perceived exertion mechanisms (Williamson et al. 2001). Taken together, our results discussed above are in favor of an impairment of this brain area during prolonged CMDT. In addition, it has been shown that performing CMDT also resulted in changes in PFC activity (Mehta and Parasuraman 2014). Mehta & Parasuraman (2014) showed that PFC activity was significantly lower at the end of the CMDT (i.e. at exhaustion) compared to the control condition, suggesting a failure of this brain area with the appearance of mental fatigue. According to Tanaka & Watanabe (2012), the frontal area (including PFC and ACC) contributes to the increase in motor output to counteract the effects of supraspinal fatigue. Therefore, a failure of this cerebral area could have impacted the ability to voluntarily generate force during CMDT in the present study.

The difference of VAL between both CMDT and control condition was only observed at 100% of the shortest test duration. This result suggests that the accomplishment of a concurrent cognitive task leads to a deleterious effect only after a certain period of time. This is consistent with previous works showing that cognitive task must be performed long enough (i.e. > 10min) to induce mental fatigue (Van Cutsem et al. 2017). Consequently, when the CMDT includes a motor task that cannot be maintained for a long time, the achievement of the concurrent cognitive task does not induce mental fatigue. This hypothesis is supported by the results of Mehta & Agnew (2012) who reported that endurance time was negatively affected by mental demand for a fatiguing task performed at a low level of force (i.e. 35% MVC) that can be sustained for a long time (i.e. ~15min), but not for a high level of force (i.e. 55% MVC), that can only be sustained for a shorter time (i.e. ~5min).

Previous studies reported that the addition of a cognitive mental-math task to a motor task led to a greater increase in sympathetic drive than during control condition (Yoon et al. 2009; Mehta and Agnew 2012; Keller-Ross et al. 2014). Our results extend these observations to working memory challenges. We found a larger increase in pupil diameter and HR and a larger decrease in ln HF during 2-back condition, supporting a more elevated sympathetic drive during this condition compared to both others. However, no significant difference was observed in ln RMSSD between conditions. Although HRV parameters are valuable indicators of ANS activity, their use during exercise remains quite controversial (Prinsloo et al. 2014) as they can be influenced by several parameters (e.g. respiratory rate, artifact correction) making their interpretation difficult (Laborde et al. 2017; Rincon Soler et al. 2018). In order to overcome this limit, we also recorded pupillometry data. To our knowledge, this study is the first to use pupillometry to evaluate ANS activity during prolonged CMDT. We found a persistent (i.e. for each isotime point) higher pupil diameter during 2-back condition compared to both others, showing the sensitivity of this index to detect small adaptations in ANS activity during CMDT. Moreover, the absence of differences in pupil diameter between 1-back condition and control suggests that the mental task must induce a sufficient cognitive demand to elicit specific changes in ANS activity during CMDT.
This is consistent with the results of Yoon et al. (2009), who did not observe differences in ANS activity (i.e. HR and blood pressure) between CMDT with simple mental-math task and control condition.

Several authors suggested that changes in ANS activity could impact motor performance and contribute to decreased endurance time (Yoon et al. 2009; Mehta and Agnew 2012; Keller-Ross et al. 2014). It has been shown that increased sympathetic drive can restrict muscle blood flow during exercise, since the release of noradrenaline causes vasoconstriction of the blood vessels within the active muscles (Thomas and Segal 2004). It can be speculated that a greater activation of ANS during 2-back condition could impair leg blood flow and oxygenation and contribute to the observed reduced performance. Such assumption should be confirmed with appropriate hemodynamics measurements. On the other hand, it has been shown that an increase in sympathetic drive contributed to the increase in the force produced by the type II muscle fibers (i.e. fast twitch) but also, a reduction in the force produced by the type I muscle fibers (i.e. slow twitch) (Passatore and Roatta 2006). However, we did not observe differences in peripheral fatigue between conditions, making unlikely the hypothesis of a specific alteration in type I fiber contractility during CMDT.

Recovery of neuromuscular parameters was similar across all conditions. This is consistent with previous results during CMDT with mental-math task (Yoon et al. 2009; Mehta and Agnew 2012; Keller-Ross et al. 2014) confirming that the addition of a cognitive task to a motor task does not influence force recovery. Mehta & Agnew (2012) showed that CMDT could slow down HR recovery. In the present study, only pupillary recovery appeared to be slowed since the pupil diameter was more elevated in 2-back after 90-s recovery (i.e. R90 in Fig. 4b). This may reflect an elevated noradrenaline level during recovery (caused by the more important sympathetic activation during 2-back condition) (Mehta and Agnew 2012). The absence of differences between conditions for HR and HRV recovery could reflect, similarly to what happen during exercise, the less sensitivity of these parameters to CMDT.

Limitations and perspectives

A potential limitation of this study is that peripheral nerve stimulation technique does not allow differentiating spinal from supraspinal mechanisms implicated in central fatigue. We cannot firmly conclude that the loss of VAL reported in this study was only related to supraspinal impairment. One way to overcome this limitation would be the use of transcranial magnetic stimulation to determine cortical voluntary activation and the associated changes in corticospinal excitability and inhibition (Gruet et al. 2013). Nevertheless, we believe that the discomfort associated with the repetitive use of transcranial magnetic stimulation (i.e. several stimulations are needed to determine cortical VAL (Todd et al. 2003)) could have interfered with the psychological state of the subjects, reducing the ecological validity of the present findings. This issue seems particularly relevant for protocols manipulating cognitive/perceptual parameters along with repeated fatigue measurements (i.e. neuromuscular evaluations every 3 min in the present study). Another limitation of our study is the absence of neuroimaging data. It is well acknowledged that some brain regions, upstream to the motor cortex, exchange information and synchronize their activities during a fatiguing exercise, influencing the generation of the motor command (e.g. (Tanaka and Watanabe 2012)). As discussed above, some brain areas such as the PFC may be particularly affected during CMDT. Future CMDT studies should augment traditional neurostimulation methods with non-invasive neuroimaging (e.g. multichannel functional near-infrared spectroscopy) and corticomuscular coherence (electroencephalography-electromyography coupling) techniques.

Conclusion

The present study provides experimental evidence that motor performance is reduced when adding a concomitant demanding memory task to a fatiguing quadriceps exercise. Various neurophysiological adaptations can contribute, alone or most likely in combination, to the decrease in endurance performance during CMDT. These factors may include an exaggerated and/or early exercise-induced central fatigue or greater perturbation in ANS activity during CMDT. The accomplishment of the cognitive task, by inducing a state of mental fatigue and modifying the psychological state of the subjects may also induce a higher perception of effort contributing to earlier disengagement from the motor task.
The nature of the mechanisms underlying reduced endurance time in CMDT is probably dependent on the complexity of the concurrent cognitive task. A better understanding of these mechanisms will promote the development of new interventions to counteract fatigue, particularly in ecological situations since cognitively demanding motor tasks are relevant to functional activities in daily-life and sports.
References


Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
## Table 1

<table>
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<td>7.1±3.1*</td>
<td>0.4±1.2</td>
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* Denotes statistical significance (p < 0.05)
Fig. 1 Experimental setup with 10-grade Borg scales (a); Eye-tracker (b); Height adjustable tables (c); Computer used for the cognitive tasks (d); TV screen (e); Constant-current stimulator (f); Computer used for electromyography and force recordings (g); Computer used for eye-tracker recordings (h); MP150BIOPAC Systems (i); Computer mouse (j); Audio speaker used for the cognitive task (k).

Fig. 2 Panel A: timing of one block of dual-task (the grey areas indicate the periods of analysis for the different signals); Panel B: Overview of the fatiguing task during one session for a given subject; Panel C: Timing of delivered peripheral nerve stimulation (PNS) during one neuromuscular evaluation.

Fig. 3 Panel A: Effect of condition on endurance time; Effect of condition and time on potentiated peak twitch (Tw_p) (Panel B), maximal voluntary contraction (MVC) (Panel C) and voluntary activation level (VAL) (Panel D). Data points are presented as mean±SEM (n=18). Significant differences between *1-back and control and **2-back and control. *Significant difference with Pre. **Significant difference with TF. Pre: before exercise; 50%: 50% of the duration of the shortest test; 100%: 100% of the duration of the shortest test; TF: task failure; R150: at 150 s post-TF; R300: at 300 s post-TF. The grey areas indicate the period of fatiguing tasks.

Fig. 4 Effect of condition and time on heart rate (HR) (Panel A), pupil diameter (Panel B), natural logarithm of high frequency (ln HF) (Panel C) and natural logarithm of the root-mean-square difference of successive normal RR intervals (ln RMSSD) (Panel D). Data points are presented as mean±SEM (n=15). Significant differences between $^4$1-back and 2-back and $^2$2-back and control. $^4$Significant difference with Pre. $^2$Significant difference with TF. Total rest period: neither cognitive task nor motor task; Cognitive period: cognitive task alone; 0%: first block performed; 50%: block corresponding to 50% of the duration of the shortest test; 100%: block corresponding to 100% of the duration of the shortest test; TF: task failure; R30: at 30 s post-TF; R60: at 60 s post-TF; R90: at 90 s post-TF; R120: at 120 s post-TF. The grey areas indicate the period of fatiguing tasks.

Fig. 5 Effect of condition and time on ratings of perceived muscular exertion (leg RPE) (Panel A) and ratings of perceived mental exertion (mental RPE) (Panel B), respectively. Data points are presented as mean±SEM (n=17). Significant differences between $^3$2-back and control and $^4$1-back and 2-back. $^3$Significant main effect of time. Cognitive period: cognitive task alone; 0%: first block performed; 50%: at block corresponding to 50% of the duration of the shortest test; 100%: at block corresponding to 100% of the duration of the shortest test; TF: task failure. The grey areas indicate the period of fatiguing tasks.

Fig. 6 Effect of condition and time on cognitive performance (d') (Panel A), reaction time (RT) (Panel b) and number of non-answer (number of NA) (Panel C). Data points are presented as mean±SEM (n=15). $^5$Significant differences 1-back and 2-back. $^5$Significant main effect of time. Cognitive period: cognitive task alone; 0%: first block performed; 50%: at block corresponding to 50% of the duration of the shortest test; 100%: at block corresponding to 100% of the duration of the shortest test; TF: task failure. The grey areas indicate the period of fatiguing tasks.

Fig. 7 Effect of condition on NASA-TLX scores: Overall score (Panel A); Mental demand subscale (Panel B). Data points are presented as mean±SEM (n=18). $^3$Significant difference between three experimental conditions.

Table 1 Scores of Brunel Mood State questionnaire for the experimental conditions. Data are presented as mean±SD (n=18). *Significant difference with Pre value.