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INTERACTION OF CEREBRAL, CARDIAC AND MUSCULAR CHANGES INDUCED BY ACUTE ENDURANCE EXERCISE

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INTERACTION OF CEREBRAL, CARDIAC AND MUSCULAR CHANGES INDUCED BY ACUTE ENDURANCE EXERCISE

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- Christine MOHR, Professeure à l'Université de Lausanne

autorise, sans se prononcer sur les opinions du candidat, l'impression de la thèse de Monsieur Jérôme SPRING, intitulée :

« Interaction of cerebral, cardiac and muscular changes induced by acute endurance exercise »

Jean-Philippe LERESCHE
Doyen

Lausanne, le 28 mars 2018

Abstract

English version

Resting and premotor brain activity seems to be decisive for numerous motor behaviors. The literature has shown that acute endurance exercise may modulate the brain activity and reduce the motor performances. The aim of this thesis is to investigate the links between the modulations in resting and premotor electroencephalographic activity, and the knee-extensor neuromuscular function and the autonomic cardiovascular activity changes after an endurance exercise performed on an ergocycle. In parallel, this work aims to bring to the field of exercise sciences a new analysis method of the functional state of the resting brain, namely, the microstate analysis.

In the first article, we reported a reduction in premotor potential amplitude and maximal voluntary contraction force after exercise. The decrease in premotor brain activity shows links with the neuromuscular function and suggests that mechanisms implicated in a voluntary contraction may reside at the premotor level, even before movement arises.

In the second article, we reported a main effect of exercise on microstate C stability, which was characterized by an increase in its duration, time coverage and explained variance, and a greater percentage of transition towards this microstate. This study suggests that the modulations of microstate C may reflect a dominance of the salience resting-state network, likely under the influence of muscle afferents and endogenous stimuli, which could affect the voluntary motor command.

In the third article, we showed that the increase in microstate C mean duration and the modulations in heart rate variability persist during the 1 hour after exercise. The modifications in microstate C temporal properties may reflect the adjustment of the autonomic cardiovascular activity and/or an increase in exercise-related cardiovascular arousal.

By investigating the resting and premotor brain activity, the present thesis provides a better understanding of the motor response modulations after endurance exercise and opens up novel opportunities for exploring the interactions between the global functional state of the brain and the exercise-related physiological responses.

French version

L'activité cérébrale de repos et pré-motrice semble être déterminante pour de nombreux comportements moteurs. La littérature a montré qu'un exercice physique d'endurance aigu pouvait moduler l'activité cérébrale et réduire les performances motrices. Le but de cette thèse est d'investiguer les liens entre les modulations de l'activité électroencéphalographique de repos et pré-motrice, et les modifications de la fonction neuromusculaire des muscles extenseurs du genou et de l'activité cardiaque autonome à la suite d'un exercice d'endurance réalisé sur ergocycle. En parallèle, ce travail vise à apporter au champ des sciences de l'exercice une nouvelle méthode d'analyse de l'état fonctionnel global du cerveau au repos, à savoir l'analyse de micro-état.

Dans le premier article, nous avons observé une réduction de l'amplitude du potentiel pré-moteur et de la force maximale volontaire après l'exercice. La réduction de l'activité cérébrale pré-motrice présente des liens avec les modulations de la fonction neuromusculaire, suggérant que des mécanismes impliqués dans une contraction volontaire pourraient résider au niveau pré-moteur, avant même que le mouvement soit produit.

Dans le deuxième article, nous avons observé un effet principal de l'exercice sur la stabilité du micro-état C, caractérisé par une augmentation de sa durée, du temps couvert et de sa variance expliquée, ainsi qu'un pourcentage de transition vers ce micro-état plus important. Cette étude suggère que les modulations du micro-état C pourraient refléter une dominance du réseau de repos saillant, probablement sous l'influence d'afférences musculaires et de stimuli endogènes, qui exercerait une influence sur la commande motrice volontaire.

Dans le troisième article, nous avons montré que l'augmentation de la durée moyenne du micro-état C persiste 1 heure après l'arrêt de l'exercice, tout comme les modulations de la variabilité de la fréquence cardiaque. Les modifications des propriétés temporelles du micro-état C pourraient refléter l'ajustement de l'activité cardiaque autonome et/ou une augmentation de l'éveil cardiovasculaire lié à l'exercice.

En étudiant l'activité cérébrale de repos et pré-motrice, cette thèse fournit une meilleure compréhension des modulations de la réponse motrice à la suite d'un exercice physique d'endurance et ouvre de nouvelles opportunités pour explorer les interactions entre l'état fonctionnel global du cerveau au repos et les réponses physiologiques liées à l'exercice.

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This thesis would not have been possible without the people who have accompanied me along these five years of my PhD. I want to express my gratitude to the following individuals.

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Thank you to the volunteers who have contributed to this work by pedaling on an uncomfortable bike in a gloomy room without windows wearing this “trendy” EEG cap. A special thanks to Nicolas Jungo, the most faithful subject who participated in every single experiment from my bachelor degree to my doctoral thesis. Thank you for your extremely precious friendship.

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Abbreviations

ACC	Anterior cingulate cortex
AI	Anterior insula
AP	Action potential
BA	Brodmann area
BG	Basal ganglia
BOLD	Blood-oxygen-level dependent
BP	*Bereitschaftspotential*
BP1	*Bereitschaftspotential* 1 (-1.5 to -1 second before movement onset)
BP2	*Bereitschaftspotential* 2 (-1 to -0.5 second before movement onset)
CMAs	Cingulate motor areas
dACC	Dorsal anterior cingulate cortex
DMN	Default mode network
EEG	Electroencephalography
EMG	Electromyography
FFT	Fast Fourier transform
fMRI	Functional magnetic resonance imaging
GEV	Global explained variance
GLM	General linear model
HR	Heart rate
HR_{max}	Maximal heart rate
HRV	Heart rate variability
HT	Hypothalamus
IC	Insular cortex
ICA	Independent component analysis
MAP	Maximal aerobic power
MCC	Midcingulate cortex
MP	Motor potential
MRCP	Movement-related cortical potential
MVC	Maximal voluntary contraction
M1	Primary motor cortex
nHF	Normalized high-frequency power (0.15 - 0.40 Hz)
nLF	Normalized low-frequency power (0.04 - 0.15 Hz)

NS'	Negative slope
OFC	Orbitofrontal cortex
PAG	Periaqueductal gray
PCC	Posterior cingulate cortex
PET	Positron emission tomography
PFC	Prefrontal cortex
pI	Posterior insula
PM	Premotor area
Pt	Peak twitch
P100 Hz	Paired stimuli at 100 Hz
RMSSD	Root mean square of the successive difference between beat-to-beat intervals
RP	Readiness potential
R-R	Beat-to-beat intervals
RSN	Resting-state network
Rsp	Retrosplenial cortex
SC	Spinal cord
SMA	Supplementary motor area
S2	Secondary somatosensory cortex
TH	Thalamus
VAL	Voluntary activation level
$\dot{V}O_{2\ peak}$	Peak oxygen consumption
$\dot{V}O_{2\ max}$	Maximal oxygen consumption
VStr	Ventral striatum
VTA	Ventral tegmental area

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Part III

Part IV

Part I

Introduction

The present thesis is divided into four parts. In part I, we focus on the resting brain state and emphasize the importance of the spontaneous brain activity in determining a wide range of behaviors. Perception and action may be brain-state dependent as will be demonstrated from previous literature. Electroencephalography (EEG) is subsequently described and presented as a relevant method for quantifying resting brain activity because of its high temporal resolution. We then show that between rest and voluntary movement, a typical premotor brain activity may be collected above premotor and motor areas, which is strongly connected to the upcoming movement. This section ends with a description of the neuromuscular system and its implication in modulating voluntary contractions. In section II, the effects of acute endurance exercise on the neuromuscular function, premotor and resting brain activity are successively presented. Particular attention is paid to the post-exercise resting electrocortical activity and its putative underlying modulators. The microstate analysis is subsequently presented as a promising method for investigating post-exercise resting brain changes. Section III presents the aim of this thesis and contains a summary of our experimental results. Section IV completes this thesis work by discussing the main results and providing future perspectives.

1. Brain state as the origin of perception and action

Variations in maximal athletic and cognitive performances are apparent over the course of a day (Atkinson and Reilly, 1996; Cappaert, 1999; Drust et al., 2005); however, it is less intuitive that behavioral and cognitive performances vary over shorter periods at the second and millisecond time scales (Buzsáki, 2006). A given behavioral response may depend on the state of the brain that immediately precedes this response. A study from Palva (2005) illustrates this statement by implementing a near-threshold perception task, in which an electrical stimulation of the index finger was set so that approximately 50 percent of stimuli were perceived by the subject. The authors reported that whether the stimulus was perceived depended on the brain activity recorded approximately 50 milliseconds from stimulus onset in the somatosensory, frontal, and parietal regions. In an experiment conducted by Haig and Gordon (1998), the volunteers responded to a target auditory stimulus by pressing a button as fast as possible. Using this auditory oddball task, the authors showed that the brain synchronicity at the moment of stimulus presentation influences the reaction time performance. The reaction time was shorter

in high phase synchronicity than low phase synchronicity, which suggests that the brain state at the moment of stimulus presentation may influence the speed of the motor response (Haig and Gordon, 1998). In a pursuit rotor task that consisted of maintaining a cursor on a target as the target moved along a circle on a monitor, Wu et al. (2014) demonstrated that the resting-state cortical connectivity was a strong predictor of motor skill acquisition. The percentage of time on the target was increased after a single training session, depending on the connectivity between the primary motor cortex and the left parietal cortex. Finally, evidence suggests that the ongoing brain state at the time of memory encoding is crucial in determining if items are recalled, which indicates that transforming experiences into memories may also be brain-state dependent (Fell et al., 2001; Fernández et al., 1999a, 1999b). These results emphasize that the resting brain state is determinant for a wide range of behaviors. In general, attention, arousal, anticipation, volition, planning or preparation, are example of terms that may characterize this brain state and are associated with changes in behavioral performances and sensory input perception (Buzsáki, 2006). Because perception and action are brain-state dependent (Buzsáki, 2006), exploring spontaneous brain activity at rest is an exciting approach to better understand the basic brain functioning and the putative consequences on forthcoming behaviors.

2. Spontaneous activity of the brain

Brain activity is continuous throughout the lifespan. This is true even if is the consciousness is modified, such as during sleep, hypnosis or fainting, or when the brain state is altered, such as in a coma. The human brain is a complex and dynamic system, which is constantly changing depending on events originating from inside the body (thought, motivation) and outside (environment). The human brain represents roughly 2% of the total body weight, whereas it consumes approximately 20% of the total energy expenditure (Raichle et al., 2001). Approximately 60% to 80% of this energy is devoted to communication and neural support, which indicates that the energy used to execute cognitive or behavioral responses is rather small compared to the amount of energy needed to maintain the basal spontaneous brain state (Raichle et al., 2001; Tozzi et al., 2016). The importance of this spontaneous resting brain activity has been highlighted by advanced neuroimaging studies (Raichle and Snyder, 2007). By subtracting the brain activity of a goal-directed task by a baseline for which the subject is resting (i.e., a no-goal-directed task), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have indicated a counterintuitive reduction in brain activity during cognitively demanding tasks (Raichle, 2009; Snyder and Raichle, 2012). This unexpected

finding further highlighted the importance of the resting and spontaneous activity of the brain as it may reflect a functional intrinsic well-organized activity instead of background noise (Snyder and Raichle, 2012).

3. Investigating resting spontaneous brain activity

3.1. fMRI and resting state networks

fMRI is a principal method for exploring resting brain activity. Using a statistical procedure to extract the blood-oxygen-level dependent (BOLD) signal, fMRI detects the amount of oxygen carried by hemoglobin in the blood flow. When a population of neurons becomes active, an increase in the hemodynamic response is expected with a concomitant change in the amount of oxygen extracted from the blood circulation, thus reflecting a supposed increase in brain activity.

Raichle and colleagues conducted different studies on the resting brain and showed that spontaneous brain activity at rest is not unconstrained noise but reflects an intrinsic and self-organized activity of a default mode of brain function (Raichle and Snyder, 2007; Raichle et al., 2001). A highly correlated activity between brain regions has been identified, forming an interconnected and anatomically defined default mode network (DMN). Different brain regions are considered to be functionally connected when their respective signal fluctuations are correlated in time. The main DMN hubs include the medial prefrontal cortex, the posterior cingulate cortex/retrosplenial cortex (PCC/Rsp), and the inferior parietal lobule (Buckner et al., 2008). A multicenter fMRI study conducted on more than 1000 participants demonstrated the presence of a universal functional architecture, with a significant stability and reproducibility across individuals (Biswal et al., 2010). The exploration of the brain at rest has been replicated and has led to a consensus that assumes the brain is organized in multiple functional anatomical networks (resting state networks, RSN) (Damoiseaux et al., 2006; De Luca et al., 2006; Mantini et al., 2007). Figure 1 illustrates six RSNs identified by Mantini et al. (2007) using a data-driven approach.

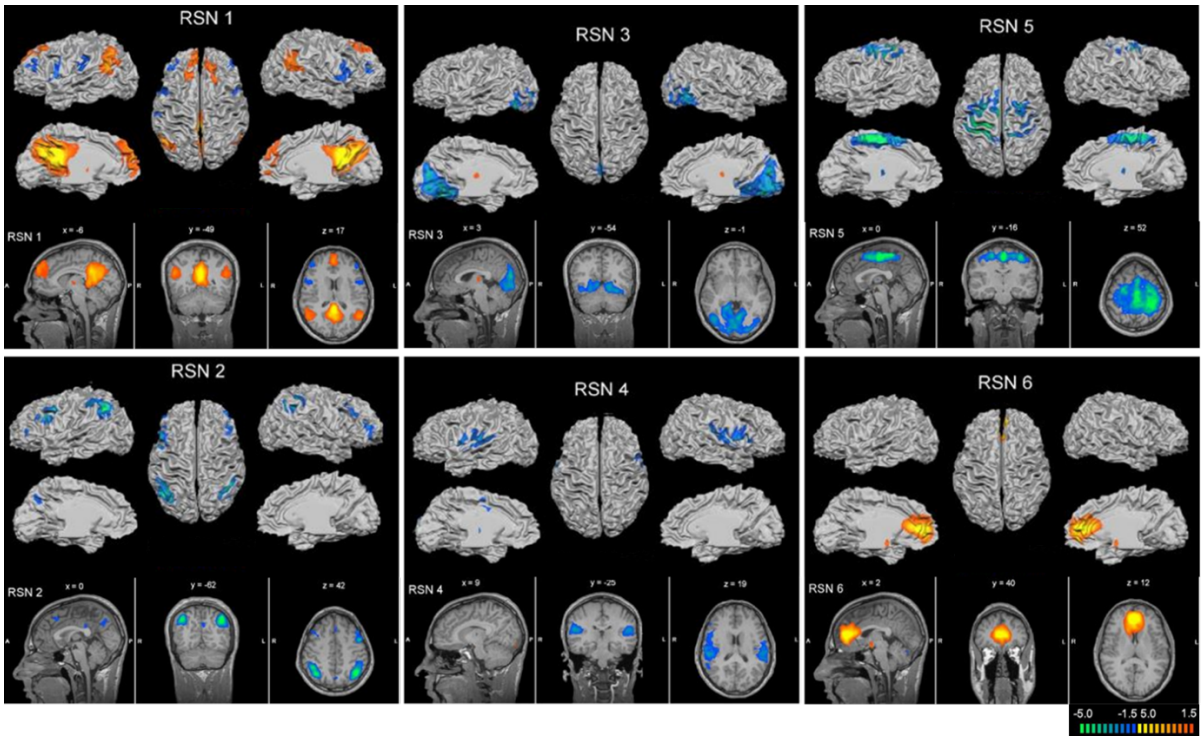

Figure 1: Cortical representation of the 6 RSNs. For each RSN, the dorsal view (upper center) is surrounded by lateral and medial views of the left (upper left) and right (upper right) hemispheres and the sagittal, coronal and axial maps (bottom). RSN1: default mode network, RSN2: dorsal attentional network, RSN3: visual network, RSN4: auditory network, RSN5: sensory-motor network; RSN6: salience network (Adapted from Mantini et al. 2007).

The brain is organized in multiple RSNs from which task-networks are dynamically assembled and modulated during different behavioral states (Mantini et al., 2007). As a result, mentation, cognition, and motor response likely emerge from a basal rhythmic activity of large-scale neuronal networks distributed across the brain (Bressler, 1995; Bressler and Menon, 2010; Buzsáki, 2006). According to Bressler (1995), "*Successful behavioral adaptation may depend on the ability of the cortex to flexibly process a wide range of complex temporal patterns*" (Bressler, 1995, p. 299). To support this flexibility, "[…] *the cortex must process time-varying sensory patterns and generate time-varying motor patterns on time scales of milliseconds to seconds.*" (Bressler, 1995, p. 299). However, the fMRI time resolution may be insufficient as the blood-flow response delay is approximately a half second after neuronal activation. As the brain works in the millisecond time range, a method with a millisecond time scale resolution is necessary to explore the spontaneous brain dynamics. In this thesis work, multi-channel surface EEG was selected to investigate the brain activity. This method is briefly presented in the next chapter.

3.2. Electroencephalography

The fact that the brain is active at rest was demonstrated more than 70 years before the development of advanced neuroimaging techniques. In 1929, Hans Berger first identified wave oscillations above the human brain. Using a galvanometer, he differentiated two bioelectrical signals (alpha and beta rhythms) that have been subsequently related to different mental states (Berger, 1929). Since the discovery of human brain waves by Hans Berger, resting brain activity has been investigated by various EEG studies, which showed that different states of the brain are characterized by typical EEG oscillation patterns (Brandeis et al., 2009). A comprehensive review of the resting brain state explored using electrocortical activity is described in a book by György Buzsáki, *Rhythms of the Brain* (2006).

3.2.1. Origin of the EEG signal

The postsynaptic potentials generated by pyramidal cells aligned perpendicularly to the cortical surface are the main sources of the surface EEG. The rapid depolarization of the neuronal membrane (flow of NA^+ inwards) followed by its repolarization (flow of K^+ outwards) generates an action potential (AP) that propagates along the neuron. When the AP arrives to the axon terminal, neurotransmitters are released into the synaptic cleft. Depending on the type of neurotransmitter released, the AP results in an excitatory postsynaptic potential (generated by the influx of Ca^{2+} and Na^+ ions though the postsynaptic membrane) or an inhibitory postsynaptic potential (generated by an influx of Cl^- and a K^+ efflux). These postsynaptic activities result in a sink-source current between the soma and the dendrites in the extracellular space around the neuron (Vion-Dury and Blanquet, 2008). The summation of a substantial number of simultaneous postsynaptic potentials of pyramidal cells generates a sufficient electrical field (current dipole) that propagates to the scalp surface as a result of volume conduction. A surface of approximately 100 mm^2 of the cortex (i.e., equivalent to the surface of dice) must be synchronously activated to detect a signal at the brain surface (Vion-Dury and Blanquet, 2008). The detection of EEG signals depends on different factors, such as the surface of the cortical area in which electrical potentials are summed, the localization and distance of the generators relative to the active electrodes, the orientation of the cortical source, and the electrical conductive properties of the tissues between the source and the active electrode.

3.2.2. Signal acquisition

EEG reflects time-varying potentials above many electrode sites disposed according to a standard positioning method (Niedermeyer and da Silva, 2005). The 10-20 international system is one of the most used methods, which supposes that the distance between adjacent electrodes is 10% or 20% of the total distance between standardized landmarks. The distance is measured in the sagittal plan between the nasion and the inion and in the frontal plan between the two preauricular points. The position of each electrode is characterized by a number that differentiates the left-side hemisphere (odd numbers) from the right-side hemisphere (even numbers). The letter more precisely indicates the relative position of each electrode above cortical regions, such as the frontopolar (Fp), frontal (F), central (C), parietal (P), temporal (T) occipital (O), and midline (Z). A high-density EEG indicates that at least 64 electrodes are placed above the brain surface, which enables good spatial resolution. However, with a sampling frequency up to 2000 Hz, the temporal resolution is the main advantage of EEG for investigating highly rapid brain processes.

3.2.3. EEG frequencies

The EEG signal represents the temporal evolution of the surface electrical field generated by brain activities at each instant, and can be visualized as a matrix with temporal information in one dimension and spatial information in the other dimension (Britz and Michel, 2011). Traditionally, an oscillation can be described by its amplitude and frequency. Accordingly, conventional analyses of resting brain activity consider the amplitude and frequency power time course at particular electrodes sites (Britz and Michel, 2011).

The human brainwaves are generally subdivided into five main physiological rhythms that have been linked with particular behavioral correlates (Vion-Dury and Blanquet, 2008). The delta oscillation is a slow wave activity (< 4 Hz, > 30 μV) observed in adults during deep sleep. The theta frequency band (4-7 Hz, ~20 μV) is observed during a specific sleep stage and the transition between sleep and vigilance. The alpha rhythm (8-13 Hz, 25-75 μV) is elicited when subjects close their eyes or enter a relaxed state. Alpha is preferably observed around the occipito-parietal region and is commonly associated with the level of alertness and cortical excitability to which it is inversely related (Pfurtscheller and Klimesch, 1992). Alpha may be segmented into alpha-1 (8-9 Hz) and alpha-2 (10-13 Hz). The beta frequency band (14-30 Hz, < 20 μV) is observed during alertness, when the subject is aroused, and may be segmented into beta-1 (13-17 Hz) and beta-2 (18-30 Hz). Finally, the gamma frequency band (> 30 Hz, < 20

μV) is associated with active information processing, such as memory consolidation (Düzel et al., 2010).

Measuring the EEG oscillations and BOLD activity at rest in a scanner revealed correlations in brain regions that partly corresponded to some of the above described RSNs (Goldman et al., 2002; Laufs et al., 2003). For example, the six RSNs described by Mantini et al. (2007) (see Figure 1, page 4) correlated with the power time course in the EEG frequency bands; however, none of the RSN could be linked to a single frequency band. Brain regions display a combination of several rhythms and the assumption that a single cerebral rhythm is associated with a specific cerebral functional network is not likely (Mantini et al., 2007).

3.2.4. EEG microstates

The topographic analyses of spontaneous resting EEG consider the spatial dimension and assesse the temporal dynamics of the surface electric field map. Unlike power modulations of the EEG oscillations, which are measures of brain activity that are reference-dependent, the EEG topography is a global and reference-free measure of momentary brain activity (Britz et al. 2011) (Figure 2). Moreover, unlike amplitude modulations of the brain oscillations, the EEG scalp topography remains quasi-stable for periods of about 80-120 millisecond before rapidly transitioning to a different topographic configuration. These periods of quasi-stability are referred to as EEG microstates (Lehmann et al. 1990).

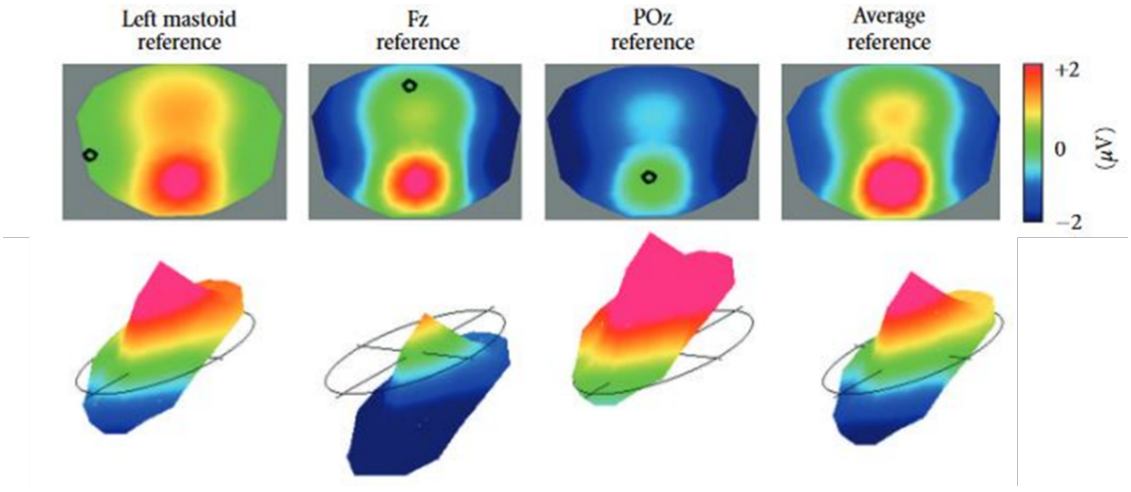

Figure 2: Contribution of the reference on the EEG topography. The upper line indicates the effect of changing the reference on a given topography. Note that the color intensity differs, whereas the shape remains identical. The bottom line indicates the respective topography displayed in relief, with the zero level depicted with the 2-D black target. Note that the shape does not change, whereas the zero target increases or decreases (Adapted from Brunet et al., 2011).

The time course of resting EEG microstates is a direct measurement of synchronized postsynaptic neural activity independent of frequency. The summation of neuronal activity propagates to the scalp surface, which results in an electric potential recorded at each electrode site. By linking the brain regions with isopotential lines at a given moment in time, a particular landscape or topography may be drawn (Figure 3).

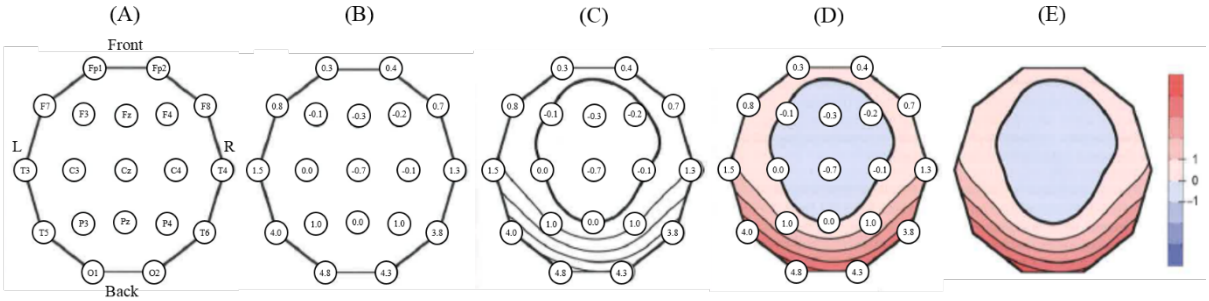

Figure 3: Construction of an electrical field map in 2-D top view. (A) Representation of the scalp electrode position. (B) Microvolts activity recorded at each electrode position at a given time point. (C) Electrodes with the same microvolts are linked with an isopotential line. (D) The color code differentiates the negative value (blue) from the positive value (red), with a color intensity that is proportional to the voltage difference from zero. (E) Final topography without microvolt values (Adapted from Michel et al., 2009).

Dietrich Lehmann and colleagues first segmented conventional EEG data in electric field maps (Lehmann, 1971; Lehmann and Skrandies, 1980; Lehmann et al., 2009). They demonstrated that the continuous stream of momentary electrical field potential distributions may be segmented into time epochs of varying durations during which the field shows a near-stable landscape (Lehmann et al., 1998). Standard resting EEG recordings may be segmented in 4 recurrent and stable topographies (Figure 4) that account for more than 70% of the variance (Khanna et al., 2015; Koenig et al., 2002). Arbitrarily named A, B, C and D, these microstates have right-frontal left-posterior, left-frontal right-posterior, midline frontal-occipital, and midline frontal topographies (Figure 4, (D)). The 4 microstates are consistent across subjects and lifespan and show reproducible temporal properties (Khanna et al., 2015; Koenig et al., 2002). The microstate temporal parameters are quantified in terms of duration (mean continuous period of time assigned to a given microstate), global explained variance (GEV) (percentage of total variance explained by a given microstate), frequency of occurrence (number of time that a map occurred in one second), and time coverage (relative percentage of times covered by a map) (Khanna et al., 2015). The temporal sequence between microstates,

the syntax, is a promising parameter for understanding human cognition (Michel et al., 2009). The syntax corresponds to the transition percentage of moving from one map to another map. Microstates are thought to combine different modes, contents, or steps of information processing and have been considered "atoms of thought" (Lehmann et al., 1998). Accordingly, Michel et al. (2009) suggests that microstate syntax "*[…] defines the appropriateness of the whole mental process, just as correct sequence of words is needed to build a proper sentence and several correct sentences to build an understandable story.*" (Michel et al., 2009, p. 115).

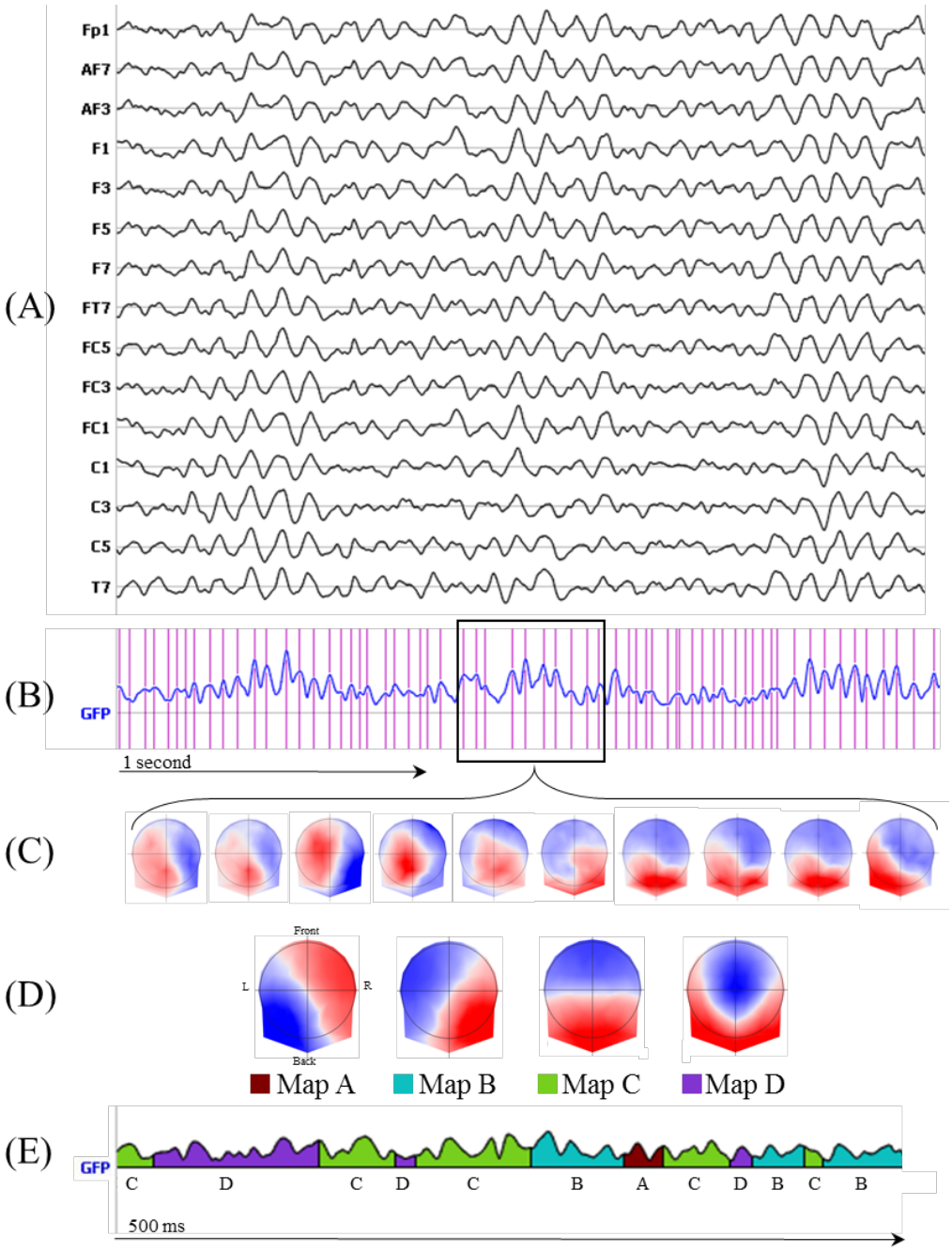

Figure 4: Graphic illustration of resting EEG microstate analysis. (A) Conventional EEG traces recorded at rest with eyes closed (i.e., only the left frontotemporal hemisphere electrodes are shown). (B) A topography is extracted at each global field power peak. (C) From the spontaneous topography sequence, the spatial K-mean clustering analysis generated the microstate topographies that explain most of the data. (D) The four conventional microstate maps obtained. (E) Using spatial correlation, each time point of the initial data set is labeled with a given microstate template to create the time series of the four resting microstates. Note that regardless of whether the field strength varies, the topography remains stable for a period of tens of milliseconds (Inspired from Tomescu et al. 2014).

Interestingly, the EEG microstates correlate with the hemodynamic fluctuations of the BOLD RSNs (Britz et al., 2010; Musso et al., 2010; Yuan et al., 2012) and may represent the electrophysiological signatures of RSNs (Britz et al., 2010; Mantini et al., 2007). Specifically, Britz et al. (2010) showed that each of the four conventional microstates corresponds to a particular RSN (Figure 5). Microstate class A was correlated with BOLD activity in the bilateral superior and middle temporal gyri. This network was associated with phonological processing. Microstate class B was correlated with BOLD activity in the bilateral extrastriate visual areas and was related to the visual network. Microstate class C was correlated with BOLD activity in the posterior part of the anterior cingulate cortex, bilateral inferior frontal gyri, right anterior insula and left claustrum. This microstate was related to the saliency network. Finally, microstate class D was correlated with BOLD activity in the right-lateralized dorsal and ventral areas of the frontal and parietal cortex and was associated with the attention network. The association between EEG microstate and fMRI resting-state networks has been strengthened by the work of Van de Ville et al. (2010), in which microstate time series have a scale-free dynamics, indicating that brain activity at the EEG and fMRI timescales may reflect the same underlying neurophysiological processes (Khanna et al., 2015; Van De Ville et al., 2010). In summary, the EEG microstates is an estimation the activity of large-scale functional networks, which provide the advantage of collecting the spontaneous brain fluctuations in sub-second time scale.

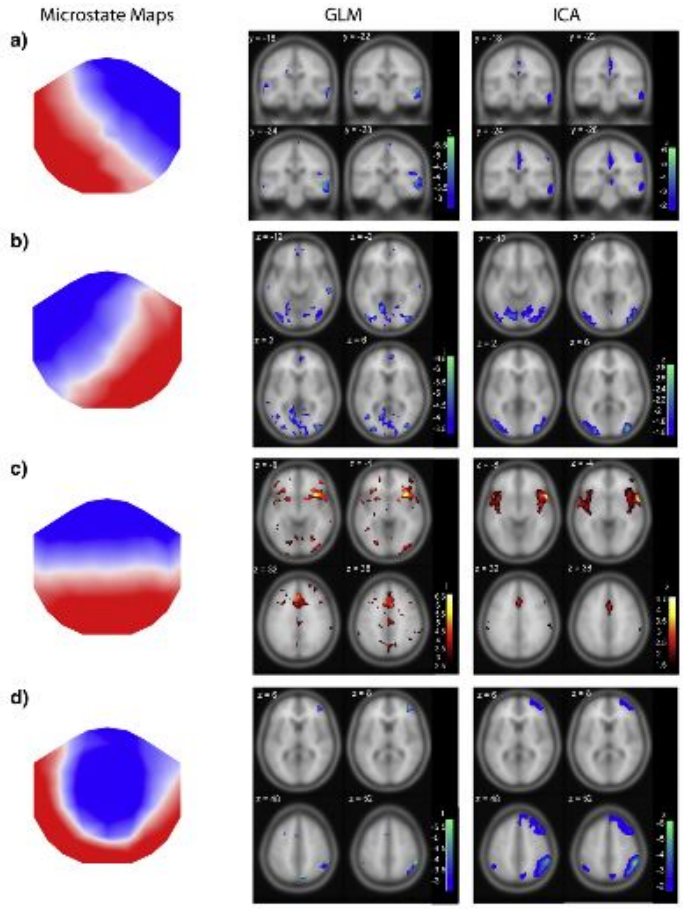

Figure 5: The four (a-d) resting EEG microstates (first column) with their corresponding fMRI BOLD correlates obtained from a general linear model (GLM) (second column) and independent component analysis (ICA) (third column) (Adapted from Britz et al.2010).

According to Pascual-Marqui et al. (2014), the canonical 4 map topographies also appear to be a fragmented version of the DMN. The slow metabolic activity of the DMN would represent a low-pass filtered version of the rapid sequence of the four maps (Pascual-Marqui et al., 2014). Using eLORETA, the authors determined the cortical distribution of the electrical activity that generates each topography. Figure 6 shows the resting EEG microstates and their corresponding eLORETA images, with the maximal activity highlighted with bright yellow color. The maximum activities of microstate maps A, B, C, and D are located in the left occipital/parietal, right occipital/parietal, anterior cingulate, and posterior cingulate areas, respectively. The four microstates have a common generator in the posterior cingulate cortex.

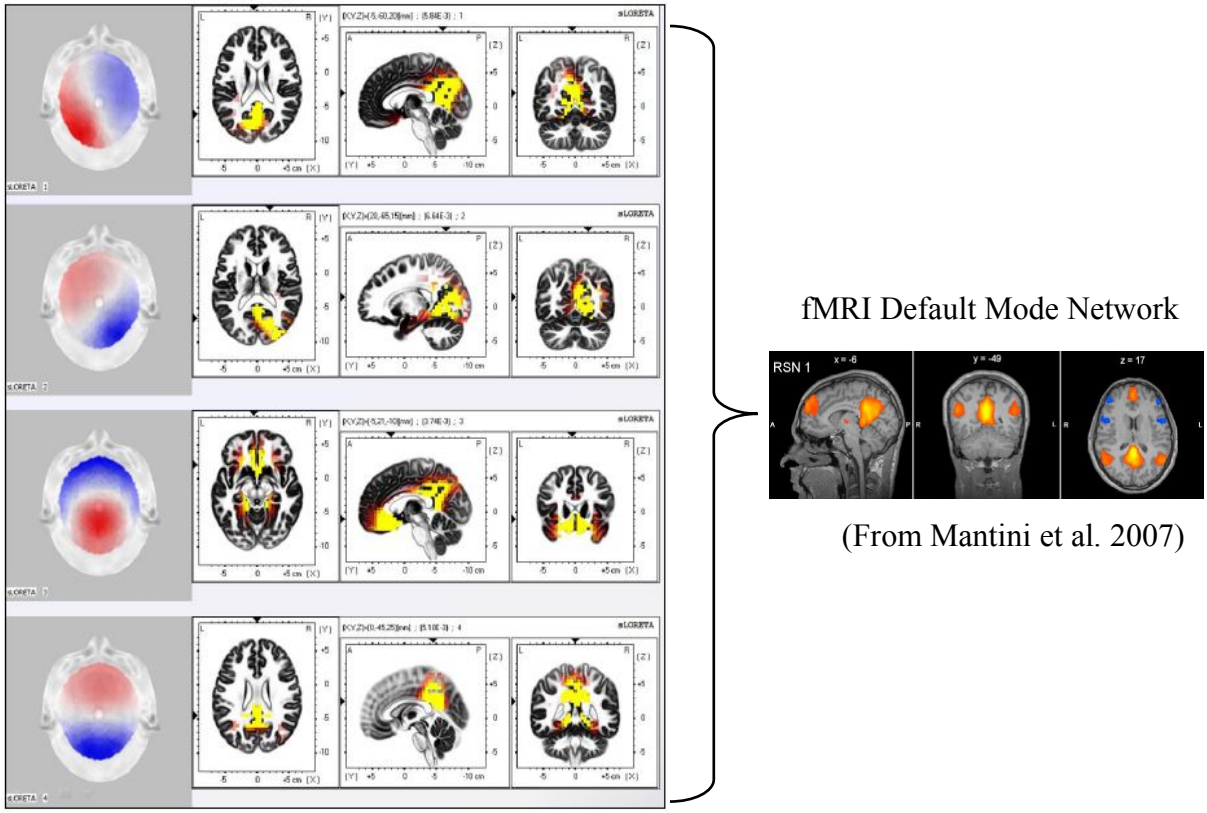

Figure 6: The four resting EEG microstates and their corresponding eLORETA images of electric neuronal activity. The bright yellow color indicates the maximum current density, and the two black triangles along the coordinate axes point to the maximum activity. The 4 map topographies correspond to a fragmented version of the resting fMRI DMN (right). Note that all microstates have a common generator in the posterior cingulate cortex (Adapted from Pascual-Marqui et al. 2014).

4. From resting spontaneous brain activity to movement onset

In the early chapters, we emphasized the importance of the resting spontaneous brain activity as it can determine the perception threshold, predict the acquisition of new motor skills, and modulate the motor performance. Focusing on voluntary movement, this chapter indicates that the origin of the behavioral response should not only be sought during the resting state but also the period that immediately precedes voluntary movement.

4.1. Movement-related cortical potential

Before movement arises, a slow rising negative potential is observed above the premotor and motor brain areas. This response-lock electrocortical potential was first described in 1965 by

Kornhuber and Deecke as the *Bereitschaftspotential* (BP) (Kornhuber and Deecke, 1965). Using a self-paced handgrip contraction task, they provided the first EEG evidence for neural activity that started 1-2 seconds before movement. This pre-movement activity may reflect the brain processes that contribute to movement genesis and the intention to act (Jahanshahi and Hallett, 2003). The classical BP paradigm requires self-initiated movements performed in simple motor tasks, such as finger movements (e.g., isometric/concentric contraction or button press), handgrip contractions, or other movements that mobilize an isolated segment of the body, thus avoiding artifacts generated by unwanted muscle activity. The BP is an event-related potential locked to the muscle contraction recorded by electromyography (EMG) or directly to the movement onset. Accordingly, even if there is no clear consensus, researchers tend to indifferently employ the terms BP or readiness potential (RP) when the pre-movement activity is locked to muscle activity, whereas the term movement-related cortical potential (MRCP) is preferentially used when brain activity is locked to the movement onset. In all cases, similar to other EEG event-related potentials, several trials must be performed and averaged to increase the signal to noise ratio.

4.1.1. Components and shape

Although the terminology may diverge across studies, the literature is fairly consistent in describing the principal components of the BP. There are three principal components that are visually identifiable in a traditional BP recording with a specific topographic distribution (Figure 7) (Shibasaki and Hallett, 2006). The early BP arises between 1500 milliseconds and 500 milliseconds before movement and corresponds to a slow increase of the negativity (upward deflection). This component is bilaterally distributed at the fronto-central midline above the supplementary motor area (SMA). Approximately 500 milliseconds before movement, an abrupt increase in slope differentiates the early BP from the late BP (also referred to as negative slope (NS')). The slope of the late BP becomes larger until it reaches its maximal amplitude above the primary motor cortex (M1) according to the somatotopic organization. This maximal negativity occurs around movement onset and is referred to as the motor potential (MP) or pre-motion negativity (Colebatch, 2007; Kornhuber and Deecke, 1965).

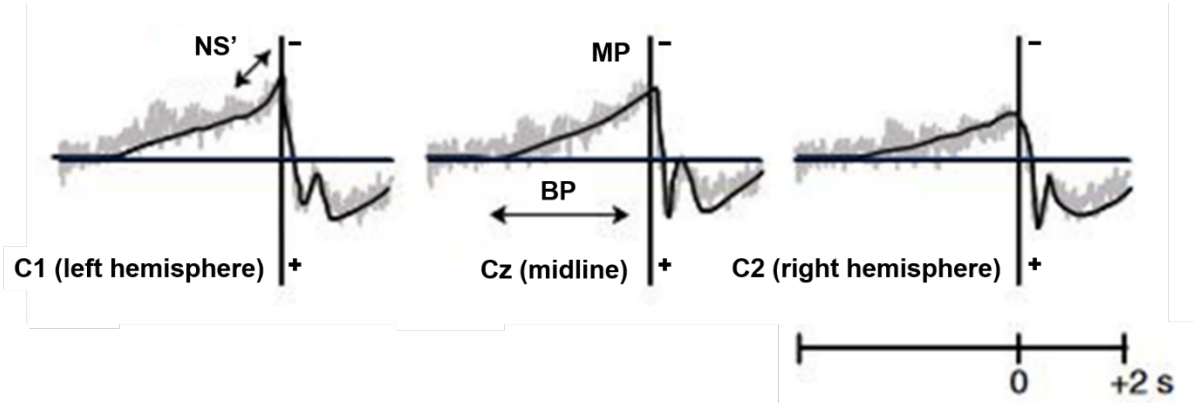

Figure 7: Movement-related cortical potential time course and main components associated with self-paced right finger movements in a right-handed subject. The early BP starts bilaterally approximately 2 seconds before movement onset, above C1 and C2. The negative slope (NS') is more pronounced over the contralateral hemisphere and occurs approximately 500 milliseconds before movement onset. The motor potential (MP) corresponds to the maximal amplitude observed during movement onset (Adapted from Shibasaki and Hallett, 2006).

4.1.2. Generators

The BP is likely generated by the increase in the extracellular K^+ concentration and the decrease in the Ca^{2+} concentration (Rösler et al., 1997), thus resulting in a slow negative shift commonly interpreted as neuronal activation (Jahanshahi and Hallett, 2003). Different neuroimaging methods, including EEG (Cui and Deecke, 1999), fMRI (Cunnington et al., 2002, 2003), magnetoencephalography (Erdler et al., 2000) or EEG and fMRI combined (Nguyen et al., 2014) have indicated the main generators of the BP. The neural structures at the origin of the early BP appear to be located in the bilateral region of the mesial frontal cortex, including the pre-SMA, SMA, and cingulate motor areas (CMAs) (particularly the anterior mid-cingulate cortex) (Jahanshahi et al., 2001). Studies also assume the participation of subcortical structures, such as the basal ganglia and thalamus (Cunnington et al., 2002; Rektor et al., 2001). The late BP is likely to have a main generator within the M1, which suggests a direct relationship with the motor command, particularly the MP that appears to reflect the activity of pyramidal tract neurons (Shibasaki and Hallett, 2006).

4.1.3. Time course and meaning

The functional significance of the BP is not clearly established. The literature has used various experimental designs and indicates that the amplitude and onset of the pre-movement brain

activity may be deeply affected by the brain state (e.g., attention, expectancy, cognitive load, action meaning, learning, perceived effort, or fatigue) and the task characteristics (e.g., bimanual vs. unimanual, speed, precision, force, rate of force development, or complexity) (Di Russo et al., 2017; Jahanshahi and Hallett, 2003; Shibasaki and Hallett, 2006). For example, the late BP is larger when movement requires precision in terms of force production or with greater perceived effort. Complex movements lead to a larger late BP amplitude and earlier BP onset, whereas faster movement is accompanied by a later BP onset (Shibasaki and Hallett, 2006). Based on the generator of the BP components, the pre-SMA, SMA, CMAs, and lateral premotor cortex may be involved in the preparatory process, whereas the primary motor cortex is more directly associated with the execution of the movement (Jahanshahi and Hallett, 2003). Taken together, the BP may reflect an index of anticipatory attention and motor preparation and is clearly associated with the upcoming movement.

In the mid-1980s, Benjamin Libet proposed a well-known experimental task to articulate the timing of the brain process and the conscious will and discussed the significance of the BP in the perspective of *free will* (Libet, 1985, 1999). A small spot revolved around a clock circle projected on a screen, and the subject had to freely decide to make a wrist flexion. Participants were instructed to note the position of the spot on the clock when they became aware of the intention to move. The BP started approximately 1050 to 550 milliseconds before the muscle activation onset, whereas the first awareness of the wish to move occurred approximately 200 milliseconds before the muscle activation onset. The results were discussed in terms of free will as the pre-movement brain activity started before the subject consciously knew that he decided to move. Benjamin Libet concluded by saying that cerebral initiation of a spontaneous voluntary act begins unconsciously (Libet, 1985, p. 529).

The long gap between negative brain activity onset and the time of intention has been confronted by researchers. Matsuhashi and Hallet (2008) reported several methodological biases in Libet's experiment (e.g., subjective recall, preparation and latency for reading the clock time) and proposed an alternative protocol to measure the timing of intention to move with minimal dependence on a subjective participant's recall. In their study, subjects had to produce a self-paced finger movement as soon as they thought of the movement while tone stimuli were presented at pseudo-random intervals. The tone was ignored when subjects were not thinking about the movement; however, if the tone was heard when subjects started thinking about the movement, the forthcoming movement had to be canceled. In this situation, the movement was canceled or executed if the tone was too close to the movement. The results showed that the early BP and late BP were slightly earlier (i.e., 2.17 seconds and 0.57 seconds

before movement, respectively); however, they were similar to previous literature. In contrast, the mean time of conscious intention to move was 1.42 seconds, which is more than 1 second earlier than in the Libet experiment. During the early BP, subjects are not aware of the movement genesis; however, when movement genesis progresses, the awareness state further increases until it may be consciously detected (Matsuhashi and Hallett, 2008). For the authors, the late BP began during the period in which subjects *could consciously access awareness of their movement genesis as intention* (Matsuhashi and Hallett, 2008, p. 2350). During this period, there is a conscious veto, in which an intention to perform a movement may be either blocked or proceed until a point of no return is reached (i.e., 0.13 s before movement). For Matsuhashi and Hallet (2008), the late BP does not appear to be responsible for the formation of intention as it started later after the conscious intention to move and it is not the final part of execution, as movement genesis may be canceled during this period. Based on these results, Shibasaki (2012) postulated that the early BP may reflect the subconscious readiness of movement, whereas the late BP appears more related to the conscious will to move.

4.1.4. The hypothesis of a spontaneous fluctuation origin

Recently, the meaning, time course, and involvement of the BP in the awareness of voluntary movements have been challenged (Schmidt et al., 2016; Schurger et al., 2012). Schmidt et al. (2016) reviewed the results from the Libet experiment and more generally the interpretation of the BP. For the authors, the early BP does not have a preparatory or a decision related origin; however, it may originate from stochastic fluctuations as previously suggested by Schurger et al. (2012). Schurger et al. (2012) assumed a connection between spontaneous fluctuation in neural activity and self-initiated movement, so that the neural decision to move is determined, in part, by spontaneous fluctuation. More precisely, the self-initiated movement is facilitated or impeded according to the fluctuation of ongoing brain activity (Schmidt et al., 2016). If this fluctuation reaches a given threshold, there is an inner feeling of an urge to act and the decision to move is likely to occur. As spontaneous movement more likely occurs during the increase in negativity, the shape of the early BP may be explained by the average procedure of the BP trials, which was termed the *slow cortical potential sampling hypothesis* by Schmidt et al. (2016). In this model, the early BP would reflect the summation of spontaneous brain activity to the decision threshold. The early BP is subsequently defined as task-unspecific slow cortical fluctuation, whereas only the late BP reflects the preparatory process. These recent findings

further highlighted the critical importance of the continuous resting brain activity as a mediator of the initiation of voluntary movement and thus the upcoming movement.

5. Neuromuscular system

In the previous chapter, it was shown that voluntary movement is strongly associated with the brain activity preceding its onset. However, a critical component of voluntary contraction includes the neuromuscular system. In this chapter, we briefly describe the neuromuscular system, the potential sites implicated in neuromuscular alterations, and a means of investigation.

5.1. From the brain to the muscle

In response to a motivational stimulus, voluntary contraction starts with an AP generated within the M1. The upper motor neurons carry the AP through the pyramidal tract. A majority of these fibers cross the midline of the body in the brain stem and continue down through the spinal cord to the lower motoneurons. The anterior gray column of the spinal cord contains the cell bodies of these neurons, which innervate the skeletal muscle fibers and thus act as a relay between upper motoneurons and muscles. When the AP reaches the terminal end of motoneurons, the signal is transmitted to the muscle through the release of acetylcholine in the neuromuscular junction (Gardiner, 2011; Marieb, 2005).

At the muscle level, acetylcholine binds its receptor located in the muscle cell membrane. The excitation of the sarcolemma induces a muscular AP that propagates down the transverse tubules into the interior of the muscle to the myofibrils. The depolarization in the T-tubule membrane results in Ca^{2+} release from the sarcoplasmic reticulum. Released Ca^{2+} binds troponin C and permits a cross-bridge formation between actin and myosin. The excitation-contraction couplings end with cross-bridge activation, muscle contraction, and force production (Allen et al., 2008; Marieb, 2005).

5.2. Potential sites implicated in neuromuscular system alterations

The mechanisms implicated in neuromuscular system alterations may be located at different sites along the previously described motor pathway from the primary motor cortex to the muscle fiber (Figure 8) (Bigland-Ritchie, 1981). In general, a distinction is made between the central mechanisms located above the neuromuscular junction and the peripheral mechanisms located

below the neuromuscular junction (Figure 8). The central component designates a decrease in voluntary activation or a reduction in neural activity that drive the muscle (Gandevia, 2001), whereas the peripheral component refers to biochemical changes within the muscle metabolic milieu that lead to an attenuated response to neural excitation (Amann, 2011).

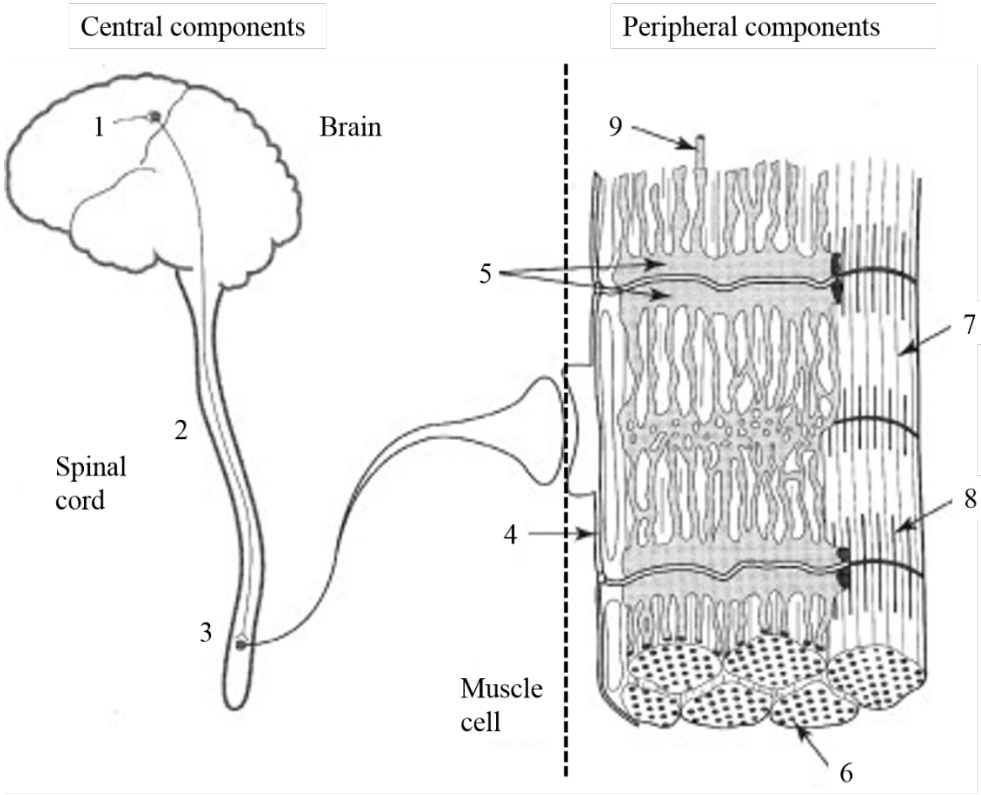

Figure 8: Potential sites that may contribute to the reduction in maximal voluntary force: 1) activation of the primary motor cortex, 2) propagation of the command from the central nervous system to the motoneurons, 3) activation of the motor units and muscle, 4) neuromuscular propagation, 5) excitation-contraction coupling, 6) availability of metabolic substrates, 7) state of the intracellular medium, 8) performance of the contractile apparatus, and 9) blood flow (Adapted from Bigland-Ritchie, 1981).

5.3. Peripheral electrical nerve stimulation

The application of an electrical pulse on a given motor nerve will evoke a contraction of the related muscle groups. For example, stimulating the femoral nerve will evoke a knee-extension. There are different types of procedures; however, electrical stimulation is typically applied on contracted and relaxed muscles in combination with EMG and force measurements. The stimulating electrode is positioned over a nerve trunk and the EMG electrodes are positioned over the muscle belly. The interpolated twitch technique was the first method to quantify the

voluntary activation level (VAL) (Merton, 1954) and remains the gold-standard technique for neuromuscular function assessment (Millet et al., 2011). Specifically, the quantification of the VAL may facilitate the detection of an altered drive to the muscle (Millet et al., 2011). The method compares the force evoked by a supramaximal electrical stimulation delivered over the nerve trunk during a maximal voluntary contraction (MVC) to the force evoked at rest by the same intensity electrical stimulus (Allen et al., 1995). In cases in which the superimposed evoked force is elicited slightly before or after the peak force, a correction may be applied using the formula from Strojnik and Komi (1998) (refer to equation below). For a more sensitive quantification of neuromuscular alteration, it is recommended to apply high-frequency paired-stimuli (P100 Hz) (Place et al., 2007). When motor units are not fully recruited or if they discharge at submaximal frequencies, the electrical stimulation delivered during the MVC will evoke a greater force (Figure 9).

$$VAL = (1 - (superimposed\ doublet\ force \times (force\ level\ at\ stimulation \div MVC\ force) \div potentied\ doublet\ force)) \times 100$$

Figure 9: Illustration of the interpolated twitch technique recorded during isometric maximal voluntary knee extension. The superimposed evoked force during MVC illustrates an incomplete motor unit recruitment and/or suboptimal motor units firing rate. The potentiated doublet force evoked at rest is used to calculate the voluntary activation level and reflects muscle contraction properties.

Delivering an electrical stimulation while the muscle is resting may be coupled with EMG and force recordings to obtain insights from peripheral components (Millet et al., 2011). By delivering a single supramaximal intensity stimulus while the muscle is resting, a complete motor unit recruitment is expected, which results in an M-wave observed in the EMG trace. The M-wave corresponds to the synchronous depolarization of all motor units (Hicks et al., 1989) and provides information regarding the sarcolemmal excitability and AP propagation/transmission. The mechanical force evoked by the same single supramaximal stimulation (i.e., peak twitch (Pt)) is also informative regarding the intramuscular components, such as Ca^{2+} release from the sarcoplasmic reticulum, myofibrillar Ca^{2+} sensitivity, or cross-bridge formation (Lepers et al., 2004; Place et al., 2010). Together, the M-wave and Pt provide information regarding the conversion of an action potential to a mechanical output (i.e., excitation-contraction coupling), as well as reliable information on intramuscular properties.

In summary, voluntary contractions rely on a set of electrochemical processes ranging from the initiation of an AP in the primary motor cortex to the sliding of actin-myosin filaments within the muscle. We briefly described the neuromuscular system and the potential sites that may modulate voluntary contraction. In the next chapter, the effect of physical exercise on the neuromuscular function will be presented, as well as the relative contribution of central and peripheral components responsible for force production.

Part II

6. Acute endurance exercise

In this section, we first show that acute endurance exercise can modulate the neuromuscular system and result in a decrease in the ability to produce a MVC. The relevant literature regarding premotor and resting EEG activity after a single-session of endurance exercise is presented, as well as the potential modulators implicated in post-exercise electrocortical changes. The section ends by proposing the resting EEG microstate analysis as a relevant method for investigating post-exercise spontaneous brain activity.

6.1. Neuromuscular function

Endurance exercise of a sufficient intensity or duration inevitably leads to neuromuscular alterations and results in a reduction in the MCV (Gardiner, 2011). In neuromuscular physiology, the exercise-induced reduction in the MVC (also termed neuromuscular fatigue) has been thoroughly investigated. Regardless of the vigorous debate regarding the origin of neuromuscular fatigue (Amann et al., 2013; Marcora, 2008; Marcora and Staiano, 2010; Noakes and Gibson, 2004; St Clair Gibson, 2004), researchers agree that central and peripheral factors are implicated and appear to be mutually dependent (Millet et al., 2011).

The consequences of endurance exercise on the neuromuscular system are multifaceted as central alterations are often associated with metabolic and structural changes within the muscle (Millet and Lepers, 2004). The relative participation of central and peripheral parameters in post-exercise neuromuscular alterations is influenced by several factors, such as the type, duration, and intensity of exercise (Millet and Lepers, 2004). In general, the MVC has been shown to decrease by approximately 5% to 16% following submaximal endurance exercises (Bentley et al., 2000; Lepers, 2012; Millet and Lepers, 2004). Specifically, after 30 minutes of cycling at 80% of maximal aerobic power (MAP), a suboptimal motor drive has been evidenced by a reduction in the VAL from 82% to 72% (Lepers et al., 2001). At the peripheral level, the mechanical evoked response appears to be systematically affected after prolonged cycling and comprises between 15-23% (Lepers et al., 2004).

As brain activity is a primary concern of this thesis, the implication of the central component in force production is of particular interest. Several studies have aimed to determine the causes of the reduction in neuronal drive, and different mechanisms have been proposed, particularly after locomotor exercise. Large-muscle group exercises imply a larger amount of muscle work,

which thereby leads to a massive increase in cardiorespiratory and physiological demands. As a result, factors such as hyperthermia (Périard et al., 2011), cerebral oxygenation (Rupp and Perrey, 2008), or catecholamine concentration (Roelands and Meeusen, 2010) play a role in central regulation (Sidhu et al., 2013a). The involvement of group III and IV muscle afferents is another important mechanism implicated in central regulation (Amann and Dempsey, 2008; Amann et al., 2011; Laurin et al., 2015). These afferents may be stimulated by mechanical and metabolic modulations associated with muscle contractions and may exert an inhibitory effect on supraspinal (and spinal) sites, which results in a reduction in voluntary activation and MVC (Gandevia et al., 1996). After sustain fatigued contractions, when the exercised muscle is held ischemic with a sphygmomanometer cuff in order to trap the metabolites in the muscle, Gandevia et al. (1996) reported that the central component and MVC did not recover. In the context of endurance cycling exercise, Sidhu et al. (2014) confirmed the involvement of muscle afferents in voluntary activation by artificially manipulating the afferent signal. After a cycling exercise to exhaustion, the authors reported a progressive reduction in the maximal force of non-exercised muscles (i.e., elbow flexor), which was attributed to central alterations. In contrast, when the type III/IV afferent fibers were inhibited via intrathecal fentanyl injection, the elbow flexor maximal force remained unchanged with exercise, which suggests that the afferent feedback has been withdrawn. According to Sidhu et al. (2014), this result indicates that the reduction in neural drive is, at least in part, a consequence of inhibitory feedback effects. Central mechanisms implicated in force production are not clearly understood because interactions among several brain structures likely participate in modulating the motor output. Figure 10 illustrates the regulating model of motor output proposed by Tanaka and Watanabe (2012). The regulation of motor output is driven by a facilitation and inhibition system that enhances and limits the recruitment and firing rate of motor units (Tanaka and Watanabe, 2012). Specifically, the facilitation system compensates for muscle fatigue in order to maintain the motor output from M1, whereas inhibitory input originating from the periphery reduces the motor output by passing through different brain structures. Accordingly, different brain regions are likely to play a role in the motor output. This hypothesis has been experimentally verified using EEG by Hilty et al. (2011). The authors reported an increase in communication between the mid/anterior insular and motor cortex while exercising. The insular cortex process various sensory stimuli from the physiological condition; however, it may also communicate with the motor cortex and thus may indirectly participate in force production (Hilty et al., 2011). This example illustrates mechanisms that temporally occur before the voluntary motor command may participate in mediating the motor output.

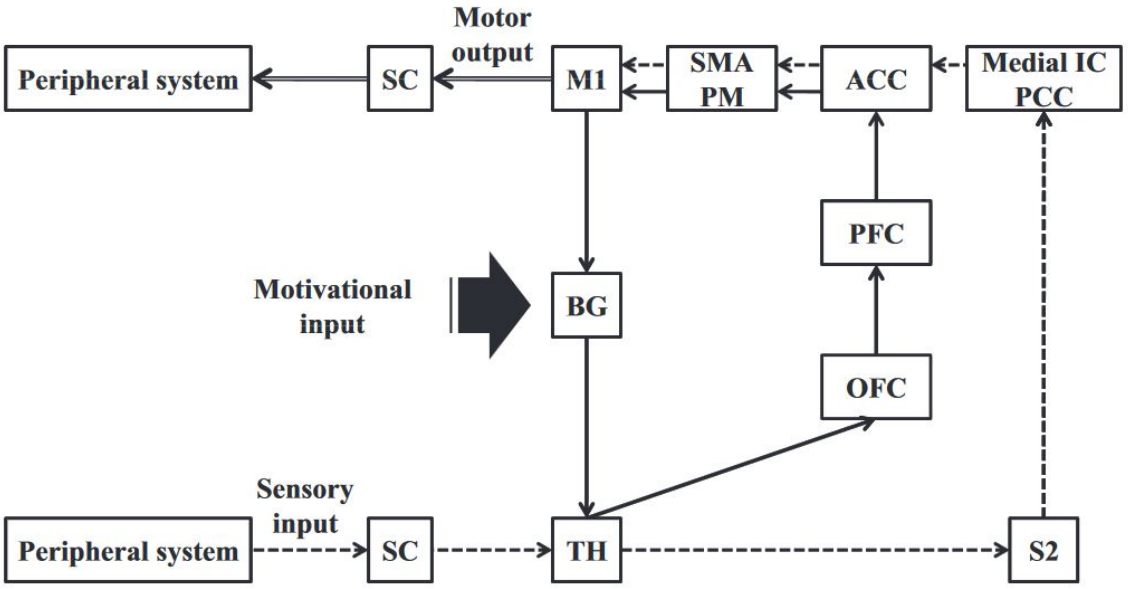

Figure 10: Simplified representation of the central mechanisms that regulate motor output. The circuit in dotted arrows represents the inhibition system. The sensory input originating from the peripheral system decreases the motor output by passing through the spinal cord (SC), thalamus (TH), secondary somatosensory cortex (S2), medial insular cortex (IC), posterior cingulate cortex (PCC), anterior cingulate cortex (ACC), premotor area (PM), supplementary motor area (SMA), and primary motor cortex (M1). The circuit in solid arrows represents the facilitation system. The M1 increases the motor output by passing through the basal ganglia (BG), TH, orbitofrontal cortex (OFC), prefrontal cortex (PFC), ACC, PM, SMA, and M1. Motivation input may also enhance this facilitation system (Adapted from Tanaka and Watanabe, 2012).

6.2. Movement-related cortical potential

Investigation of the BP after endurance exercise has generated limited attention. To the best of our knowledge, there is only one study that has investigated the BP after acute endurance exercise (Thacker et al., 2014). In this research, participants performed a 20-minute bout of recumbent cycle ergometer exercise at 70% of the age-predicted maximal heart rate (HR_{max}), which corresponded to an average of 136 beats/min. The estimated HR_{max} was obtained by subtracting the age from the constant 220. The BP task consisted of self-paced wrist extensions every 3-6 seconds for 8 minutes. The post-exercise measurements were obtained immediately after exercise and 40 minutes post-exercise, which corresponded to the duration for the heart rate (HR) to return to the pre-exercise value. Compared to baseline, there was no change in the BP immediately after exercise. The amplitude and the latency of the BP components were not

altered with exercise. In contrast, after the HR recovered, the onset of the increase of the early BP occurred earlier. The authors suggested that these long-term changes in the early BP may originate from the increase in neurotransmission activity in the basal ganglia and SMA network and may reflect alterations in the participant's preparatory state (Thacker et al., 2014).

The BP is obtained by repeating the same movement or contraction several tens of times to generate the typical BP trace. However, depending on the force exerted, this type of protocol alters the voluntary motor command and mechanisms in force production. It has been reported that the BP amplitude increases with the number of repetitions, likely because of the fatigue induced by the repetitive task (Berchicci et al., 2013; Johnston et al., 2001; Schillings et al., 2006). For some authors, a greater BP amplitude would reflect an increase in cortical activity to compensate for the muscle fatigue (Freude and Ullsperger, 1987; Johnston et al., 2001). Nevertheless, as some authors did not report significant peripheral muscle fatigue, it has been suggested that an increase in BP may compensate for a decrease of cortical efficiency (Schillings et al., 2006) or may reflect cognitive processes, such as the perceived/anticipated effort, concentration, or attention (Freude and Ullsperger, 1987; Schillings et al., 2006; Slobounov et al., 2004). Even if the mechanisms are not well understood, these findings confirm that premotor brain activity may be related to central mechanisms in voluntary force production and the upcoming movement. Because an acute endurance exercise of a sufficient intensity or duration inevitably leads to a reduction in the MVC (Gardiner, 2011), investigating the BP in association with the neuromuscular function would provide relevant insights regarding this issue.

6.3. Spontaneous brain activity

6.3.1. Preliminary studies

Following the discovery of human brain oscillations by Hans Berger in 1929, nearly 30 years passed before the first experiment that employed resting EEG in the context of physical exercise. The study from Beaussart et al. (1959) first reported an increase in the alpha amplitude in boxers the following minutes after boxing matches and marked the beginning of studies on EEG oscillations after exercise. Several years later, Pineda and Adkinsson (1961) used an incremental treadmill exercise until exhaustion with a duration that ranged from 35 to 70 minutes. The authors identified a greater amount of absolute alpha at the frontal cortex 5-10 minutes post-exercise and a return to the pre-exercise value after approximately 30 minutes of

recovery. More precisely, there were more alpha in the left hemisphere in the frontal and central regions than the right hemisphere. An increase in the alpha amplitude was reported in a published abstract from Wiese and al. (1983). The authors implemented a control-group design in which participants performed an exercise on a cycle ergometer of 40 minutes duration, which was split into two exercises of 25 minutes and 15 minutes at an intensity of 40% of maximal oxygen consumption ($\dot{V}O_{2\ max}$) and 60% of $\dot{V}O_{2\ max}$, respectively. Compared to the control group (i.e., non-exercising group), the exercising group showed a significant increase in alpha power. In a crossover design, Boutcher and Landers (1988) compared a control condition (reading) to an exercise condition that comprised 20 minutes of running at 80-85% of the age-related HR_{max} in runners and nonrunners. An increase in absolute alpha power was also reported after exercise for both groups, thus confirming previous findings on the alpha frequency band.

6.3.2. Modulations extend to the whole power spectra

Since the early 1990s, studies have extended their power analyses to wider frequency bands and showed that post-exercise electrocortical changes were not limited to the alpha frequency band or the frontal regions. In an article published by Youngstedt et al. (1993), the relative and absolute EEG power were computed for theta, alpha and beta frequency bands above occipital sites (O1 and O2). The physical task consisted of an underwater cycling exercise for 20 minutes at 70% of peak oxygen consumption ($\dot{V}O_{2\ peak}$) in a thermoneutral (32-35°C) versus cold (18-23°C) environment. Data were collected during 5 minutes before, between 10-15 minutes, and 20-25 minutes after the end of exercise. The authors reported a significant time effect for the alpha, beta, and theta frequency bands. In both conditions, the relative alpha power increased from pre-exercise to 15 minutes post-exercise before returning to baseline 25 minutes after exercise cessation. Interestingly, the relative beta power also increased in the 15 minutes post-exercise condition; however, it continued to increase 25 minutes after the end of exercise. In contrast, the relative theta power decreased in the 15-minute post-exercise condition and remained at approximately the same value 25 minutes after exercise cessation. The authors indicated that the statistical results were similar when the absolute data were analyzed.

In contrast to preliminary studies, Mechau et al. (1998) did not identify an increased alpha power after exercise; however, the authors highlighted the notion of exercise intensity on the EEG response and identified a tipping point that occurred around a lactate threshold (approximately 2-3 $mmol \cdot l^{-1}$) during exercise. Even if this article focused on EEG activity

during exercise, the authors provided interesting findings that deserve to be presented. In an incremental field test, leisure-time athlete participants performed 5 to 6 stages of running (increasing speed by 0.3 m/s at each stage) interspersed by short periods of rest for data collection. The protocol was designed so that the first three stages were run in a steady state condition prior to lactate accumulation, whereas the remaining stages were performed above the lactate accumulation threshold. Modulations in the delta, theta, alpha and beta frequency bands were investigated before and during exercise, as well as 15 and 30 minutes after exercise cessation. The authors identified a relative increase in alpha and beta power during the first stages of exercise compared to baseline, followed by a reduction in alpha power after passing the lactate threshold. The delta and theta frequency bands were increased during the entire exercise period. Compared to values obtained during the last stages of exercise, global (i.e., frontal, temporal, central, and occipital) decreases were identified after 15 minutes recovery in the delta, theta and alpha-2 frequency bands. However, when the 15 minutes post-exercise condition is compared to baseline, only the delta frequency band was higher in the central (C3, Cz, C4) and frontolateral (F3, F4) areas. No other changes were reported in other frequency bands after exercising.

According to the results obtained by Youngstedt et al. (1993), Oda et al.(1999) identified a relative (i.e., percentage of total 2-30 Hz power) increase in alpha and beta power after 60 minutes of gymnastic exercises in warm water (34°C) at a low/moderate intensity above central electrodes. However, they reported a relative decrease in delta and theta frequency bands between 10-15 and 15-20 minutes post-exercise, which did not corroborate the increase in delta power observed by Mechau et al. (1998). In another moderate intensity exercise protocol (40 minutes at approximately 130-150 beats per minute), Smit et al. (2005) identified an increase in relative alpha and beta-1 power and reduction in beta-2 power. The authors did not clearly justify why only the beta band was split in two sub-frequencies and were differently modulated by exercise.

Similar to moderate intensity exercises, maximal intensity exercises are followed by electrocortical changes that extend to several frequency bands. Moraes et al. (2007) used a maximal incremental (+15-30 watts/min) test on a cycle ergometer. They collected absolute alpha and beta activity across 20 electrode sites before and after exercise. In contrast to previous studies, even if the absolute alpha tended to increase at frontal sites, the results were not significant. Nevertheless, beta power in the frontal (Fp1, F3, F4) and central (C4) electrodes were significantly increased, which extends previous findings reported in a moderate intensity

protocol. In another maximal increment cycling test, Bailey et al. (2008) reported an overall increase in EEG power with no dominant hemisphere response. Specifically, the authors reported a relative increase from baseline in theta, alpha-1, alpha-2, beta-1 and beta-2 across the frontal and central regions and a return to baseline within 10 minutes post-exercise.

The diversity of the electrocortical response may be explained by the type of exercise performed. Schneider et al. (2009a) investigated the resting EEG activity before and after physical exercises of different intensities in volunteers who were regular runners. The exercise consisted of cycling during 21 to 60 minutes (individualized to match participant preference) at 50-55% $\dot{V}O_{2\ max}$, 80-85% $\dot{V}O_{2\ max}$, or a preferred intensity. EEG was collected before, immediately after, and 15 minutes after exercise cessation. The absolute power was averaged across 19 electrodes for delta, theta, alpha-1, alpha-2, beta-1, beta-2, and gamma. After low intensity exercise, the alpha-1 power increased, followed by a decrease to pre-exercise value within 15 minutes of recovery. In the high intensity condition, the alpha-1 did not change after exercise; however, it was reduced after 15 minutes of recovery. In the preferred and high intensity conditions, beta-2 decreased immediately after exercise and remained lower in the post-15 condition. No differences were identified for theta and gamma frequency bands. The article from Schneider et al. (2009) is interesting as they provided the spectral changes for the 19 electrodes collected across alpha and beta bands at three different intensities. The results are subsequently presented and show the modulations electrode-by-electrode immediately after exercise cessation and after 15 minutes of recovery (Table 1). These findings illustrate the diversity of the power spectra response and challenge the relevance of using classical power analysis after exercising.

Table 1: Results reported by Schneider et al. (2009) showing changes in the EEG activity on each single electrode immediately (POST) and 15 minutes (POST15) after a cycling exercise of approximately 40 minutes at 50-55% $\dot{V}O_{2\ max}$ (low), preferred intensity (pre), and 80-85% $\dot{V}O_{2\ max}$ (high).

	Fp1	F3	F7	C3	T7	P3	P7	O1	Pz	Cz	Fz	Fp2	F4	F8	C4	T8	P4	P8	O2
low							↑	↑									↑		↑
pre															↑			↑↑	
high							↓↓	↓↓											↓↓
low																			↑
pre													↑						↓3
high	↑	↓3											↑↓						↓3
low														↓↓		↓↓			
pre							↓							↓3		↓↓15			↓3
high							↓↓							↓3		↓↓15			↓↓15
low			↓																
pre			↓↓	↓↓											↓↓			↓3	↓↓
high			↓↓15	↓↓											↓↓			↓↓	↓↓

↓ indicates a significant decrease in measurement POST. ↓↓ indicates a significant decrease in measurement POST and POST15. ↓3 indicates a significant decrease in measurement POST15 only. ↓15 indicates an ongoing significant decrease from measurement POST to POST15. ↑ indicates an increase in measurement POST. ↑↓ indicates an increase in POST followed by a decrease in POST15. No changes in any of the electrodes were identified for the gamma and theta frequency bands (Adapted from Schneider et al., 2009a).

In summary, although many researchers have identified an increase in alpha power after exercise (Bailey et al., 2008; Fumoto et al., 2010; Oda et al., 1999; Schneider et al., 2009a; Youngstedt et al., 1993), some researchers did not report an effect in the alpha frequency band (Mechau et al., 1998; Moraes et al., 2007; Schneider et al., 2009a). When the analysis is extended to other frequency bands, an increase in beta power is identified in many studies (Bailey et al., 2008; Moraes et al., 2007; Oda et al., 1999; Youngstedt et al., 1993) and may be accompanied by changes in delta and theta frequency bands (Fumoto et al., 2010; Mechau et al., 1998; Oda et al., 1999). Finally, even if the effect of exercise on a given frequency cannot be highlighted, an effect appears to persist for approximately 15 to 25 minutes post-exercise (Oda et al., 1999; Schneider et al., 2009a; Youngstedt et al., 1993).

6.3.3. Emotional and affective response to exercise

Abundant literature has used the resting electrocortical activity to provide an objective, reliable measurement of the affective response to exercise. Experimental studies have reported that

acute exercise increases positive mood (vigor) (Moraes et al., 2011; Oda et al., 1999) or reduces negative mood (tension/ anxiety, depression/dejection, or mood disturbance) (Fumoto et al., 2010; Moraes et al., 2011) and state anxiety (Boutcher and Landers, 1988). A direct relationship between EEG and mood/anxiety has been effectively identified by researchers (Moraes et al., 2011; Schneider et al., 2009a). For example, Schneider et al. (2009a) reported significant correlations between the elevated alpha power after exercise and the increase in perceived physical state (Kleinert, 2006), as well as between the increase in beta and gamma power and the decrease in perceived motivational state. However, the link between exercise, brain activity, and emotional response may be tenuous as some studies did not identify an effect of exercise on anxiety and vigor (Fumoto et al., 2010; Youngstedt et al., 1993) or a direct correlation between exercise-induced mood and EEG changes (Oda et al., 1999).

In parallel to former descriptive studies, researchers have quantified the frontal EEG asymmetry to provide neurophysiological outcomes on how exercise interacts with the emotional and affective state. This research field was based on the Davidson's model of frontal asymmetry (Davidson, 1993, 2004; Davidson et al., 1990; Tomarken et al., 1990), which assumes that the frontal hemispheres are specialized in a particular behavior and affective process. A greater relative activation in the right anterior region would be related to avoidance behavior and negative affect, whereas a greater relative activation in the left anterior regions is related to approach behavior and positive affect. In this context, frontal EEG asymmetry in the alpha frequency band has been used to quantify prefrontal lobe activation. Steven Petruzzello and his group published numerous papers regarding the exercise-affect relationship, assuming that information regarding emotion and affect may be obtained from EEG alpha asymmetry (Hall et al., 2000, 2007; Petruzzello and Landers, 1994; Petruzzello and Tate, 1997; Petruzzello et al., 2001). The asymmetry is computed by the difference between the right hemisphere alpha power minus the left hemisphere alpha power. Alpha activity is negatively related to the activity within the underlying region, which indicates that an increase in frontal alpha power is interpreted as a lower cortical activity. Relying on the Davidson's model, several studies have shown that pre-exercise frontal EEG asymmetry may predict the affective response to exercise (Hall et al., 2007, 2010; Petruzzello and Landers, 1994; Petruzzello and Tate, 1997; Petruzzello et al., 2001). Moreover, a significant inverse correlation has been identified between frontal asymmetry and anxiety after exercise (Moraes et al., 2011). The relationships between frontal asymmetry and the affective response appear dependent on the exercise intensity (Hall et al., 2000; Petruzzello and Tate, 1997; Woo et al., 2009), duration (Woo et al., 2009) and the level

of fitness (Petruzzello et al., 2001). However, other studies did not identify a frontal asymmetry (Fumoto et al., 2010; Schneider et al., 2009a) or a relationship between frontal asymmetry and affective response (Hall et al., 2010; Lattari et al., 2014). Different elements may explain these discrepancies. For example, it has been postulated that adults interpret emotions using two major dimensions, valence (positive/negative) and arousal (high/low), and each dimension may rely on different brain regions (Heller, 1990). The parietal region plays a role in the mediation of arousal, whereas the frontal regions play a role in emotional valence (Heller, 1990). Consequently, an increase in the feeling of arousal after exercise is not necessarily associated with changes in the affective valence (Crabbe and Dishman, 2004; Crabbe et al., 1999). Moreover, regarding the classical spectral changes, the asymmetry does not appear to be restricted to the alpha band or the frontal region (Lattari et al., 2014; Woo et al., 2009). According to the recent systematic review from Lattari et al. (2014), the mood state after a single-session of exercise may be partially mediated by an asymmetric pattern in the frontal regions; however, it is not possible to define a clear relationship between mood changes and modulation in cortical activity. Nevertheless, the frontal asymmetry model proposed during the 1990s has undoubtedly drawn the attention of researchers to the frontal cortex for several years, thus likely leading to hasty conclusions in the EEG-exercise literature.

6.3.4. Source localization

With the development of new EEG methods that enable the localization of the origin of the surface electrocortical activity, additional knowledge has emerged regarding our understanding of the post-exercise brain state. From a standardized surface EEG recording, the electric neuronal activity distributions are identified from a probabilistic head model composed of several hundred solution points (voxels). The inverse solution algorithm used in exercise literature enables an approximation of the generators of the surface brain activity and allocates the current density to their corresponding Brodmann area (BA) or cerebral gyri. Schneider et al. (2010) were the first to use an EEG source localization method in the context of physical exercise. In their preliminary study, regular runners performed an incremental running test until exhaustion with EEG collected before, after, and 15 minutes post-exercise. They identified an increase in the alpha-1 activity in the left frontal area (BA 8) immediately after exercise, which was associated with an increase in delta widely spread across the cortex. The theta activity also increased in the left and right temporal regions (BA 21, 22, 37). Compared to baseline, after 15 minutes of recovery, the alpha-2 activity decreased in the temporal cortex (BA 20), the beta-1

activity decreased in left inferior, middle and superior temporal gyri (BA 20-22), and the gamma activity decreased in the occipital lobe (BA 18, 19). In a similar incremental test of approximately 15 minutes, the same group investigated the relaxing effects of exercise according to the individuals' physical activity history and preference (Schneider et al., 2009b). The EEG responses of regular runners were compared after arm crank, bicycle, or treadmill exercise. The EEG was collected before and 2, 15, and 30 minutes post-exercise. In the 2-minute post-exercise condition, source localization analyses showed an increase in the alpha activity in the frontal (BA 6, 8, 9) and limbic (BA 24, 32) regions and an increase in the beta activity in the parietal region (BA 7) after treadmill exercise. After the biking exercise, the results indicated increases in the alpha and beta activities in the parietal (BA 7) and limbic (BA 23, 31) lobes, whereas after the arm crank exercise, increases in the alpha and beta activities were identified in the frontal and parietal regions (BA 45 and BA 7, 40, respectively). The results from Schneider et al. (2010) indicated a different long-lasting effect depending on the type of exercise performed. In 15- and 30-minute post-exercise conditions, the alpha and beta were not different after treadmill, whereas in the biking condition, the alpha activity increased in the frontal (BA 6, 9) and limbic (BA 24,32) regions after 15 minutes without a change in the beta band. After the arm crank exercise, there were increases in alpha and beta in the left and right temporal lobe after 15 minutes post-exercise. After 30 minutes post-arm crank exercise, the beta frequency band remained different from baseline in retrosplenial region and dorsal posterior cingulate (BA 30/31). Schneider et al. (2009b) assumed that the increase in alpha mainly in the frontal cortex immediately after treadmill exercise may reflect the individuals' physical activity preference, as subjects were regular runners and the frontal region is involved in emotional processing.

In a submaximal intensity exercise protocol that comprised 20 minutes of cycling at 80% of the age-predicted HR_{max}, global increases in alpha (BA 24, cingulate gyrus), beta-1 (BA 33, anterior cingulate) and beta-2 (BA23, posterior cingulate) were identified immediately after exercise, with no changes in the delta and theta frequency bands (Moraes et al., 2011). The authors did not provide an explanation concerning their divergent results compared to the results of Schneider et al. (2009b), with the exception of the implication of variables related to the experimental design, such as the room temperature, type of exercise (intensity and duration), or time frame of measurement after exercise.

In a protocol composed of submaximal and maximal intensity exercise, the group of Schneider investigated the effects of exercise mode and intensity on the EEG response (Brümmer et al., 2011) and tested their previous dose-response relationship hypothesis and the individuals'

physical activity history and preference hypothesis (Schneider et al., 2009b, 2009a). The protocol was composed of two experiments. In the first experiment, 12 runners performed three types of exercises on a treadmill, bicycle, and arm crank at 50% $\dot{V}O_{2\ peak}$ or 80% $\dot{V}O_{2\ peak}$. The duration was 30 minutes for biking and running and 3 x 10 minutes separated by 3 minutes rest for the arm crank where subjects had to perform 3 x 30 wrist flexions at 50% or 80% of the maximal capacity. EEG was collected before and approximately 20 minutes after exercise cessation. In the second experiment, five hand-cycling athletes completed an incremental arm crank test until exhaustion with EEG collected before and immediately after exercise cessation. In the first experiment, alpha increased after all types of exercise in the 50% $\dot{V}O_{2\ peak}$ condition compared to the pre-exercise value (Table 2). The increase in alpha was located in the parietal cortex after treadmill (BA 1, 2) and cycling exercise (BA 5, 7, 40) and in the frontal cortex (BA 6, 9) after arm crank. Concerning the beta frequency band, an increase was reported in the parietal cortex (BA 30, 31) after cycling. In the 80% $\dot{V}O_{2\ peak}$ intensity condition, alpha tended to decrease in the frontal cortex after treadmill; however, it did not reach significance. Concerning the beta frequency band, a decrease in the frontal cortex (BA 11, 25, 47) was reported after running. No other changes were identified. The results are summarized in Table 2. In the second experiment, after the maximal incremental arm crank exercise, alpha decreased in the frontal cortex (BA 11), whereas beta slightly decreased in the frontal cortex but was not significant.

Table 2: Summary of the main results from Brümmer et al. (2011). Significant changes in the alpha (7.5-12.5 Hz) and beta (12.5-35 Hz) frequency bands after three exercise modes (treadmill, bicycle, arm crank) at 50% $\dot{V}O_{2\ peak}$ and 80% $\dot{V}O_{2\ peak}$.

	Alpha frequency band		Beta frequency band	
	50%	80%	50%	80%
Treadmill	↗ BA 1, 2 (parietal)	NS	NS	↘ BA 11, 25, 47 (frontal)
Bicycle	↗ BA 5, 7, 40 (parietal)	NS	↗ BA 30, 31 (parietal)	NS
Arm crank	↗ BA 6, 9 (frontal)	NS	NS	NS

BA, Brodmann area; NS, not significant; ↗ and ↘, increase and decrease of activity (Adapted from Brümmer et al. 2011).

According to Brümmer et al. (2011), when the exercise intensity is moderate, there is an increase in alpha in either somatosensory brain regions after familiar exercise (treadmill exercise) or in emotional brain regions after unfamiliar exercise (arm crank exercise). When the exercise intensity is high, preferred exercise results in a reduction in beta in the prefrontal cortex, which was interpreted as a deactivation of frontal cortex. For the authors, this deactivation may reflect a specific cortical pattern after familiarization and adaptation to certain exercises and intensities. This result suggests that the EEG response to exercise depends on the exercise mode and intensity and likely the individuals' physical activity preference.

In summary, after reviewing the relevant literature on EEG and exercise, we conclude that exercise modulates the brain electrocortical state; however, no persistent EEG pattern may be emphasized. The interpretation of the oscillations, amplitude, and power changes after exercise is sometimes confusing in articles, especially when authors use the terms of brain activation and deactivation to discuss their findings. With the multiplication of studies and the variety of protocols, analysis methods, and designs, varying findings have emerged. Increasing the number of frequency bands, splitting them into sub-frequencies, and extending the regions of interest have been considered to provide a global view of spectral changes across the whole scalp. Unfortunately, instead of providing reliable conclusions, it leads to further inconsistencies. In this scattered literature, the meta-analysis published by Crabbe and Dishman (2004) facilitates a clearer vision of this issue. The most consistent result concerned the absolute alpha frequency band that appears to increase with exercise compared to a baseline or control condition (Crabbe and Dishman, 2004). However, when alpha changes are expressed relative to the total power, evidence does not indicate that exercise results in a specific effect in this frequency band or changes in alpha are restricted to the frontal cortex (Crabbe and Dishman, 2004). When the power analysis was extended to wider frequencies, the modulations were similar in size to those observed in the absolute alpha activity (Crabbe and Dishman, 2004; Mechau et al., 1998; Schneider et al., 2010). It appears that there is no more than an overall cortical activation after exercise, which makes it difficult to examine frequency-specific characteristics (Schneider et al., 2010).

7. Interpreting the post-exercise resting electrocortical activity

The vast majority of the literature regarding resting EEG and exercise did not directly assess the putative mechanisms that underlie the modulations in electrocortical activity. Although assumptions have been formulated, the relationships between changes in brain activity and objective physiological measurements have not been extensively investigated. The initial assumption regarding the increase in alpha oscillations was interpreted as a possible state of decreased cortical activation, indicative of fatigue or relaxation (Boutcher and Landers, 1988; Crabbe and Dishman, 2004). A widely accepted view supposes that an activated/aroused behavioral state is characterized by a low amplitude high frequency, whereas a high amplitude, slow frequency appears in resting and quiet conditions. Accordingly, spontaneous brain oscillations have been supposed to shift from high frequencies to slow frequencies with exercise. However, the subsequent published studies reported a global increase in power in nearly all frequencies, which makes it difficult to interpret the specific frequency-dependent changes. In this chapter, we present the principal modulators raised by the EEG-exercise literature.

7.1. Potential modulators

7.1.1. Cardiovascular activity

The cardiovascular system has been proposed as an important mechanism in exercise-induced electrocortical changes under the influence of skeletal muscle activity (Crabbe and Dishman, 2004; Hatfield, 1991; Hatfield and Landers, 1987). The specificity of large-muscle group exercise is the massive cardiovascular response that copes with the increase in the metabolic requirement, thus ensuring the delivery of dioxygen to working muscles and the removal of carbon dioxide. The optimal physiological balance in the body and the successful cardiovascular control of blood pressure and distribution of blood flow are under the control of the autonomic nervous system (Christensen and Galbo, 1983; Fisher, 2014; Murphy et al., 2011). The cardiac autonomic response is predominately modulated by three distinct neural mechanisms: the central command, the exercise pressor reflex, and the arterial baroreflex (Figure 11). The central command is a descending feed-forward process that coordinates muscle activation with cardiovascular adjustment. The other mechanisms comprise feedback information from muscle sensory afferents, as well as baroreceptor afferents that primarily originate from the carotid sinus and aortic arch (Iellamo, 2001; Spranger et al., 2015).

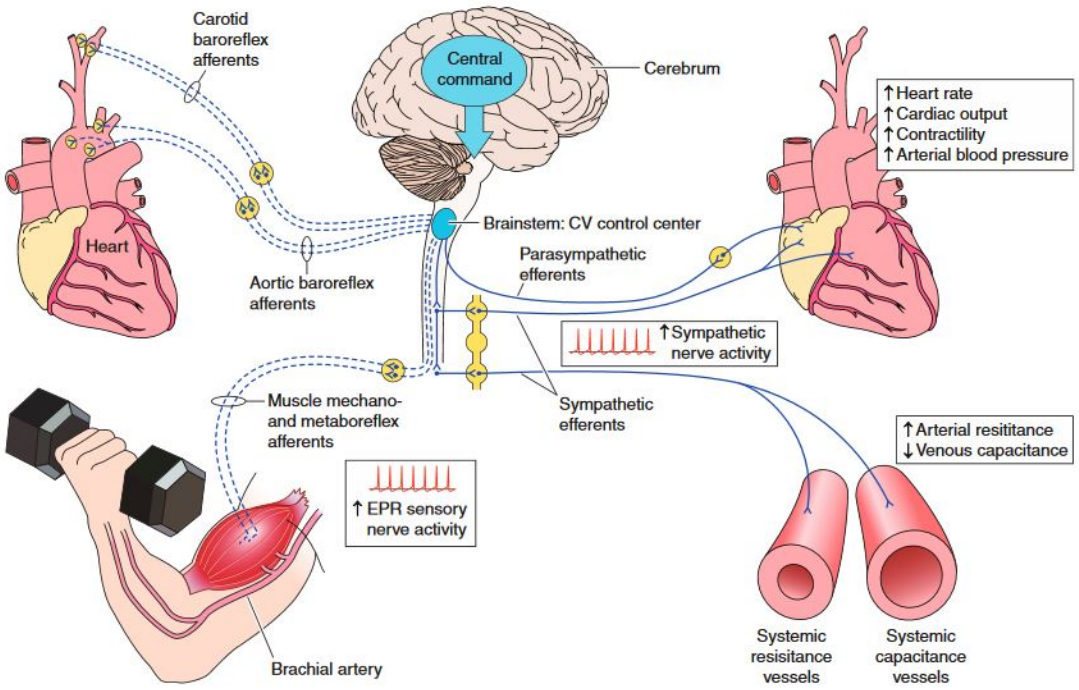

Figure 11: Autonomic cardiovascular regulation during exercise. Three mechanisms mediate cardiovascular adjustment during exercise: the central motor command, the arterial baroreflex and the skeletal muscle exercise pressor reflex. When exercise starts, a neural signal from the central command stimulates both the motor units for muscle contraction and the cardiovascular control center in the brain stem. The activation of mechanically and metabolically sensitive skeletal muscle afferents and carotid baroreflex afferents stimulates, in turn, the cardiovascular control center that coordinates the sympathetic and parasympathetic autonomic adjustment (Adapted from Spranger et al. 2015).

In the context of physical exercise, the muscle afferents are of particular interest because of the substantial amount of work produced by muscle contractions. The physiological conditions, including the mechanical, thermal, chemical, metabolic and hormonal status of skin, muscle, joints, and viscera, are conducted by small-diameter type III/IV afferent fibers (Craig, 2003). Mechanoreceptors are stimulated by the muscle contraction, whereas metaboreceptors are stimulated by chemical by-products of muscle contraction (Figure 12) (Murphy et al., 2011). Once activated, this feedback converges on neural structures within the brain stem and sub-cortical areas (e.g., insula and thalamus) (Menon, 2015; Shoemaker et al., 2012) that are implicated in cardiovascular regulation (Lacey and Lacey, 1978). Consequently, post-exercise electrocortical changes have been suggested to be related to cardiovascular regulation during exercise and its subcortical structures (Crabbe and Dishman, 2004). Specifically, the insula is involved in cortical regulation of autonomic cardiac activity and has been associated with

exercise intensity in a dose-dependent manner (Williamson et al., 1997). As the insular cortex has projections to the thalamus, which, in turn, exerts excitatory and inhibitory control on cortical regions, the cardiovascular system has been proposed as a moderator in post-exercise electrocortical changes (Crabbe and Dishman, 2004). For Woo et al. (2009), this autonomic afferent feedback may explain why brain activity more likely involves the frontal cortex. Based on the work from Craig (2005), Woo et al. (2009) presupposed a lateralization of the autonomic afferents to the anterior insula, in which the left anterior insula primarily receives parasympathetic input, whereas the right anterior insula preferentially receives input from sympathetic afferents. As a result, the activity of the insula would impact the power activity of the dorsal lateral prefrontal cortex and thus the above frontal electrodes (Woo et al., 2009). Depending on the sympatho-vagal balance, a dominant activation of the related cerebral hemisphere is subsequently expected, which is in favor of a specific effect of exercise on the prefrontal cortex as previously presented.

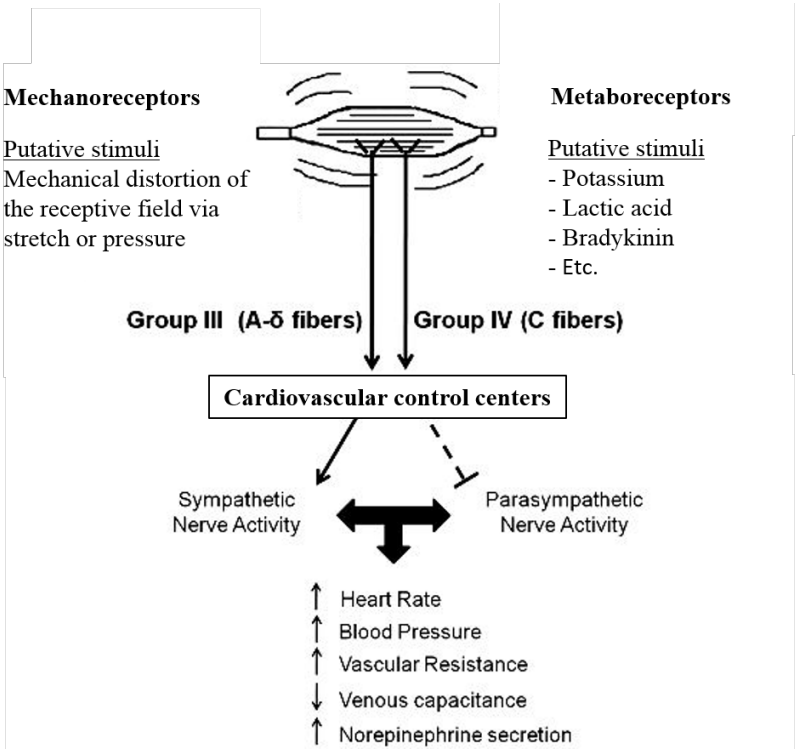

Figure 12: The exercise pressor reflex. Skeletal muscle contraction excites the terminal end of type III/IV afferents through several putative stimuli. Potential mechanoreceptors are predominantly located on group III afferents, whereas potential metaboreceptors are more likely located on group IV afferents. These afferents transmit the information to the cardiovascular control centers located within the medullary region of the brain stem, which promote an increase in sympathetic tone and withdrawal of the parasympathetic tone (Adapted from Murphy et al. 2011).

The cardiovascular control is guided by the parasympathetic and sympathetic autonomic balance (Figure 13) (Furlan et al., 1993; Mourot et al., 2004; Perkins et al., 2016; Seiler et al., 2007). The initial increase in HR at the beginning of exercise is driven by parasympathetic withdrawal and is subsequently driven by the increase in the sympathetic tone (Tulppo et al., 1996). In the immediate post-exercise period, it is generally agreed that the parasympathetic system plays a major role in decreasing HR in the first minute after exercise cessation (Perini et al., 1989) in combination with a reduction of the sympathetic tone in the following hour (Arai et al., 1989; Furlan et al., 1993; Martinmäki and Rusko, 2007). The measurement of heart rate variability (HRV), which corresponds to the variability between beat-to-beat intervals, is an indirect approach to investigate the autonomic cardiovascular response induced by exercise, and, more precisely, the sympathetic and parasympathetic components of the autonomous nervous system (Malik, 1996).

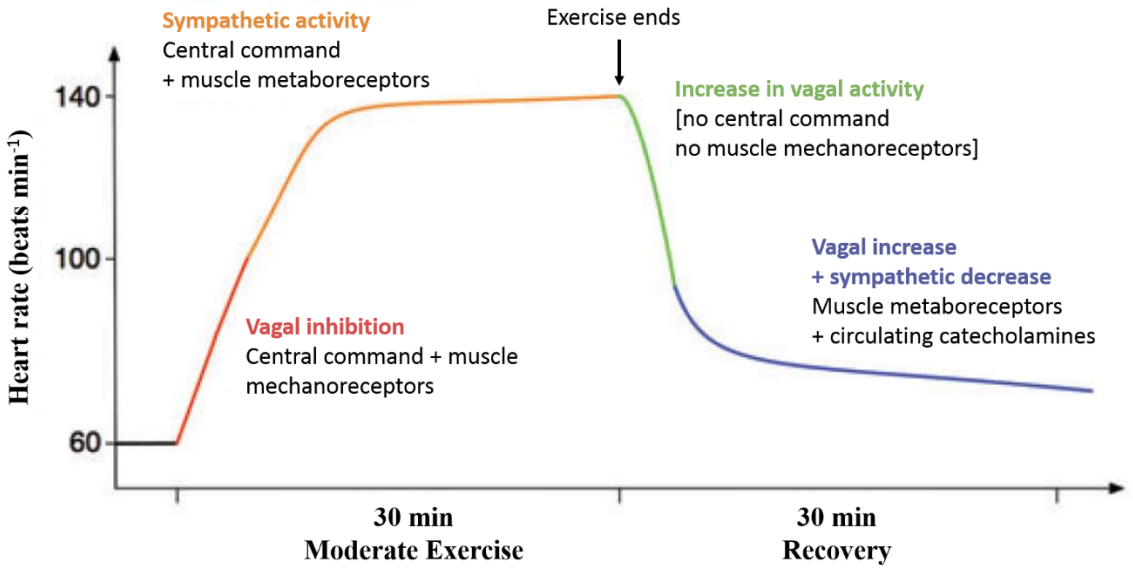

Figure 13: Heart rate kinetics during and following exercise. The graph illustrates the modification in heart rate from rest, during 30 minutes of moderate dynamic exercise, and during the 30 minute recovery period. The contribution of the cardiac sympatho-vagal balance and the underlying mechanisms are schematically indicated (Adapted from Coote, 2010).

7.1.2. Somatic afferents

The hypothesis of somatic afferent modulations is closely related to the afferent pathway described in the previous subchapter. In both situations, the muscular milieu appears at the origin of the increase in peripheral afferent activity. Even if the implication of a somatic afferent has not been directly investigated in the EEG and exercise literature, this hypothesis has been suggested by researchers (Mechau et al., 1998; Youngstedt et al., 1993). For example, the decrease in cortical activity has been related to blood lactate accumulation that may modulate the afferent system (Mechau et al., 1998). In their meta-analysis, Crabbe and Dishman (2004) referred to a study from Stock (1996), who reported a positive correlation between the noradrenaline plasma level and the increased alpha-1 and beta-1 power density after resistance exercise. The noradrenaline level increases as a function of the intensity of exercise and is associated with nociceptive and mechanoceptive responses to exertion, which may influence electrocortical activity (Crabbe and Dishman, 2004). Finally, the modulation in alpha activity recorded at centroparietal electrode sites above the sensorimotor cortex (e.g., Brümmer et al., 2011; Schneider et al., 2009b)) and within the BA receiving body afferent projections, such as the cingulate cortex (Moraes et al., 2011), indirectly support a mediating effect of somatic afferents on electrocortical activity.

7.1.3. Hyperthermia

A local increase in temperature in skeletal muscle, cardiac muscle, or internal organs may have an action on brain activity (Nielsen et al., 2001). It has been shown that the spectral power appears inversely correlated with the temperature; thus, the high frequency EEG spectrum decreases as temperature increases (Dubois et al., 1980). The hyperthermic sate promotes synchronous firing of the hypothalamic neurons, which results in slow-wave high amplitude EEG (Hatfield, 1991). In the context of physical exercise, it has been proposed that a hypothalamic modulation of brain activity may be mediated by an increase in body temperature as a result of exercising (Nielsen et al., 2001). A strong correlation has been reported between EEG power and core temperature (Nybo and Nielsen, 2001a), in which an increase in temperature was associated with a gradual slowing of brain oscillations (Nielsen et al., 2001; Nybo and Nielsen, 2001a; Rasmussen et al., 2004). Hyperthermia is thought to have a direct action on the brain and/or an indirect effect because of the afferent signals that originate from skeletal and cardiac muscles or internal organs in response to an increasing local temperature (Nielsen et al., 2001). Nevertheless, when submaximal intensity exercise was performed during

1 hour in a thermoneutral environment (18°C), the core temperature stabilized at approximately 38°C (Nybo and Nielsen, 2001b). Although we cannot exclude an effect of hyperthermia on the EEG signal, we are confident that other mechanisms may explain the exercise-related cortical changes when exercise is performed in thermoneutral conditions.

7.1.4. Catecholamines

Additional mechanisms related to intrinsic brain and body modulations have been proposed, such as an increase in catecholamine concentration. The study from Fumoto et al. (2010) is one of the few studies that collected surface EEG with objective physiological measurements. The blood serotonin concentration was measured before, immediately after exercise, and after 30 minutes of recovery, whereas EEG was collected only before and after exercise. The exercise consisted of a moderate cycling task of 15 minutes at a rating of perceived exertion of 12-13, which corresponded to 93 ± 5 watts. The results showed an increase in the relative power of high frequency alpha and a reduction in the theta band after exercise at the Cz and Pz positions with no difference between electrode sites. The whole blood serotonin level increased immediately after exercise and returned to baseline within 30 minutes. The increase in the serotonin concentration has been suggested to cause a shift to low frequency oscillations because of inhibition of the basal forebrain via a cholinergic pathway (Fumoto et al., 2010).

7.2. Limitations of classical power spectral analysis in physical exercise studies

Based on previous literature, EEG power spectral analysis showed an effect of acute exercise on spontaneous resting brain activity. Recent FFT-based (Fast Fourier Transform) source localization studies have been informative as they identified the potential generators of the surface spectral changes. The overall picture of brain oscillations across electrode sites, or BA, provides valuable insights regarding the post-exercise electrocortical state, and different modulators have been identified, such as the duration, intensity, familiarity of the task, or subjects' level of fitness. However, classical power analyses have failed to show consistent results, and investigators have struggled to explain and interpret the post-exercise changes according to the different frequency bands. Brain oscillations have been commonly considered in tight frequency bands in an independent manner. The postulation that a single cerebral rhythm is associated with a particular brain state appears unlikely, and recent findings have

emphasized the importance of analyzing multiple frequency bands simultaneously rather than limiting the analysis to one or two EEG bands (Mantini et al., 2007).
Classical surface power and FFT-based source localization analysis presumes that the signal is stationary over time (i.e., as periods of time are averaged) and does not benefit from the temporal resolution of EEG. Moreover, the generators of EEG have also been considered independently instead of as a part of a large-scale brain networks. This point of view contrasts with the current literature emphasized at the beginning of the present thesis, which visualized the resting brain as a dynamic system of large-scale distributed networks. Consequently, one goal of the present thesis is to apply an innovative analysis method of the spontaneous electrocortical signal that copes with the previously described issues.

8. EEG microstates as an innovative approach

The brain is organized in large-scale functional networks that display electrophysiological oscillations in multiple frequency bands, which are likely coupled to mediate brain operations (Mantini et al., 2007). Accordingly, the microstate analysis is a global measure of momentary brain activity that provides several benefits in terms of EEG methodological issues. First, as previously mentioned in chapter 3.2.4. (Figure 2, page 7), the microstate topographies are reference-independent (Lehmann and Skrandies, 1980) and avoid the critical question of reference selection (Lei and Liao, 2017). Second, the overall electrode sites are used in the analysis, and there is no a priori with respect to a specific location to investigate. Third, the traditional frequency bands are considered as a whole, and the raw signal is bandpass filtered in broad spectra. Fourth, the microstates reflect indirectly superficial and deep brain processes (Lehmann et al., 1998) with a time resolution that enables to capture of extremely rapid brain processes. Finally, the investigation of the electric field map enables assumptions regarding the neuronal generators of the related topographies (Michel and Murray, 2012).

We have seen that EEG microstates correlate with large-scale neuronal networks. In the context of this thesis work, microstate map C and its associated salience RSN are of particular interest. The salience RSN is anchored around two main structures, the anterior insula and cingulate cortex, which receive inputs from multisensory modalities, including somatosensory afferents (Menon, 2015). These brain nodes also respond to internal signals that originate from autonomic processes and mediate cardiovascular arousal and interoceptive awareness (Bechara

and Naqvi, 2004; Critchley et al., 2000; Eckert et al., 2009; Pollatos et al., 2007), which makes microstate map C and its related RSN a major element to explore.

In the previous chapters, we reported that feedbacks from cardiac activity and somatic afferents are two important mechanisms underlying the brain changes in response to exercise (Hatfield, 1991). Specifically, muscle contraction during exercise induces mechanical and chemical modulations that activate the terminal end of type III/IV muscle afferents, which, in turn, project to different sites within the central nervous system (Amann et al., 2015; Craig, 2003; Laurin et al., 2015). This input is processed at higher cortical levels, such as the cingulate and insular cortices, whose output may modulate sensorimotor activities (Craig, 2003; Liu, 2003) and mechanisms in force production (Amann et al., 2015; Gandevia, 2001; Taylor et al., 2000) and may participate in autonomic cardiovascular control (Fisher et al., 2010, 2013; Murphy et al., 2011; Thayer et al., 2012). There are cortical and subcortical contributions to the autonomic cardiovascular regulation (Critchley et al., 2003), and the variation in autonomic cardiac activity measured by HRV has been related to activation within the cingulate and insula (Chang et al., 2013; Critchley et al., 2003; Menon, 2015). Taken together, these results confirm the particular interest of microstate map C and its related salience RSN, as well as potential interactions with the neuromuscular and cardiovascular systems.

Part III

Aim

In the introduction, we emphasized the importance of the resting ongoing brain activity in determining a wide range of behavioral abilities and reported that the premotor brain activity is crucial, as it may be associated with the voluntary motor response. After exercise, the literature has shown modulations in brain electrocortical activity, alterations within the neuromuscular system, and a reduction in the maximal voluntary contraction force. These modifications are modulated by the whole-body physiological response, which is likely under the influence of skeletal locomotor muscle activity. If voluntary motor action is brain state dependent, one may wonder whether modulations in resting and premotor brain activities may be related to the neuromuscular system and mechanisms in force production following exercise. Furthermore, exploring the brain state at rest and before movement onset provides a better understanding of the motor response variation after exercise. To overcome several limitations of the classical power analysis, this thesis proposed to grasp the resting brain activity via the EEG microstate temporal properties with the assumption that a given microstate (map C) would show a particular responsiveness to exercise.

The present thesis is founded on four objectives:

1. Investigating the effect of acute large-muscle group exercise on premotor brain activity - ***Article 1***
2. Quantifying the effect of acute large-muscle group exercise on resting brain activity by applying microstate analysis as an innovative EEG method in exercise physiology - ***Article 2***
3. Investigating the association between post-exercise resting EEG microstates and MRCP changes with the knee-extensor neuromuscular function and the mechanisms in force production - ***Article 1 and Article 2***
4. Describing the resting EEG microstates and autonomic cardiac activity recovery dynamic after exercise - ***Article 3***

Summary of the results

9. Article 1

Movement-related cortical potential amplitude reduction after cycling exercise relates to the extent of neuromuscular fatigue.

Spring JN, Place N, Borrani F, Kayser B, Barral J

Published in Frontiers in Human Neuroscience 10:25, 2016

We assessed the movement-related cortical potential (MRCP) during 60 self-generated knee extensions and the knee extensor neuromuscular function in sixteen endurance-trained male athletes before (PRE), between (POST1), and after (POST2) a sequence of 30-minute heavy submaximal cycling exercise followed by a severe intensity 10-km all-out time trial.

The exercise resulted in a decrease in the knee extensor maximal voluntary contraction force (MCV) after the first (-10 ± 8%) and second (-21 ± 9%) exercise ($p < 0.01$). The voluntary activation level (VAL; -6 ± 8% and -12 ± 10%), peak twitch (Pt; -21 ± 16% and -32 ± 17%), and paired stimuli force (P100 Hz; -7 ± 11% and -12 ± 13%) were also reduced after the heavy 30-min exercise and the time trial respectively ($p < 0.05$). The M-wave amplitude was significantly reduced only after the time trial (-10 ± 15%) ($p < 0.01$).

Following the first exercise, MRCP reductions were identified for the BP2 (-1000 to -500 milliseconds), NS' (-500 milliseconds to movement onset), and MP (peak amplitude) components above the FC1-FC2 electrodes and the BP2 component above the C2 electrode ($p < 0.05$). The sequence of the two cycling exercises resulted in a significant reduction of the four MRCP components (BP1 (-1500 to -1000 milliseconds), BP2, NS' and MP) above the FC1-FC2 and C2 electrodes ($p < 0.05$).

Following the severe intensity exercise, the reduction in P100 Hz was correlated with the reduction in the NS' ($r = 0.61$) and MP ($r = 0.61$) components above the FC1-FC2 electrodes. Above the C2 electrode, the decreases in BP1 ($r = 0.57$), BP2 ($r = 0.65$), NS' ($r = 0.72$) and MP

($r = 0.64$) were correlated with the reduction in the VAL, and the reduction in MP was correlated ($r = 0.64$) with the reduction in the MVC (all $p < 0.05$).

We showed that large-muscle group exercise reduces the MRCP amplitude. This reduction was associated with reductions in the maximal voluntary contraction force, peripheral muscle alterations, and central mechanisms in force production. An influence that originates from muscle afferent activity is suggested as a putative modulator of MRCP changes.

Contribution

Led the conception and design of the protocol and conducted data collection and analysis. Led the interpretation, writing of the manuscript, and editorial process.

10. Article 2

A single-bout of endurance exercise modulates EEG microstates temporal features

Spring JN, Tomescu MI, Barral J

Published in Brain Topography 30(4), 461-472, 2017

We assessed EEG resting microstate temporal features and knee-extensor neuromuscular function in twenty endurance-trained male athletes before (PRE), between (POST1) and after (POST2) a sequence of a heavy 30-minute submaximal cycling exercise followed by a severe intensity 10-km all-out time trial.

The maximal voluntary contraction force (MVC) decreased after the heavy exercise (-9 ± 8%) and the time trial (-20 ± 8%) ($p < 0.01$). The paired stimuli force (P100 Hz) and the voluntary activation level (VAL) were reduced after the heavy (-7 ± 11% and -5 ± 9%, respectively, $p < 0.05$) and severe intensity exercise (-12 ± 13% and -10 ± 11%, respectively, $p < 0.001$).

The four canonical microstates were obtained, and explained more than 84% of the global variance. The global explained variance (GEV), mean duration, and time coverage significantly increased for microstate class C after the heavy exercise and the time trial ($p < 0.001$). No differences were identified between the heavy and severe intensity exercises. The 30-minute exercise also resulted in modulation of the observed microstate syntax, in which the probability of transition increased from map A to C (+12%, $p = 0.04$), map B to C (+15%, $p = 0.02$), and map D to C (+11%, $p < 0.001$). After the time trial, the microstate syntax remained higher and different compared to PRE, with an increased probability of transition from map A to C (+13%, $p = 0.02$), map B to C (+20%, $p < 0.001$), map D to C (+14%, $p < 0.001$), and map C to D (+15%, $p = 0.01$).

The resting-state power analysis revealed a reduction in delta power (0.5-3.5 Hz) in POST1 compared to PRE ($p = 0.006$), whereas in POST2, global increases were identified for theta (3.5-7.5 Hz), alpha (7.5-12.5 Hz), and beta (12.5-35 Hz) power (all $p \leq 0.01$).

The increase in the map C GEV was significantly correlated with the reduction in P100 Hz (r = -0.50) after the heavy submaximal exercise, whereas the increase in the map C mean duration was correlated with the reduction in the MVC force (r = -0.50) and the P100 Hz (r = -0.49) after the severe intensity time trial (all $p < 0.05$).

We showed that EEG microstates are modulated by a single-bout of endurance exercise with a main effect on microstate map C, which was associated, in part, with the neuromuscular alterations. We postulated that the post-exercise temporal reorganization of microstate map C could be modulated by an afferent pathway and indirectly participates in modulating the maximal voluntary contraction force.

Contribution

Led the conception and design of the protocol and conducted data collection and analysis. Led the interpretation, writing of the manuscript, and editorial process.

11. Article 3

Resting EEG microstate and heart rate variability do not return to baseline one hour after a submaximal exercise

Spring JN, Bourdillon N, Barral J

Frontiers in Neuroscience, section Autonomic Neuroscience (in review)

We assessed the EEG resting microstate temporal features and conventional heart rate variability (HRV) parameters in thirty-eight young adults (22 females and 16 males) before (BSL), and 5 (P05), 15 (P15), 30 (P30), 45 (P45), and 60 (P60) minutes after a 25-minute constant-load exercise at an intensity that was subjectively perceived as "hard".

Compared to BSL, the mean HR, the ratio between the low-frequency and high-frequency power (LF/HF ratio), and the normalized low-frequency power (nLF) significantly increased, whereas the root mean square of the successive difference between beat-to-beat intervals (RMSSD) and the normalized high-frequency power (nHF) significantly decreased after exercise at P05 (all $p < 0.001$). All HRV parameters remained different from BSL during the hour following exercise cessation (all $p \leq 0.004$), with the exception of the LF/HF ratio that returned to baseline at P45.

After exercise, the duration of microstate map B significantly increased ($p = 0.04$) and the frequency of occurrence of map D significantly decreased ($p = 0.005$) before returning to baseline at P15. In contrast, the duration of microstate map C increased and remained higher and different from BSL during the 60-minute post-exercise conditions (all $p < 0.001$). The time coverage of microstate map C also increased after exercise (all $p \leq 0.03$) and returned to the baseline value after 45 minutes of recovery. Compared to BSL, the observed microstate syntax showed an increased transition from A to C (+17%, $p < 0.001$), B to C (+18%, $p < 0.001$), D to C (+16%, $p < 0.001$), and C to B (+14%, $p = 0.002$) in the P05 condition. The transition A-C, B-C and D-C remained significantly higher and different from BSL during the 60 minutes post-exercise conditions (all $p \leq 0.04$). The deltas in the mean HR and microstate map C mean duration between the BSL and P60 conditions were significantly correlated ($r = 0.42$, $p < 0.05$).

A long-lasting effect of exercise was identified for the map C mean duration and nearly all HRV parameters, which indicates an uncomplete recovery of the microstate and autonomic cardiovascular system during at least one hour after exercise cessation. The implication of an exercise-related common afferent pathway is suggested as a potential modulator of the autonomic regulation of heart rate and the resting EEG microstate.

Contribution

Led the conception and design of the protocol and conducted data collection and analysis. Led the interpretation, writing of the manuscript, and editorial process.

Part IV

Discussion

In this section, we discuss the main findings of the present thesis, further deepen relevant results, and present several limitations. Rather than repeating the information previously elaborated in the articles, the discussion aims to further develop the interpretation of our results and substantiate the underlying hypotheses. In the first two articles, the premotor and resting brain activities were examined after two exercises of different intensities. These post-exercise brain changes were associated with the neuromuscular function and mechanisms in force production evaluated with transcutaneous electrical nerve stimulation. In the third article, the resting EEG microstate and autonomic cardiovascular activity recovery dynamic were described after a constant-load submaximal exercise.

12. Movement-related cortical potential amplitude reduction after cycling exercise relates to the extent of neuromuscular fatigue - *Article 1*

The MRCP amplitude was investigated in trained participants before, between, and after two successive cycling endurance exercises, at heavy and severe intensities, interspersed by 15 minutes rest for data collection. After exercise, the MVC decreased and was accompanied by alterations at the central (i.e., VAL) and peripheral (i.e., Pt, P100 Hz, M-wave) levels. We identified an unexpected reduction in the MRCP amplitude obtained during 60 self-generated isometric knee extensions at 20% of the MVC. As the generator of early and late BP components are thought to differ and reflect different brain processes (Jahanshahi and Hallett, 2003), it appears relevant to differentiate at least the initial part of the MRCP (termed BP1 and BP2 in our study) recorded above the SMA (i.e., FC1-FC2 electrodes) from the final part of the MRCP (termed NS' and MP in our study) recorded above M1 (i.e., C2 electrode). After the heavy exercise, a global reduction in the MRCP was identified above the SMA without a reduction of the late BP above M1. Thus, the late preparation process is modulated only when exercise is substantial, as reflected by the reduction in the final part of the MRCP above M1 after the second exercise.

12.1. MRCP modulations

Previous studies have frequently reported an increased MRCP amplitude associated with neuromuscular fatigue (Berchicci et al., 2013; Guo et al., 2017; Johnston et al., 2001; Liu et al., 2005; Schillings et al., 2006). Accordingly, because our exercise protocol was thought to induce neuromuscular fatigue (i.e., defined as a reduction in the MVC force), an increase in the MRCP amplitude was expected after exercise as a result of the central mechanisms that must compensate for the attenuated muscle response to neural excitation (Freude and Ullsperger, 1987; Johnston et al., 2001) or the decrease in cortical efficiency (Schillings et al., 2006). However, the MRCP amplitude decreased after the heavy and severe intensity exercises. There are different methodological considerations that may explain this discrepancy. In our protocol, the neuromuscular fatigue was induced by large-muscle group exercise, whereas in previous literature, fatiguing protocols were not dissociated from the MRCP procedure (Berchicci et al., 2013; Freude and Ullsperger, 1987; Johnston et al., 2001; Liu et al., 2005; Schillings et al., 2006). Participants performed tens of contractions, and the MRCP amplitude changes were compared between early and late blocks of contractions. The study of Morree et al. (2012) is one of the few studies in which a specific fatiguing protocol was implemented; however, no modification in the premotor brain activity was reported. Furthermore, in most experimental designs, a real-time visual force feedback was presented on a computer screen to monitor the force produced by the subjects (Berchicci et al., 2013; Guo et al., 2017; Johnston et al., 2001; Schillings et al., 2006). This procedure mobilizes supplementary cognitive resources and leads to an increase in attentional load, which may be a confounding factor in MRCP changes (Berchicci et al., 2013; Freude and Ullsperger, 1987). Moreover, many studies implemented MRCP protocols that consisted of contractions performed with a heavy load (≥70% of the MVC) (Johnston et al., 2001; Liu et al., 2005; Schillings et al., 2006). However, the brain must compensate for muscular fatigue only if an additional amount of activation is needed to continue lifting the load over contractions. In our MRCP task, the force exerted was small (20% MVC), and the mobilization of additional resources appears unlikely. Moreover, some authors did not observe an increase in the premotor potential amplitude after repeated contractions at 20% of the MVC despite a strength loss of 35% (Morree et al., 2012). Future research should investigate the difference between single-joint and large-muscle group fatiguing exercises on the MRCP by differentiating the effect of the experimental intervention from the MRCP task.

The increase in premotor slow-wave negativity has been commonly interpreted as neuronal activation (Jahanshahi and Hallett, 2003). Thus, a reduction in negativity would be interpreted as a decrease in activation. For Freude and Ullsperger (1987, 2000), a reduction in the MRCP may occur when mental or motor activities are repeated because of habituation and learning processes or when intentional involvement decreases. In contrast to previous MRCP-induced fatigue protocols, we attempted to differentiate the confounding factors related to the repeated contraction task from the effect of acute exercise. In our protocol, the self-generated knee-extension consisted of lifting a weight platform with no visual feedback, the number of repetitions was small (e.g., 60 repetitions in our study vs. 150 to 250 trials in Freude and Ullsperger (1987)), and the participants were familiarized with the procedure prior to starting the protocol. Although we cannot rule out a bias related to learning or habituation processes, we are confident that the modulations observed may be explained primarily by mechanisms related to the exercise intervention rather than mechanisms related to the MRCP procedure.

12.2. MRCP and the neuromuscular function

12.2.1. MRCP decreases after exercise

The MRCP task was performed in the same condition before and after exercise, which indicates that the force produced is supposed to have remained constant across contractions and time of measurement. Nevertheless, a reduction in the MRCP amplitude was identified after exercise. In fatigued condition, during single-joint contractions, an increase in the excitability of the corticospinal cells has been reported by transcranial magnetic stimulation studies (Gandevia et al., 1996; Sidhu et al., 2013a). During voluntary contraction, the cell membrane potential increases and additional motoneurons are brought close to their firing threshold (Sidhu et al., 2013a). As a consequence, the motoneurons require less stimulus strength to fire. This excitability increases from rest to approximately 50-75% MVC in knee extensor muscles and leads to a better efficacy of the voluntary motor command (Sidhu et al., 2013a). Accordingly, an increase in corticospinal excitability after exercise may explain the absence of motor output modification despite the reduction in premotor activity. This assumption deserved to be verified by investigating the corticospinal excitability and the premotor potential changes during submaximal contractions.

12.2.2. MRCP and voluntary contraction

The physical exercise implemented in this study altered the mechanisms in force production and resulted in a decrease in the MVC force of 10% to 21% depending on the exercise intensity, which corroborates previous findings reported in similar exercise protocol studies (Lepers et al., 2001). The significant impairment in the maximal voluntary neural drive to knee-extensor muscles was accompanied by an alteration of AP transmission-propagation and a reduction of evoked force, which indicate that both central and peripheral mechanisms participate in the reduction of MVC. The absence of significant correlations between MVC force loss and indices of neuromuscular function does not allow the determination of a predominant participation of central or peripheral components.

After the severe intensity exercise, a significant correlation was identified between the reduction in the late BP (i.e., MP) amplitude above M1 and the reduction in the motor output. We assumed that the final part of the MRCP may be reduced so that the premotor brain activity is insufficient to fully activate the motor command during MVC; thus, the force exerted decreases. This assumption is supported by the significant correlation between the ability to maximally activate the muscle and the reduction in the MRCP amplitude. The reduction in the VAL was significantly correlated with the reduction in all MRCP components recorded above M1, consolidating the potential association between the premotor brain activity and central mechanism in force production. Taken together, these results indicate that the final part of motor preparation above M1 may be indirectly associated with the reduction in MVC and central mechanisms in force production.

12.2.3. MRCP and peripheral alterations

The physical exercise implemented in this study induced peripheral muscle alterations, as indicated by the reduction in Pt and P100 Hz after the heavy intensity exercise and the reduction in Pt, P100 Hz, and M-wave after the severe intensity exercise. After the first exercise, we showed that the muscle excitability was preserved as no significant modification in the M-wave were identified. In contrast, after the second exercise, the intensity was sufficient to reduce the M-wave amplitude, which is likely to reflect motor unit depolarization and indicates alterations in neuromuscular transmission/propagation (Gardiner, 2011). The reductions in Pt and P100 Hz evoked force reported after both exercises reflect intramuscular alterations and confirm that peripheral modulations participate in the reduction in the MVC force. These alterations may be

attributed to a decrease in the amount of Ca^{2+} release by the sarcoplasmic reticulum, a decreased sensitivity of the myofilaments to Ca^{2+}, a reduction in the force produced by actin-myosin cross-bridges, or changes in metabolite concentrations (H^{+}, Pi), which together lead to an impairment of the excitation-contraction coupling (Allen et al., 2008; Gardiner, 2011). After the severe intensity exercise, when neuromuscular alterations are substantial, the relative changes between the P100 Hz and the MRCP amplitude (i.e., NS' and MP) recorded above the SMA were significantly correlated. This finding indicates a potential association between peripheral alterations and modulation in the MRCP amplitude.

12.3. Hypothesis outlined

By combining MRCP recordings with neuromuscular measurements, we showed that modulations of the premotor potential may be related to the neuromuscular system and mechanisms in force production. Specifically, the premotor potential recorded above the SMA appears more related to changes at the peripheral level, whereas the final part of motor preparation recorded above M1 is more likely related to the motor drive and voluntary motor output.

The reduction in MVC and the suboptimal motor drive from the motor cortex have been associated with the activation of type III/IV afferent fibers (Amann and Dempsey, 2008; Amann et al., 2011; Laurin et al., 2015). Modulations within the muscular metabolic milieu alter the excitation-contraction coupling, induce peripheral fatigue (Allen et al., 2008), and stimulate the terminal ends of type III/IV afferents. Group III/IV afferents are sensitive to mechanical and metabolic modulations associated with muscle contraction and provide feedback to the spinal cord and the brain (Blain et al., 2016). During muscle exercise, group III and IV afferents represent more than 50% of the total muscle afferents and contribute to motor command adjustment by informing the central structures regarding mechanical, chemical and thermal events (Laurin et al., 2015). Group III afferents respond more to force production, muscle stretch, and local intramuscular pressure and are subsequently more sensitive to mechanosensitive stimuli (Hayes et al., 2006; Laurin et al., 2015; Rotto and Kaufman, 1988). Group IV afferents are more sensitive to metabosensitive stimuli as they start discharging after a delay during contraction and continue to discharge until the withdrawal of muscle metabolites (Hayes et al., 2006, 2009; Laurin et al., 2015). Together, this afferent feedback may facilitate or inhibit via an excitatory or inhibitory influence the motor unit recruitment by acting on the

α-motoneuron excitability, spinal motor reflex, and motor cortex and may thus regulate mechanisms in force production (Amann et al., 2011; Gandevia, 2001; Laurin et al., 2015).

Type III/IV muscle afferents may have an inhibitory effect on the central motor drive (Amann, 2011; Amann et al., 2015), and recent findings emphasize the significant involvement of this afferent feedback in the development of central alteration during large-muscle group exercise (Amann et al., 2015; Sidhu et al., 2013a). For example, when group III/IV muscle afferents are pharmacologically attenuated by fentanyl, the motoneuronal output and muscle activation are increased (Amann et al., 2009; Sidhu et al., 2014). The implication of this afferent pathway in central alterations has been also demonstrated using ischemia (i.e., cuff-induced ischemia) to prevent recovery from muscle fatigue at the end of exercise (Gandevia et al., 1996). In this situation, the afferents continue to discharge after sustained MVC exercise and the reduction in VAL persists as long as the muscle remains ischemic (Gandevia et al., 1996; Taylor et al., 2006). Using transcranial magnetic stimulation, it has been shown that the size of the motor evoked potential (short latency excitatory response of cortical neurons) and the duration of the salient period (reflects the effectiveness of intracortical inhibitory interneurons) rapidly return to pre-exercise values even if the muscle is held ischemic, which indicates that the neurons in the pathway from the motor cortex to the muscle appear to recover (Gandevia et al., 1996; Taylor et al., 2006). However, in this situation, the output from the motor cortex remains insufficient to fully activate the muscle as the VAL remains reduced during ischemia. This finding suggests that impairments in voluntary activation may not occur at the motoneuron level or the motor cortical output; rather, it suggests that muscle afferents may act upstream of the motor cortex (Sidhu et al., 2008; Taylor et al., 2006). According to Sidhu et al. (2013b), in exercise-induced fatigue conditions, the III/IV afferents are likely to increase the amount of intracortical inhibition, decrease the excitability of the corticospinal cells, and reduce the cortical drive from the motor cortical cells. These mechanisms are complex and do not necessarily change in parallel. Nevertheless, based on the results of the literature and the correlation between the increase in peripheral alterations and the reduction in MRCP after exercise, we hypothesized that an increase in afferent activity may participate in modulating the early premotor brain activity after a single-bout of exercise.

The SMA is implicated in the central processing of incoming afferent signals (Cadoret and Smith, 1997; Korotkov et al., 2005), plays a role in the development of intention to act (Baker et al., 2011; Goldberg, 1985), and is activated several milliseconds before activity within the pyramidal tracts (Eccles, 1982). The SMA comprises connections to the M1 and spinal cord and may thus influence the control of movement at both the cortical and spinal levels (Dum and

Strick, 1991). Because the SMA is closely related to motor output (Herz et al., 2012), the modulation in MRCP components recorded above the SMA may influence the final MRCP components above M1, which results in reductions in the VAL and force production. The early BP may consist as part of nonspecific "preparation" for movement and may facilitate cortical and subcortical motor pathways (Colebatch, 2007). It may be expected that after exercise, alterations in early preparatory brain components will impact the final process of movement preparation above M1 and subsequently the motor output during MVC. Taken together, the association between the reduction in MRCP and alterations in the neuromuscular function suggests that the mechanisms in force production may reside at the premotor level, even before movement occurs.

12.4. Limitations

One methodological concern in this study refers to the procedure of MRCP data collection. The MRCP measurements were not obtained directly before producing the knee-extension MVC; they were collected after several minutes. Participants cannot perform a maximal contraction several tens of times (to obtain the MRCP trace) without dramatically altering the voluntary motor command. It is important to dissociate the unwanted effect induced by the MRCP procedure from the effect of endurance exercise alone. Furthermore, as the EEG procedure requires approximately 10 minutes of recording, we are aware that the neuromuscular condition measured immediately after exercise does not exactly reflect the state of the neuromuscular system at the moment of EEG recording.

We discussed the early and late BP processes based on their temporal courses (early BP occurs between -1500 milliseconds and -500 milliseconds and late BP occurs from -500 milliseconds to movement onset) and recording positions (above SMA for the early BP and above M1 for the late BP). To use of arbitrary defined periods to differentiate the main MRCP components is debatable. However, the measurement of the different MRCP components may be difficult because identifying the change in steepness between the early and late BP is not always possible in individual recordings. Moreover, some generators of the MRCP are located in relatively deep regions, and subcortical activations are part of the premotor potential (Colebatch, 2007; Rektor, 2002; Rektor et al., 2001). Consequently, it is unclear the extent to which the scalp-recorded premotor potential effectively reflects the potential that arises from the underlying generators (Jahanshahi and Hallett, 2003).

13. A single-bout of endurance exercise modulates EEG microstates temporal features - *Article 2*

In article 1, we showed that modulations of the mechanisms implicated in force production may be related to the variation in brain activity preceding voluntary movements. Because self-initiated movement originates from a resting brain state, we make a step backwards by exploring whether mechanisms in force production may be associated with resting brain activity, from where voluntary movement emerges. The second objective was to quantify the post-exercise resting brain dynamics by applying EEG microstate analysis.

13.1. Microstate modulations

Using the same protocol as described in study 1, we demonstrated that the four well-known resting EEG microstate topographies (A, B, C and D) prevailed after a single-bout of endurance exercise and explained the majority of the variance of the EEG distribution (> 70%). We quantified conventional microstate parameters and syntax and showed for the first time that microstate temporal properties are reorganized after a single-bout of large-muscle group endurance exercise.

In this study, modulations in microstates were not dependent on the exercise intensity. This finding does not corroborate previous results on the spectral power (Brümmer et al., 2011; Schneider et al., 2009a). The difference in intensity between the two exercises was not likely sufficient to differentially affect the microstates, or a possible maximum plateau of intensity had been reached after the first exercise. Our complementary power spectral analysis indicated a decrease in absolute delta power only after the heavy intensity exercise, whereas after the severe intensity exercise, global increases were identified in the absolute power for the delta, theta, alpha, and beta frequency bands across the frontal, central and parieto-occipital regions. These findings indicate a global increase in nearly all frequency bands independent of the region of interest.

The main finding of this study was the significant increase in the GEV, mean duration, and time coverage of map C. The mean duration appears to reflect the stability of a given microstate, whereas the GEV and time coverage are thought to reflect the relative time coverage of a microstate compared to the other microstates (Khanna et al., 2015). Based on our results, it may reasonably be supposed that map C becomes more stable after exercise and that the fraction of total recording time of this particular microstate increased. The dominance of map C is strengthened by the microstate syntax analysis. After exercise, the transition percentage

between the four microstates was different from random transition process and was characterized by an increase in transition percentage towards map C, which suggests that microstate map C may become an *attractor*. This study shows for the first time a reorganization of the microstate temporal properties and sequence after a single-bout of endurance exercise in healthy volunteers.

13.2. Microstate and the neuromuscular function

The neuromuscular modulations behaved in the same way in article 1 and article 2 as the experimental setting was identical in these two studies. The MVC was reduced after exercise and the origin of this force loss originates from the reduction in descending motor drive as indicated by the reduction in the VAL, as well as from alterations in the intramuscular milieu as indicated by the reduction in P100 Hz. After the heavy and severe intensity exercise, the index of peripheral alteration was correlated with the increase in the map C GEV and mean duration, respectively. After the severe intensity exercise, the increase in the map C duration was correlated with the reduction in the MVC. These findings indicate that changes in the map C duration may be related to peripheral muscle alterations and the reduction in motor output when the exercise intensity becomes substantial. In general, changes in resting spontaneous brain activity after exercise may be indirectly associated with neuromuscular function and motor output modulations.

13.3. Hypothesis outlined

The functional significance of each microstate is not clearly established. Nevertheless, based on the brain networks and structures associated with each map, assumptions may be formulated regarding the related brain function. Pascual-Marquis et al. (2014) identified the anterior cingulate cortex as the main generator of map C, and Britz et al. (2010) associated this specific topography with the salience RSN, organized mainly around the anterior cingulate and the insula. Menon (2015) described an integrative model of the salience network that comprises an ascending pathway originating from the body condition and a direct efferent pathway sending connections to motor-related brain regions (Figure 14). The afferent/efferent organization of the salience network is relevant in the context of the present study as it provides a potential framework for interpreting the relationship between microstate changes and the neuromuscular system highlighted in the present experimental results.

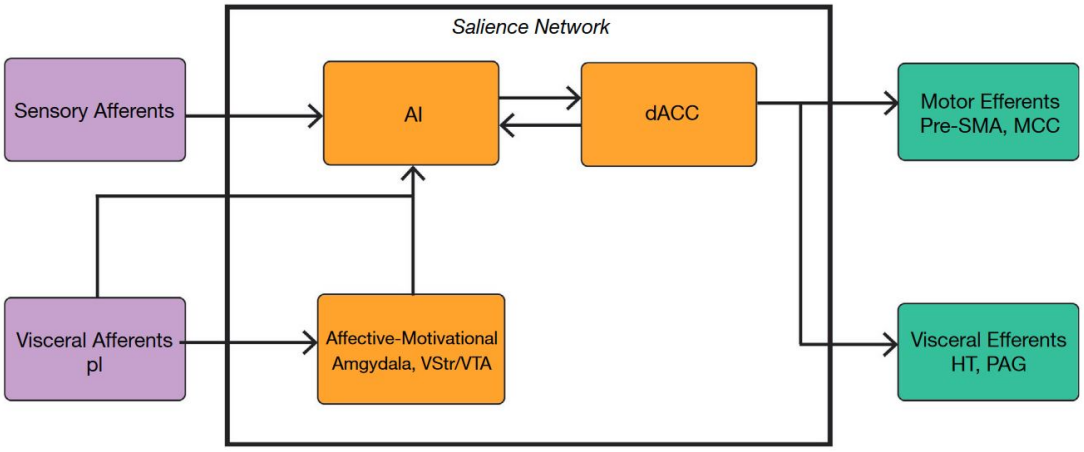

Figure 14: Salience network organization and pathways. The anterior insula receives convergent multisensory inputs, whereas the dorsal anterior cingulate cortex plays a role in response selection, guiding overt behavior and modulating autonomic reactivity. AI, anterior insula; dACC, dorsal anterior cingulate cortex; HT, hypothalamus; MCC, midcingulate cortex; PAG, periaqueductal gray; pI, posterior insula; VStr, ventral striatum; VTA, ventral tegmental area (Adapted from Menon 2015).

The conjoint activation of the AI and dACC is at the interface of afferent and efferent paths and contributes to various of complex brain functions by identifying biologically and cognitively relevant endogenous and external stimuli to adaptively guide behavior (Menon, 2015; Nieuwenhuys, 2012). The AI has been associated with the affective state, interoceptive process, and several specific feelings from the body (e.g., pain, temperature, muscular and visceral sensations) (Craig, 2003; Singer et al., 2009). In previous chapters, it has been reported that the terminal end of muscle afferents may be stimulated because of mechanical and chemical modulations induced by muscle contractions. Thus, the previously described afferent pathway may be an integral part of the model proposed by Menon. At the spinal level, lamina I neurons are activated by changes in the physiological status of the tissues of the body, such as fluctuations in temperature, mechanical or thermal stress or damage, and muscle exercise (Craig, 1995; Wilson et al., 2002). Specifically, these neurons may be excited by muscle afferents, noxious muscle stimulation, and muscle contraction (Wilson et al., 2002). From the superficial layer of the dorsal spinal horn, an afferent pathway caries information through the lateral spinothalamic tract to the thalamus (ventromedial nucleus) (Craig, 2002, 2003), where projections relay the information from thalamic nuclei to the mid/posterior dorsal insula, which, in turn, projects to the AI (Menon and Uddin, 2010). The AI is the key node of the salience network, and it mediates afferent signals that originate from the periphery. This well-described

pathway may support the implication of the map C related salience network after exercise and, more specifically, the correlation between exercise-induced peripheral alterations and the increase in the map C GEV and mean duration.

Compared to the AI, the dACC node is more directly associated with cognitive control and action (Botvinick et al., 2004; Menon, 2015). The ACC and associated dorsomedial prefrontal cortex send strong motor output and have direct control over action (Menon, 2015). The ACC is linked with the midcingulate cortex, supplementary motor cortex, and other motor areas (Rudebeck et al., 2008; Vogt, 2009). The cingulate regions regulate specific aspects of behavioral control and are implicated in the generation of motor intention, behavioral selection, and monitoring of behavioral outcomes (Vogt, 2009). Moreover, the functional coupling between the AI and dACC appears to facilitate rapid access to the motor system (Menon, 2015). The efferent organization of the salience network supports the correlation between the increase in the map C duration and the reduction in the MVC after severe intensity exercise.
Taken together, the dominance of microstate map C after exercise may reflect an increase in endogenous stimuli and a greater "awareness" of the physiological state that together may affect voluntary motor behavior.

13.4. Limitations

The functional interpretation of the microstate may rest on the notion that different topographies are generated by different neural assemblies, which, in turn, may be related to different brain processes. The association between the microstate map C and the salience RSN brain nodes relies on previous findings, and we did not objectively demonstrate the implication of a specific neural correlate after exercise. FFT-inverse solution studies have highlighted the implication of the cingulate cortex after exercise (Brümmer et al., 2011; Moraes et al., 2011; Schneider et al., 2010), which suggests that source localization analysis may be relevant to support our interpretation. The combination of source localization and resting EEG microstate (Custo et al., 2017) would substantiate the implication of an interoceptive network after exercise.

Although fMRI RSNs may be coupled with the power fluctuations of EEG rhythms (Mantini et al., 2007), the temporal spectral power fluctuations do not appear to be correlated with the different microstates (Britz et al., 2010). EEG microstates show more distinct correlations with fMRI RSNs (Britz et al., 2010). In the present study, no associations were identified between

modulations in microstate and power spectral after exercise. Therefore, it is difficult to discuss our microstate results with previous exercise studies using power analysis. The relationships between the characteristics of EEG oscillations and microstates deserved to be explored by methodological studies in standardized resting conditions first, prior to the addition of an experimental condition that may modulate this link.

14. Resting EEG microstate and heart rate variability do not return to baseline one hour after a submaximal exercise - *Article 3*

In the introduction, the cardiovascular system was shown to be a candidate that may influence the post-exercise electrocortical activity (Crabbe and Dishman, 2004; Hatfield, 1991). In article 2, we reported a main effect of exercise on microstate map C. As map C-related brain nodes are implicated in autonomic cardiovascular regulation (Macefield and Henderson, 2015; Sander et al., 2010; Williamson, 2015; Williamson et al., 2003) and mediate cardiovascular arousal (Bechara and Naqvi, 2004; Critchley et al., 2000; Eckert et al., 2009; Menon, 2015; Pollatos et al., 2007), we quantified the autonomic cardiovascular response and microstate changes during the post-exercise recovery period in article 3. We hypothesized that exercise-induced modulations of map C would persist as long as the autonomic cardiovascular response had not returned to baseline.

14.1. Microstate modulations

We identified an increase in the microstate map C duration and time coverage immediately after exercise cessation, accompanied by an increased transition percentage of moving mainly toward map C. This finding partially supports the results from study 2 and strengthens the implication of map C after exercise. As a novelty, we identified an increase in the map B mean duration and a reduction in the map D occurrence immediately after exercise. During the following hour, the only changes concerned the map C time coverage that returned to baseline after 45 minutes and the map C duration that remained elevated and different from baseline during one hour post-exercise. Our results showed a short-term microstate temporal reorganization after exercise that was different from the long-lasting microstate configuration characterized by persistent changes in map C temporal properties only. Interpreting the modifications in maps B and D is tricky as the literature on exercise and EEG microstates is inexistent. Nevertheless, as different microstates are thought to reflect different mental

processes (Michel et al., 2009) and perceptual and behavioral performances may vary as a function of ongoing microstate activity (Britz and Michel, 2010, 2011), we questioned whether the microstate could comprise a type of neural substrate underlying post-exercise cognitive changes. There are many reviews showing an effect of exercise on cognitive performances (e.g., Chang et al., 2012; Lambourne and Tomporowski, 2010; McMorris and Hale, 2015; Tomporowski, 2003). In particular, better information processing has been reported the first minutes after a similar submaximal cycling exercise; however, it disappeared the next several minutes after exercise cessation (Audiffren et al., 2008). In the present study, the microstate modulations in map B and D were different from baseline only in the post-5 minute exercise condition. The significant modifications in the duration of map B and occurrence of map D indicate that the stability and propensity of the underlying neural assemblies to become active may have changed. Thus, it may be assumed that the modifications in the availability of the neuronal resources immediately after exercise may affect the underlying cognitive process. Our interpretation is merely speculative, as we have no cognitive data to support this hypothesis.

14.2. Microstate map C and the heart rate variability

The large-muscle group exercise implemented in our protocol resulted in a massive cardiovascular response as reflected by the changes in all HRV parameters. The mean HR increased in the immediate post-exercise period, and it was accompanied by a parasympathetic withdrawal characterized by a reduction in the RMSSD and nHF, as well as a sympathetic overdrive as reflected by the increased LF/HF ratio. During the hour following exercise, there was an incomplete recovery of the global autonomic cardiovascular activity, as the mean HR, RMSSD, nHF, and nLF remained significantly different from baseline 1 hour after exercise cessation. However, the data did not enable the differentiation of the parasympathetic reactivation from the reduction in sympathetic activity.

The microstate map C mean duration and HRV parameters (mean HR, RMSSD, nHF, and nLF) did not recover 1 hour after exercise, which suggests a similar recovery pattern between the map C and autonomic cardiovascular system. There were no correlations between microstate changes and the parameters related to the sympatho-vagal balance; however, we identified a significant correlation for the global recovery between the mean HR and microstate map C mean duration. Participants who better recovered in terms of HR after 1 hour represent those who better recovered in terms of the map C mean duration, thus confirming a potential link

between microstate map C and HR recovery. As the recovery of HR is related to the level of fitness (Peçanha et al., 2014), our result raises the question of physical fitness on microstates. To verify this hypothesis, we correlated the ΔBSL-P60 mean HR with the Sport index obtained with the Baecke questionnaire. We identified significant correlations that indicated individuals who reported to be more active before starting the protocol are the ones who recover the best in terms of HR. Following the reasoning, 2 subgroups of 19 subjects were created based on the mean HR difference obtained between baseline and after 1 hour of recovery. The individuals with the smaller difference were attributed to the "more-active" group, whereas the individuals with the larger difference in the mean HR were attributed to the "less-active" group. When these two groups were compared on the map C duration using an unpaired *t*-test, the "less-active" group had a mean duration that remained significantly higher 1 hour post-exercise (+7.61 seconds) than the "more-active" group (+0.29 seconds) ($t(26) = 3.8$, $p < 0.05$). These results are promising and require further investigation in future work.

Connections between the brain and the HRV have been described in the literature (Thayer et al., 2012). Specifically, the insula and cingulate cortices are implicated in the neural mechanisms that regulate the cardiac autonomic activity (Sander et al., 2010; Williamson, 2015; Williamson et al., 2003), and several neuroimaging studies have reported a functional association between the HRV and salience network brain nodes (Critchley et al., 2003; Lane et al., 2009; Napadow et al., 2008; Thayer et al., 2012). For example, Chang et al. (2013) identified the neural correlates of autonomous nervous system states measured by HRV and emphasized the implication of the dACC (and amygdala). The implication of the salience network brain nodes in autonomic cardiac regulation further supports the link between post-exercise autonomic cardiovascular recovery and the post-exercise microstate map C changes.

14.3. Hypothesis outlined

A potential mechanism implicated in the autonomic cardiac regulation during exercise is the exercise pressor reflex (Fisher et al., 2013; Kaufman and Hayes, 2002; Secher and Amann, 2012). The mechanical and chemical stimuli associated with muscle contraction activated the terminal ends of small-diameter type III/IV afferent fibers, which relay information to the cardiovascular control centers within the brain stem, where it is integrated and processed (Menon and Uddin, 2010; Murphy et al., 2011). Consequently, the cardiovascular response is adjusted according to the metabolic demand of working muscles (Kaufman and Hayes, 2002;

Murphy et al., 2011). In the post-exercise period, the autonomic cardiovascular reactivation was thought to predominately depend on the accumulation of stress metabolites likely under metaboreceptor feedback (Stanley et al., 2013). The accumulation of metabolites within the active muscle (e.g., H^+ and lactate) stimulates afferent fibers, which, in turn, activate the metaboreflex (Boushel, 2010; Coote, 2010; Hartwich et al., 2011; Peçanha et al., 2014, 2016). The metaboreflex subsequently stimulates the cardiac sympathetic activity and delays the parasympathetic reactivation (Fisher et al., 2013; Fisher, 2014). Other mechanisms participate in delaying the HR recovery, such as reduced afferent input from the baroreceptor because of a drop in blood pressure or circulating catecholamines; however, modification of the exercise pressor reflex is likely a crucial process in regulating the autonomic cardiovascular response and recovery (Kaufman and Hayes, 2002; Stanley et al., 2013). When a post-exercise blood flow restriction is applied to occlude the blood supply and trap the metabolites in the active muscle (i.e., post-exercise ischemia), experimental studies have showed that the exercise-induced increase in HR (Fisher et al., 2013; Macefield and Henderson, 2015), sympathetic activity (Victor and Seals, 1989), and/or withdrawal of cardiac parasympathetic tone (Fisher et al., 2013) are partially maintained. In the same vein, the post-exercise HR recovery may be delayed depending on the blood acidosis and blood lactate level (Ba et al., 2009).

The autonomic response to post-exercise ischemia indicates an increase in BOLD signals within the insular cortex (Macefield and Henderson, 2015; Sander et al., 2010). According to Macefield et al. (2015), an afferent feedback associated with the exercise-induced physiological changes has been supposed to contribute to the increase in the signal intensity within the insula. Taken as a whole, an afferent pathway stimulated by the intramuscular milieu has been shown as a main process in the autonomic cardiovascular response and recovery and may involve key nodes of the salience network. Therefore, the similar recovery pattern of the map C duration and autonomic cardiac parameters and the significant correlation between the global HR and map C mean duration recovery suggest that microstate map C temporal properties may reflect the adjustment of autonomic cardiovascular activity and/or the increase in cardiovascular arousal, likely via a common increase in exercise-induced afferent activity.

14.4. Limitations

In article 3, we identified effects of exercise on microstate maps B and D that were not observed in article 2, even if the exercise was similar between these two studies (i.e., approximately 30-minute at a heavy intensity). This difference may be explained by effects related to gender,

level of fitness, or exercise load, which differ between the two articles. In article 2, there was only men, whereas in article 3 the group was composed of 22 women and 13 men. The volunteers in article 2 were more active (total Baecke score 9.27±0.7 vs. 8.26±1.5, respectively) and the exercise load was higher (mean power 228±22 watts vs. 136±37 watts, respectively). In the future, these effects must to be controlled.

An important limitation of this article, as well as article 2, is the lack of cognitive measurements. The microstates, or more precisely the underlying brain activity, may subserve human cognitive processes (Seitzman et al., 2016), and behavioral and cognitive manipulations have partially verified their roles in specific cognitive functions (Milz et al., 2016; Seitzman et al., 2016). In the present thesis, there are no cognitive data that could have been associated with the observed microstate changes. As acute endurance exercise has been associated with modulations in cognitive performances, we hypothesized that microstate modulations could reflect the neurophysiological substrate that underlies some cognitive performances. The implementation of simple reaction tasks (e.g., discrimination task) and attentional tasks (e.g., switching task) would help to better understand the interaction between exercise and cognition and, and more generally, the significance of microstate changes after exercise.

The literature has described a homeostatic afferent pathway that represents the physiological condition of all tissues of the body (Craig, 2003). There is a feeling arising from the skin, muscle and joints and a more diffuse feeling associated with autonomic afferents that together create the interoception (Craig, 2003; Menon, 2015). The brain-state, to a large extent, is internally coordinated (Buzsáki, 2006), and the present thesis focuses on a potential increase in endogenous stimuli after exercise to interpret the electrocortical modulation. Nevertheless, large-muscle group exercise involves the coordination of an assembly of systems (e.g., skeletal, cardiovascular, respiratory, and integumentary systems) that participate in preserving the body homeostasis (Larry et al., 2015). Accordingly, physical exercise affects the whole body condition at the musculoskeletal, metabolic, biochemical, and neurochemical levels, and it is very unlikely that a single mechanism may be responsible of the observed electrocortical variations. The preliminary findings reported in this thesis must be replicated by additional studies, and other potential mechanisms, such as brain perfusion, brain metabolism, neurotransmitter activity, or cerebral oxygen delivery, must be considered in the future.

15. Strength and weakness of this interdisciplinary thesis

This thesis relied on an interdisciplinary approach and required the development of broad knowledge on several systems (brain, neuromuscular, and cardiovascular). The acquisition of these competencies can be achieved only at the expense of sharp knowledge in each field. Consequently, each system could not have been studied in depth at both the theoretical and experimental levels, which may constitute a weakness of this work. However, the general purpose was to identify the potential relationships between the changes in different physiological signals, as well as demonstrate and provide reasoning with the results before placing them in a broader context. The strength of this thesis is to have shown that each discipline, method and investigation technique may become complementary and thus may contribute to a better understanding of an interdisciplinary issue. The results were discussed in a larger framework and interpretations have been suggested according to previous literature. This thesis provides conclusive preliminary results and generates encouraging perspectives across multiple research areas.

Perspectives

16. Exploring the implication of an afferent pathway in microstate modulations

The exploration of the hypothesis of an afferent modulation involving microstate class C may be achieved using a neuromuscular electrical stimulation protocol. The application of a series of intermittent electrical stimuli over the muscle, or nerve trunk, induces peripheral muscle contractions. The resulting massive recruitments of sensory afferents may influence somatosensory brain regions (Beaulieu and Schneider, 2013, 2015). Even if this procedure does not directly solicit the voluntary motor command, fMRI studies have reported a widespread brain activation located in several brain regions, such as the primary motor cortex, sensory cortices, secondary somatosensory area, supplementary motor area, prefrontal cortex, and cingulate gyrus (Blickenstorfer et al., 2009; Francis et al., 2009; Wegrzyk et al., 2017). Interestingly, there is a dose-response relationship between the stimulation intensity and the brain activation pattern (Smith et al., 2003). Moreover, when this stimulation is applied directly on a nerve, it generates more discomfort and results in higher activation of pain-sensitive regions, such as the anterior cingulate cortex (Niddam et al., 2002; Wegrzyk et al., 2017). The increased activity related to the somatosensory integration appears to be modulated by several factors, such as attention, unpleasant feelings, anticipation of pain, or discomfort (Backes et al., 2000; Blickenstorfer et al., 2009; Wegrzyk et al., 2017). The investigation of the effect of neuromuscular electrical stimulation on microstates, with the assumption that class C temporal properties would be preferentially affected, will address the hypothesis of peripheral-related afferent modulations.

17. Combining resting EEG microstate and MRCP

In the introduction, the hypothesis of a spontaneous fluctuation origin of the MRCP was presented. Schurger et al. (2012) assumed a connection between spontaneous fluctuations in neural activity and self-initiated movement, so that the neural decision to move is determined, in part, by spontaneous fluctuation. The timing of self-initiated action is a product of bottom-up and top-down processes (Schmidt et al., 2016). At rest, there is a slow cortical potential fluctuation, which is considered a moderator of action initiation (bottom-up). When a given

threshold is reached, there is an upcoming intention to act and a conscious decision to release or withhold the intention (top-down). In this model, the early BP is attributed to more frequent occurrences of self-initiated movement during negative deflections of slow cortical potentials (i.e. slow cortical potential sampling hypothesis). The subject will feel more "ready" to act during this slow negative potential (Schmidt et al., 2016). Perceptual and behavioral performances may vary as a function of ongoing microstates (Britz and Michel, 2011; Khanna et al., 2015), and self-initiated movement may be facilitated or impeded according to the fluctuation of ongoing brain activity (Schmidt et al., 2016). A very interesting perspective is to further explore how the brain transitions from spontaneous microstate fluctuations to self-initiated movement, particularly after exercise as we reported modulation in the early MRCP component.

18. Microstates and health exercise-intervention program

Participation in regular physical activity has significant benefits for heath, and abundant evidence supports the advantages of an acute exercise-intervention program in the management of several physiological and psychological disorders. For example, physical exercise is a promising nonpharmacological approach that provides valuable benefits in mental health (Callaghan, 2004; Paluska and Schwenk, 2000) and neuropsychiatric diseases, such as anxiety (Asmundson et al., 2013; Wegner et al., 2014), depression (Ranjbar et al., 2015; Tavares et al., 2014), or schizophrenia (Dauwan et al., 2016; Firth et al., 2017).

Microstate time series may provide insights regarding the neurophysiological processes that underlie the functional state of the brain in health, as well as in disease (Khanna et al., 2015). A growing body of literature has reported specific parameters change in certain neuropsychiatric disorders. In schizophrenia research, substantial work has been performed and supports the investigation of the microstate (Rieger et al., 2016) as it may provide a novel approach for evaluating illness severity or treatment efficacy (Khanna et al., 2015). Furthermore, the present thesis shows that resting EEG microstates represent a valuable method that may be implemented in an exercise protocol. Consequently, resting EEG microstates may provide a novel approach for monitoring exercise-intervention programs through objective neurophysiological biomarkers. The quantification of microstate parameters may be applied to evaluate exercise efficacy in neuropsychiatric disease or design individual exercise intensity protocols in this population.

Conclusion

This thesis contributes to a better understanding of resting and premotor brain activity in the context of physical exercise. Specifically, we highlighted associations between changes in resting and premotor brain activities and alterations within the neuromuscular system, which suggest that early brain processes occurring before movement onset may be involved in the mechanisms in force production following exercise. One thesis goal was to overcome several limitations of the classical power analysis previously used in EEG and exercise studies by applying an advanced electrical neuroimaging method. EEG microstate analyses grasp the temporal dynamics of large-scale distributed networks, and the present findings showed that this method is relevant in the context of physical exercise. We report a reorganization of microstate temporal properties after exercise with a main effect on microstate class C. Interestingly, the duration of microstate map C and the autonomic cardiovascular activity remained changed 1 hour after exercise cessation. Modulations within this specific map may reflect a dominance of the salience resting-state network under the influence of endogenous stimuli, including the adjustment of the autonomic cardiac activity. The implication of an afferent pathway is proposed as a putative mechanism by which exercise modulates the resting microstate and likely the awareness of the physiological state (Menon, 2015).

These preliminary results provide novel opportunities to explore the interaction between the global functional state of the brain and physiological responses to acute exercise. By exploring the brain state at rest and before movement onset, we provide a better understanding of the modulation of motor responses following exercise. Furthermore, the implementation of an exercise procedure provides an excellent method to improve understanding of how the brain activity and different systems respond and interact. From an application perspective, the resting EEG microstates may provide a promising approach for monitoring exercise-intervention programs through objective neurophysiological biomarkers.

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Annex I – *Article 1*

Spring J.N., Place N., Borrani F., Kayer B., and Barral J. (2016). Movement-related cortical potential amplitude reduction after cycling exercise relates to the extent of neuromuscular fatigue. Front. Hum. Neurosci. *10*, 1-12.

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Movement-Related Cortical Potential Amplitude Reduction after Cycling Exercise Relates to the Extent of Neuromuscular Fatigue

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Exercise-induced fatigue affects the motor control and the ability to generate a given force or power. Surface electroencephalography allows researchers to investigate movement-related cortical potentials (MRCP), which reflect preparatory brain activity 1.5 s before movement onset. Although the MRCP amplitude appears to increase after repetitive single-joint contractions, the effects of large-muscle group dynamic exercise on such pre-motor potential remain to be described. Sixteen volunteers exercised 30 min at 60% of the maximal aerobic power on a cycle ergometer, followed by a 10-km all-out time trial. Before and after each of these tasks, knee extensor neuromuscular function was investigated using maximal voluntary contractions (MVC) combined with electrical stimulations of the femoral nerve. MRCP was recorded during 60 knee extensions after each neuromuscular sequence. The exercise resulted in a significant decrease in the knee extensor MVC force after the 30-min exercise (−10 ± 8%) and the time trial (−21 ± 9%). The voluntary activation level (VAL; −6 ± 8 and −12 ± 10%), peak twitch (Pt; −21 ± 16 and −32 ± 17%), and paired stimuli (P100 Hz; −7 ± 11 and −12 ± 13%) were also significantly reduced after the 30-min exercise and the time trial. The first exercise was followed by a decrease in the MRCP, mainly above the mean activity measured at electrodes FC1-FC2, whereas the reduction observed after the time trial was related to the FC1-FC2 and C2 electrodes. After both exercises, the reduction in the late MRCP component above FC1-FC2 was significantly correlated with the reduction in P100 Hz ($r = 0.61$), and the reduction in the same component above C2 was significantly correlated with the reduction in VAL ($r = 0.64$). In conclusion, large-muscle group exercise induced a reduction in pre-motor potential, which was related to muscle alterations and resulted in the inability to produce a maximal voluntary contraction.

Keywords: EEG, fatigue, *Bereitschaftspotential*, peripheral nerve stimulation, maximal voluntary contraction

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INTRODUCTION

Prolonged exercise increases the difficulty to perform voluntary motor actions by altering motor control or the capacity to sustain an ongoing effort or to generate maximal force or power (Allen et al., 1995; Jaric et al., 1997, 1999; Gandevia, 2001; Bottas et al., 2004) in other words, fatigue develops. Exercise-induced muscle fatigue can be defined as a reduction in the maximal voluntary contraction force (MVC; Gandevia, 2001) and can be related to alterations occurring at different sites along the motor pathway, from the cortex to the muscle fiber. It is typical to distinguish between the central factors located before the neuromuscular junction (central fatigue), which refers to the neural activity that drives the muscle, and the peripheral factors located after the motor plate at the muscle level (peripheral fatigue).

Standardized investigative methods for examining neuromuscular function, such as peripheral nerve stimulation or transcranial magnetic stimulation, have been extensively used to explore the complex relationship between exercise and fatigue (Gandevia, 2001; Taylor et al., 2006; Barry and Enoka, 2007). Peripheral nerve stimulation provides relevant information about muscular properties such as excitation-contraction coupling. This method also allows for the quantification of suboptimal motor drive for force production by the twitch interpolation technique (Merton, 1954). However, peripheral nerve stimulation alone remains ineffective for differentiating between supraspinal and spinal adaptations. Transcranial magnetic stimulation is an alternative stimulation method that consists in applying an electromagnetic field above the motor cortex and/or the cervicomedullary junction, to evoke motor evoked potentials, and as such transcranial magnetic stimulation provides information about changes in corticospinal excitability/inhibition (Goodall et al., 2012). By combining transcranial magnetic stimulation and peripheral nerve stimulation, it is possible to assess the neuromuscular pathway and to gain insight into potential site(s) of impairment during or following exercise (Gruet et al., 2014).

Because any voluntary physical effort begins and ends in the brain (Kayser, 2003), voluntary contraction is not limited by the motor command *per se* but also by processes upstream from the motor cortex that might limit motor drive and thus contribute to central fatigue (Gruet et al., 2013). Indeed, during voluntary movement, cortical and subcortical regions are involved in the final motor output (Ball et al., 1999; Shibasaki, 2012; Tanaka and Watanabe, 2012). For example, peripheral afferents send projections to the cingulate anterior cortex, the premotor area, the lateral prefrontal cortex and the orbitofrontal cortex (Liu et al., 2002; Liu, 2003; Hilty et al., 2011a; Robertson and Marino, 2016) and thereby participate in modulating motivational and executive processes. Hence, the brain integrates the internal state and the perceptual information to finally modulate the motor output.

Electroencephalography (EEG) appears to be a relevant method for investigating exercise-induced changes in brain activity, particularly because it reflects the spontaneous and immediate activity of neural networks from a wide range of brain systems. During voluntary muscle contraction tasks, EEG allows for investigation of the spontaneous cortical activity related to movement production. The movement-related cortical potential (MRCP) is an event-related potential locked to the onset of movement (Shibasaki and Hallett, 2006). It reflects the preparatory brain activity, taking into account the time factor. First described by Kornhuber and Deecke; (1965a, b) as the *Bereitschaftspotential*, this EEG pattern is characterized by a slow negative shift, starting ∼2 s before movement, and reflects neural processes involved in preparing the motor command. The MRCP is composed of two main components, distinguished by their change in slopes occurring ∼500 ms before movement onset (Deecke, 1996; Shibasaki and Hallett, 2006). The first component (BP: Bereitschaftspotential) has a moderate steepness and is bilaterally distributed at the fronto-central midline above the supplementary motor area (SMA). BP occurs between 1500 and 500 ms before movement onset. The second component (NS′: negative slope) is contralateral dominant, more pronounced above the primary motor cortex (M1) and occurs between 500 ms before movement onset and movement onset. Within NS′, the motor potential (MP) is observed at movement onset and corresponds to the MRCP peak amplitude.

Performing voluntary movement implies dynamic processes that involve multiple areas within the brain, likely with overlapping activities. At present, there is a divergence in opinion regarding whether the MRCP components reflect different processes (Jahanshahi and Hallett, 2012). Nevertheless, the identification of the generators of MRCP and their related intracortical connections suggest different stages in the movement-generating procedure. The main generator of MRCP is not limited to the primary motor cortex; the pre-SMA, SMA, and cingulate cortex have a major contribution (Jahanshahi and Hallett, 2012). The activity of additional subcortical structures, such as the thalamus, the caudate, the putamen, and the pallidum also participate in scalp surface-recorded activity and cannot be excluded. By manipulating motor preparation with specific tasks, neuroimaging studies have shown increased activity in the SMA in self-generated movement, compared with movements directed by external cues (Deiber et al., 1991, 1996; Jenkins et al., 2000). Some evidence supports sequential activation within this neural network, in which the SMA has a driving effect on M1 (Herz et al., 2012). Thus, we can assume that the early MRCP generated above the SMA represents the cognitive process related to the decision to perform a movement and the preparation, whereas the late MRCP recorded above the primary motor cortex is more likely to represent the motor part of movement production (Arai et al., 2012; Hoffstaedter et al., 2013). Although the functional distinction between components is still unclear, their segmentation helps to differentiate the processes related to movement planning from motor execution.

The MRCP is obtained by repeated single-joint contractions protocols. Some studies have reported that repetitions of the same movement are accompanied by an increase in MRCP amplitude (Johnston et al., 2001; Schillings et al., 2006; Morree et al., 2012), which has been interpreted as a way

to compensate for peripheral fatigue, whereas others have reported no modifications (Siemionow et al., 2004; Liu et al., 2005). Schillings et al. (2006) observed an increase in MRCP after handgrip contractions, but did not find any correlation between MRCP modulations and the reduction in force or with any peripheral fatigue parameters. The authors postulated that the increase in the motor cortical activity compensated for a reduction in central efficiency. In their study, Freude and Ullsperger (1987) asked participants to perform self-paced contractions for 30 min (i.e., 150–250 contractions) at 20, 50, and 80% of their MVC. MRCP increased when fatigue developed during exercise at 20 and 80% of MVC but decreased when the contractions were performed in the absence of fatigue at 50% of MVC. The increase in MRCP amplitude was interpreted differently according to the intensity of effort. At 80% of MVC, the results suggested an increase in cortical activation to compensate for peripheral fatigue, whereas at 20% of MVC, they indicated the high degree of concentration and attention required to properly perform the task. Conversely, at 50% of MVC, the reduction in MRCP was interpreted as a decrease in intentional involvement because of task monotony. More recently, Berchicci et al. (2013) investigated simultaneously MRCP, perception of effort, muscle twitch force and EMG activity. Eighteen subjects performed four blocks of isometric knee extension at 40% of MVC (240 2 s long contractions). After averaging the early (block 1–2) and late blocks (block 3–4), the authors performed a cluster analysis to create two groups based on the rating of perceived exertion (RPE) and the peripheral fatigue (muscle twitch force loss). MRCP increased in the group with higher RPE and in the group with greater peripheral fatigue. They also observed higher positive activity in the prefrontal cortex in the group with greater RPE. According to the authors, the protocol used required high cognitive effort to properly perform the task, which could explain the frontal positivity. In summary, MRCP modulations appear to be related to global exercise-induced fatigue, not only to peripheral fatigue. Some factors might influence the modulations of the pre-motor potential, such as the cognitive load and the perceived effort (Freude and Ullsperger, 1987; Slobounov et al., 2004).

The present study is the first to investigate the effects of an acute endurance exercise on a motor task (voluntary knee extensions) by combining pre-motor brain activity and neuromuscular measurements. Our strategy was to use a specific fatigue-generating procedure, different from the task used to quantify MRCP changes, to avoid bias from additional and unwanted mental weariness. We asked our subjects to perform a large-muscle-group exercise (cycling) and to participate in a specific MRCP task (repeated knee extension; before and after cycling) to quantify the changes induced by the fatiguing exercise. The aim of the study as two-fold: (1) to assess the effect of cycling exercise intensity (heavy and severe) on MRCP modulations and (2) to relate the exercise-induced MRCP modulations to the extent of central and peripheral fatigue. We hypothesized that large-muscle-group exercise would induce neuromuscular fatigue and an increase in MRCP amplitude above the premotor and motor area.

MATERIALS AND METHODS

Participants

Twenty well-trained male athletes were enrolled in the study after having been informed of the experimental procedure. All the participants completed the Baecke questionnaire to ensure that they were physically active (Baecke et al., 1982). The protocol was approved by the local ethics committee (CERVD: protocol 153/14) and was in agreement with the Declaration of Helsinki. Each subject provided written consent before participation.

Experimental Protocol

The volunteers visited the laboratory on two occasions, for the pre-participation session and for the experimental session. During the pre-participation session, preliminary medical screening confirmed that participants were in good health and had no disorders that could interfere with the experimental procedure. Upon inclusion, they performed a maximal ramp exercise protocol on a cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) to measure the first ventilatory threshold (SV_1), peak oxygen consumption ($\dot{V}O_2$ *peak)*, and maximal aerobic power (MAP). The participants warmed up for 6 min at 60 W, after which power was incremented by 30 W/min until voluntary exhaustion. Maximality was considered to have been reached when at least three of the following criteria were met: $\dot{V}O_2$ plateau; respiratory quotient (QR) >1.1; maximal heart rate (HR_{max}) >90% of theoretical HR_{max} (i.e., 220-age); or a pedaling rate below 60 rotations per minute despite strong verbal encouragement.

The experimental session occurred within 4 weeks after the pre-participation session. The volunteers were instructed to maintain their usual diet and to avoid severe exercise the day before. They had to avoid alcohol and caffeine consumption over the 12 h preceding the session. The last meal had to be taken at least 2.5 h before the beginning of the test, and water was provided *ad libitum* during the experimental session.

The protocol consisted of heavy exercise, followed 15 min later by severe exercise (**Figure 1**). The heavy exercise consisted in pedaling at a freely chosen cadence on a cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) for 30 min at an intensity of ∼60% of MAP. The severe exercise was a 10-km all-out time trial (TT) performed on a road bike with the rear wheel mounted on a home trainer (CycleOps, Madison, USA). The objective was to complete the distance as fast as possible, with the distance displayed on a bike computer fixed to the handlebar. The resistance of the roller increased automatically with the force exerted by the cyclist to reproduce field-like sensations. A fan was placed in front of the subject to avoid excessive sweating, and the wind speed was adjusted upon request. A power meter in the rear hub (PowerTap, CycloOps, Madison, USA) allowed the power output to be recorded. Perceived exertion was assessed with the 6–20 Borg scale at the end of the heavy exercise and the TT. Knee extensor neuromuscular function was investigated through the quantification of several parameters. The MVC represents the maximum force that a subject could produce in the isometric knee exercise. The voluntary activation level (VAL) was chosen

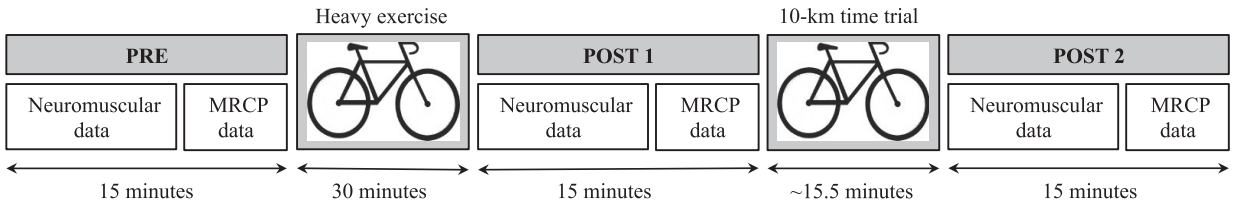

FIGURE 1 | Experimental session design and timeline. PRE, pre-exercise condition; POST1, post-heavy exercise condition; POST2, post-time trial condition; MRCP, movement-related cortical potential.

as an index of central fatigue and is believed to reflect the ability of the motor cortex to drive muscle. The M-wave was recorded to explore neuromuscular transmission/propagation. The paired stimuli force at 100 Hz (P100 Hz) and the muscle twitch force (Pt) reflected the muscle properties and were both chosen as indices of peripheral fatigue. Neuromuscular data were collected before the fatiguing task (PRE), immediately after the 30-min exercise (POST1) and immediately after the 10-km TT (POST2). The MRCP data were recorded after each neuromuscular assessment for these three time points.

Neuromuscular Data Collection

Neuromuscular Assessment

The session began with a warm-up of 10 submaximal voluntary isometric knee extensor contractions (4–5 s) between 20 and 90% of the estimated MVC. After a short recovery period, the participants performed two MVCs (4 s duration) with the right leg, separated by a 1-min rest period. For both trials, P100 Hz were delivered at maximal force, followed by a P100 Hz and a Pt in 2-s intervals.

Evoked Contractions

A constant-current stimulator (DS7AH, Digitimer, Hertfordshire, UK) was used to deliver electrical pulses. The cathode (5 cm diameter) and the anode (5 × 10 cm, Dermatrode, American Imex Irvine, CA) were placed over the femoral nerve at the femoral triangle level below the inguinal ligament and on the lower part of the gluteal fold opposite the cathode, respectively. The optimal intensity for electrical stimulation was determined after the warm-up period by progressively increasing the stimulus intensity in 10-mA increments until there was no further increase in the amplitude of the mechanical or electrical (M-wave) responses. A 20% supplementary increment was added to ensure supramaximal stimulation intensity (Neyroud et al., 2013).

Force Recording

Voluntary and evoked force exerted by the right knee extensors were recorded using an isometric ergometer consisting of a custom-built chair equipped with a strain gauge (Universal Load Cell, model 9363-C3, linear range 0–250 N•m, output sensitivity 2.0 mV•V^{-1}, Vishay, Malvern, US). The calibrated strain gauge was fixed to the chair and strapped to the ankle with a custom-made mold. Subjects were seated with a 90-degree knee angle, the trunk was attached at a 100° angle to the chair back panel with a harness belt, and the arms had to be crossed on the chest to minimize upper body movement. The force signal was recorded at 1 kHz using an AD converter system (MP 150, BIOPAC Systems, Goleta, CA).

EMG Recording

The EMG activity of the right vastus lateralis (VL) was recorded with a pair of silver chloride (Ag/AgCl) circular (1 cm) surface electrodes (MediTrace 100, Kendall, Canada) positioned lengthwise over the middle of the muscle belly according to SENIAM recommendations (Hermens et al., 2000); the inter-electrode distance was 2 cm. The reference electrode was placed over the patella. The VL was chosen as representative of quadriceps muscle activity (Place et al., 2007). Low resistance (<5 kΩ) was obtained by shaving, abrading and cleaning the skin. EMG signals were amplified (gain = 1000) over a frequency bandwidth of 10–500 Hz and digitized at a sampling frequency of 2 kHz using an AD converter system. Force and EMG data were analyzed offline using the software Acknowledge (Biopac System, Santa Barbara CA, USA).

MRCP Data Collection

EEG Recording

Continuous EEG was recorded at a sampling rate of 2048 Hz with a 64-channel Biosemi Active two-amplifier system (Biosemi, Amsterdam, the Netherlands) mounted according to the 10–20 International System. All channels were referenced to the CMS-DRL ground, which functioned as a feedback loop driving the average potential across the montage as close as possible to amplifier zero (Biosemi, Amsterdam, the Netherlands). Impedance was kept below 5 kΩ by using conducting gel. Participants wore the EEG cap during the entire protocol. Post-exercise recordings started 5.5 ± 0.5 min after the end of the heavy exercise and the TT, and lasted 10 min. Offline analyses were performed with BrainVision analyzer software (Brain Products Gmbh, Munich, Germany).

The MRCP data were collected using the same ergometer device used for neuromuscular assessment. To avoid any unknown disturbances induced by the neuromuscular stimulation, the other leg was used for the MRCP task. A string attached to the left ankle ran over a pulley to a free-hanging weighted platform. The subjects were instructed to lift this weight 60 times, equivalent of 20% of their MVC force, by ∼10 cm. The contraction duration was not strictly controlled, but participants were instructed to produce a 2-s

contraction: a 1-s concentric contraction to lift the weight, and 1-s eccentric contraction to put it down (metronome). The onset of movement was automatically reported on the EEG recording by the release of a trigger placed behind the heel of the subjects. The contractions were self-generated, but to ensure that the duration of the task was identical for each participant in each condition, a beep sounded every 10 s. The participants were instructed to perform the contraction spontaneously between two beeps. The subjects were also instructed to keep their eyes closed during the task to avoid excessive attentional load and artifacts generated by visual feedback.

Data Analysis

Gas Exchange

Breath-by-breath pulmonary gas-exchange data were collected during the maximal ramp test with a metabolic cart (OxyconPro, Jaeger, Germany) and averaged over consecutive 10-s period. The $\dot{V}O_2$ *peak* was taken as the highest value attained during the last 30 s before the subject's volitional exhaustion. SV_1 was determined from the combination of different measurements, including the first disproportionate increase in $\dot{V}CO_2$ from visual inspection of individual plots of $\dot{V}CO_2$ vs $\dot{V}O_2$, an increase in expired ventilation $\dot{V}E/\dot{V}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$, and an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The intensity for the 30-min exercise was obtained by adding 20% of the difference between SV_1 and MAP to the power reached at SV_1.

Force Data

MVC force of the knee extensors was reported as the force produced during the maximal voluntary contractions (i.e., peak to peak). Resting P100 Hz and Pt amplitude were analyzed for the trials yielding the highest MVC. The VAL during MVCs was estimated with the superimposed and the potentiated doublets according to the formula proposed by Strojnik and Komi (1998).

$$VAL = (1 - (\textit{superimposed } 100 \textit{ Hz doublet force} \times (\textit{force level at stimulation } / \textit{MVC force}) / \textit{superimposed } 100 \textit{ Hz doublet force})) \times 100$$

EMG Data

The EMG signals recorded during the highest MVC were used for analysis. M-wave peak-to-peak amplitude was measured from the EMG response after the single stimulation.

MRCP Data

The raw EEG data were first down-sampled from 2048 to 512 Hz to reduce computational load and band-pass filtered from 0.1 to 5 Hz. The low-pass filter was set at 5 Hz (Thacker et al., 2014) to avoid bias for alpha rhythm induced by the eyes-closed procedure and to avoid unwanted activity generated by spontaneous physiological and rolandic *mu* rhythms. EEG signals were segmented into 60 epochs of 3000 ms each (from 2500 ms before movement onset to 500 ms after movement onset). All trials were baseline-corrected with −2500 ms to −2000 ms as a reference and averaged using a semi-automatic artifact rejection procedure with a ±80 μV criterion. Artifacted electrodes were interpolated when necessary with a spherical 3D spline, and trials containing periods of muscular artifacts were also rejected. On average, 53 ± 9, 52 ± 11, and 52 ± 9 of 60 trials were available for analysis for the PRE, POST1, and POST2 conditions, respectively. The MRCPs were segmented into four sequential components (Shibasaki and Hallett, 2006; Jahanshahi and Hallett, 2012). The *Bereitschaftspotential* was divided into BP1 and BP2. The BP1 corresponds to the average amplitude between −1500 and −1000 ms. The BP2 corresponds to the average amplitude between −1000 and −500 ms. The third component was the negative slope (NS′), which corresponds to the average amplitude from −500 ms to the onset of movement. The last component was the motor potential (MP), taken as the maximal peak amplitude recorded between −500 ms and movement onset. Those components were calculated for two regions of interest. The first region corresponded to the mean activity of the FC1 and FC2 electrodes and the second region was the mean activity above the C2 electrode. Those electrodes were chosen because they correspond to the area known to generate MRCP, namely the SMA (represented by FC1-FC2 mean activity) for the first part of MRCP and the primary motor cortex (M1; represented by C2 activity) for the late part of MRCP (Shibasaki and Hallett, 2006).

Statistical Analysis

One-way repeated measures ANOVAs with factor Time were used to compare the neuromuscular and MRCP variables between the different times of measurement (PRE, POST1, and POST2, respectively). When ANOVA revealed significant interactions, pairwise contrasts were performed using the Bonferroni correction. Friedman ANOVAs with follow-up Wilcoxon signed rank tests were used in a few cases in which conditions for using parametric tests were not reached. To better understand the mechanisms responsible for the MVC loss, we performed Pearson correlation analyses by using the deltas between PRE-POST1 and PRE-POST2 on the factor MVC explained by VAL and P100 Hz.

Because of the non-normal distributions of EEG variables, Spearman correlations were used to explore the relationship between neuromuscular and MRCP modulations. The three neuromuscular parameters used for the correlation analyses were the reduction in MVC, VAL, and P100 Hz, which were chosen as global, central and peripheral indices of fatigue, respectively. Those parameters were correlated with the four MRCP components modulations (i.e., BP1, BP2, NS′, MP) at the FC1-FC2 and C2 electrodes. All statistical analyses were performed using the software Statistica 12.6 (Statsoft, Tulsa, USA). The level of significance was set to $p < 0.05$. The results are presented as mean ± standard deviation.

RESULTS

Participants

Data from four of the original 20 recruited participants had to be excluded because of heavily artifacted EEG signals. The mean age of the 16 remaining participants was 29 ± 7 (years ± SD), and their body mass index was 22.9 ± 1.6 ($kg \bullet m^{-2}$). All of them were

active road cyclists and/or triathlon athletes, had a total score of 9.2 ± 0.7 on the Baecke questionnaire, and reached a MAP of 385 ± 47 W at a $\dot{V}O_2$ *peak* of 63.8 ± 5.9 ml•min^{-1}•kg^{-1} during the incremental cycling test.

Exercise Data

The mean power output during the 30-min exercise was 231 ± 97 W. The mean duration for the TT was 15.7 ± 1.6 min and the average power was 279 ± 31 W. The RPE was 14.9 ± 1.7 at the end of the heavy exercise, whereas the RPE reached 19.7 ± 0.5 at the end of the TT.

Neuromuscular Data

Force

The sequence of the two cycling exercises caused a significant reduction in the MVC force measured at POST1 and POST2 [$F_{(2,\ 30)} = 55.34$, $p < 0.001$; **Table 1**]. **Figure 2** shows two representative recordings of a superimposed MVC with a 100 Hz-potentiated doublet in the PRE (A) and POST2 (B) conditions.

Peripheral and Central Fatigue

The peripheral indices of fatigue measured by the Pt force and P100 Hz were significantly reduced [$F_{(2,\ 30)} = 36.95$, $p < 0.001$ and Chi^2 ($N = 16$, df = 2) = 6.5, $p = 0.039$], respectively after POST1 and POST2 (**Table 1**). For the M-wave amplitude, Friedman ANOVA revealed a significant effect of Time [Chi^2 ($N = 16$, df = 2) = 9.5, $p = 0.008$]. However, although no reduction was observed between PRE and POST1 ($p = 0.3$), the difference in the M-wave amplitude between PRE and POST2 was significant ($p = 0.007$). Concerning central fatigue, a Time effect [$F_{(2,\ 30)} = 14.21$, $p < 0.001$] was observed for VAL. For all the significant main Time effects, *post-hoc* tests revealed a significant difference between PRE and POST1 (except for M-wave) and between PRE and POST2 (All $p < 0.033$; **Table 1**).

Correlations

At POST1, Pearson correlation analysis showed a trend toward a relationship between the reduction in MVC force and the reduction in P100 Hz ($r = 0.44$, $p = 0.08$), but without reaching the level of significance. No relationship was found between MVC and VAL reduction ($r = 0.32$, $p = 0.22$). At POST2, the results indicated no significant relationship between the decrease in MVC and P100 Hz ($r = 0.27$, $p = 0.3$), whereas a positive relationship was found between the reduction in MVC and VAL ($r = 0.5$, $p = 0.047$).

MRCP Data

The MRCP grand averages between the PRE, POST1, and POST2 conditions at FC1-FC2 and C2 are shown in **Figures 3A,B**. The common MRCP shape can be observed, with a typical increase in slope at ∼1000 and 500 ms before movement onset.

Mean Activity at FC1-FC2

ANOVA revealed an effect of Time for FC1-FC2 mean activity for the components BP1 [$F_{(2,\ 30)} = 3.93$, $p = 0.03$], BP2

TABLE 1 | Neuromuscular indices of central and peripheral fatigue measured before the fatiguing task (PRE), after the heavy exercise (POST1), and after the 10-km time trial (POST2).

	PRE	POST1		POST2	
	***Mean* ± SD**	***Mean* ± SD**	**Δ PRE % ± SD**	***Mean* ± SD**	**Δ PRE % ± SD**
MVC (N)	306 ± 54	276 ± 55**	−10 ± 8	244 ± 51**	−21 ± 9
VAL (%)	90.9 ± 5.1	85.6 ± 9.5*	−5.9 ± 8.4	80.5 ± 11.5**	−11.6 ± 10.4
Pt (N)	93.3 ± 21.3	72.7 ± 19.4**	−20.9 ± 15.8	61.2 ± 14.2**	−32.4 ± 16.7
P100 Hz (N)	132.7 ± 30.1	122.6 ± 28.8**	−6.9 ± 10.69	116.5 ± 30.9**	−12.1 ± 13.2
M-wave (mV)	4.8 ± 2.3	4.6 ± 2.3	−3.9 ± 15.2	4.3 ± 2.3**	−10 ± 15.5

*MVC, maximal voluntary contraction; VAL, voluntary activation level with reference to the 100 Hz resting peak doublet. Pt, muscle twitch; P100 Hz, resting peak doublet at 100 Hz; M-wave, peak to peak M-wave amplitude. Δ PRE, percentage of difference from PRE. Significant differences from PRE: *p < 0.05; **p < 0.01.*

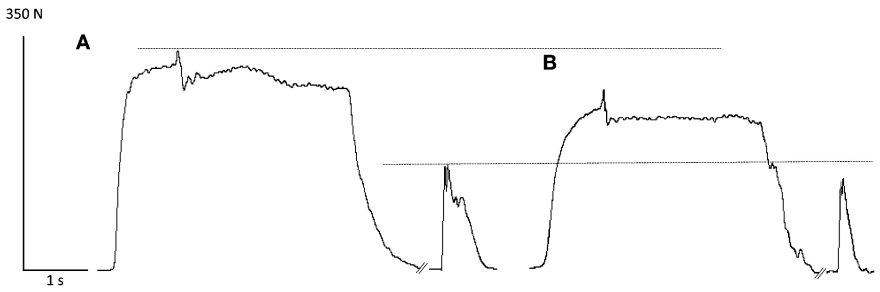

FIGURE 2 | Representative recordings of a superimposed maximal voluntary contraction with a 100 Hz-potentiated doublet in the PRE (A) and POST2 (B) conditions. Note the decrease in MVC force and P100 Hz after the completion of the two fatiguing exercises.

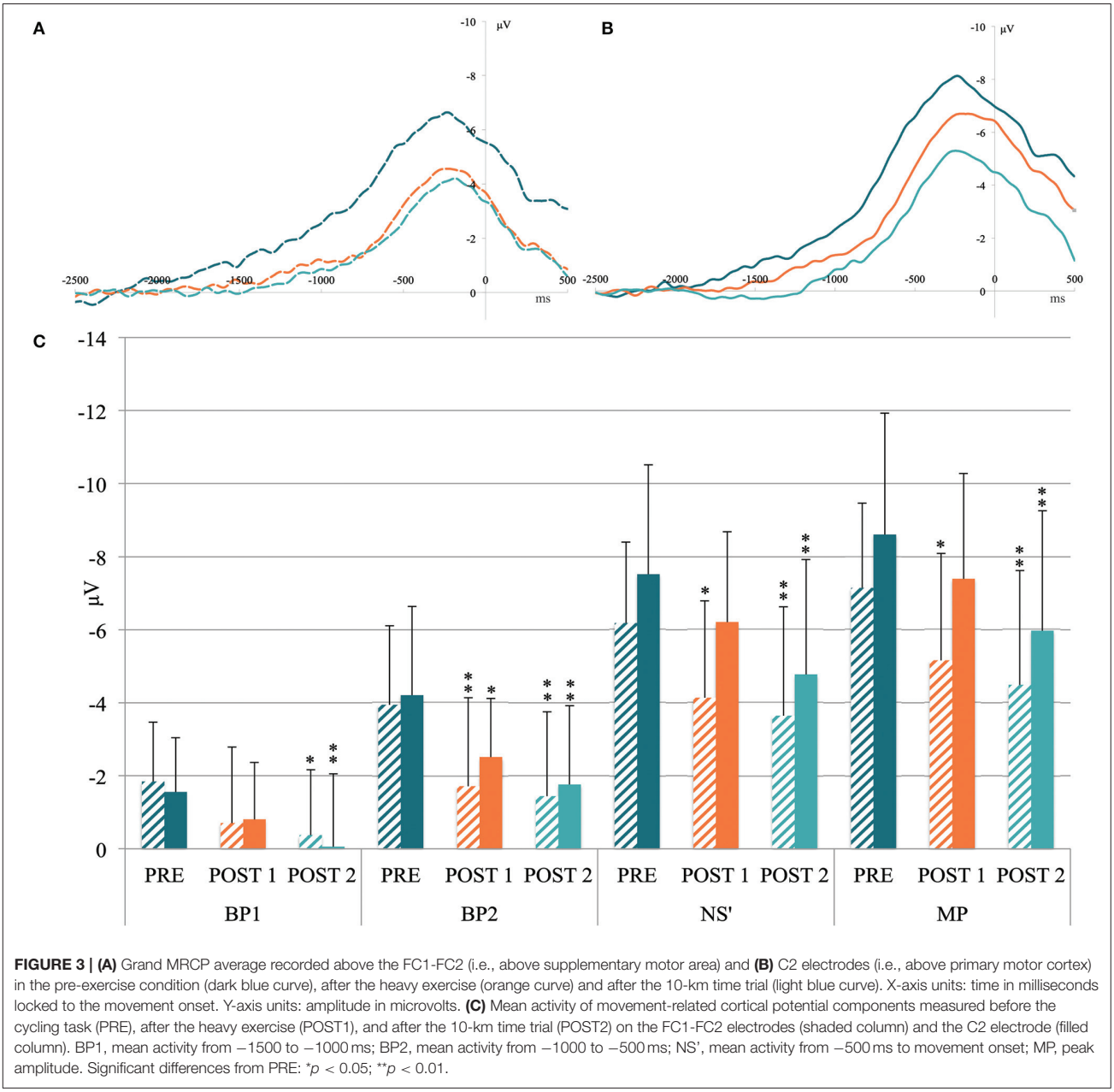

FIGURE 3 | (A) Grand MRCP average recorded above the FC1-FC2 (i.e., above supplementary motor area) and **(B)** C2 electrodes (i.e., above primary motor cortex) in the pre-exercise condition (dark blue curve), after the heavy exercise (orange curve) and after the 10-km time trial (light blue curve). X-axis units: time in milliseconds locked to the movement onset. Y-axis units: amplitude in microvolts. **(C)** Mean activity of movement-related cortical potential components measured before the cycling task (PRE), after the heavy exercise (POST1), and after the 10-km time trial (POST2) on the FC1-FC2 electrodes (shaded column) and the C2 electrode (filled column). BP1, mean activity from −1500 to −1000 ms; BP2, mean activity from −1000 to −500 ms; NS', mean activity from −500 ms to movement onset; MP, peak amplitude. Significant differences from PRE: $^{*}p < 0.05$; $^{**}p < 0.01$.

[$F_{(2,\ 30)} = 7.73$, $p = 0.019$], NS′ [$F_{(2,\ 30)} = 7.17$, $p = 0.003$], and MP [$F_{(2,\ 30)} = 7.39$, $p = 0.002$; **Figure 3C**]. The *post-hoc* tests indicated a significant reduction between PRE and POST1 for BP2 ($p = 0.007$), NS′ ($p = 0.012$), and MP ($p = 0.016$). At POST2, the reduction was significant for the four MRCP components (All $p < 0.04$; **Figure 3C**).

Mean Activity at C2

ANOVA revealed a Time effect on the C2 mean activity for the component BP1 [$F_{(2,\ 30)} = 4.91$, $p = 0.014$], BP2 [$F_{(2,\ 30)} = 8.83$, $p < 0.001$], NS′ [$F_{(2,\ 30)} = 9.14$, $p < 0.001$], and MP [$F_{(2,\ 30)} = 8.43$, $p = 0.001$]. The *post-hoc* tests indicated a significant decrease between PRE and POST1 only for B2 ($p = 0.01$), whereas the reduction was significant for the four components between PRE and POST2 (all $p < 0.012$; **Figure 3C**).

Neuromuscular and MRCP Correlations

Bonferroni *post-hoc* tests revealed significant changes for the MRCP components between PRE-POST1 and between PRE-POST2 conditions, whereas no changes were observed between POST1 and POST2 (see **Figure 3**). Therefore, the correlation analyses between MRCP and neuromuscular modulations were based solely on the PRE-POST1 and PRE-POST2 differences.

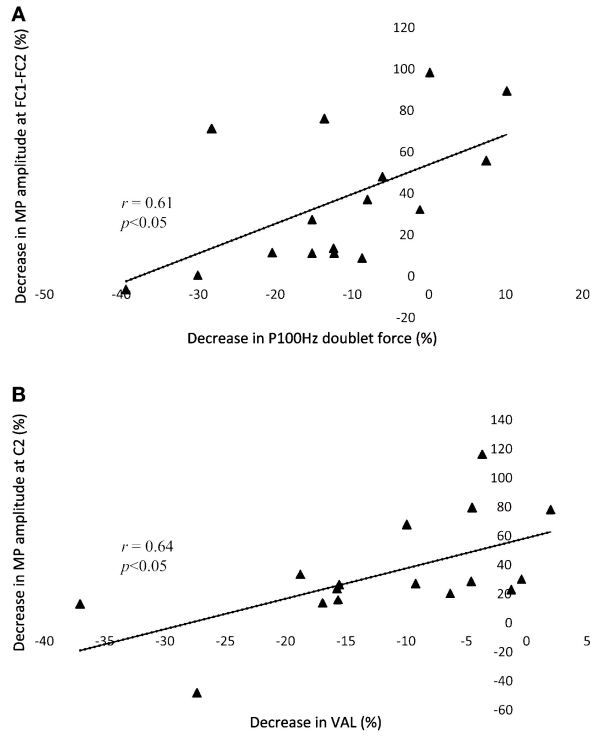

FIGURE 4 | Correlation analyses between the P100 Hz doublet force and the motor potential above the C2 electrode (A) and between the voluntary activation level and the decrease in the motor potential above the FC1-FC2 electrodes (B). Both correlations are based on the differences between the PRE and POST2 conditions. Note that the pre-motor potential is a negative value, and thus, a reduction in the amplitude represents a change toward zero (i.e., the value becomes less negative). MP, motor potential; VAL, voluntary activation level.

No correlation was found between PRE and POST1. In contrast, between PRE and POST2, the reduction in P100 Hz was correlated with the decrease in NS′ ($r = 0.61$) and the MP ($r = 0.61$) amplitude above FC1-FC2 (all $p < 0.05$), whereas the reduction in VAL was correlated with the decrease in BP1 ($r = 0.57$), BP2 ($r = 0.65$), NS′ ($r = 0.72$), and MP ($r = 0.64$) above C2 (all $p < 0.05$). The correlations between the reduction in VAL and MP above FC1-FC2 and between the reduction in P100 Hz and MP amplitude above C2 are illustrated in **Figure 4**. Note that the pre-motor potential is a negative value, and thus, a reduction in the amplitude represents a change toward zero (i.e., the value becomes less negative).

DISCUSSION

The current study was designed to induce neuromuscular fatigue by using two successive cycling exercises, at heavy and severe intensities, and to assess the related effects on MRCP. The second aim was to relate the exercise-induced MRCP modulations with neuromuscular alterations. Although the large-muscle-group exercise induced neuromuscular fatigue, the results indicated a reduction in MRCP amplitude instead of an increase as expected. The cycling exercise induced both peripheral alterations (as indicated by the decreased P100 Hz and Pt) and central impairments (as indicated by the reduction in VAL). The exercise intensity difference between the heavy exercise and the TT was indicated by a higher perception of effort and a greater strength loss after the second exercise. After heavy exercise, MRCP was reduced mainly above the FC1-FC2 electrodes, whereas the MRCP reduction observed at the end of the TT was associated with the FC1-FC2 and C2 electrodes. The relationship found between the reduction in the late MRCP components (i.e., NS′, MP) and P100 Hz above FC1-FC2 and with VAL above C2 indicated a close interaction between neuromuscular fatigue and pre-motor brain activity.

Physical Exercise and Neuromuscular Fatigue

An MVC loss of 10% was observed after the heavy exercise. As expected, this reduction was associated with peripheral alterations characterized by a decrease of 20% in Pt force, without changes in the VL M-wave amplitude and with central fatigue, as reflected by the decrease in VAL. Such neuromuscular changes are very similar to those reported by Lepers et al. (2001). After a cycling exercise lasting 30 min at 80% MAP among trained athletes, the authors reported a decrease of 13% in knee extension force, accompanied by a reduction of 20% in the Pt without changes in the M-wave properties, suggesting alteration of processes located beyond action potential propagation/transmission. Such a reduction in Pt force may be related to intracellular disturbances, such as reduced Ca^{2+} release from the sarcoplasmic reticulum, decreased sensitivity of myofilaments to Ca^{2+}, changes in metabolite (H^+, inorganic phosphate) concentrations within the muscle, and/or reduced force produced by each active cross-bridge (Allen et al., 2008). The absence of significant correlations between MVC force loss and central or peripheral markers of fatigue between PRE and POST1 does not allow for a clear determination of the origin of fatigue. However, relying on other studies demonstrating that peripheral fatigue develops early during such exercise (Decorte et al., 2010), we believe that the trend observed between the MVC force loss and the reduction in P100 Hz in POST1 favors a major role for peripheral alterations.

After the severe intensity exercise, knee extensor MVC force was decreased by 21%. This strength loss is comparable to the results of Lepers et al. (2001), who reported a reduction of 16% after 30 min of cycling at 80% of PMA. As at POST1, peripheral and central alterations participated in knee extensor force impairment at POST2. The additional peripheral fatigue (−32% in Pt at POST2 vs. −21% at POST2) can be attributed to alteration in action potential transmission/propagation, as indirectly indicated by the 10% reduction in VL M-wave amplitude at POST2. The finding that the VL M-wave amplitude decreased at POST2 but not at POST1 confirms the results of Lepers et al. (2002, 2004) suggesting that cycling exercise must be of sufficient intensity and duration to affect muscle excitability.

The purpose of performing the 10-km time trial after having previously performed a heavy exercise lasting 30 min at 60% of MAP (i.e., 67% $\dot{V}O_2$ *peak*), with only a 15-min break (i.e., for data collection), was also to generate greater central fatigue. Indeed, VAL was 91% in PRE condition and decreased to 80% at POST2, in accord with the literature (Lepers et al., 2001; Millet and Lepers, 2004). This finding suggests altered CNS functioning leading to a limitation of descending motor drive. Overall, our results indicate that peripheral mechanisms were mainly involved in the development of fatigue at POST1, whereas central fatigue played a major role in force reduction at POST2.

MRCP Data

Before, between and after exercise, we observed MRCPs with a shape and amplitude similar to those reported in the literature (Shibasaki and Hallett, 2006). The MRCPs were characterized by a slow negative shift starting between 1500 and 2000 ms before movement onset, with a typical change in slope occurring at approximately −500 ms (**Figure 3**). Our data indicate that this inflection occurred slightly earlier (around −750 ms), likely because the trigger was set at the onset of movement instead of the onset of EMG activity. Both fatiguing cycling tasks resulted in a decrease in MRCP amplitude recorded during 60 spontaneous knee extensions at 20% of MVC. A long-term effect on brain activity was already reported by Thacker et al. (2014), who showed MRCP modulations 1 h after the end of an endurance exercise. Our results extend these findings by showing that the large-muscle-group exercise led to distinctive changes in the pre-motor potential during the two 15-min periods post-exercise (POST1 and 2) compared with the fresh condition (PRE).

The present findings do not confirm that pre-motor brain activity increases with muscle fatigue, as expected and as previously reported in repeated single-joint contraction protocols. Our study used a dynamic large-muscle-group-fatiguing task quite different from the task used to quantify changes in MRCP. It is therefore difficult to compare our results with those of previous single-joint contractions protocols. Several authors have asked their experimental subjects to perform repetitive blocks of contractions and have compared pre-motor potential amplitudes between the first and the last blocks. In such designs, fatigue is not experimentally manipulated through a specific fatiguing task. Another difference from our design is the potential for mental load. In most studies, participants have had to achieve a given force level during contraction by using visual feedback. A high degree of concentration must be maintained to correctly perform the task throughout the block of repeated contractions. However, it has been suggested that an increase in attentional load and the mobilization of cognitive resources could be a confounding factor resulting in an increase in MRCP amplitude (Freude and Ullsperger, 1987; Berchicci et al., 2013). Another factor to consider is the contraction force level used during the MRCP task. In the studies of Johnston et al. (2001) and Schillings et al. (2006), the MRCP task consisted of contractions at 70% of the MVC. By comparing the premotor potential amplitude between the first and last blocks of contractions, the authors reported an increase in MRCP. According to the authors, this result suggested that the cortical activity compensated for the reduction in strength capacities (i.e., to provide the same level of force, the brain had to mobilize more resources). In the present study, the load lifted during the MRCP task was kept low (20% of MVC force) and additional cortical activation to compensate for peripheral fatigue did not appear to be required. In a similar vein, Morree et al. (2012) did not observe any increase in pre-motor potential after repeated contractions at 20% of MVC force despite a final maximal force loss of 35%.

The difference in the effects on pre-motor potential observed in this study and others (i.e., a reduction in pre-motor vs. an increase, respectively) may arise from the differing fatiguing task features. The effect of single-joint contraction tasks on motor cortex excitability measured by transcranial magnetic stimulation has been reported to be different from the effect observed for locomotor exercises. The excitability of the motor cortex increases after a fatiguing single-joint contraction task, but after a 30-min steady-state sustained cycling exercise, Sidhu et al. (2012) reported no increase in the responsiveness of the motor cortex. Modulation of cortical excitability is likely task-specific and may be related to the systemic physiological consequences of large-muscle-group exercise. The reduction in MRCP could be related to input from group III and IV muscle afferents to the brain. Indeed, fatiguing voluntary contraction lengthens the cortical silent period, as measured by transcranial magnetic stimulation, which is believed to reflect intracortical inhibitory activity (Gruet et al., 2013). However, when the activation of group III and IV muscle afferents are artificially blocked with an anesthetic solution (fentanyl) injection, the cortical silent period is not prolonged, indicating a modulation of intracortical inhibitions (Hilty et al., 2011b). Similarly, maintaining muscle afferent activity at the end of a fatiguing task with ischemia reduces motor cortex output that maximally activates the muscle (Gandevia et al., 1996). More generally, endurance exercise modulates several additional parameters that might play a role in the reduction of the pre-motor potential, such as cerebral oxygenation (Ide and Secher, 2000), brain catecholamines (Nybo and Secher, 2004), or hyperthermia (Périard et al., 2011).

To the best of our knowledge, only one study has investigated MRCP modulations after a large-muscle-group endurance exercise (Thacker et al., 2014). The exercise consisted in pedaling for 20 min at 70% of the age-predicted maximum heart rate on a cycle ergometer. To remove confounding and fatigue factors, the authors used an MRCP task involving the upper limbs (i.e., wrist extension). The results indicated no changes in MRCP amplitude or onset immediately after the end of exercise. We assume that the absence of modification was caused by insufficient exercise intensity or durations and/or because the muscle group used for the MRCP task was different from that mobilized for the fatiguing task.

In our protocol, a reduction in MRCP was observed during movement preparation, initiation, and movement onset, as reflected by the reduction in the BP, NS′, and MP components, respectively. The heavy exercise induced a decrease in MRCP amplitude for the components BP2, NS′, and MP above FC1-FC2, whereas only BP2 decreased significantly above C2. After the TT, all MRCP components decreased significantly from the PRE condition above the FC1-FC2 and C2 electrodes. Our results

indicate that heavy exercise affects the MRCP differently if it is recorded above SMA or M1. The SMA is connected to the subcortical region, relays sensory feedback received from the muscle and sends direct signals to the M1 (Dum and Strick, 1991; Cadoret and Smith, 1997; Colebatch, 2007), such that the neurons in the SMA are activated several milliseconds before activity within the pyramidal tract (Eccles, 1982). Thus, it appears that the modulations observed above the SMA are more closely related to the integration of peripheral mechanisms, whereas the modulations above the primary motor cortex are more likely to reflect the cortico-motor command. The positive correlation between the increase in peripheral fatigue measured by P100 Hz and the reduction in MRCP (i.e., NS′ and MP) above the SMA strengthens the assumption that this area may be under the modulatory influence of muscle activity, likely via type III and IV afferents. This assumption is also supported by the studies of Gandevia (2001) and Amann et al. (2011) which showed that type III/IV muscle afferents are likely to exert an inhibitory effect on central motor drive during whole-body exercise.

At POST1, the components related to movement preparation are first altered without affecting the execution period of movement production, as reflected by no differences in NS′ and MP amplitude above M1. When neuromuscular fatigue is more pronounced, as observed at POST2, the components related to movement preparation and execution are both modulated, showing a growing impact of fatigue on all the components involved in movement production. The activation of the corticospinal tract via the primary motor cortex is the final step of movement production. Thus, when exercise-induced fatigue decreases the MP above the SMA and M1, it is possible that the motor cortex does not reach the level of activity required to produce the same level of voluntary activation, resulting in a greater decrease in MVC force, as observed at POST2. The positive correlation between the decrease in VAL and the reduction in MRCP above M1 support this assumption, as well as the positive correlation between the drop in MP amplitude above M1 and the reduction in MVC force. Interestingly, some authors have reported neural plasticity between the SMA and M1. By using transcranial magnetic stimulation, Arai et al. (2012) showed that the motor-evoked potential generated by stimulating M1 can be modulated by an SMA-conditioning stimulation procedure. Recently, Bajaj et al. (2015) also showed that the neural connectivity between the SMA and M1 could be modulated by therapy in stroke survivors. It is not unlikely that the acute fatiguing task performed in our study reorganized the connectivity between the SMA and M1 and that the neural impulses sent from SMA to M1 were reduced under the influence of afferent projections.

The limits of our study concern the factors related to movement characteristics, such as strength, accuracy, or rate of force development, all of which are known to modulate the MRCP (Shibasaki and Hallett, 2006). In our protocol, the range of motion was the same between trials. However, the movement duration was not controlled, and we cannot exclude that the duration between the onset of muscle activity and the onset of movement did not change with fatigue. In addition, the MRCP modulations reported in this study are specific to well-trained athletes and cannot be extended to the general population. Because neuroelectric activity could be related to physical fitness (Kamijo et al., 2010), further studies have to be repeated in different populations to determine the impact of fitness on MRCP, especially after endurance exercises.

In conclusion, the MRCP reflects the intention to move and the preparatory period for the intended movement. The results of our study indicate that a cycling exercise induces peripheral and central fatigue and reduces MRCP amplitude. The MRCP components related to movement planning and initiation above the SMA area first altered by heavy exercise-related fatigue, likely by peripheral muscle activity. When neuromuscular fatigue is substantial, as observed after the TT, the overall reduction in MRCP, especially the reduction in the brain component related to movement execution above the primary motor cortex (i.e., MP), is associated with the reduction in the maximal voluntary level, resulting in a decrease in maximal voluntary force.

Finally, large-muscle-group exercise induces neuromuscular fatigue, resulting in an alteration of the corticospinal command. Because this study indicates that this command is modulated by pre-motor cortical activity, we now suggest taking a step backwards to investigate post-exercise resting state electro-cortical dynamics, from which the intention to move emerges.

AUTHOR CONTRIBUTIONS

JNS, FB, JB: Contributions to the conception of the work. JNS, FB, NP, BK, JB: Contributions the acquisition and interpretation of data. JNS, FB, NP, BK, JB: Revising the content. JNS, FB, NP, BK, JB: Final approval of the version.

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Annex II – *Article 2*

Spring J.N., Tomescu M.I., and Barral J. (2017). A single-bout of endurance exercise modulates EEG microstates temporal features. Brain Topogr. *30*, 462-472.

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ORIGINAL PAPER

A single-bout of Endurance Exercise Modulates EEG Microstates Temporal Features

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Abstract Electrical neuroimaging is a promising method to explore the spontaneous brain function after physical exercise. The present study aims to investigate the effect of acute physical exercise on the temporal dynamic of the resting brain activity captured by the four conventional map topographies (microstates) described in the literature, and to associate these brain changes with the post-exercise neuromuscular function. Twenty endurance-trained subjects performed a 30-min biking task at 60% of their maximal aerobic power followed by a 10 km all-out time trial. Before and after each exercise, knee-extensor neuromuscular function and resting EEG were collected. Both exercises resulted in a similar increase in microstate class C stability and duration, as well as an increase in transition probability of moving toward microstate class C. After the first exercise, the increase in class C global explained variance was correlated with the indice of muscle alterations (100 Hz paired stimuli). After the second exercise, the increase in class C mean duration was correlated with the 100 Hz paired stimuli, but also with the reduction in maximal voluntary force. Interestingly, microstate class C has been associated with the salience resting-state network, which participates in integrating multisensory modalities. We speculate that temporal reorganization of the brain state after exercise could be partially modulated by the muscle afferents that project into the salience resting-state network, and indirectly participates in modulating the motor behavior.

Keywords Resting-state network · Neuromuscular · Cycling · Fatigue · Microstate

Introduction

Surface electroencephalographic (EEG) studies have reported modulations in the resting brain activity after acute physical exercise (Crabbe and Dishman 2004). Investigating the ongoing electro-cortical signal in resting-state condition offers the opportunity to better understand the spontaneous brain functioning. Most EEG studies that were interested in the resting brain before and after exercise have used resting-state power analysis and mainly focused on the alpha frequency band (8–12-Hz) (Crabbe and Dishman 2004). Since the original study from Beaussart et al. (1959) who first reported an increase in low frequency amplitude in boxers after combat, a consensus has emerged stipulating that exercise-related increase in alpha frequency amplitude, reflects a state of relaxation and/or fatigue. Several years later, the meta-analysis from Crabbe and Dishman (2004) concluded that a selective effect of exercise on the alpha waves is not strictly established. Indeed, if absolute alpha amplitude increases compared to the pre-exercise condition, no evidences indicate that this effect is specific to the alpha waves when expressed in relative to total power (Crabbe and Dishman 2004). It is likely that the electrical brain activity is stimulated in response to the global increase in metabolic arousal induced by physical exertion (Dishman et al. 1998). At the brain level, by using source localization method immediately after exercise, some authors identified the generators of the scalp surface activity in the

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frequency bands that are thought to dominate at rest after exercise. Different regions were identified within the brain, whose activity varies depending on the frequency band, the exercise mode and intensity, the exercise preference, or the duration between the end of exercise and the time of measurement (Brümmer et al. 2011; Hall et al. 2010; Schneider et al. a, 2009b; Woo et al. 2009). A large number of Brodmann Areas appeared to be affected (Moraes et al. 2011; Schneider et al. 2010), but for now, the current literature is insufficient to state about the regions that are specifically modulated by physical exercise. Changes in the frontal cortex and in the parietal cortex were frequently reported, and have been related with emotional processing and the integration of somatosensory afferents respectively (Brümmer et al. 2011; Schneider et al. 2009a, b). Nevertheless, it is questionable if specific frequency bands can be assigned to a particular cognitive state, especially after physical exercise where the literature is still sparse. Moreover, the meaning of oscillation changes according to the different frequency bands is not fully elucidated yet and has to be cautiously interpreted.

So far, the resting-state power analyses have considered the brain structures independently, in tight frequency bands, and have neglected the dynamic of brain function. A promising approach to further investigate the resting-state activity is to consider the overall surface activity in broad range frequencies, and the related intra-cortical generators as part of neural networks instead of an isolated activity. By using recent electrical neuroimaging techniques, like the microstate analysis, it makes possible to explore continually and non-invasively the overall brain electric filed with a high time resolution (Michel 2009). At each instant, the summation of the activity of superficial and deep brain structures generates an electrical potential that can be visualized, at the scalp surface, as a series of brain electric field maps that varies over time (Lehmann et al. 1998). As different spatial configuration is generated by different neural elements, the topographical analysis of the continuous brain field would reflect the functional state of neurocognitive networks (Koenig et al. 2002). Studies have shown that four recurrent and dominant microstate classes are observed at rest and remain stable for around 80–100 ms (Koenig et al. 2002; Lehmann et al. 2009; Lehmann and Michel 2011). These microstates are consistent across subjects and can be identified during the entire lifespan (Koenig et al. 2002). As the four brain states might represent the basic blocks of human information processing (Lehmann 1990), microstate analysis offers the opportunity to better understand the temporal dynamics of the brain .

Interestingly, the four EEG microstates (named A, B, C and D) were associated respectively to the auditory, visual, salience and attention fMRI resting-state networks (RSNs) (Britz et al. 2010; Damoiseaux et al. 2006; Musso et al. 2010; Van De Ville et al. 2010). Among these four networks, the salience RSN is of particular interest as it is anchored in the anterior insula and cingulate cortex, two main structures receiving inputs from multisensory modalities including the somatosensory afferents (Menon 2015). With exercise, muscle contractions induce mechanical and chemical modulations that activate the terminal end of type III and IV muscle afferents, that in turn project to various sites within the central nervous system (Amann et al. 2015; Craig 2003). For instance, it has been shown that the insula and cingulate are activated by fatiguing muscles stimulated by exercise and by the increasing level of muscle pain during sustained contractions (Craig et al. 1996; Liu 2003; van Duinen et al. 2007). Moreover, the interaction between insula and cingulate is also sensitive to autonomic process, such as respiration and heartbeat (Critchley et al. 2013; Menon 2015; Singer et al. 2009). Based on the aforementioned findings, we assume that the map C topography is likely to be modulated by an acute physical exercise.

By quantifying the temporal dynamics of the brain immediately after exercise, microstate analysis provides innovative knowledge on the relationship between exercise and brain functioning. This study aims to investigate the effect of acute large-muscle group exercise on the temporal dynamics of the EEG resting-state. More specifically, the purpose of this study is to identify the effect of two exercises of different intensities on the four conventional microstates described in the literature and its related parameters (i.e., mean duration, global explained variance, time coverage, occurrence rate, transition probabilities). As the neuromuscular fatigue modulates the brain activity (Gandevia et al. 1996; Sidhu et al. 2013), especially via muscle afferents (Amann 2011; Amann et al. 2011), and in order to investigate a possible relationship between resting microstates and neuromuscular fatigue (Gandevia 2001; Kent-Braun 1999), conventional neuromuscular measurements were collected. Assuming that acute exercise modulates the activity within the insula and cingulate cortices by increasing the somatosensory afferent activity, we hypothesize that the post-exercise brain activity would show modulations of microstate class C.

Materials and Methods

Participants

Twenty endurance-trained male athletes participated in the study. All volunteers completed the Baecke questionnaire (Baecke et al. 1982) to evaluate their habitual physical activity. All of them were active road cyclists and/or triathletes. The protocol was approved by the local ethics committee and was in agreement with the declaration of

Helsinki. Each volunteer was informed of the experimental procedure and gave a written consent before participation.

Procedure

The protocol was composed of two sessions. The first session included a medical screening to confirm that participants were in good health and had no disorders that may interfere with the exercise protocol. During this pre-participation session, the volunteers performed a maximal ramp exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), consisting in 6 min warm-up at 60 watts, followed by a linear resistance increase of 30 watts/min until voluntary exhaustion. During the maximal test the first ventilatory threshold (SV_1), the peak oxygen consumption ($\dot{V}O_2$ *peak*) and the maximal aerobic power (MAP) were collected.

The second session was conducted within 4 weeks after the medical screening. The participants were instructed to avoid severe exercise 24 h before the test, to keep their usual diet, and to avoid alcohol and caffeine consumption during the 12 h preceding the test session. Water was provided ad libitum during the entire protocol. The experimental procedure was composed of two sequential endurance exercises, separated by an inactive period of 15 min, required for neuromuscular and EEG data collection (Fig. 1). The first exercise consisted in a 30-min heavy exercise performed at 60% of MAP (i.e., $SV_1 + 20\%$ of the difference between SV_1 and MAP) on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The second exercise was a severe intensity exercise consisting in a 10-km all-out time trial (TT) on a road bike. The rear wheel was mounted on a home trainer (CycleOps, Madison, USA) and the roller resistance increased automatically with the force exerted by the participant to reproduce field-like sensations. Power output was recorded with power meter fixed in the rear hub (PowerTap, CycloOps, Madison, USA) and the distance was displayed on a bike computer fixed on the handlebar. Rate of perceived exertion was collected at the end of the heavy exercise and the TT using the 6–20 Borg scale. Knee extensor neuromuscular function and spontaneous EEG activity were collected before (PRE), between (POST1), and after the exercises (POST2).

Gas-Exchange Acquisition

Breath-by-breath pulmonary gas-exchange data ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$) were collected during the maximal ramp test with a metabolic cart (OxyconPro, Jaeger, Germany) and averaged over consecutive 10 s period. The $\dot{V}O_2$ *peak* corresponds to highest value attained during the last 30 s before the subject's volitional exhaustion. SV_1 was determined from the combination of different measurements, including: the first disproportionate increase in $\dot{V}CO_2$ from visual inspection of individual plots of $\dot{V}CO_2$ versus $\dot{V}O_2$; an increase in expired ventilation $\dot{V}E/\dot{V}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$; an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The intensity for the 30-min heavy exercise was standardized for each participant and corresponded to 20% of the difference between the power reached at SV_1 and the MAP.

Neuromuscular Data

Knee extensor neuromuscular function was investigated using maximal voluntary contractions combined with electrical stimulations of the femoral nerve in the PRE condition and immediately after the heavy (POST1) and the severe (POST2) exercise. The conventional indices of neuromuscular fatigue were collected through the maximal voluntary contraction force (MVC), the voluntary activation level (VAL) and the supramaximal paired stimulus at 100 Hz (P100Hz). These indices were chosen because their reduction are thought to reflect the global (i.e., MVC), central (i.e., VAL) and peripheral (i.e., P100Hz) indices of neuromuscular fatigue respectively (Gandevia 2001; Kent-Braun 1999). The MVC force is the maximal force (i.e., peak-to-peak maximal amplitude) exerted by the subject in the isometric knee exercise. The VAL reflects the ability of the central nervous system to drive the muscle and was estimated with the superimposed and the potentiated doublets (100 Hz) during the MVC according to the formula proposed by Strojnik and Komi (1998) (see below). Finally, the post-exercise P100Hz is thought to reflect the muscular properties alterations (Allen et al. 2008).

$$VAL = (1 - (superimposed\ 100\ Hz\ doublet\ force \times (force\ level\ at\ stimulation \div MVC\ force) \div potentiated\ doublet\ force)) \times 100$$

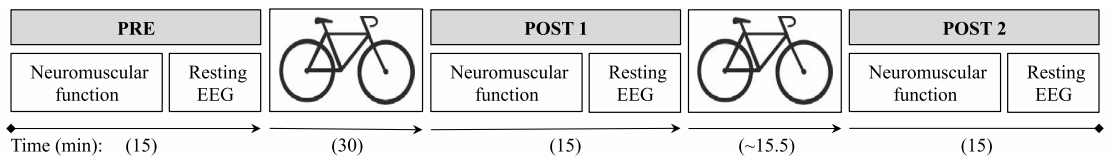

Fig. 1 Experimental design and protocol timeline. *PRE* pre-exercise condition, *POST1* post-heavy exercise condition, *POST2* post-time trial condition. *EEG* surface electroencephalography

The evoked force was obtained with an electrical pulses delivered with a constant-current stimulator (DS7AH, Digitimer, Hertfordshire, UK). The cathode (5 cm diameter) and the anode (5×10 cm, Dermatrode, American Imex Ivrine, CA) were placed over the femoral nerve at the femoral triangle level below the inguinal ligament and on the lower part of the gluteal fold opposite to the cathode, respectively. The intensity for the electrical stimulation was determined by using the following conventional procedure. After the warm-up, the stimulus intensity was progressively increased by 10-mA increments, until there was no further increase in the mechanical response amplitude. To ensure a supramaximal stimulation intensity, a 20% supplementary increment was added to the value obtained (Neyroud et al. 2014).

EEG Data

EEG was recorded immediately after each neuromuscular data collection for the PRE, POST1 and POST2 conditions. Participants were seated in the same ergometer used for the neuromuscular data collection, and were instructed to relax and to keep their eyes closed for 3 min of recording. All the recordings were conducted during the afternoon or the early evening. Continuous EEG was recorded at a sampling rate of 2048 Hz with a 64-channels Biosemi Active two amplifier system (Biosemi, Amsterdam, Netherlands). The cap had to be worn throughout the entire exercise protocol. Unwanted brain activity generated by exercise-induced sweating artifacts was controlled by placing a fan in front of the subject with the wind speed adjusted by the experimenter. The impedance was also checked (<5 kΩ) before and during each EEG data collection. Post-exercise recordings started 1.5 ± 0.5 min after the end of the heavy and the severe exercises.

Offline analyses were performed with the Cartool software by Denis Brunet (brainmapping.unige.ch/cartool). The three initial datasets (i.e., PRE, POST1, POST2 conditions) were pre-processed separately. EEG raw signal was band-pass filtered between 1 and 40 Hz and visually inspected. The high pass filter was set at 1 Hz to exclude slow waves activity possibly generated by sweating and skin potentials (Thompson et al. 2008). Period of muscular artefacts were manually excluded and infomax-based independent component analysis (ICA) was applied to remove eye-blinks and cardiac artefacts based on the topography, the waveform, and the time course of the ICA component (Jung et al. 2000). The signal was down-sampled to 128 Hz, bad electrodes were interpolated using a 3-D spherical spline and recomputed to the common average reference. Since the GFP peak is considered as more representative of a given microstate in term of signal-to-noise ratio (Pascual-Marqui et al. 1995), only the data at GFP peaks were kept for further analysis. GFP reflects the strength of the scalp potential field and corresponds to the standard deviation of all electrodes at a given time point (Brunet et al. 2011; Michel et al. 1993). GFP peaks visually identifiable within periods of residual artefacts were manually excluded. The three pre-processed datasets were then concatenate into one file and submitted to a k-means clustering to identify the map topographies that maximally explain the variance of the map topographies (for technical issues, see Michel 2009). The best representative microstates of each participant were then used to compute a grand clustering to obtain the mean classes for the concatenate dataset (i.e., the four conventional map topographies). These four maps were then used as templates. The spatial correlation was computed between the map topographies within the initial pre-processed dataset of each participant in the three conditions (PRE, POST1 and POST2), and the four representative templates identified by the grand clustering. Each topography was labelled to the microstate class that correlated the best (Brunet et al. 2011). Temporal smoothing parameters (windows ((half) size) = 3; strength (Besag)10) were fixed to avoid excessive artificial interruptions in segments because of a low GFP. All the procedure described above allowed to generate the functional microstate parameters for the four classes, as the Global Explained Variance (GEV, sum of the explained variances of each microstate weighted by the global field power), the mean duration (average time in millisecond covered by a given microstate), the time coverage (percentage of time covered by a given microstate), and the frequency of occurrence (mean number of distinct microstate that occurs within 1 s). To investigate the EEG microstates syntax, we considered the transition of microstate as Markov chain. To compute the probability of transition from one map (i.e., A) to another map (i.e., B), we assessed the number of transitions between the two (from A to B) normalized by the total transitions from the initial map (i.e., A). We thus obtained a total of 12 pairs of transition (i.e., A-B, A-C, A-D, B-A, B-C, B-D, C-A, C-B, C-D, D-A, D-B, D-C). In order to verify that the microstate syntax observed after exercise cannot be reduced to changes in occurrences, a randomized procedure, previously described by Lehmann et al. (2005), was used to test the transition matrices against a random transition probability model. Individual expected and observed transition percentages were averaged and the overall difference between the mean observed transitions and the mean expected transitions was assessed using the Chi square distance (Lehmann et al. 2005; Nishida et al. 2013). A random permutation (5000 permutations) between individual observed and expected probabilities was conducted to obtain a chi-squared distance

compatible with the null hypothesis. The observed data were then compared against this null hypothesis. A significant result indicates that the observed microstate syntax is different from a randomness transition probability.

EEG Resting-State Power Analysis

Resting-state power spectral analysis was performed with BrainVision Analyzer (Brain Products, München, Germany). The pre-processed datasets were segmented into epochs of 4 seconds and analysed by a Fast Fourier Transformation (FFT) (Hanning window with a 10% overlap). The absolute power (μV^2) was obtained for delta (0.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–12.5 Hz) and beta (12.5–35 Hz) frequency bands, and averaged across frontal (Fp1, Fpz, Fp2), central (C1, Cz, C2) and parieto-occipital (PO3, POz, PO4) regions.

Statistical Analyses

Statistical analyses were performed with Statistica 12.6 Software (Statsoft, Tulsa, USA). Results are given in mean ± SD and the data normality was checked using a Kolmogorov–Smirnov test. One-way ANOVAs were used to compare neuromuscular and resting-state power data between the three time of measurement (PRE, POST1 and POST2). For the microstate analysis, two-way ANOVAs 3(*TIME*)×4(*MAPS*) were used to compare the different microstate parameters (GEV, mean duration, time coverage, frequency of occurrence) between time of measurements and maps. To test the microstate syntax, especially whether the transition percentage of moving from one map to another is modulated by exercise, we performed a two-way ANOVA 3(*TIME*)×12(*PAIRS*). Bonferroni post-hoc tests were applied in case of significant interaction. In order to identify relationships between neuromuscular and microstate parameters changes, we correlated the deltas for the pair of variables that showed a significant change between conditions. Significant microstates changes were also correlated with the absolute power changes in delta, theta, alpha and beta frequency bands between PRE-POST1 and PRE-POST2 conditions. The level of significance was set at $p < 0.05$.

Results

Exercise

The subjects' characteristics and exercise performances are shown in Table 1.

Neuromuscular Data

The MVC and VAL from one subject had to be excluded from analysis because the maximal force produced in PRE condition was 30% lower than the MVC exerted by the subject in POST1, and thus was not considered as maximal.

MVC force decreased significantly between PRE-POST1 (−9 ± 8%), PRE-POST2 (−20 ± 8%) and POST1-POST2 (−12 ± 8%) (all $p < 0.01$). The P100Hz was also significantly reduced between PRE-POST1 (−7 ± 11%, $p = 0.012$) and PRE-POST2 (−12 ± 13%, $p < 0.001$), but was not reduced between POST1-POST2 ($p = 0.14$). Concerning the central fatigue, the VAL decreased significantly between PRE-POST1 (−5 ± 9%, $p = 0.03$) and PRE-POST2 (−10 ± 11%, $p < 0.001$), but was not significantly reduced between POST1-POST2 (−5 ± 8%, $p = 0.11$) Fig. 2.

EEG Data

The four microstates obtained in the present study (see Fig. 4) are similar to the four dominant topographies identified in the literature (Koenig et al. 1999, 2002, Lehmann et al. 2005, Britz et al 2010, Nishida et al 2013, Tomescu et al. 2014). Those microstates explained more than 84% of the global variance and were labelled as classes A, B, C and D in accordance with previous studies.

The ANOVA showed a significant *TIME* effect for the GEV ($F(2,38) = 39.5$, $p < 0.001$) and the time coverage ($F(2,38) = 11.5$, $p < 0.001$), and also a significant *MAPS* effect for the GEV ($F(3,57) = 62.6$, $p < 0.001$), mean duration ($F(3,57) = 38.1$, $p < 0.001$), time coverage ($F(3,57) = 42.8$, $p < 0.001$) and frequency of occurrence ($F(3,57) = 31.1$, $p < 0.001$).

Results from the ANOVA indicated a significant interaction 3(*TIME*)×4(*MAPS*) for the GEV ($F(6,114) = 13.3$,

Table 1 Participants (n = 20) characteristics

Variable	Mean ± SD
Age (years)	30.8 ± 6.9
Weight (kg)	73.1 ± 6.0
Height (cm)	179 ± 5
Total Baecke score	9.27 ± 0.73
VO_2 peak (ml min^{-1} kg^{-1})	63.1 ± 7.1
Maximal aerobic power (watt)	381.9 ± 43.7
Heavy exercise mean power (watt)	227.9 ± 21.8
RPE at the end of heavy exercise	14.9 ± 1.6
Time trial mean power (watt)	279.2 ± 32.1
RPE at the end of time trial	19.7 ± 0.5

RPE rate of perceived exertion (Borg scale 6–12); *SD* standard deviation

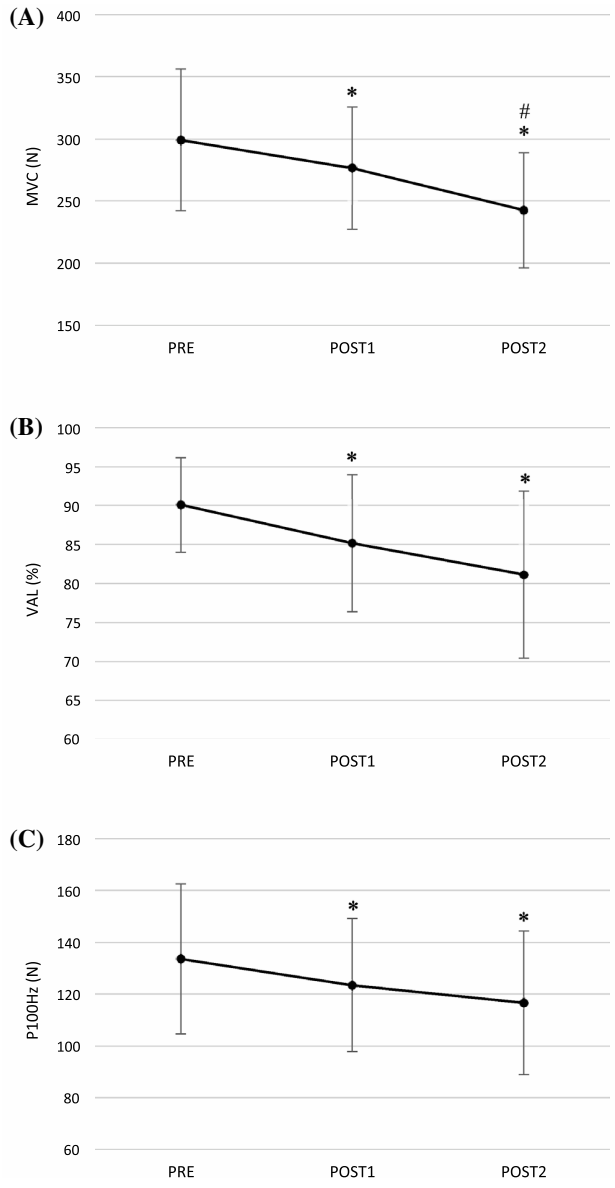

Fig. 2 Neuromuscular changes observed for (**A**) the maximal voluntary contraction (MVC) force, (**B**) the voluntary activation level (VAL), and (**C**) the 100 Hz resting doublet (P100Hz) before the exercise session (PRE), after the 30-min heavy exercise (POST1), and after the 10-km time trial (POST2). *Error bars* indicate the standard deviation. * indicates a significant difference from PRE, # indicates a significant difference from POST1, *p*-value at <0.05

$p < 0.001$), mean duration ($F(6,114) = 7.5$, $p < 0.001$) and time coverage ($F(6,114) = 7.5$, $p < 0.001$). The post hoc tests revealed a significant increase for the GEV, mean duration and time coverage in POST1 and POST2 conditions when compared with PRE condition for the microstate class C only (all $p < 0.001$) (Fig. 3). No difference was observed between POST1 and POST2.

Concerning the microstate syntax, ANOVA showed a simple effects for *TIME* ($F(2,38) = 21.5$ $p < 0.001$) and *PAIRS* ($F(11,209) = 32.5$, $p < 0.001$), and also a significant interaction 3(*TIME*)×12(*PAIRS*) ($F(22,418) = 3.3$, $p < 0.001$) on the observed transition percentage. Post-hoc tests indicated an increase probability of transition from class A to C (12%, $p = 0.04$), from class B to C (15%, $p = 0.02$) and from class D to C (11%, $p < 0.001$) between PRE-POST1 condition. In POST2 condition, the microstate syntax was similar as in POST1 with an increase transition percentage between microstate classes A-C (13%, $p = 0.02$), B-C (20%, $p < 0.001$) and D-C (14%, $p < 0.001$). The transition percentage to move from class C to D also increased between PRE-POST2 (15%, $p = 0.01$) (Fig. 4). The randomization tests on overall transitions were significant after the 30-min heavy exercise ($p < 0.001$) and the 10-km time trial ($p < 0.001$). To further explore the increase transitions towards map C, each pair of transition was corrected from randomness by subtracting the expected transitions from the observed ones. The corrected pairs of variable were then submitted to the ANOVA 3(*TIME*) x 12(*PAIRS)*. Results still show a significant interaction ($F(22,418) = 1.89$, $p = 0.009$) along with a tendency to transit more often from map B to map C in POST2 condition when compared to PRE ($p = 0.07$).

Resting-state power analysis revealed a significant *TIME* effect for delta ($F(2,36) = 5.74$, $p = 0.007$), theta ($F(2,36) = 4.9$, $p = 0.01$), alpha ($F(2,36) = 40.1$, $p < 0.001$) and beta ($F(2,36) = 11.8$, $p < 0.001$) frequency bands. No significant *TIME* effect was observed after the first exercise, except for delta power that decreased in POST1 condition ($p = 0.006$) before returning to baseline in POST2. After the second exercise, we reported a global increase in all frequency bands independently of the region of interest. Indeed, the post-hoc tests revealed that theta ($p = 0.011$), alpha ($p = 0.001$) and beta ($p < 0.001$) power increased in POST2 condition when compared to PRE.

Correlations Between Neuromuscular and EEG Microstates

As the correlations were based on significant neuromuscular and microstates changes, the analyses were performed only between the increase in microstate class C parameters (mean duration, GEV, time coverage) and the reduction in MVC, VAL and P100Hz. No relation was found between VAL and the microstate class C. In contrast, the correlation between PRE-POST1 changes showed a significant relationship between the increase in GEV and the reduction in P100Hz ($r = -0.50$, $p < 0.05$) (Fig. 5, panel (A)). Between the PRE and POST2 condition, the increase in class C mean duration was correlated with the reduction in MVC

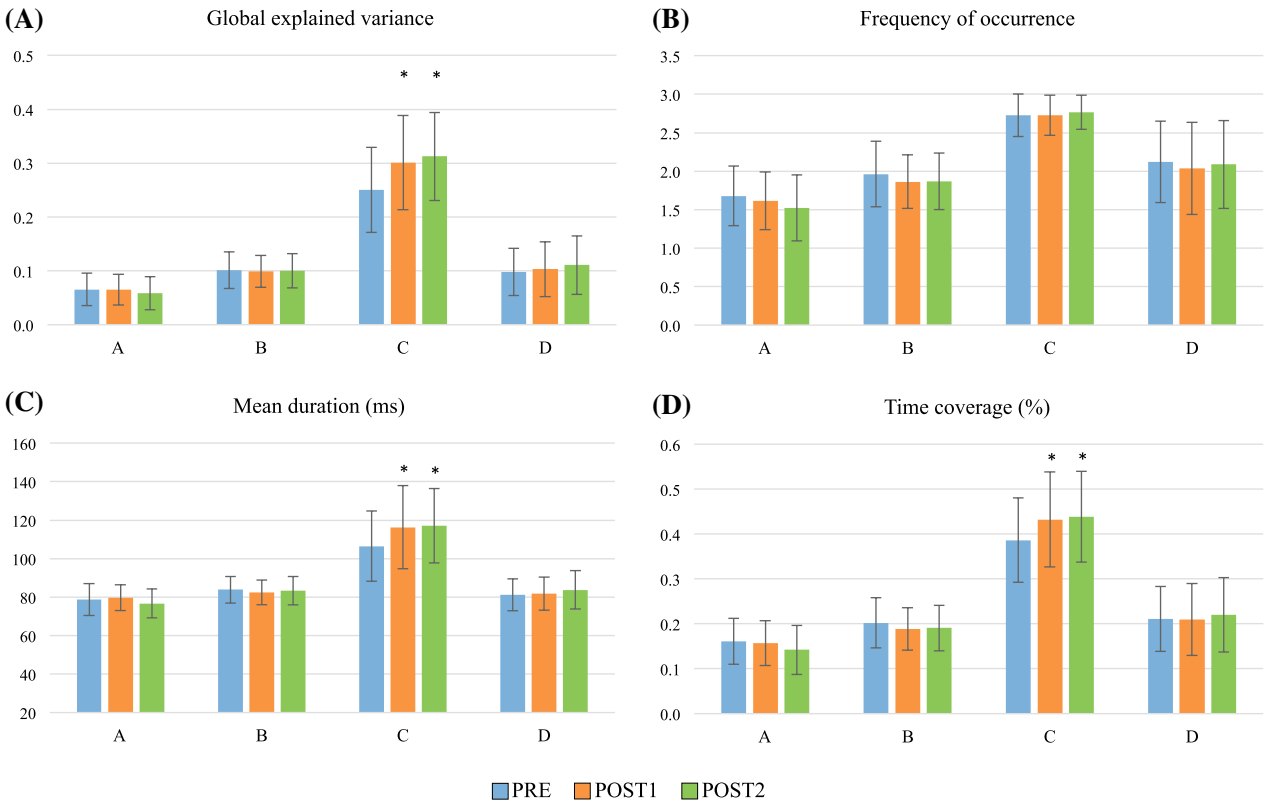

Fig. 3 Microstate parameters of classes A, B, C and D, recorded before the exercise session (PRE, *blue bars*), after the 30-min heavy exercises (POST1, *orange bars*), and after the 10-km time trial (POST2, *green bars*). The global explain variance (**A**), the mean duration (**C**) and the time coverage (**D**) of microstate class C were significantly increased after both exercises. No difference was found for the frequency of occurrence (**B**). *Error bars* indicate the standard deviation. * indicates a significant difference from PRE with *p*-value at <0.05. (Color figure online)

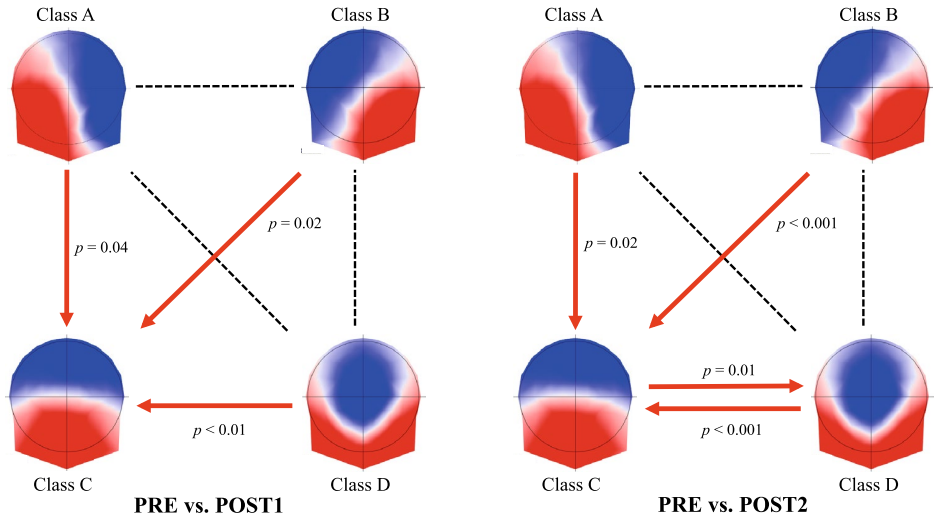

Fig. 4 Microstate maps topographic configuration and syntax. Each map has 3-transition possibilities for a total of 12 possible transitions. PRE versus POST1, the probability of moving from microstate class A, B and D to microstate class C were more important after the 30-min heavy exercise (POST1) compared to pre-fatigued condition (PRE). PRE versus POST2, the probability of moving from microstate class A, B and D to microstate class C were increased after the 10-km time trial, as well as the probability to move from microstate class C to D. *Red arrows* show the significant transition changes. (Color figure online)

($r = -0.55$, $p < 0.05$) (Fig. 5, panel (B)) and the reduction in P100Hz ($r = -0.49$, $p < 0.05$) (Fig. 5, panel (C)).

Correlations Between Resting-State Power Data and EEG Microstates

Spearman correlations between EEG microstate changes and the absolute power changes in delta, theta, alpha and beta frequency bands, across frontal (Fp1, Fpz, Fp2), central (C1, Cz, C2) and parieto-occipital (PO3, POz, PO4) regions revealed no significant relationship.

Discussion

The present study examined the effect of two consecutive acute endurance exercises of different intensities (i.e., heavy and severe) on EEG resting microstates. In order to investigate a putative relationship between brain changes and neuromuscular fatigue, knee-extensor neuromuscular function was investigated using the percutaneous nerve stimulation method. The results showed that acute large-muscle group exercise modulates the EEG resting microstates dynamics. After the 30-min heavy exercise, the GEV, mean duration and time coverage increased significantly only for microstate class C. The sequence of heavy exercise followed by the severe intensity TT was accompanied by a similar microstates pattern. Indeed, the GEV, mean duration and time coverage remained significantly higher in the POST2 condition. The transition probability between the four microstate classes computed after both exercises is significantly different from random transition process. After exercise, transition percentage towards map C is increased and is partly independent of changes in microstates occurrence. Results from neuromuscular data indicated that both exercises induced neuromuscular fatigue, as evidenced by the significant reduction in the MVC force. The force loss was accompanied by central and peripheral alterations as reflected by the decrease in VAL and P100Hz respectively, which corresponds to reductions previously reported by other researchers in similar fatiguing protocol (Decorte et al. 2012; Gandevia 2001; Lepers et al. 2001). The origin of this force loss might come from the reduction in descending motor drive as revealed by the reduction in VAL, and also from alterations in the excitation–contraction coupling process as revealed by the reduction in the P100Hz amplitude.

The main effect in this study was the significant increase in GEV, mean duration, and time coverage for the microstate class C. In their EEG-fMRI study, Britz et al. (2010) have found association between this microstate class and BOLD activity in the posterior part of the anterior cingulate cortex, the bilateral inferior frontal gyri, the right anterior insula and the left claustrum. Interestingly, the functional connection between the anterior right insula and the anterior cingulate cortex may be involved in emotional saliency monitoring (Taylor et al. 2009). In

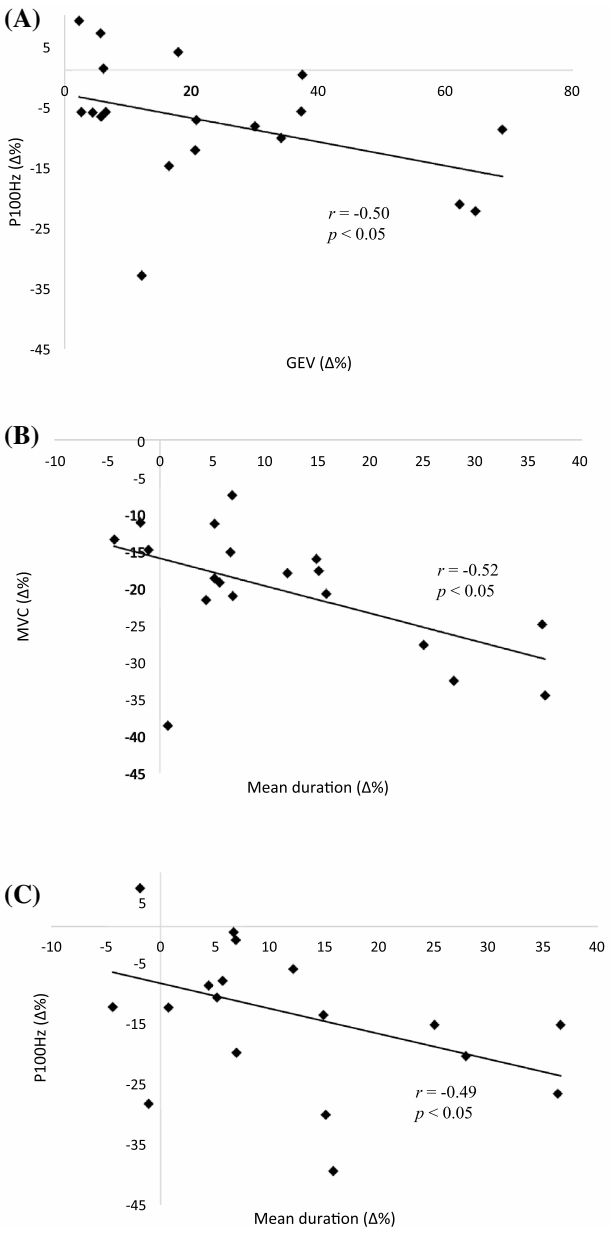

Fig. 5 Significant correlations between neuromuscular and microstate class C changes, between the pre-exercise session (PRE) and the post 30-min heavy exercises (POST1) or the post 10-km time trial (POST2). Spearman correlations show significant association between: (**A**) the reduction in the 100 Hz resting doublet (P100Hz) and the increase in global explained variance (GEV) between PRE-POST1; (**B**) the reduction in the maximal voluntary contraction force (MVC) and the increase in mean duration between PRE-POST2; (**C**) the reduction in P100Hz and the increase in mean duration between PRE-POST2

particular, the anterior insula has been associated with affective tasks involving pain and introspective process (Craig 2003; Henderson et al. 2007; Menon 2015), and appeared to be sensitive to internal signal associated with autonomic process such as heartbeat, blood pressure, skin conductance and respiration (Critchley et al. 2013; Menon 2015; Singer et al. 2009). The convergence of the exercise-related autonomic and muscle afferent activity to the insular region may explain the microstate class C modulations reported in the present study. This assumption is supported by the significant correlation between the muscular properties alterations (i.e., reduction in P100Hz) and the increase in microstate class C mean duration. Insofar as the reduction in P100Hz force have been related to impairment in action potential, muscle damage and/or intracellular disturbance (Place et al. 2010), it is likely that the structures within the salience RSN are informed about the muscle condition through this afferent activity. On the other hand, the salience RSN has efferent projections that might modulate the motor and behavioural response. According to Menon (2015), the dorsal anterior cingulate cortex has efferent outputs that modulates response selection and guiding overt behaviour. Furthermore, in a constant load cycling task, Hilty et al. (2011) reported that the mid/anterior insula do not only integrates the sensory information, but also act in communication with the motor cortex. Thus, we assume that the post-exercise neuronal configuration observed in our study might be related to the reduction in MVC force via this efferent pathway. The significant correlation between microstate class C mean duration changes and the reduction in MVC force supports this assumption. This hypothesis ought to be investigated by further research, by looking at the relationship between resting microstates and the motor command in different fatigued conditions.

Modulation within the cingulate cortex has already been reported by EEG source localisation studies after different endurance exercises. After an incremental biking exercise of 15 min, Schneider et al. (2009b) found an increase in alpha (7.5–12.5 Hz) amplitude in dorsal anterior cingulate (Brodmann area (BA) 32). In an constant load cycling exercise during 20 min at 80% of the age-predicted maximal heart rate, Moraes et al. (2011) identified an increase in alpha (8–12Hz) and beta-1 (13–18Hz) amplitude in the cingulate region (BA33). In a longer duration exercise (40 min at 50–55% of $\dot{V}O_2$ *peak)*, Brümmer et al. (2011) also reported an increase within the same location in the beta frequency band (12.5–35 Hz). Taking together, these results strongly suggest that the cingulate cortex, and probably the related neural assembly within the salience RSN, is sensitive to exercise. It is likely that these structures work as a relay between inputs from body afferents and the output connections to other cortical areas, and indirectly participate in modulating the motor output.

It has been suggested that part of the activation in the insula after sustained contraction could be due to muscle pain (van Duinen et al. 2007) and that the magnitude of insular activation varies with exercise intensity, which may be related to the level of perceived effort (Williamson et al. 1999). Our results failed to show an effect of exercise intensity on EEG microstates, despite greater force reduction and increased perception of effort after the severe exercise. This finding could be due to an already established maximum plateau of intensity during the initial heavy exercise. Investigating the effect of a moderate exercise on EEG microstates would provide relevant information regarding this issue, especially because lighter exercise (i.e., 40–79% MAP) seems to affect differently the brain when compared to heavy intensities (McMorris et al. 2015; McMorris and Hale 2015).

Modifications in microstate parameters and transition patterns have been associated with several neuropsychiatric diseases like schizophrenia (Lehmann et al. 2005) or frontotemporal dementia (Nishida et al. 2013). For example in schizophrenia, mostly shortened duration of microstate class D alongside an increase occurrence of class C are interpreted as an impairment in the normal networks activities, such as alteration in connectivity and decrease in function organization (Khanna et al. 2015). However, modifications in terms of temporal dynamics of EEG microstates are not necessarily a sign of alterations, but also vary in healthy people across developmental stages (Koenig et al. 2002) or conscious state (Khanna et al. 2015). The significance of microstates changes in health must be interpreted with caution and has to be further extended by studying the behaviour of microstates during different stimulus-induced conditions. In this context, the present study showed that a single-bout of exercise results in a momentary increase in duration of microstates class C and an assumed increased stability within the related neural assemblies. The absence of shortening certain microstate may indicate no premature termination of a specific brain-related neural activity, but the sequential activation towards different microstates suggest a temporal reorganization of the brain state after exercise. Because the microstate class C has been associated with a neural network involved in interoceptive-autonomic processing, it is likely that several acute physiological adaptations induced by exercise participate in modulating the dynamic of the spontaneous brain activity. Additional physiological measurements are now necessary to explore in deep the interaction between EEG microstates and physical exercise. Investigating the autonomic response in association with microstate temporal features would give relevant knowledge regarding this issue.

In accordance with previous researches, resting state power analysis revealed a global increase in absolute theta, alpha and beta power after an endurance exercise (Crabbe and Dishman 2004; Kubitz and Mott 1996; Kamijo et al. 2004; Bailey et al. 2008). Moreover, this increase was not limited to a specific brain region. The present results are in line with the conclusion of Crabbe and Dishman (2004), reporting that a selective effect of exercise on a specific frequency band cannot be established. The overall increase in almost all frequency bands make the interpretation of post-exercise resting state power analysis still difficult to discuss, strengthening the importance of developing and applying new EEG methods in exercise physiology.

Conclusion

In conclusion, this study showed the feasibility of using EEG microstates analysis to detect the effect of acute exercise on the resting EEG brain dynamics. We reported for the first time that the four convention microstates topographies identified after an acute large-muscle group exercise are reorganized. After exercise, the duration and stability of the microstate class C is increased, as well as the transition probability of moving towards microstate class C. Based on the significant correlations between the peripheral muscle alterations and the increase in map C mean duration, we speculate that the post-exercise microstate pattern could be partially changed by the muscle afferents that project into the two main structures of the salience RSN (i.e., the cingulate and the insula). We suggest that some properties of this resting-state network—interconnected with the motor cortex—can be associated with the motor output, as shown by the correlation between the increase in map C mean duration and the reduction in maximal voluntary force. To better read the post-exercise microstates, it would be interesting to relate the brain state changes with other physiological consequences of endurance exercise, such as the autonomic system activity.

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Annex III – *Article 3*

Spring J.N., Bourdillon N., and Barral J. (in review). Resting EEG microstates and autonomic heart rate variability do not return to baseline one hour after a submaximal exercise. Front. Neurosci. .

Resting EEG microstates and autonomic heart rate variability do not return to baseline one hour after a submaximal exercise

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EEG, microstates, HRV, exercise, recovery

Abstract

Recent findings suggest that an acute physical exercise modulates the temporal features of the EEG resting microstates, especially the microstate map C duration and relative time coverage. Microstate map C has been associated with the salience resting state network, which is mainly structured around the insula and cingulate, two brain nodes that mediate cardiovascular arousal and interoceptive awareness. Heart rate variability (HRV) is dependent on the autonomic balance; specifically, an increase in the sympathetic (or decrease in the parasympathetic) tone will decrease variability while a decrease in the sympathetic (or increase in the parasympathetic) tone will increase variability. Relying on the functional interaction between the autonomic cardiovascular activity and the salience network, this study aims to investigate the effect of exercise on the resting microstate and the possible interplay with this autonomic cardiovascular recovery after a single bout of endurance exercise. Thirty-eight young adults performed a 25-minutes constant-load cycling exercise at an intensity that was subjectively perceived as "hard". The microstate temporal features and conventional time and frequency domain HRV parameters were obtained at rest for 5 minutes before exercise and at 5, 15, 30, 45, and 60 minutes after exercise. Compared to the baseline, all HRV parameters were changed 5 minutes after exercise cessation. The mean durations of microstate B and C, and the frequency of occurrence of microstate D were also changed immediately after exercise. A long-lasting effect was found for almost all HRV parameters and for the duration of microstate C during the hour following exercise, indicating an uncompleted recovery of the autonomic cardiovascular system and the resting microstate. The implication of an exercise-induced afferent neural traffic is discussed as a potential modulator of both the autonomic regulation of heart rate and the resting EEG microstate.

1. Introduction

At rest, the brain continually integrates information from external and internal sources, resulting in rapid changes in the distribution of neural activation at the cortical and sub-cortical levels (Michel et al., 2009). The summation of this electrical activity propagates to the scalp surface, resulting in specific map topographies. Assuming that different map topographies are generated by different neuronal configurations, resting microstate analysis enables the quantification over time of spontaneous brain activity from the surface electric fields (Michel et al., 2009; Brunet et al., 2011). Conventional multichannel electroencephalographic recordings (EEG) are segmented into a sequence of discrete map topographies with archetypal and reproducible temporal properties (Lehmann et al., 2009; Koenig et al., 2002; Khanna et al., 2015). Four map topographies (A, B, C, and D) can be extracted from an EEG signal, and explain approximately 70-80% of the total topographic variance (Khanna et al., 2015). Each of these maps has been associated with a group of interconnected brain nodes that are organized into a network and active at rest (Britz et al., 2010). Map A has been associated with the visual resting state network (RSN), map B with the auditory RSN, map C with the salience RSN, and map D with the attentional RSN (Britz et al., 2010).

A single session of dynamic whole-body exercise modulates microstate temporal features (Spring et al., 2017). Specifically, duration and relative time coverage of map C significantly increased following a 30-minute cycling exercise in trained participants. The predominance of microstate C was supported by changes in the microstate syntax, where the propensity to transit toward map C was greater after exercise. The authors postulated that post-exercise microstate might be related to an afferent signaling pathway via projections to the salience RSN (Spring et al., 2017). Indeed, the salience network integrates information such as pain and muscular sensation (Craig, 2003; Singer et al., 2009) and correlation exists between peripheral muscular alterations and the increase in microstate map C mean duration (Spring et al., 2017). However, the salience network also responds to internal signals coming from autonomic processes and mediates cardiovascular arousal (Bechara and Naqvi, 2004; Critchley et al., 2000; Eckert et al., 2009; Menon, 2015; Pollatos et al., 2007). To our knowledge, no study has explored the potential links between autonomic cardiovascular responses and microstates changes during post-exercise recovery.

The specificity of large-muscle group exercise is the massive cardiovascular response that copes with the increase in the metabolic requirement. The optimal physiological balance in the body and the successful cardiovascular control of blood pressure and distribution of blood flow is

under the control of the parasympathetic and sympathetic autonomic nervous system (Christensen and Galbo, 1983; Murphy et al., 2011). At exercise onset, parasympathetic withdrawal drives the sudden increase in heart rate (HR). Increase in the sympathetic tone drives the subsequent increase in HR (Tulppo et al., 1996). At cessation of exercise, parasympathetic reactivation principally determines the fall in HR (Coote, 2010; Perini et al., 1989). HR then decreases slowly in an exponential manner (Savin et al., 1982), depending on different factors such as the level of fitness for instance (Darr et al., 1988). Depending on the exercise intensity and duration, this autonomic reactivation can last several hours (Furlan et al., 1993; Mourot et al., 2004; Perkins et al., 2017; Seiler et al., 2007; Stanley et al., 2013) and seems to reflect the time required for a set of physiological and metabolic parameters to return to homeostasis (Stanley et al., 2013). The sympathetic and parasympathetic components can be indirectly assessed using the heart rate variability (HRV) obtained from a conventional electrocardiogram (ECG) recording. HRV is defined as the variability between beat-to-beat (R-R) intervals and reflects the autonomic background of the fluctuation in heart rate (Malik, 1996). Standards from the task force of the European Society of Cardiology more precisely differentiate the relative participation of the sympathetic and parasympathetic components (Malik, 1996). Accordingly, the root mean square of the successive differences between R-R intervals (RMSSD) reflects the rapid fluctuations in parasympathetic activity. The high-frequency power (HF: 0.15-0.40Hz) is modulated by respiration-induced variations in HR and reflects mainly parasympathetic activity. The low-frequency power (LF: 0.04-0.15Hz) reflects both the sympathetic and parasympathetic activities. Consequently, it is assumed that the LF/HF ratio provides information about the sympatho-vagal balance (Malik, 1996).

The salience network is a connected large-scale network anchored in the anterior insula (AI) and dorsal anterior cingulate (dACC) cortex (Menon, 2015; Menon and Uddin, 2010). The cardiac autonomic activity involves cardiovascular control centers within the brain stem, but also cortical and subcortical structures (Craig, 1995, 2002, 2003; Menon and Uddin, 2010). For instance, the insula and cingulate cortices have been shown to play an important role in cortical regulation of cardiac autonomic activity (Macefield and Henderson, 2015; Sander et al., 2010; Williamson, 2015; Williamson et al., 2003). During static handgrip exercise, an fMRI study showed an increase in neurovascular coupling in the sensorimotor and insular cortices, and a decrease in the midcingulate cortex (Sander et al., 2010). More specifically, neuroimaging studies have associated HRV with fluctuations in brain connectivity within different neural structures including the dACC and AI (Chang et al., 2013; Critchley et al., 2003; Lane et al.,

2009; Napadow et al., 2008; Thayer et al., 2012). Together, these studies confirm the implication of the salience network in the autonomic cardiac regulation, further supporting a putative link between the autonomic cardiovascular activity and the post-exercise microstate C changes.

The present study aimed to further explore the implication of map C-related salience RSN after exercise and the possible association with the autonomic cardiovascular response. The objective was to describe the microstates and HRV time-courses after a single-bout of endurance exercise and during the 60-minutes recovery period in non-athlete males and females. We predicted that the exercise-induced modulation of map C would persist as long as the autonomic HR modulation had not returned to baseline.

2. Materials and Methods

2.1. Participants

The forty-two healthy right-handed volunteers enrolled in this study completed the French version of the Physical Activity Readiness Questionnaire (PAR-Q) to exclude obvious risk factors for adverse cardiac events during exercise. The exclusion criteria included recent musculoskeletal pathology that may interfere with exercise; a personal history of psychiatric, neurological or cardiovascular disorders; alcohol consumption above 3 units per day; asthma; and current drug usage. All of the volunteers completed the Baecke questionnaire (Baecke et al., 1982) for the evaluation of habitual physical activity using a score between 1 to 5 for sport, work, and leisure activities, and also a total score obtained from the sum of the 3 previous domains. Before starting the protocol, all participants provided written informed consent. The study was approved by the local ethic committee (CER-VD: 2016-01730) and conducted in compliance with the applicable legal requirements.

2.2. Experimental protocol

According to the literature, an exercise duration of approximately one half hour at an intensity around the anaerobic threshold is enough to induce HRV changes for several minutes after exercise cessation (Seiler et al., 2007; Terziotti et al., 2001). Therefore, the exercise consisted of a 25-minutes exercise on a cycle-ergometer (Lode Excalibur Sport, Groningen, The Netherlands) at a subjective intensity of 15 on the Borg scale of 6-20. The intensity was individualized based on the subjective effort perception, assuring that exercise results in a similar relative physiological response between subjects. A score of 15 corresponds to a perceived physical effort considered as “hard” and is thought to be close to the ventilatory threshold (Purvis and Cukiton, 1981; Hill et al., 1987). The exercise started with an incremental protocol starting at 60 watts with an increase of 15 watts every minute. At the end of each step, subjects were asked to provide a score between 6 (no exertion) and 20 (maximal exertion). When a score of 15 was reached, the increment in workload ceased, and the 25-minute constant-load exercise began. The time required to reach 15 was considered as the warm-up period. If RPE deviated by more than ±1 unit during the first 5 minute of constant-load cycling, the load was adjusted (increased or decreased) to ensure a stable perceived effort during the entire 25 minutes. The experimental protocol lasted 3 hours. Participants were asked to avoid severe exercise 24 hours before the test. Alcohol and caffeine consumption were proscribed for the 12 hours preceding the test and water was provided *ad libitum* during the protocol.

2.3. EEG and ECG data acquisition

An EEG and an ECG were simultaneously recorded in light-shielded room for 5 minutes before (BSL) and 5 minutes after the exercise session (P05), as well as 15 (P15), 30 (P30), 45 (P45) and 60 (P60) minutes after the end of exercise. Participants were comfortably seated on a chair with their chin rested on a chin support to reduce head movement artifacts. The only instruction given was to close the eyes and relax during the recording. The EEG was collected using a 64-chanel BioSemi Active two amplifier system (Biosemi, Amsterdam, Netherlands) mounted according to the International 10-20 recommendations. The ECG was collected using two flat-type electrodes (BiomSemi, Amsterdam, Netherlands) placed under the right clavicle on the midclavicular line, and near the 5^{th} left intercostal space on the anterior axillary line. Both signals were referenced to the active CMD-DRL ground system incorporated in the BioSemi device. EEG and ECG data were simultaneously collected at a sampling frequency of 2048 Hz, and the EEG electrode impedance was kept below 10 kΩ. The experiment was conducted in a cool air-conditioned room (temperature: 19ºC, humidity: 30%, CO_2: 550 ppm), and a breathable Lycra cap (BiomSemi, Amsterdam, Netherlands) was used to help dissipate heat. A fan was also placed in front of the subject during the exercise protocol to ensure minimal artifacts of sweat on the collected signals.

2.4. EEG and ECG data processing

The EEG and ECG analyses were performed using the Cartool software (brainmapping.unige.ch/cartool) and MATLAB (version 8.5.0, MathWorks, Natick, MA, USA). The ECG signal was extracted from the EEG and processed separately using custom MATLAB scripts. The ECG signal was down-sampled at 256 Hz and ECG R peaks were identified from the ECG using an automatic extrema detection method. Ectopic beats were compensated using means of interpolation to calculate normal-to-normal intervals (R-R). From this signal, the HRV parameters were obtained. In the time domain, we computed the mean heart rate during the 5-minute recording (mean HR) and the root mean square of the successive differences between R-R intervals (RMSSD). For the analysis in the frequency domain, the R-R interval time series were resampled at 4 Hz using cubic spline interpolation. The low-frequency (nLF) (0.04-0.15 Hz) and the high-frequency (nHF) (0.15-0.40 Hz) spectra were obtained using the Fast Fourier Transform on the resampled RR intervals and normalized to the total power. The LF was divided by the HF to compute the LF/HF ratio.

The EEG signals were band-pass filtered between 1 Hz and 40 Hz to exclude unwanted slow wave activities generated by sweating and skin potentials and also to avoid high frequency

muscular artifacts and non-cortical electrical sources. The data were visually inspected, and an infomax-based independent component analysis (ICA) was applied. Based on the waveform, the time course, and the topography of each ICA component (Jung et al., 2000), residual eye-twitching and cardiac artifacts were removed. The reconstructed signal was down-sampled to the same sampling rate as for the ECG (256Hz), bad electrodes were interpolated with a 3-D spherical spline, and the signal was recomputed to the common average reference. The microstate analysis followed a conventional procedure applied in previous studies (Britz et al., 2010; Brunet et al., 2011; Spring et al., 2017; Tomescu et al., 2014) and implemented in the Cartool software (Brunet et al., 2011; Michel et al., 2009). To summarized, the pre-processed datasets collected in BSL, P05, P15, P30, P45 and P60 were used to obtain the 4 expected convention microstate templates. The topographies at Global Filed Power (GFP) peaks were submitted to a k-means clustering to identify for each participant in each condition the four maps that maximally explained the topographic variance. The GFP is a reference-free measure of total filed strength and corresponds to the standard deviation of the potential field (Skrandies, 1990). After applying the clustering to individuals, a second clustering was applied using the 4 best individual topographies to obtain the best representative microstate map for the group across the 6 periods of measurement. Based on multiple criteria selection, the software automatically generated the best cluster for the group, which corresponded to the expected 4 maps. These maps were spatially similar to those described in previous studies (Michel and Koenig 2017) and thus were labelled as map A to D (Koenig et al. 1999). Finally, individual pre-processed EEG recordings were allocated to one of these 4 maps based on their spatial correlation. This back-fitting process allows to compute for each microstate the mean continuous period of time assigned to a given microstate (mean duration), the number of time that a map occurred in one second (frequency of occurrence), the relative percentage of time covered by a map (time coverage), and the transition percentage of moving from one map to another (syntax). In the present study, the observed transition percentage corresponds to the transition percentage of moving from a given map to another, normalized by the total transition from the initial map. To further explore this microstate syntax and to make the transition percentage changes independent from modifications in the microstate occurrence after exercise, the observed transition percentages were corrected by the expected transitions, which were computed according to Lehmann et al. (2005) and as previously used by Spring et al. (2017).

A possible association between microstate and HRV parameters changes was tested as follows. First, to identify if microstate and HRV responses were associated with exercise, we computed the difference between BSL and P05 (ΔBSL-P05) for microstate and HRV parameters before

using these differences as correlation variables. Second, to explore a putative association during the post-exercise recovery, we proceeded in similar manner by computing the difference between P05 and P60 (ΔP05-P60). Finally, we computed an index of global recovery, which corresponds the difference between BSL and P60 (ΔBSL-P05).

2.5 Statistical analyses

Statistical analyses were performed with Statistica 12.6 software (Statsoft, Tulsa, USA). Normality of each variable was evaluated by Shapiro-Wilk tests and significance was set at $p<0.05$. The data are presented as the mean ± *SD* in the text and the mean ± *IC* (95%) in the figures. The mean HR, RMSSD, nLF, nHF and LH/HF ratio were analyzed using one-way repeated measures ANOVA (rmANOVA) to compare the 6 periods of measurement (BSL, P05, P15, P30, P45, and P60). We compared the 4 microstate maps changes across time using two-way rmANOVA 6(*TIME*) x 4(*MAP*). These analyses were conducted for the microstate mean duration, time coverage, and frequency of occurrence. The microstate syntax was investigated using a two-way rmANOVA 6(*TIME*) x 12(*PAIRS*) to identify a difference between the 12 pairs of transition across BSL, P05, P15, P30, P45, and P60. This rmANOVA was also performed on the transition percentages corrected for occurrence. Bonferroni post-hoc tests were applied for significant interactions. The presumed association between microstates map C and HRV parameters changes were explored using Pearson correlations on the absolute difference between BSL and P05 (ΔBSL-P05), P05 and P60 (ΔP05-P60), and BSL and P60 (ΔBSL-P60).

3. Results

3.1 Participants

Forty-two volunteers completed the protocol, and four participants were excluded from analysis. Two subjects had artifacts due to important eyelid twitching or involuntary movements, and two subjects were anxious because of personal reasons. The remaining 38 volunteers (22 females and 16 males) were 24±4 years old. Their average height, body mass, and body mass index were 173±9 cm, 68±13 kg and 22±3 $kg \cdot m^{-2}$, respectively. Participants were physically active as demonstrated by a total Baecke score of 8.3±1.5, with a value of 2.1±0.7 for work, 3.2±0.9 for sport, and 2.9±0.6 for leisure.

3.2. Exercise data

The warm-up period, which corresponded to the time required for reaching 15 on the Borg scale of 6-20 was 7 ± 2 minutes. The total duration of exercise was 32 ± 2 minutes, the mean HR during the 25 minutes of constant-load exercise was 160 ± 19 bpm, and the mean RPE was 15.1 ± 0.6. The HR and RPE time courses during the cycling exercise are shown in Table 1.

Table 1: Heart rate and Borg rating of perceived exertion scale (6-20) during the 25-minute cycling exercise.

	BSL	Exercise duration (min)				
		5	10	15	20	25
	Mean ± SD	*Mean* ± SD	*Mean* ± SD	*Mean* ± SD	*Mean* ± SD	*Mean* ± SD
HR (bpm)	66 ± 11	155 ± 20	159 ± 20	159 ± 20	163 ± 18	164 ± 18
Borg scale	- ± -	15.0 ± 0.7	15.0 ± 0.8	15.1 ± 1	15.1 ± 0.8	15.3 ± 0.9

BSL, baseline; HR, heart rate; SD, standard deviation.

3.3. HRV data

The endurance exercise resulted in a significant *TIME* effect for mean HR ($F(5,185) = 194.35$, $p < 0.001$). In BSL, the mean HR was 66 ± 11 bpm and increased significantly by 41±15% immediately after the end of exercise in P05 ($p < 0.001$). Compared to P05, the mean HR decreased by 10 ± 4%, 17 ± 5%, 21 ± 6% and 23 ± 6% in P15, P30, P45, and P60, respectively (all $p < 0.001$). In P60, the HR remained significantly higher than BSL (8 ± 7%, $p < 0.001$), indicating an incomplete recovery (Figure 1, panel A). The rmANOVA showed a *TIME* effect for RMSSD ($F(5,185) = 37.7$, $p < 0.001$). Compared to BSL, RMSSD was reduced to 67 ± 27% after exercise in P05 ($p < 0.001$). During the post-exercise recovery period, RMSSD was

different from P05 in P15, P30, P45, and P60 (all $p \leq 0.004$) and remained different from BSL in P60 ($p < 0.03$) (Figure 1, panel B).

The frequency-domain HRV analysis revealed a similar *TIME* effect for the nLF and nHF ($F(5,185) = 22.44$, all $p < 0.001$). Compared to BSL, nLF and nHF were altered after exercise in P05 and remained different from BSL across all time points measured (all $p < 0.001$). In P30, P45 and P60, the nLF and nHF were significantly lower than in P05 (all $p < 0.001$) (Figure 1, panels C and D). A significant *TIME* effect was found for the LF/HF ratio ($F(5,185) = 15.3$, $p < 0.001$). After a significant increase induced by exercise (from 1.13 ± 0.9 in BSL to 2.8 ± 1.9 in P05, $p < 0.001$), the ratio remained around a similar value in P15 (2.7 ± 2.1), before returning to baseline in P45. Indeed, from P45 to P60 the LH/HF ratio was different from P05 (all $p \leq 0.04$), but not greater from BSL (Figure 1, panel E).

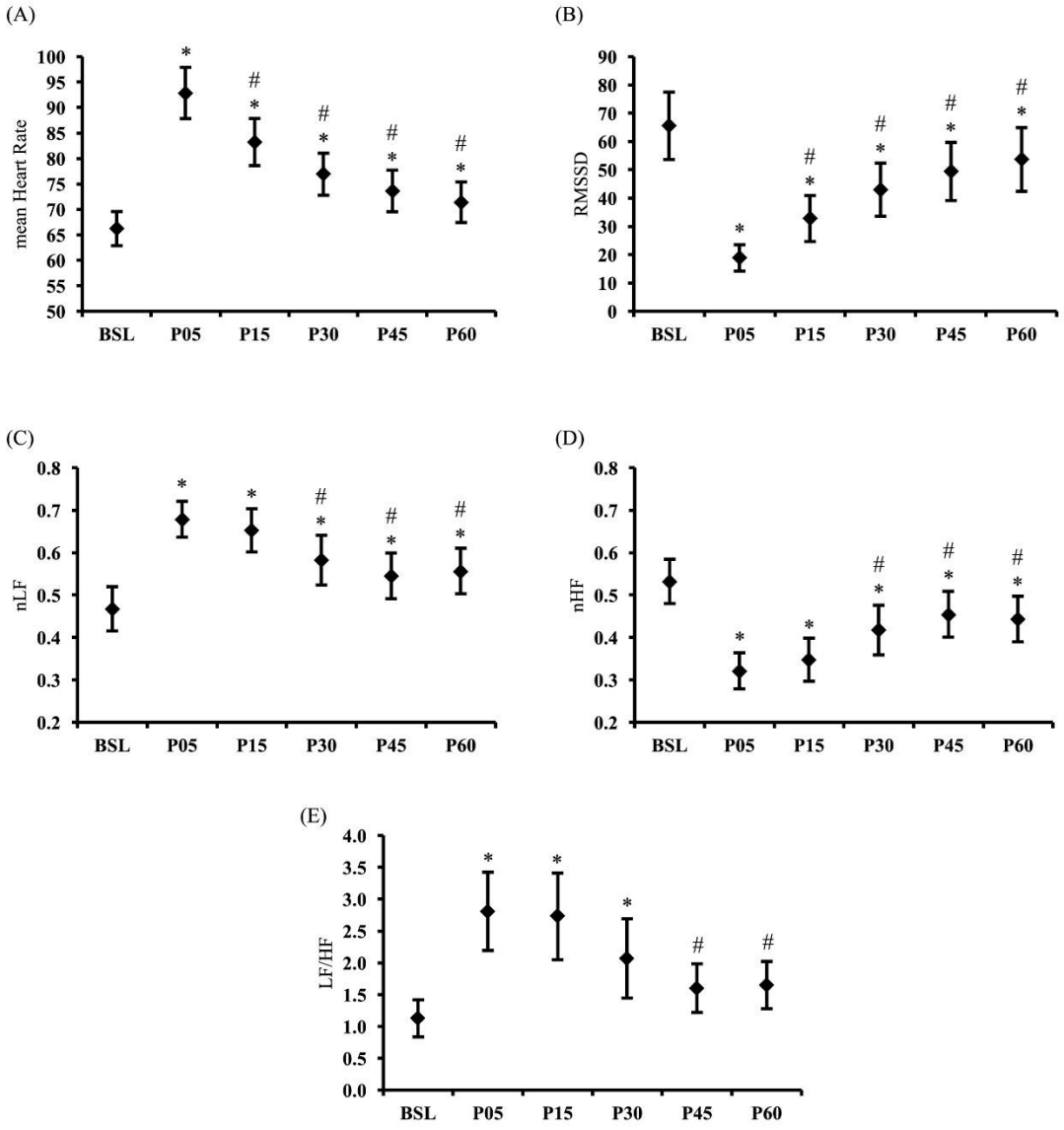

Figure 1: HRV analysis results. Time course of **(A)** mean Heart Rate, **(B)** RMSSD, **(C)** nLF, **(D)** nHF, and **(E)** LF/HF ratio, recorded at baseline (BSL) and 5 (P05), 15 (P15), 30 (P30), 45 (P45), and 60 (P60) minutes after exercise cessation. Error bars indicate the 95% confidence interval. * indicates a significant difference from BSL, # indicates a significant difference from P05; p-value < 0.05.

3.4. EEG data

The 4 best representative topographies of all individuals across conditions explained 85% of the total variance and were labelled as map A, B, C and D (Figure 2).

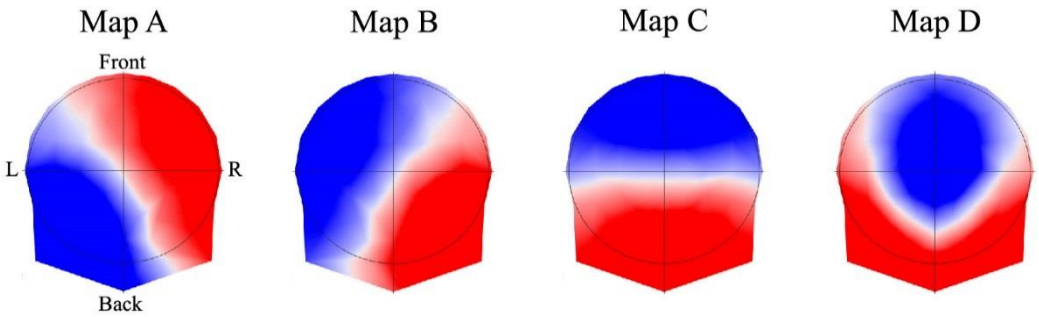

Figure 2: The four resting EEG microstate topographies. The maps obtained across condition were labelled in class A, B, C, and D, according to previous studies. Note that EEG microstate analysis ignores the topographic polarity.

There was a significant interaction 6(*TIME*) x 4(*MAP*) for the microstate mean duration ($F(15,555) = 3.79$, $p < 0.001$). Post-hoc analyses revealed that the mean duration of map C was significantly increased (+8.7%) by exercise and remained higher compared to BSL during all recovery time points (all $p < 0.001$). In P60, the map C duration had decreased (-3.4%) from P05 ($p=0.007$), but did not return to BSL ($p < 0.001$) suggesting an uncomplete recovery. A short-term increase (+4.2%) in the map B duration was also found in P05 ($p = 0.04$) and returned to the pre-exercise value in P15 ($p < 0.05$) (Figure 3, panel A).

There was a significant 6(*TIME*) x 4(*MAP*) interaction for the microstate time coverage ($F(15,555) = 3.7$, $p < 0.001$). Post-hoc tests demonstrated an increase in the time coverage only for map C in P05 ($p < 0.001$), P15 ($p = 0.03$), and P30 ($p < 0.002$) when compared to BSL. In P45 and P60 the time coverage tended to be different from BSL ($p = 0.07$ and $p = 0.08$, respectively) and thus indicated a return to the pre-exercise value (Figure 3, panel B).

A significant 6(*TIME*) x 4(*MAP*) interaction was also found for the frequency of occurrence ($F(15,555) = 1.99$, $p = 0.014$). Bonferroni post-hoc tests indicated a significant change for map D immediately after exercise in P05 ($p = 0.005$). At P15 and subsequent time points, the map D occurrence was not different from BSL (Figure 3, panel C).

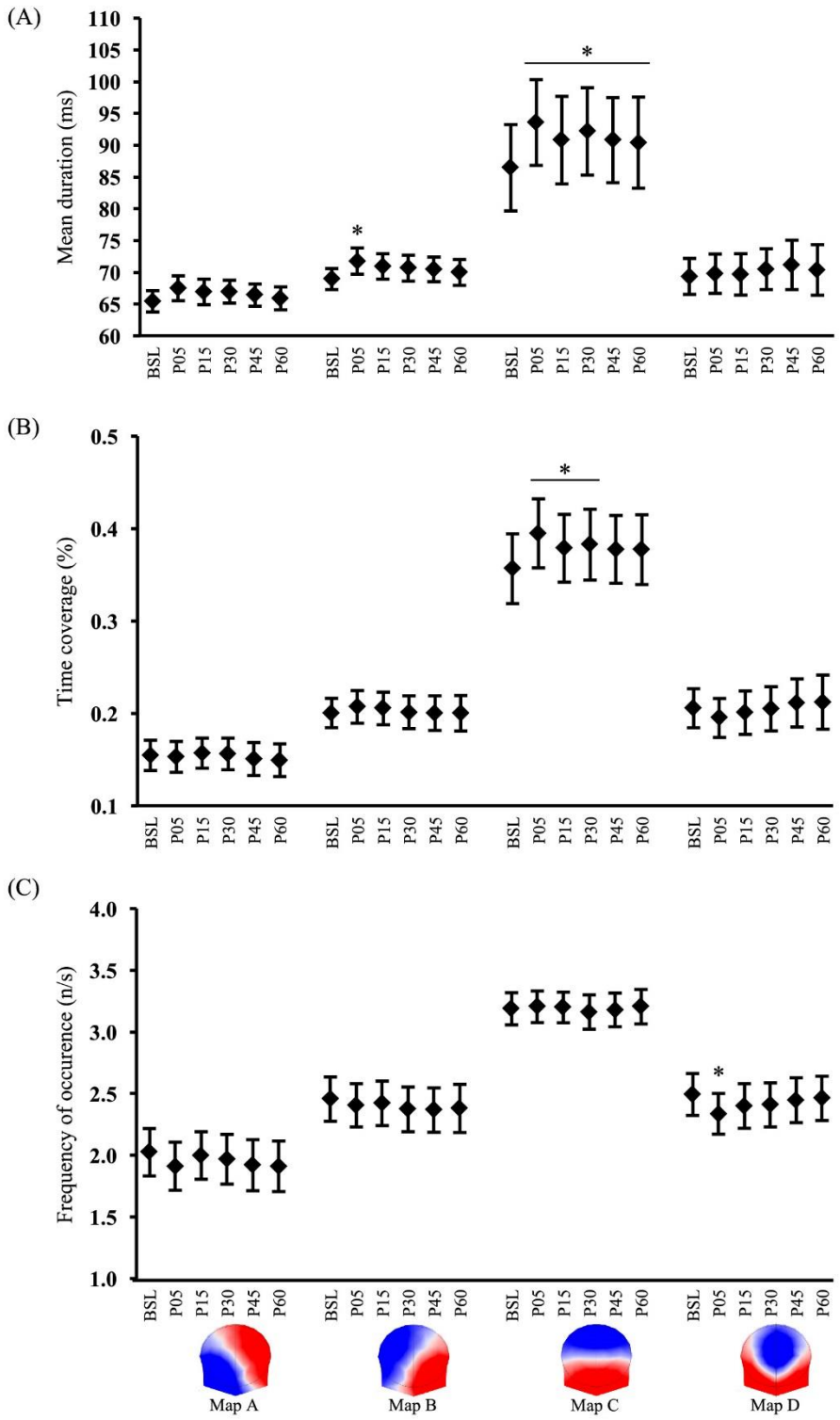

Figure 3: Microstate analysis results. Time course of microstates **(A)** mean duration, **(B)** time coverage, and **(C)** frequency of occurrence, computed in baseline (BSL) and 5 (P05), 15 (P15), 30 (P30), 45 (P45), and 60 (P60) minutes after exercise cessation. Error bars indicate the 95% confidence interval. * indicates a significant difference from BSL; p-value < 0.05.

The rmANOVA showed a significant 6(*TIME*) x 12(*PAIRS*) interaction for the observed transition percentage ($F(55,2035) = 2.86$, $p < 0.001$) (Figure 4). Compared to BSL, the transition percentages between A-C, B-C, C-B and D-C was higher in P05 (all $p \leq 0.001$). During the 60 minutes post-exercise, the transitions between A-C, B-C and D-C remained significantly different from BSL ($p \leq 0.04$) and the transition between B-C in P60 was different from P05 ($p = 0.02$). When the syntax was corrected for changes in occurrence, we still found a significant interaction ($F(55, 2035) = 1.69$, $p = 0.001$) (Figure 4). The follow-up tests showed an increased transition between B-C ($p < 0.001$) and D-C ($p = 0.046$) in P05. In P60, the transition between D-C was also different from BSL ($p = 0.02$).

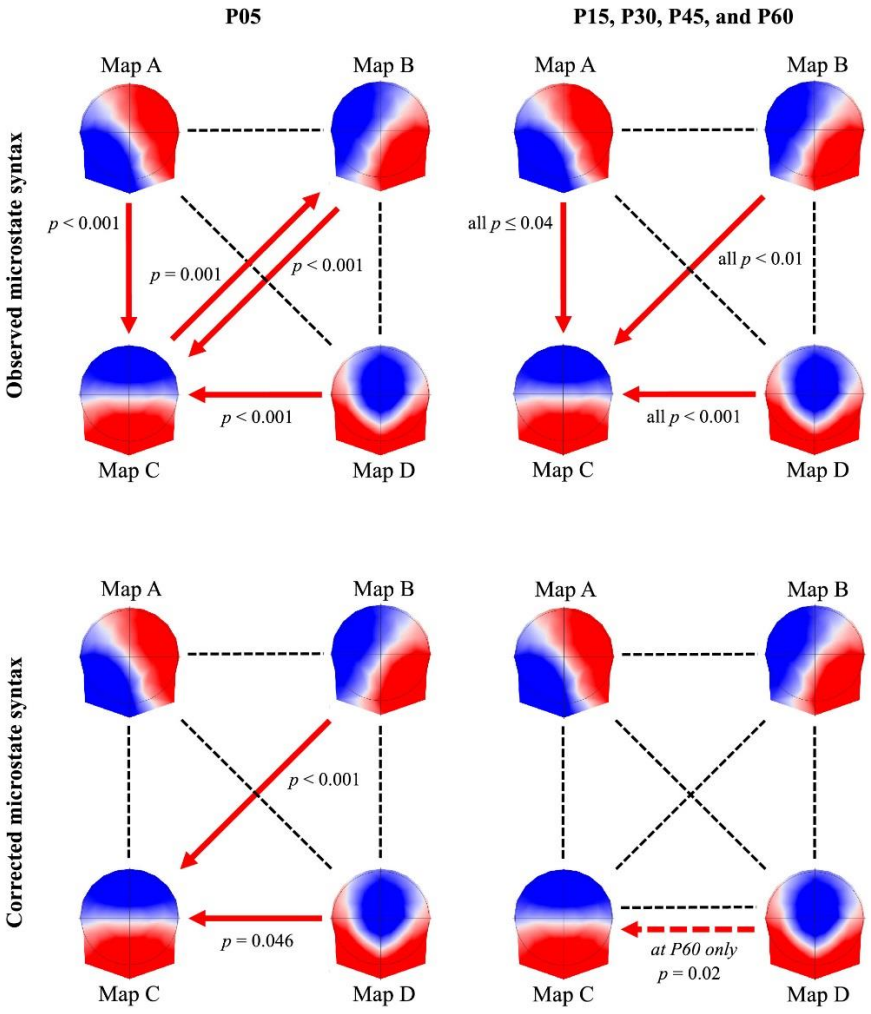

Figure 4: Microstate syntax. Microstate syntax changes from baseline, reported 5 minutes (P05) after exercise cessation (first column), and from 15 minutes (P15) to 60 minutes (P60) post-exercise (second column). The first row shows the observed microstate syntax between the four microstates, whereas the second row shows the

microstate syntax independent for changes in microstate occurrence. The red arrows indicate the direction of the significant changes. Note that the dotted red arrow indicates a significant difference in P60 condition only.

3.5. HRV and EEG correlations

Pearson correlations indicated that the exercise-induced microstate map C (mean duration, time coverage, and frequency of occurrence) and HRV parameters (mean HR, RMSSD, nLF, nHF, LF/HF ratio) changes were not correlated between BSL and P05(ΔBSL-P05), and between P05 and P60 (ΔP05-P60). However, between BSL and P60 (ΔBSL-P60), the microstate map C mean duration was significantly correlated with the ΔBSL-P60 mean HR ($r = 0.42$, $p < 0.05$) (Figure 5, panel A). Because the HR recovery is related to the level of fitness, we decided to correlate *a posteriori* the ΔBSL-P60 mean HR with the Sport index collected with the Baecke questionnaire. We found a significant negative correlation ($r = -0.49$, $p < 0.05$), indicating that people who reported to be more active before starting the protocol are those who better recover (Figure 5, panel B).

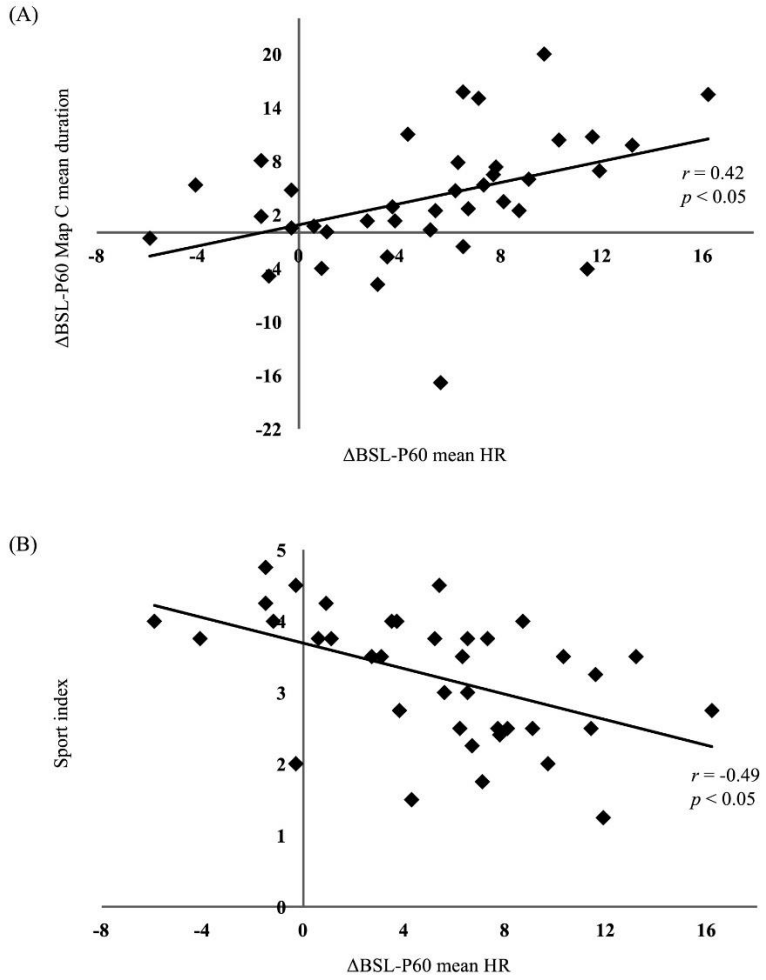

Figure 5: Correlation results. (A) Pearson correlation computed on the absolute differences (ΔBSL-P60) between baseline (BSL) and the post-60 minutes exercise condition (P60) for mean heart rate (HR) and microstate map C mean duration. The significant positive association indicates that the higher the difference in heart rate at P60, the longer the duration of map C remains. **(B)** Pearson correlation computed on the differences between BSL and P05 (ΔBSL-P05) for mean HR and the Sport index collected with the Back questionnaire. Note that people who have a lower Sport index are those who have a greater HR difference, and thus have less well recovered 60 minutes after the end of exercise.

4. Discussion

The present study aimed to describe the resting EEG microstate and HRV changes after a single-bout of physical exercise as well as the specific implication of the microstate map C after exercise. In accordance with a similar study conducted in trained male athletes (Spring et al., 2017), our results partly confirm that an acute submaximal endurance exercise of approximately 30 minutes modulates the microstate map C mean duration and time coverage, and is accompanied by an increased probability of transition mainly toward map C. The new outcome is that the microstate temporal change is not transient, but persists for at least one hour after

exercise cessation, suggesting a long-lasting effect of exercise on this specific microstate. In contrast to Spring et al. (2017), a short-term effect of exercise was found for the duration of map B and the frequency of occurrence of map D. According to the literature, the mean HR, RMSSD, nLH, nHF, and LF/HF ratio parameters were modulated by exercise. Whereas the LF/HF ratio returned to the pre-exercise value 45 minutes after the end of exercise, the mean HR, RMSSD, nLF, and nHF were still altered in P60, indicating an incomplete recovery of the autonomic balance after one hour. The correlation analyses performed on the delta between BSL and P60 showed a significant relationship between the microstate map C mean duration and mean HR, indicating that people who better recover in term of HR are also those who better recover in term of map C mean duration. In summary, when young adults performed a submaximal constant-load exercise of approximately 30 minutes, one hour is not sufficient for both the autonomic cardiovascular activity and resting microstate to return to baseline.

4.1. Microstate data

Acute exercise increased the microstate map C duration and time coverage without modifying the frequency of occurrence in the immediate post-exercise period. These modulations are in line with the results reported by Spring et al. (2017). Similarly, the increased transition probability to move from map A, B, and D to the map C, initially described by Spring et al. (2017), was also found in the present study confirming that the map C becomes a predominant attractive microstate after exercise. During the hour following exercise cessation, even if the map C duration slightly decreased, the value was still significantly different from the pre-exercise condition, indicating that the microstate changes had not fully recovered.

During the immediate post-exercise measurement, we reported a transient increase in map B mean duration, a reduction in map D occurrence, and an increased transition from map B and D to the map C. This short-term microstate temporal reorganization in P05 was different from the long-lasting microstate configuration characterized by persistent changes in map C temporal properties only.

The literature on exercise and EEG microstates is almost nonexistent, making difficult the interpretation of these findings. However, as microstate may correspond to particular classes of mentation, and perceptual and behavioral performances may vary as a function of ongoing microstate activity (Britz and Michel, 2011), we questioned if post-exercise brain modulations could comprise a kind of neural substrate underlying cognitive changes. In a similar exercise protocol consisting of 35 minutes of cycling at 90% of the ventilator threshold (i.e., at a HR between 142 to 152 beats/min and RPE between 12 and 15), Audiffren et al. (2008) reported

changes in cognitive performance (i.e. information processing) only in the post-exercise period (between 1-6 minutes), but no effect after 15 minutes of recovery. Generally speaking, exercise of moderate intensity (40-80% VO_{2max}) can have a positive effect on cognitive performance (Lambourne and Tomporowski, 2010; Chang et al., 2012; McMorris and Hale, 2015), including visual task performance (Bullock and Giesbrecht, 2014; Chang et al., 2012; Lambourne and Tomporowski, 2010). To explain this improvement, Bullock and Giesbrecht (2014) referred to the theory of visual attention (Bundesen et al., 2011), in which the size of the neural assemblies mobilized to encode a relevant object appear to be determinant. As microstate B has been associated with the visual RSN (Britz et al., 2010), the increase in the map B duration after exercise may reflect an increased stability within the related neural assemblies, proving supplementary brain resources that might be mobilized for a visual task. In the other hand, the better availability of attentional resources suggests a beneficial effect on cognition, and it has been hypothesized that the improvement in simple information processing after acute moderate exercise relies on increased arousal (Davranche and Audiffren, 2004; McMorris and Graydon, 2000; McMorris et al., 2015; Tomporowski, 2003). As microstate D has been associated with the dorsal attentional RSN and is likely to be involved in switching and reorientation of attention (Britz et al., 2010; Corbetta and Shulman, 2002), we thus questioned if modulation in map D occurrence might reflect modulations in complex switching attentional processes. Obviously, the above interpretation is merely speculative as no cognitive measurements have been undertaken. Nevertheless, it raises interesting perspectives about the brain mechanisms that may underlie post-exercise cognitive changes. Investigating the association between microstates and cognitive performance after exercise deserve to be explored.

4.2. HRV data

Sympathetic and parasympathetic HRV parameters were modulated immediately following exercise. RMSSD and nHF decreased, reflecting a parasympathetic withdrawal, whereas the increased in the LF/HF ratio indicated of a shift towards sympathetic overdrive (Malik, 1996). The amount of time required for the autonomic cardiovascular system to recover depends on exercise type (Esco et al., 2015), intensity and duration (Hautala et al., 2001; Seiler et al., 2007). In the present study, 25 minutes at an intensity perceived as “hard” resulted in modifications in the HRV that lasted at least 1 hour. These findings agree with previous research conducted in young adults using a similar exercise protocol. Esco et al. (2015) found that after a cycling exercise of 30 minutes at slightly lower intensity (65% VO_2 reserve), HRV parameters (nHF, nLF, LH/HF) were still different from baseline 30 min after exercise cessation. Terziotti et al.

(2001) implemented a steady-state exercise of 20 minutes on a cycle ergometer at 50% or 80% of the anaerobic threshold and measured the HRV parameters up to 180 minutes after exercise. After 60 minutes, even if some subjects displayed signs of sympathetic activation, no significant differences were observed in the HRV parameters compared to baseline. The authors concluded that 1 h of rest is sufficient to fully recover after an exercise bout of 20 minutes at 50% of the anaerobic threshold, and just sufficient to recover after an exercise bout at 80% of the anaerobic threshold. In our study, the intensity was similar to the 80% condition, but the duration was longer, which certainly extended the time required to fully recover. As the nHF and RMSSD remained lower and the nLF greater during the entire recovery period, we infer that both autonomic components were responsible for the persistent changes in HR.

4.3. Microstate and HRV correlations

In contrast to resting EEG studies showing an association between the electrocortical signal and a specific component of the autonomic cardiovascular system (Duschek et al., 2015; Triggiani et al., 2016), the microstate parameters changes after exercise (ΔBSL-P05) and during recovery (ΔP05-P60) were not correlated with HRV. Dushek et al. (2015) reported a significant correlation between the R-wave-to-pulse interval, which is thought to reflect the sympathetic control of HR, and frontal beta power (13-30 Hz). The correlation was rather weak (r = -0.28) considering the sample size (50 subjects), and no correlations were found between the EEG power and the respiratory sinus arrhythmia (RSA, variation in R-R interval during the breathing cycle) or the baroreflex sensitivity, two other parameters reflecting the cardiovascular control. Using EEG source localization method, Triggiani et al. (2016) found a negative correlation (r = -0.42) between the resting central Rolandic cortical source in the low-frequency beta band (13-20 Hz) and the LF power, suggesting that Rolandic beta rhythms are related to sympathetic activity. In an interventional task, Chang et al. (2011) investigated the relationships between changes in the spectral EEG and the HRV when the body position is changed from the supine to the upright position. When passing from the supine to the upright position, the theta rhythms (0.4-8 Hz) were associated with the HR, the alpha (8-14 Hz) and beta (14-35 Hz) bands with the vagal modulation, and the gamma band (35-45 Hz) with the sympathetic modulation. Even if an association between the electrocortical activity and some cardiovascular parameters can be identified at rest, this relationship seems to be tenuous, especially after physical exercise. The variability of the exercise-induced physiological response and the different temporal fluctuation of the heart and the brain activities probably make this interaction difficult to observe.

Interestingly, the microstate C duration and almost all HRV parameters remained higher one hour after exercise cessation, confirming that both the autonomic cardiac activity and the resting EEG microstate did not fully recover. Furthermore, a significant correlation was found on ΔBSL-P60 between the mean HR and microstate map C mean duration. After 1 hour of passive recovery, the higher the difference in HR, the greater the difference of microstate map C mean duration. This correlation is in accordance with the functional association between the HRV and the main brain nodes of the salience network. Several neuroimaging studies have related HRV with fluctuations in brain connectivity within different neural structures including the insula and cingulate (Critchley et al., 2003; Lane et al., 2009; Napadow et al., 2008; Thayer et al., 2012). For instance, Chang et al. (2013) described covariations between resting HRV and several brain regions, including the dorsal anterior cingulate cortex and anterior insula, whereas Critchley et al.(2003) reported that the dorsal anterior cingulate is involved in autonomic control during cognitive processing, but also during a motor task consisting of isometric handgrip contractions. Consequently, we infer that the post-exercise microstate C and mean HR recovery may rely on the functional connection between the salience RSN and the autonomic cardiovascular system.

The post-exercise autonomic cardiovascular reactivation is thought to largely depends on accumulation of stress metabolites (e.g. H^+, lactate) likely driven by metaboreceptor feedbacks (Boushel, 2010; Coote, 2010; Hartwich et al., 2011; Peçanha et al., 2014, 2016; Stanley et al., 2013). The reflex inputs from metaboreceptors and mechanorecptors in the active muscles has been postulated as an important mechanism in cardiovascular and ventilatory regulation (Fisher et al., 2013; Kaufman and Hayes, 2002; Secher and Amann, 2012). The mechanical and chemical stimuli associated with muscle contraction activate the terminal ends of small-diameter type III and IV afferent fibers that stimulate the lamina I neurons within the spinal cord. From the superficial layer of the dorsal spinal horn, an afferent pathway caries information through the lateral spinothalamic tract to the thalamus (Craig, 2002, 2003), where projections relay the information from thalamic nuclei to the mid/posterior dorsal insula that in turn project to AI (Menon and Uddin, 2010). The implication of this feedback was supported by several experimental manipulations. For instance, the lowering of the afferent feedback during exercise by a pharmacological blockade attenuate the increase in blood pressure and cardiac output (Secher and Amann, 2012). In post-exercise condition, when a blood flow restriction is applied in order to occlude the blood supply and to trap the metabolites in the active muscle (i.e. post-exercise ischemia), the exercise-induced increase in HR (Fisher et al., 2013; Macefield and Henderson, 2015), sympathetic activity (Victor and Seals, 1989), and/or withdrawal of cardiac

parasympathetic tone (Fisher et al., 2013) do not return to baseline. Furthermore, the autonomic response to post-exercise ischemia indicates an increase in BOLD signal within the insular cortex, and a contribution of an afferent feedback associated with the exercise-induced physiological changes has been postulated (Macefield and Henderson, 2015; Sander et al., 2010). Although the intramuscular metabolites have not been quantified in the present article, previous study reported higher circulating lactate value 50 minutes after eight, 5-min bouts of cycling at 80% of maximum workload, suggesting long-lasting metabolic disturbance (Sidhu et al., 2008). Yet, depending on the blood acidosis and blood lactate level, the post-exercise HR recovery would be delayed (Ba et al., 2009). Taken together, modifications in afferent activity after exercise may participate in modulating the autonomic cardiovascular response and recovery, and potentially the microstate map C temporal properties.

5. Conclusion

The present study investigated the resting EEG microstates and HRV before and during one hour after a constant-load cycling exercise perceived as “hard” in healthy males and females. Acute exercise modulates the microstate C duration with an effect that persists for at least one hour after exercise, suggesting that this specific topography may be considered as an electrocortical index of exercise-related brain modulation. As for the duration of map C, the markers of cardiac autonomic activity (mean HR, RMSSD, nHF, nLF) were modulated immediately after exercise and for one hour post-exercise. The HR recovery correlates with the recovery of microstate C duration, indicating a possible interaction between the cardiovascular system and microstate map C. The functional association between HRV and salience network brain nodes reported in the literature (Thayer et al., 2012) further support our finding. We assumed that the microstate C and autonomic cardiovascular activity changes are likely mediated by a common increase in exercise-induced afferent activity. The metabolic byproducts of fatiguing muscular exercise stimulates the metaboreceptor afferents and may modulate the autonomic regulation of heart rate after exercise. As the autonomic and muscle afferents seems to converge to the salience RNS nodes (Craig, 2003; Menon, 2015), an increase in afferent neural traffic after exercise might be considered as a mediator of both the autonomic regulation of heart rate and the resting EEG microstate.

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