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Examining the relationship between fitness, cortical excitability, and neurochemistry of the brain (GABA, glutamate, and NAA)

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Ce mémoire intitulé

Examining the relationship between fitness, cortical excitability, and neurochemistry of the brain (GABA, glutamate, and NAA)

Présenté par

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Résumé

L'exercice aérobique (AE) est associé à de nombreuses modifications fonctionnelles et anatomiques dans le cerveau humain. Par exemple, il a été démontré que l'EA modulait l'excitabilité corticale et la neurochimie immédiatement après l'exercice. Les effets d'une activité physique répétée et soutenue sur les fonctions cérébrales restent toutefois mal compris. En effet, peu de données sont disponibles permettant de déterminer si les personnes ayant une bonne condition physique présentent des modifications persistantes de l'excitabilité corticale et du métabolisme cérébral malgré les changements rapportés dans la matière grise et la matière blanche. Dans la présente étude, 20 personnes sédentaires en bonne santé (< 2 heures/semaine d'activité physique) ont été comparées à 20 personnes actives (> 6 heures/semaine d'activité physique) sur la base de mesures de l'excitabilité corticale (rMT, courbe I/O, SICI, ICF) et de la concentration de métabolites (GABA, Glx, NAA) dans la représentation corticale de la main droite. L'épaisseur corticale de la représentation du cortex moteur primaire de la main droite et la densité apparente des fibres de la voie corticospinale (CST) ont également été évaluées. L'aptitude cardiorespiratoire (VO_{2max}) était significativement plus élevée chez les athlètes que chez les sédentaires, ce qui n'était pas le cas de l'indice de masse corporelle. Aucune différence entre les groupes n'a été constatée en ce qui concerne les mesures du rMT, du SICI et de l'ICF. Les valeurs de la courbe I/O étaient significativement plus élevées et la courbe I/O était plus prononcée chez les individus actifs. Aucune différence significative n'a été observée pour l'épaisseur corticale, la concentration de métabolites et les valeurs de diffusion de la CST. La pente de la courbe I/O était positivement corrélée à la VO_{2max}. Les présentes données suggèrent que des niveaux élevés de capacité aérobique sont associés à une excitabilité corticale accrue dans la représentation de la main du cortex moteur primaire.

Mots clés: Condition physique aérobie ; VO_{2max} ; Excitabilité corticospinale ; Imagerie par résonance magnétique de diffusion ; Spectroscopie par résonance magnétique ; Cortex moteur

Abstract

Aerobic exercise is associated with widespread functional and anatomical modifications in the human brain. For example, AE has been shown to modulate cortical excitability and neurochemistry immediately after exercise. The effects of repeated and sustained physical activity on brain function, however, remain poorly understood. Indeed, little is known about whether individuals with high levels of fitness display persistent modifications in cortical excitability and brain metabolism despite reported changes in grey and white matter. In the present study, 20 healthy sedentary individuals (< 2 hours/week AE) were compared to 20 active individuals (> 6 hours/week AE) on measures of cortical excitability (rMT, I/O curve, SICI, ICF) and metabolite concentration (GABA, Glx, NAA) in the cortical representation of the right hand. Cortical thickness of the primary motor cortex representation of the right hand and corticospinal tract (CST) apparent fiber density (AFD) were also assessed. Cardiorespiratory fitness (VO_{2max}) was significantly higher in athletes compared to sedentary individuals whereas body mass index was not. No group differences were found on measures of rMT, SICI and ICF. I/O curve values were significantly higher, and the I/O curve was steeper in active individuals. No significant differences were observed between the groups for cortical thickness, metabolite concentration and CST diffusion values. I/O curve slope was positively correlated with VO_{2max}. The present data suggest that high levels of aerobic fitness are associated with increased cortical excitability in the hand representation of the primary motor cortex.

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Abbreviations and acronyms

AE: aerobic exercise

AFD: apparent fiber density

BDNF: brain-derived neurotrophic factor

BMI: body mass index

CRF: cardiorespiratory fitness

CSE: corticospinal excitability

CSF: cerebrospinal fluid

CST: corticospinal tract

CS-TS: conditioning stimulus- test stimulus

CT: cortical thickness

dMRI: diffusion magnetic resonance imaging

DTI: diffusion tensor imaging

DWI: diffusion-weighted imaging

EMG: electromyographic

FA: fractional anisotropy

FDI: first dorsal interosseus

FOV: field of view

GABA: γ-Aminobutyric acid

Glx: glutamate + glutamine

GM: grey matter

ICF: intracortical facilitation

I/O curve: input/output curve

M1: primary motor cortex

MEP: motor evoked potential

MRI: magnetic resonance imaging

MRS: magnetic resonance spectroscopy

NAA: N-acetylaspartate

PA: physical activity

rMT: resting motor threshold

ROI: region of interest

SICI: short interval cortical inhibition

TMS: transcranial magnetic stimulation

TE: echo time

TI inversion time

TR: repetition time

VO_{2max}: Maximal oxygen consumption

WM: white matter

Dedication and acknowledgement

I would like to thank my family and friends for all the supports I received during my master's journey. For all the laughter, relaxing moments, and encouragements that motivated me along the way and brought me here. I would also like to thank my supervisors François and Hugo for their supports. My master's was a great learning experience both stimulating and rewarding that I would like to describe using John Dewey's quote: "I believe finally, that education must be conceived as a continuing reconstruction of experience; that the process and the goal of education are one and the same thing."

Introductory Chapter

It is well known that physical activity (PA) is associated with a diverse range of physical and mental health benefits. Just to name a few, PA reduces risks of cardiovascular diseases such as coronary heart disease and stroke (Lavie et al., 2015). It has also been shown to contribute to the prevention and management of cancer (Cohen et al., 2020; Thomson et Garcia et al., 2014) and diabetes (Yen et al., 2022). In terms of mental health benefits, it lowers the risks of dementia (Tari et al., 2019), depression, and anxiety (Martinsen et al., 2008). Parts of its benefits are believed to be due to its positive effect on cardiorespiratory fitness (CRF) demonstrated by a more effective delivery of oxygen throughout the circulatory and respiratory systems (Voss et al., 2016; Barnes and Corkery, 2018). Currently, the golden standard to measure CRF is the VO_{2max} or maximal oxygen consumption. VO_{2max} is determined using a protocol during which an individual is put to a supramaximal exercise beyond which no additional increments are present (Hawkins et al., 2007). CRF measured by VO_{2max} is positively associated with brain function and structure which could explain the beneficial effect of PA on cognition, particularly in older adults (Espana-Irla, 2021).

Physiological effects of AE

The physiological effect of PA has been examined in acute aerobic exercise (AE) protocols. Numerous studies have shown an increase in corticospinal excitability (CSE) immediately after AE using transcranial magnetic stimulation (TMS) measures (MacDonald et al., 2019; Opie and Semmler, 2019; Nicolini et al., 2020; for review see Nicolini et al., 2021). This effect is seen both in exercised and non-exercised muscles (Yamazaki et al., 2019). Notably, the intensity of AE and the fitness level of the participants are mediating factors. Low intensity and moderate intensity AE are sometimes effective at inducing CSE changes (McDonnell et al., 2013; Opie et al., 2019; MacDonald et al., 2019) whereas high intensity AE is more consistent in inducing CSE modulation (Andrews et al., 2020; Nicolini et al., 2020). Nicolini and collaborators (2021) suggested that in order to see an effect on CSE, low fitness participants might require a higher intensity AE than athletic participants. For intracortical excitability, results have been inconsistent for acute AE studies. Whereas some studies have reported reduced GABA-mediated short-interval intracortical inhibition (SICI; Stravinos & Coxon, 2017; Opie & Semmler, 2019)

and increased glutamate-mediated intracortical facilitation (ICF; Neva et al., 2017; Morris et al., 2020), others have not (Mooney et al., 2016; Andrews et al., 2020).

Beyond acute AE protocols, studies have also examined whether the reported physiological effects persist permanently in individuals engaging in regular PA. Compared to sedentary individuals, athletes have a 35% steeper input/output (I/O) curve, as first reported by Cirillo and collaborators (2009). In other words, regular AE is associated with a higher baseline level of CSE suggesting that the effect of acute AE observed in laboratory persisted in high fitness individuals. Later, opposite findings were reported with lower I/O curve amplitude and slope in groups of young and old adults with high compared to low PA (Hassanlouei et al., 2017). Hand et al. (2021) reported no effect of long-term endurance training on CSE in cyclists. Additionally, VO_{2max} is not correlated with CSE in postmenopausal women (Harasym et al., 2020), and I/O curve amplitudes in young adults (MacDonald et l., 2019). For intracortical excitability, Harasym et al. (2020) reported a significant correlation between TMS measures and VO_{2max} whereas no association was found for measures of SICI and ICF in a sample of postmenopausal women. Similarly, Hand and collaborators (2021) reported no effect of AE training on SICI and ICF.

Taken together, data suggests that acute AE increases CSE but has no effect on intracortical excitability. The long-term physiological effects of regular PA, however, remain to be fully characterized.

Anatomical effects of AE

Many studies have investigated the anatomical correlates of CRF, namely white matter (WM) and grey matter (GM). Starting with WM, a positive correlation between VO_{2max} and fractional anisotropy (FA) in a number of white matter tracts in older adults has been reported, including the left middle cingulum segment (Marks et al., 2011), the corpus callosum (Johnson et al., 2012), and pre-motor and sensory tracts (Harasym et al., 2020). This suggests that CRF is associated with white matter microstructure. In addition, compared to low fit, sedentary individuals, older fit adults with a history of engaging in PA also show higher FA (Tseng et al.,

2013; Carson-Smith et al., 2016). However, AE intervention programs ranging from 12 weeks to 5 years have failed to modify white matter microstructure in this population (Voss et al., 2013; Clark et al., 2019; Sexton et al., 2020; Pani et al., 2022). Studies of younger adults are scarcer. Hayes and collaborators (2015) found that VO_{2max} was correlated with FA in a number of WM tracts in older adults (55-82) but not in young adults (18-31 years). Subsequently, Mace and collaborators (2021) suggested that the benefits of PA on brain structure and function increase with advancing age, permitting a better response to cognitive decline. This was tested in a study examining the link between CRF and WM microstructure in large groups ($n = 499$) of older (60-85 years) and younger (20-59) adults. Findings supported their hypothesis with a greater and more distributed association between VO_{2max} and FA in older compared to younger participants. One study has focused on the younger population examining the link between WM microstructure and CRF in adolescents between the age of 15-17 years (Herting et al., 2018). In line with previous studies, participants that reported high levels of fitness did not show differences in FA compared to low-fitness individuals, nor was VO_{2max} correlated with FA values. However, the number of streamlines was higher in high-fit individuals in the corticospinal tract (CST) and FA correlated negatively with VO_{2max} in the CST. Interestingly, the significant correlations between WM tracts and VO_{2max} max in younger adults included the corticospinal tract in the study of Mace and collaborators (2021).

With regards to the association between GM structure and CRF, the older adult population is again better documented than its younger counterpart. In studies of older adults, findings generally support a positive association between CRF and GM volume and thickness (e.g. Colcombe et al., 2006; Erickson et al., 2010,2011; Hayes 2014; Wood et al., 2016; Jonasson et al., 2017; Williams et al., 2017; Wittfeld et al., 2020; Nicolini et al., 2021). On the other hand, results are mixed for younger and middle-aged adults. In a large study of young adults (22 -36 years old), no association between measures of fitness and cortical thickness (CT) was reported (Best et al., 2020). However, another study found a negative correlation between VO_{2max} and CT in the occipital cortex, the parietal cortex, and the cuneus of young adults 16 to 25 years old (Wade et al., 2020). For middle-aged healthy adults, Tarumi and collaborators (2021) reported an absence of correlation between VO_{2max} and CT. Nevertheless, a large-scale study of 1037 participants aged 45 years found a positive correlation between VO_{2max} values and CT in the

temporal cortex, prefrontal cortex, and parahippocampal gyrus (d'Arbeloff et al., 2020). Similarly to WM microstructure, age seems to be a mediating factor in the relationship between CRF and CT. William and collaborators (2017) reported a positive correlation between VO_{2max} and CT in older healthy adults (55-82 years), whereas a negative correlation was found in younger adults (18-31years) in areas that included the precentral gyrus. Likewise, a population-based cohort study reported a stronger association between GM volume and cardiorespiratory fitness in individuals 45 years and older (Wittfeld et al., 2020). When directly comparing sedentary and athletic middle-aged adults (45-64 years), a thicker cortex was found in the precentral, postcentral, and visual cortex in athletic individuals (Tarumi et al., 2021).

Taken together, PA-related anatomical changes in cortical structure (GM and WM) seem to be moderated by age. Beneficial effects on WM microstructure and CT are more consistently reported in older adults.

Metabolic effects of AE

Studies using magnetic resonance spectroscopy (MRS) have examined the effects of PA on neurotransmitter systems. Initial findings showed that exercise led to acute increases in lactate concentration in supraventricular (Dalsgaard et al., 2004) and occipital (Maddock et al., 2011; Dennis et al., 2015) cortex whereas no changes were reported for N-acetylaspartate (NAA), creatine and choline (Caglar et al., 2005; Maddock et al., 2011). Later studies examined mainly three neurotransmitters: NAA, γ-Aminobutyric acid (GABA), and glutamate + glutamine (Glx).

Starting with NAA, while acute exercise did not affect its concentrations in a sample of teenagers (Caglar et al., 2005), studies have shown that chronic exercise does have an effect. In endurance athletes, higher NAA levels were found compared to sedentary individuals in the frontal cortex, but no difference was reported in the occipitotemporal cortex (Gonzales et al., 2013). In older adults, lifetime physical activity is associated with NAA/choline ratio and better neuronal integrity (Engeroff et al., 2019). Additionally, Erickson and collaborators (2012) found that age-related decreases in NAA were less important in individuals with higher VO_{2max}, which was also associated with better working memory. This supports the idea that higher levels of

fitness mitigated the effects of aging on NAA concentration in the frontal cortex which could have beneficial effects on cognition.

Exercise also seems to affect GABA concentrations. Following intense exercise, an increase in GABA levels was reported in the visual cortex (Maddock et al., 2016). More closely related to TMS data, the impact of PA has been investigated in the sensorimotor cortex as well. With an MRS voxel centered on the hand representation of the primary motor cortex, where TMS measures are obtained, Coxon et al. (2018) reported an acute increase in GABA concentration immediately following a single session of high-intensity interval training in young adults. However, the fitness level measured with VO_{2max} was not found to correlate with GABA in a sample of postmenopausal women with a similar voxel position over the primary motor cortex hand area (Harasym et al., 2020). Those preliminary data suggest a differential effect of acute and chronic PA on GABA levels.

For glutamate, Maddock et al. (2016) reported an increase immediately after exercise. Later studies did not find any significant change in Glx level following exercise (Dennis et al., 2015; Coxon et al., 2018). In terms of long-term PA, a negative correlation between glutamate and a proxy measure of fitness was found in one study (Dennis et al., 2015). However, in another study examining a sample of postmenopausal women, glutamate level was not correlated with VO_{2max} (Harasym et al., 2020).

Taken together, MRS studies suggest that long-term PA potentially increases NAA levels, but its effect on GABA and Glx concentrations remains understudied and unclear.

Objective

In the current literature, it remains unclear whether the physiological, anatomical, and metabolic effects of acute PA, more precisely acute AE, persist with long-term practice. Furthermore, their effects remain poorly understood in younger individuals. The present study aimed to characterize the neurophysiological, neuroanatomical, and neurometabolic correlates of intense, repeated physical activity in young adults. Fit, young adults were compared with sedentary individuals and analysis was restricted to the hand representation of the primary motor cortex to ensure

appropriate comparisons between different measures. Neurophysiological effects were examined using TMS: more precisely corticospinal (input/output curve) and intracortical (SICI, ICF) excitability. Magnetic resonance imaging (MRI) was used to measure neuroanatomical variables, namely white matter microstructure (FA, apparent fiber density: AFD) and grey matter structure (cortical thickness). In terms of neurometabolic variables, MRS was used to assess the concentration of three metabolites: NAA, GABA, Glx.

Hypotheses

Our hypotheses are based on the literature which suggests that individuals with high fitness levels have a neurophysiologic and anatomical profile that differs from that observed following an acute bout of exercise. In other words, the effects observed in the literature after an acute bout of exercise are not preserved in young athletic individuals at rest. Since neurometabolic change depends on the neurotransmitter measured, different hypotheses are formulated regarding NAA, GABA, and Glx levels. More precisely, when comparing individuals with high levels of fitness compared to sedentary individuals we expect:

Primary hypotheses

1) Higher levels of corticospinal excitability (TMS: input/output curve)

2) Similar levels of intracortical excitability (TMS: SICI, ICF)

3) Similar FA, AFD and CT values (MRI: GM, WM)

4) Higher levels of NAA (MRS)

5) Modification of GABA and Glx concentrations

Secondary hypotheses

1) Cardiovascular fitness (VO_{2max}) will be positively correlated with corticospinal excitability (I/O curve)

2) Cardiovascular fitness (VO_{2max}) will be positively correlated with NAA concentration (MRS).

Article

Physiological, anatomical, and metabolic correlates of aerobic fitness in human primary motor cortex: a multimodal study

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Introduction

Physical activity (PA) is associated with a variety of health benefits that include lower risk of cardiovascular disease (Lavie et al., 2015), reduced risk of dementia (Tari et al., 2019) and better cognition (Ludyga et al., 2016). The benefits of PA can be partly explained by its effect on cardiorespiratory fitness (CRF; Voss et al., 2016; Barnes and Corkery, 2018), which reflects the delivery of oxygen to circulatory and respiratory systems. The gold standard in assessing CRF is VO_{2max}, which is a measure of the maximum amount of oxygen used during supramaximal exercise beyond which no additional increments are present (Hawkins et al., 2007). An association between VO_{2max} and brain function and structure has been shown and may mediate the effect of PA on cognition, particularly in older adults (Espana-Irla, 2021).

Numerous studies have shown that a single session of aerobic exercise (AE) can increase corticospinal excitability (CSE), measured with transcranial magnetic stimulation (TMS; e.g. MacDonald et al., 2019; Opie and Semmler, 2019; Nicolini et al., 2020; for review see Nicolini et al., 2021), in exercised and non-exercised muscles (Yamazaki et al., 2019). These effects are dependent upon the intensity of the exercise (El-Sayes et al., 2020) and fitness level of the individual (Lulic et al., 2017; El-Sayes et al., 2020). Intracortical excitability has also been investigated in response to acute AE, but results have been inconsistent. Whereas some studies have reported reduced GABA-mediated short-interval intracortical inhibition (SICI; Stravinos & Coxon, 2017; Opie & Semmler, 2019) and increased glutamate-mediated intracortical facilitation (ICF; Neva et al., 2017; Morris et al., 2020), others have not (Mooney et al., 2016; Andrews et al., 2020).

Whether the reported effects of acute AE in primary motor cortex persist through regular physical activity remains unclear. Cirillo and collaborators (2009) first reported that the slope of the input/output (I/O) curve was 35% steeper in physically active individuals compared to sedentary participants, suggesting increased CSE associated with aerobic fitness. Subsequently, *lower* I/O curve amplitude and slope were reported in groups of young and old adults with high compared to low PA (Hassanlouei et al., 2017). No effect of long-term endurance training on CSE, SICI and ICF has also been reported (Hand et al., 2021). Some studies have assessed the link between aerobic fitness and cortical excitability of the primary motor cortex by correlating

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excitability measures with VO_{2max}. Harasym and collaborators (2020) found no association between VO_{2max} and measures of CSE, SICI and ICF in a sample of postmenopausal women. In young individuals, MacDonald et al. (2019) reported no significant correlation between VO_{2max} and I/O curve amplitudes.

The effects of PA on neurotransmitter systems have also been investigated with magnetic resonance spectroscopy (MRS). Initial findings showed that exercise led to acute increases in lactate concentration in supraventricular (Dalsgaard et al., 2004) and occipital cortex (Maddock et al., 2011; Dennis et al., 2015) whereas no changes were reported for NAA, creatine and choline (Caglar et al., 2005; Maddock et al., 2011). More recently, GABA concentrations were assessed in visual cortex and were found to increase following intense exercise along with glutamate (Maddock et al., 2016). Similarly to what was done with TMS, the long-term effects of regular PA have also been investigated. In older adults, it was reported that higher levels of fitness mitigated the effects of aging on NAA concentration in frontal cortex, where the age-related decreases in NAA were less important in individuals with higher VO_{2max} (Erickson et al., 2012). Similar results were found in endurance athletes, which showed higher NAA levels than sedentary individuals in frontal cortex but no difference in occipitotemporal cortex (Gonzales et al., 2013). More closely related to TMS data, the impact of PA on metabolic activity has been investigated in sensorimotor cortex. With a MRS voxel centered on the hand representation of the primary motor cortex, where TMS measures are obtained, Coxon et al. (2018) reported that a single session of high-intensity interval training in young adults led to acute increases in GABA concentration while no changes were found for Glx. However, with a similar voxel position over the hand area, fitness levels measured with VO_{2max} did not correlate with either GABA or glutamate in a sample of postmenopausal women (Harasym et al. 2020).

The anatomical correlates of CRF have also been investigated. For example, it has been shown that CRF is associated with white matter microstructure in older adults in the form of a positive correlation between VO_{2max} and fractional anisotropy (FA) in a number of white matter tracts (Marks et al., 2011; Johnson et al., 2012; Oberlin et al., 2016; Harasym et al., 2020). The effects of PA on WM microstructure have been less investigated in younger individuals. It has been suggested that the benefits of PA on brain structure and function increase with advancing

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age, permitting a better response to cognitive decline (Mace et al., 2021). This hypothesis was directly tested in a study comparing the link between cardiorespiratory fitness and WM microstructure in large groups ($n = 499$) of older (60-85 years) and younger (20-59) adults (Mace et al., 2021). The association between VO_{2max} and FA was found to be significantly greater and more distributed in older compared to younger participants. Interestingly, the few WM tracts in which VO_{2max} max correlated with microstructure in younger adults included the corticospinal tract (Mace et al., 2021). Similar findings were obtained by Hayes et al. (2015), who showed that VO_{2max} was correlated with FA in a number of WM tracts in older adults (55-82) whereas that was not the case in young adults (18-31 years). Another study in younger individuals assessed the link between WM microstructure and cardiorespiratory fitness in adolescents (15-18 years; Herting et al., 2014). Participants that reported high levels of fitness did not show differences in FA compared to low-fit individuals nor was VO_{2max} correlated with FA values. The number of streamlines, however, was higher in high-fit individuals in the CST and FA correlated negatively with VO_{2max} in the CST.

The effects of PA on grey matter (GM) structure in older adults have also been well documented and generally support a positive association between cardiorespiratory fitness and GM volume and thickness (e.g. Colcombe et al., 2006; Erickson et al., 2010,2011; Wood et al., 2016; Jonasson et al., 2017; Williams et al., 2017; Wittfeld et al., 2020; Nicolini et al., 2021). In younger adults, published data are inconsistent. In a large study of 1065 young adults, Best and collaborators (2020a) reported no association between measures of fitness and cortical thickness (CT), in line with data showing no correlation between VO_{2max} and CT in middle-age healthy individuals (Tarumi et al., 2021). A negative correlation between VO2max and cortical thickness has been reported, however, in young adults (16-25 years) in occipital cortex, parietal cortex and cuneus (Wade et al., 2020) whereas higher VO_{2max} values were shown to be positively correlated with CT in temporal cortex, prefrontal cortex and parahippocampal gyrus in a sample of 1037 healthy participants aged 45 years (d'Arbeloff et al., 2020).

Taken together, studies suggest that an active lifestyle is associated with acute and long-lasting, wide-ranging plastic changes in the healthy human brain, particularly in older individuals. Whether these effects are already present in younger individuals with a history of

regular aerobic exercise is unclear. The aim of the present study was to characterize the neurophysiological, neurometabolic and neuroanatomical correlates of intense, repeated physical activity in young adults. To this end, corticospinal (input/output curve) and intracortical (SICI, ICF) excitability, metabolite concentration (GABA, Glx, NAA), white matter microstructure (FA, apparent fiber density) and grey matter structure (cortical thickness) were assessed in groups of physically active and sedentary young adults. To better relate the different measures of brain function and anatomy, analysis was restricted to the hand representation of the primary motor cortex. Thus, in addition to TMS measures of excitability, metabolite concentrations were measured in a voxel centered on the hand knob region of the precentral sulcus, where CT data were analyzed, and WM diffusion was assessed for the corticospinal tract originating in the hand area of primary motor cortex. Cardiorespiratory fitness was measured with VO_{2max} to validate group membership and allow correlational analysis.

Methods

Participants and procedure

Forty healthy, right-handed (self-reported) young adults were recruited for the study (see Table 1 for sample characteristics and statistics). Following completion of an activity questionnaire, participants were assigned to the *high fitness* group if they engaged in sustained and intense cardiovascular activity for at least 8 hours/week during the last 5 years (*n*=20) or the *low fitness* group if they engaged in sustained and intense cardiovascular activity for 2 hour/week or less during the last 5 years (*n*=20). The activity questionnaire was administered by a member of the research team and included the following information for the last 5 years: periods when participant engaged in PA/sports; types of sports/activities; frequency of PA; average duration of PA. Furthermore, participants were asked if they had engaged in intensive PA or participated in elite sporting activities prior to the 5-year period and if they had engaged in contact sports in their lifetime.

Table 1. Demographic characteristics

	Low-fit	High-fit
Sex	9F/11M	5F/15M
Age	23.3 (2.4)	27.9 (4.1)[*]
Age range	19-35	18-28
Education	16.7 (2.3)	17.6 (2.8)
BMI	23.0 (3.2)	22.5 (2.3)
Cardio (hrs/week)	0.4 (0.6)	11.6 (6.2)[*]
VO2Max	43.1 (7.6)	59.5 (6.3)[*]

Mean (SD); F: female; M: male; *: $p < 0.001$

Exclusion criteria were the following: participation in a contact sport, history of traumatic brain injury, neurological or psychiatric disorders, fainting or seizures, substance abuse, smoking, current psychoactive medication intake and contraindication to MR scanning or transcranial magnetic stimulation. Participation in a contact sport was included as an exclusion criterion because of the known effects of repeated subconcussive hits to the head on brain function and anatomy (Koerte et al., 2012,2015,2016; Di Virgilio et al., 2016). Prior to each experimental session, participants were asked to avoid coffee, alcohol, and strenuous exercise for at least 12 hours. Participants underwent three sessions of testing on separate days: VO_{2max}, neuroimaging and transcranial magnetic stimulation. Experiments were performed with the approval of the local ethics committee (*Comité d'éthique de la recherche vieillissement-neuroimagerie, Centre intégré Universitaire de santé et de services sociaux du Centre-Sud-de-l'Île-de-Montréal*) in accordance with the Declaration of Helsinki. All participants gave written informed consent prior to testing.

Fitness measure: VO_{2max}

The aerobic fitness level of each participant was determined by measuring maximal oxygen uptake (VO_{2max}) using a computerized indirect respiratory calorimetry system (Cosmed, Quark CPET) during an incremental exercise protocol, where treadmill velocity was increased from a

light jog (8-10 km/h) by 1 km/h every two minutes until volitional exhaustion. Averages for oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER; the ratio between carbon dioxide and oxygen) were assessed every 30 s, using a mixing chamber. In addition, heart rate was measured throughout the exercise protocol using a Polar heart rate monitor (Polar WearLink®+ 31, Polar Electro, Finland) and ratings of perceived exertion were assessed every 2 min using the Borg centiMax scale (Borg & Borg, 2002).

VO_{2max} is defined as when oxygen consumption remained at a steady state despite an increase in workload. Relative peak oxygen consumption was based upon maximal effort as evidenced by (1) a plateau in oxygen consumption corresponding to an increase of <2 mL/kg/min despite an increase in workload, (2) a peak heart rate >185 beats per minute (American College of Sports Medicine, 2000) accompanied by a heart rate plateau, i.e., an increase in work rate without a concomitant increase in heart rate (Freedson & Goodman, 1993), (3) RER >1.0 (Bar-Or, 1983), and/or (4) ratings on the OMNI scale of perceived exertion >80 (Borg & Borg, 2002). Participant safety was continually monitored using established safety procedures.

Transcranial magnetic stimulation

Participants were seated comfortably with their arms relaxed and palms facing upwards. Resting motor threshold (rMT), single-pulse TMS and paired pulse TMS data were collected using procedures in compliance with established safety protocols (Rossini et al., 2015). Electromyographic (EMG) activity was recorded using self-adhesive surface electrodes (muscle belly-tendon configuration) placed on the right *first dorsal interosseus* muscle (FDI). A bandwidth of 20-1000 Hz was used to filter EMG signals, which was digitized at a sampling rate of 4 kHz using a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA). MEPs were recorded using LABCHART7 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis. Measures were collected for the left hemisphere.

TMS was delivered with monophasic pulses through an 8-cm figure-of-eight coil connected to a Magstim 200^2 stimulator (Magstim Company Ltd, Spring Gardens, UK). The coil was positioned with the handle pointing backward (posterior-anterior position), flat on the head

at a 45º angle from the midline. The optimal site of stimulation was defined as the coil position which elicited an MEP of greatest amplitude in the FDI muscle (Rossini et al., 2015). This was achieved by first positioning the center of the coil approximately 5 cm lateral to Cz. This position was marked on the scalp and 3 TMS pulses were administered at each location of a grid centered on this point (2cm divisions) until the location at which MEPs were of greatest amplitude was identified. All TMS pulses were delivered at a frequency of 0.1 – 0.2 Hz to avoid inducing changes in cortical excitability (Chen et al., 1997). The resting motor threshold (rMT) was then determined by gradually decreasing or increasing stimulator intensity by 1% steps, starting at 50% maximum stimulator output, until the presence of motor evoked potentials (MEPs) of 50 µV in 5 times out of 10 consecutive stimulations at the optimal stimulation site.

Cortical excitability was assessed by constructing an input-output (I/O) curve, where 20 TMS pulses were delivered at each intensity level based on the participant's rMT (100%, 110%, 120%, 130%, 140%) in a semi-randomized order for a total of 100 MEPs. Changes in cortical excitability were assessed by measuring and averaging peak-to-peak amplitudes of the MEPs at each intensity level. The slope of the I/O curve was then computed using a standard function $(a = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sum(x_i - \bar{x})^2}$, mean MEP values as y values and percentages of the rMT as x values).

Paired-pulse TMS was applied with a subthreshold (70% of the rMT) conditioning stimulus (CS) followed by a supra-threshold (intensity inducing MEPs of 1mV) test stimulus (TS) at varying interstimulus intervals (ISI) (Kujirai et al., 1993). After delivering twenty control TMS pulses at the TS level, SICI and ICF (ICF) were assessed with 20 paired CS-TS pulses at ISIs of 2ms and 10ms, respectively, delivered in semi-random order. For each measure, the average peak-to-peak amplitude of the CS-TS and TS MEPs were averaged separately and a ratio of the CS-TS average over the TS average was computed for each participant as an inhibition or facilitation coefficient. The TMS intensity required to induce MEPs of 1mV peak-to-peak amplitude (1mV%MSO) was used as a measure of corticospinal excitability.

MRI data acquisition

For magnetic resonance image acquisition, a Siemens Trio 3T whole body MRI system (Siemens, Erlangen, Germany) with a 32-channel receive-only head coil was used. In terms of

anatomical acquisitions, a T1-weighted MPRAGE sequence was performed in compliance with the following parameters: repetition time (TR) = 2300 msec, echo time (TE) = 2.98 msec, inversion time (TI) = 900 msec, flip angle = 9 degrees, the field of view (FOV) = 256 mm, matrix = 256 · 256 · 176, voxel size = 1 · 1 ·1mm^3, 176 slices. Then, a diffusion weighting gradient sequence was executed to obtain the DWI data using adequate parameters, namely TR= 9300 msec, TE = 94 msec, FOV= 192mm, voxel size = 2 · 2 ·2mm3, 64 directions with b = 1000mm2/sec and one b =0mm2/sec.

Diffusion and cortical thickness MRI data processing

The dMRI data was processed by TractoFlow (Theaud, et al., 2020; https://tractoflow-documentation.readthedocs.io/en/latest/) pipeline using the following parameters: DWI was corrected for Gibbs ringing artefact, 10 seeds per voxel were used for the particle filtering tracking with a white matter seeding mask as well as a local FA-based tracking (threshold above 0.05) with 5 seeds per voxel. TractoFlow is a robust dMRI processing pipeline based on Nextflow (Di Tommaso, et al., 2017) and containers as Singularity (https://sylabs.io/) or Docker (https://www.docker.com/). This dMRI pipeline processes data from the raw DWI to the tractography. The classic diffusion tensor imaging (DTI) metric of fractional anisotropy (FA) is an output of TractoFlow.

For the fODF task of the pipeline, there was a maximum spherical harmonic order of 8 and the fiber response function was set to [15, 4, 4] mm^2/s. Then, RBx-Flow pipeline (Garyfallidis et al., 2018; https://github.com/scilus/rbx_flow) was used to virtually extract the right pyramidal tract (PyT) bundles from the TractoFlow tractograms. As for TractoFlow, RBx-Flow is based on Nextflow and containers, and uses the 2 PyT model bundles (doi.org/10.5281/zenodo.4630660) and are anatomically defined according to Chenot et al. (2019). The brainnetome atlas was extracted from the native T1 of each participant and non-linearly registered to diffusion space. The brainstem and A4ul (upper limb) regions were used to extract the right-hand bundles (Fan et al., 2016). Each bundle was also visually validated. Finally, tractometry (Cousineau et al., 2017) (https://github.com/scilus/tractometry_flow) was computed on the 2 extracted bundles: right PyT and hand bundles with the fixel-based AFD

(AFDfixel; Raffelt et al., 2012). In addition, A4ul regions were projected onto the ribbon using Freesurfer to extract mean cortical thickness within these regions for each participant.

For quality control purposes, the outputs of TractoFlow were visually validated by three raters using dMRIQC-Flow (https://github.com/scilus/dmriqc_flow). Briefly, the dMRI sampling scheme, the raw diffusion-weighted images as an animated GIF, T1-weighted images, DTI measures (FA, residuals, principal e-vector), and whole brain tractograms were visually inspected and subjects were ''passed'', ''warned'', ''failed''.

Magnetic resonance spectroscopy data acquisition

Spectroscopic measurements were acquired by positioning the voxel of interest (27 x 24 x 32 mm^3 over the left motor cortex corresponding to the right-hand using two anatomical landmarks (Yousry et al., 1997). Shimming was done using FAST(EST)MAP (Gruetter & Tkáč, 2000) to ensure a water linewidth under 10 Hz. A MEGA-PRESS sequence which edits the γ-CH_2 resonance of GABA at 3 ppm and suppresses the water signal was performed to acquire metabolite signals (Mescher et al., 1996, 1998). Data were acquired in blocks of 32 'EDIT OFF' and 32 'EDIT ON' interleaved scans (4 blocks; 12-min acquisition time) with frequency adjustments performed before each block. Individual free induction decays (FIDs) were stored for offline processing. The same MEGA-PRESS sequence (without MEGA and VAPOR water suppression) and voxel coordinates were used to acquire the water signal, which serves as a reference for metabolite quantification. Acquisition was centered on water at 4.7 ppm (d frequency=0). The following metabolites were assessed: Glu+Gln (Glx) (2.1-2.4 ppm), GABA (2.28 ppm), and N-acetylaspartate (NAA) (2.01 ppm) and creatine (CR) (3.0 ppm).

MRS data processing

Prior to analyzing spectra, tissue segmentation to correct for fractional volume composition of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) within voxels was performed using FreeSurfer 5.3.0 to allow for the correction of relaxation and partial volume effects on water-referenced metabolite concentrations. Water attenuation was computed using the fractional volume of each compartment (Gasparovic et al., 2006). The T1 and T2 water relaxation times used in the attenuation factor calculations were taken from published reports

[T1(GM)=1.29 s, T1(WM) =0.87 s, T1(CSF)=4 s, T2(GM) =110 ms, T2(WM)=80 ms, and T2(CSF) =400 ms] (Wansapura et al. (1999); Rooney et al. (2007)). Individual averages were frequency and phase corrected offline and then averaged independently for 'EDIT OFF' and 'EDIT ON' acquisitions, to generate the 'EDIT OFF' and 'EDIT ON' subspectra. Small frequency errors between 'EDIT OFF' and 'EDIT ON' subspectra were manually corrected in LCModel 6.2-1A (Provencher, 1993, 2001). Spectra with GABA Cramér-Rao lower bounds (CRLB) > 40% were excluded from further analysis. Basis set simulations for 'EDIT OFF' spectra based on known chemical shifts and J couplings (Govindaraju, Young, & Maudsley, 2000) were executed on home-written software using density matrix formalism in MATLAB (Henry et al., 2006). The basis set included simulated spectra of the following brain metabolites: Glu+Gln (Glx) (2.1-2.4 ppm), GABA (2.28 ppm), and N-acetylaspartate (NAA) (2.01 ppm). Since at 3 T glutamate cannot be resolved from glutamine, [Glx] was computed ([Glu]+[Gln]) and interpreted as reflective of [glutamate].

Statistical analysis

Statistical analysis was performed using SPSS 25 (IBM, NY, USA). The significance level was set at $p \leq 0.05$ for all analyses and a Bonferroni correction was used when necessary. When appropriate, a Greenhouse Geisser correction was applied for sphericity violations. Unpaired t-tests for independent samples (high fitness *vs* low fitness) were conducted for the following measures: VO_{2max}, rMT, ICF, SICI, 1mV%MSO, slope of I/O curve, cortical thickness (hand area of left motor cortex), white matter diffusion (FA and AFD of the CST projecting to the right hand), and the concentration of GABA, Glx, and NAA in the hand area of left motor cortex. A 2X5 mixed ANOVA was performed for I/O data with *Group* (high fitness, low fitness) as the between-subjects factor and *Intensity* (100%, 110%, 120%, 130%, 140%) as the within-subjects factor. Variables where a significant difference between the high and low fitness groups was found were tested for correlations with VO_{2max} using Pearson's correlation coefficient.

Results

Participants and fitness measure

One participant was excluded (high fitness group) from white matter and grey matter analysis due to file corruption. Four participants (1 high fitness group, 3 low fitness group) were fully excluded from MRS analysis due to lipid contamination of the signal and an additional two subjects (high fitness group) were excluded from GABA concentration analysis because of high Cramér-Rao lower bounds values. Thus, there were 40 participants for VO_{2max} and TMS analysis, 39 participants for MRI structural analysis, 36 participants for Glx and NAA concentration and 34 participants for GABA concentration analysis.

Individual characteristics of all participants are presented in Table 2. As expected, the weekly average of cardiovascular activity (t_{38} = 7.50, $p < 0.001$; Figure 1A) and VO_{2max} (t_{38} = 7.14, $p < 0.001$; Figure 1B) values were higher in the high fitness group compared to the low fitness group. Age- and sex-matched average percentile for the VO_{2max} values based on the *Normative-referenced percentile values for physical fitness among Canadians* (Hoffmann et al., 2019) were 86.7 and 43.5 for the high- and low- fitness groups, respectively (Table 1). When values were above the 95th percentile or lower than the 5th percentile, data were computed as 95 and 5, respectively. The high fitness group was significantly older than the low fitness group ($t38$ = 4.37, $p < 0.001$; Figure 1C) whereas body mass index ($t38$ = 0.50, $p = 0.62$; Figure 1D) and sex distribution ($X2$ = 1.76, $p = 0.19$) were not different between groups.

Table 2. Individual characteristics

	Sex	BMI	Cardio	VO_{2Max}	Pctl	Sport
HF1	M	23.9	10.5	52.5	80	Running, swimming, badminton, squash
HF2	M	23.2	9	58.5	>95	Running, cycling
HF3	M	19.9	9	62.2	>95	Running
HF4	M	22.1	11	55.6	>95	Running, climbing
HF5	M	26.4	10.25	50.4	80	Running, cycling
HF6	M	20.3	9	64.6	>95	Running, cycling, swimming
HF7	M	23.6	35	54.2	90	Running, cycling, swimming, tennis
HF8	M	26.1	9	59.5	>95	Cycling, swimming, rugby
HF9	F	20.7	10	60.0	>95	Running
HF10	F	19.6	11	65.0	>95	Running, track and field
HF11	M	26.7	20	54.8	>95	Triathlon

HF12	M	21.8	10	54.0	90	Running, swimming, track, and field
HF13	F	22.2	15	61.0	>95	Cycling, climbing
HF14	M	21.2	7.5	63.3	>95	Running
HF15	M	20.2	10.5	76.7	>95	Triathlon/Ironman
HF16	F	19.6	9	52.5	90	Triathlon/Ironman
HF17	M	24.5	7	66.0	>95	Triathlon
HF18	F	22.3	10	56.0	>95	Triathlon
HF19	M	21.7	10	63.0	>95	Cycling
HF20	M	24.9	10	49.9	60	Running, swimming, cycling

	Sex	BMI	Cardio	VO$_{2Max}$	Pctl	
LF1	F	27.4	0	27.5	<5	-
LF1	F	21.2	0	43.5	80	-
LF1	F	24.5	0	38.8	30	-
LF1	F	20.9	0	33.4	<5	-
LF1	M	24.4	0	55.9	95	-
LF1	F	25.6	1	45.0	80	Cardio
LF1	F	19.9	0.5	31.0	<5	Walking
LF1	F	30.1	1	34.8	10	Cardio
LF1	M	18.6	0	49.3	50	-
LF1	M	24.6	0	41.0	20	-
LF1	M	21.6	0.75	45.5	30	Running
LF1	M	25.8	0	52.4	80	-
LF1	M	18.9	0	52.0	70	-
LF1	F	20.1	2	45.2	80	Hiking
LF1	F	19.8	1	37.0	10	Running
LF1	M	18.8	0.5	48.8	60	Running
LF1	M	24.6	0	45.7	60	-
LF1	M	25.7	1	40.3	10	Hiking
LF1	M	22.9	0.5	45.1	30	Badminton
LF1	M	24.1	0.75	49.2	60	Walking

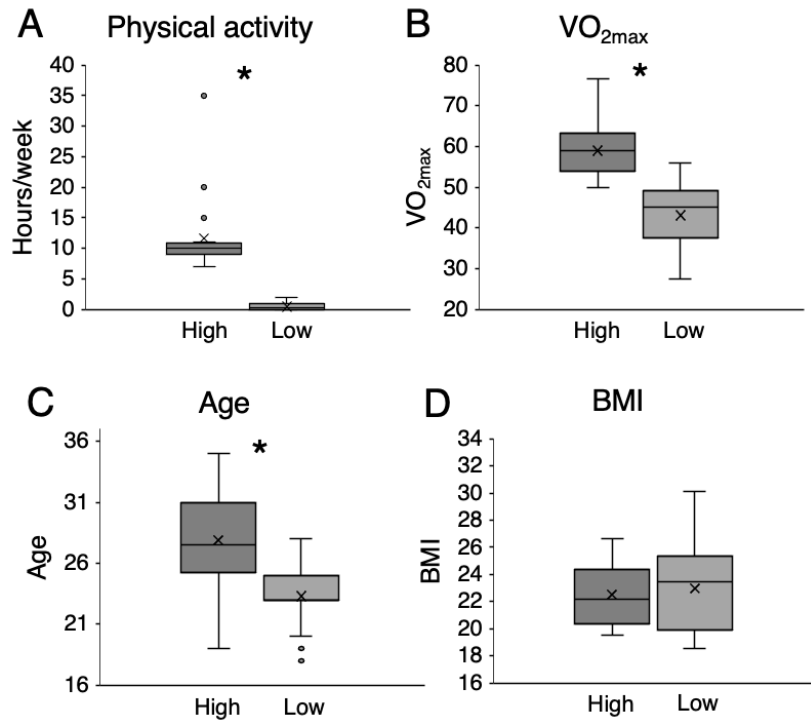HF: high fitness; LF: low fitness; Pctl: percentile

Figure 1. Boxplots showing group data for (A) physical activity, (B) VO$_{2max}$, (C) Age, (D) BMI. * < p < 0.05.

Transcranial magnetic stimulation

There was no significant difference between groups for rMT (t_{38} = 1.10, p = 0.278; Figure 2A), 1mV%MSO (t_{38} = 1.41, p = 0.46; Figure 2B), SICI (t_{38} = 1.08, p = 0.29; Figure 2C) and ICF (t_{38} = 1.34, p = 0.43; Figure 2C).
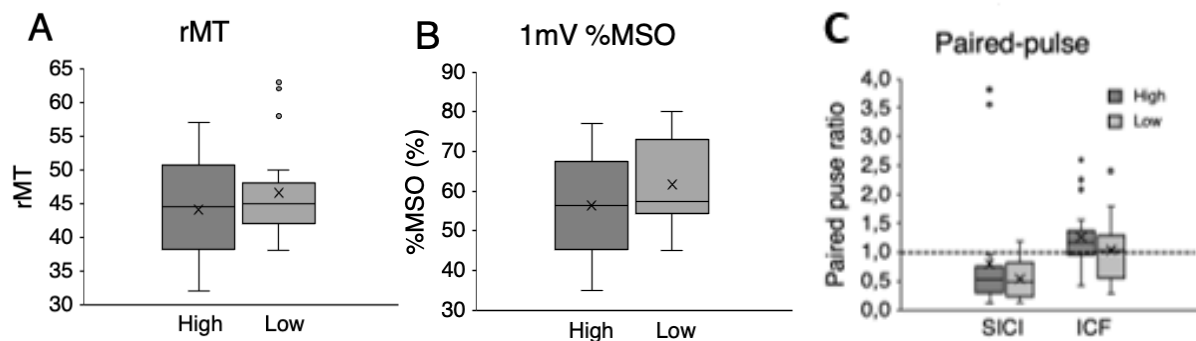

Figure 2. Boxplots showing TMS data for (A) rMT, (B) magnetic stimulator output required to induce MEPs of 1mV peak-to-peak amplitude, (C) paired-pulse measures of SICI and ICF.

For the I/O curve (Figure 3A,B), mixed ANOVA revealed significant main effects of *Group* ($F_{1,38} = 5.03$, p = 0.03) and *Intensity* (Greenhouse-Geisser: $F_{1.73,65.76} = 31.20$, p < 0.001) and a significant interaction between factors (Greenhouse-Geisser: $F_{1.73,65.76} = 3.68$, p = 0.04). Post-hoc t-tests revealed significant differences between groups that did not survive Bonferroni correction for multiple comparisons (0.05/5 = 0.01): 100% rMT ($t_{38} = 2.68$, p = 0.011), 110% rMT ($t_{38} = 1.32$, p = 0.195), 120% rMT ($t_{38} = 1.97$, p = 0.056), 130% rMT ($t_{38} = 2.19$, p = 0.035) 140% rMT ($t_{38} = 2.30$, p = 0.027). When age was added as a covariate, the *Group* effect was no longer significant ($F_{1,37} = 1.19$, p = 0.28). Additional unplanned analysis revealed that VO_{2max} values were positively correlated with age in the full sample (r = 0.45, p = 0.004).

The slope of the I/O curve was significantly steeper in the high fitness group compared to the low fitness group ($t_{38} = 2.24$, p = 0.031; Figure 3C).
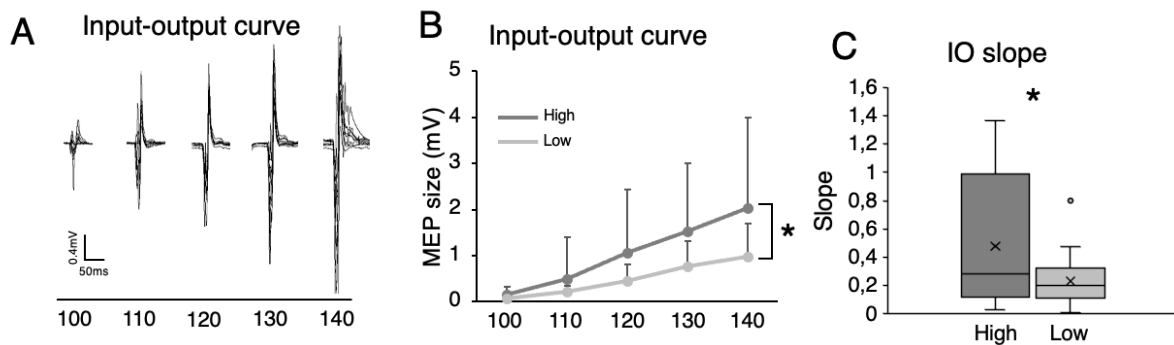

Figure 3. Corticospinal excitability measures. (A) MEP traces in a single participant for the different TMS intensities. (B) TMS Input-output curves for the two groups. (C) Boxplots showing TMS data for I/O slope * < p < 0.05.

White matter diffusion and cortical thickness

For diffusion measures in the CST originating in the left M1 hand representation and projecting to the right hand, there was no significant difference in fractional anisotropy ($t_{37} = 0.29$, p = 0.77; Figure 4A) or apparent fiber density ($t_{37} = 0.51$, p = 0.61; Figure 4B) between the high fitness and low fitness groups. Similarly, there was no significant difference in cortical thickness for the right hand representation in left M1 between groups ($t_{37} = 0.18$, p = 0.86; Figure 4C)
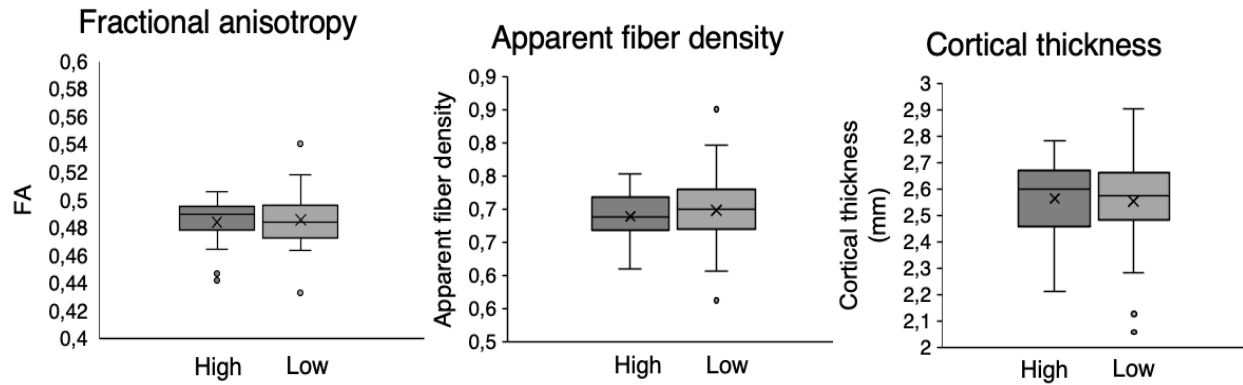
Figure 4. Boxplots showing structural imaging data for (A) fractional anisotropy, (B) apparent fiber density, (C) cortical thickness.

An exploratory, unplanned analysis was performed to determine whether group differences in white matter diffusion could be present along the length of the CST. The CST was divided in 5 equal segments and a 2X5 mixed ANOVA was performed with *Group* (high fitness, low fitness) and *Segment* (1,2,3,4,5) as factors. For FA, there was a main effect of *Segment* (Greenhouse-Geisser: $F_{1.94,71.60} = 139.10$, $p < 0.001$), no main effect of *Group* ($F_{1,37} = 2.50$, $p = 0.12$) and no interaction between factors (Greenhouse-Geisser: $F_{1.94,71.60} = 1.02$, $p = 0.36$; Figure 3A). For AFD, there was a main effect of *Segment* (Greenhouse-Geisser: $F_{2.10,77.79} = 49.81$, $p < 0.001$), no main effect of *Group* ($F_{1,37} = 0.53$, $p = 0.47$, $\eta^2 = 0.01$) and no interaction between factors (Greenhouse-Geisser: $_{2.10,77.79} = 0.89$, $p = 0.42$).

Magnetic resonance spectroscopy

No significant difference was found between groups for GABA ($t_{32} = 0.74$, $p = 0.47$; Figure 5A), Glx ($t_{34} = 2.02$, $p = 0.052$; Figure 5B) and NAA ($t_{34} = 0.54$, $p = 0.60$; Figure 5C).
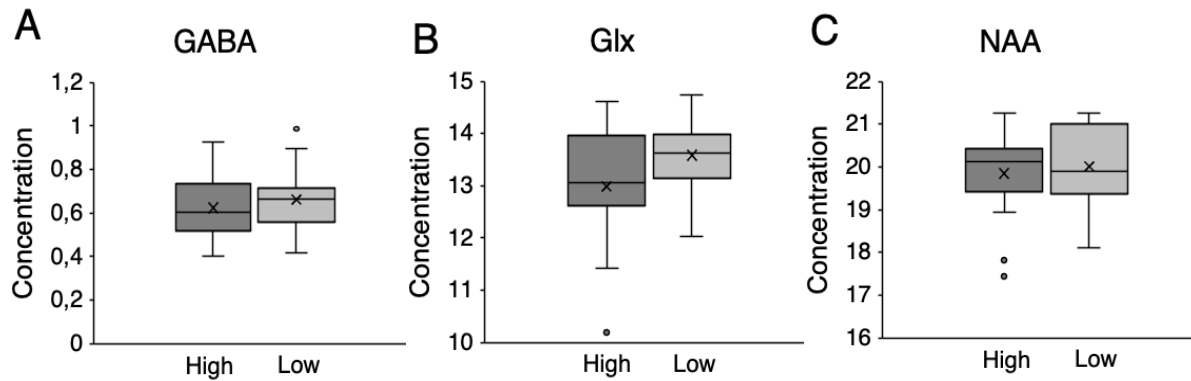
Figure 5. Boxplots showing MRS data for (A) GABA, (B) Glx, (C) NAA

Correlational analysis

Pearson's correlation analysis revealed a significant positive correlation between VO_{2max} values and the slope of the I/O curve ($r = 0.37$, $p = 0.018$; Figure 6A). Because the ICF group difference was marginally significant ($p = 0.05$), an exploratory correlation analysis was performed. ICF and VO_{2max} values were positively correlated ($r = 0.39$, $p = 0.013$; Figure 6A).
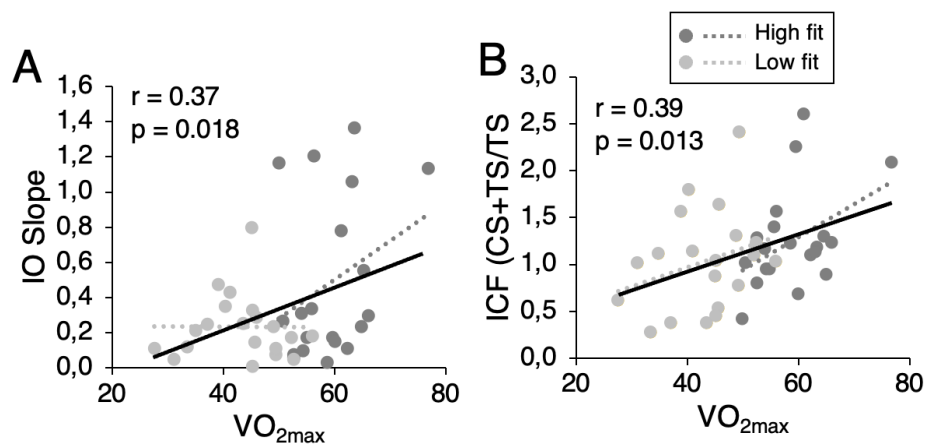

Figure 6. Correlation between VO_{2max} and (A) input/output curve slope, (B) ICF.

Discussion

The aim of the present study was to determine whether repeated physical activity was associated with long term metabolic and anatomical changes in the brain of healthy young adults. Multimodal imaging of the hand representation of primary motor cortex revealed increased CSE

at rest in highly active athletes compared to sedentary individuals. Intracortical excitability, cortical thickness and neurometabolite concentration in M1, as well white matter microstructure of the CST, were not significantly different between groups.

Corticospinal excitability indexed with I/O curve amplitude and slope was the sole measure that differed between high and low fitness individuals. This finding is in line with a previous study that reported higher I/O slope in physically active participants when data acquired before and after paired associative stimulation (PAS) were combined (Cirillo et al., 2009). Acute increases in CSE following exercise (Lulic et al., 2017) and PAS-induced plasticity (Cirillo et al., 2009) have also been shown to be dependent upon individual levels of PA, suggesting the presence of long-term changes in M1 excitability. Conversely, however, Hassanlouei and collaborators (2017) showed that I/O curve amplitude and slope were *lower* in high- compared to low-PA individuals, but this effect was subsequently shown to be limited to the trained muscle (Rozand et al., 2019). Furthermore, no difference in I/O curve amplitude has been reported between individuals with low and high levels of PA (Lulic et al., 2017; Hand et al., 2021). MacDonald and collaborators (2019) also reported an absence of correlation between CRF level and CSE in young, healthy individuals. In the present study, CSE was assessed in a hand muscle that is not specifically involved in the activity in the participants are engaged (e.g. running, cycling and swimming). This suggests that PA-induced increases in CRF may contribute to changes in CSE, which is supported by the fact that VO_{2max} was positively correlated with the I/O slope.

The specific mechanism by which regular PA may lead to long-lasting changes in CSE is unclear. Models have been suggested to account for the general effect of PA on neuroplasticity involving contributions from molecular, cellular, structural and functional components that interact to induce plasticity-related changes in cognition and behavior (El-Sayes et al., 2019; Nicolini et al., 2021). One of the often-cited candidates accounting for this link is the brain-derived neurotrophic factor (BDNF). Meta-analyses have shown that exercise training (Dinoff et al., 2016; Garcia-Suarez et al., 2021; Wang et al., 2022; Zhou et al., 2022) and acute exercise (Dinoff et al., 2017; Fernandez-Rodriguez et al., 2022) are associated with increased circulating BDNF levels. In athletes, data are scarce and baseline BDNF levels have been shown to be both

lower (Babaei et al., 2014), and higher (Correira et al., 2011) than that of sedentary individuals. Furthermore, long-term physical activity (Chan et al., 2008; Cho et al., 2012) and VO$_{2max}$ (Currie et al., 2009; Jung et al., 2011; Cho et al., 2012; Babaei et al., 2014) have been shown to be negatively correlated with BDNF levels. The idea that the effects of PA on CSE may be related to decreased BDNF levels is supported by studies showing that high frequency repetitive TMS, which is believed to increase CSE (Maeda et al., 2000), decreases BDNF serum levels (for meta-analysis, see Jiang and He., 2019) in primary motor cortex (Gaede et al., 2014) and dorsolateral prefrontal cortex (Schaller et al., 2014).

The present study found no evidence for group differences in other TMS measures. Absence of PA-related effects on motor thresholds have been reported in physically active individuals (Hassanlouei et al., 2017; Lulic et al., 2017; Rozand et al., 2019; Hand et al., 2021) and after acute exercise (Yamazaki et al., 2019; El Sayes et al., 2020; Nicolini et al., 2020). Exercise has been shown repeatedly to reduce SICI immediately after various levels of exercise intensities (Yamaguchi et al., 2012; Singh et al., 2014; Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; Opie and Semmler, 2019; Yamazaki et al., 2019; Neva et al., 2021; for a review see Alibazi et al., 2021). However, similarly to the present data, studies that have measured SICI in active and sedentary individuals reported no group differences (Cirillo et al., 2009; Lulic et al., 2017; Hand et al., 2021). With regards to the effects of acute exercise on ICF, results have been mixed, as studies have reported increases (Singh et al., 2014; Neva et al., 2017; Morris et al., 2020), decreases (Lulic et al., 2017; El-Sayes et al., 2019) and no change (Andrew et al., 2020; Nicolini et al., 2020, see a review by Nicolini et al., 2021). The long-term effects of PA, however, seem to follow the pattern found in the present study, where no group differences were observed between active and sedentary individuals (Lulic et al., 2017; Hand et al., 2021).

Taken together, the present data show that regular participation in cardiovascular exercise is associated with changes in primary motor cortex excitability that differ from those associated with acute exercise (Alibazi et al., 2021; Nicolini et al., 2021). This suggests that repeated PA has cumulative effects on M1 neurophysiology that go beyond baseline levels. Indeed, the present data may partly explain the greater response in plasticity (Cirillo et al., 2009) and CSE

(Lulic et al., 2017) that occurs following a single session of aerobic exercise in highly active compared to sedentary individuals.

Two measures of CST white matter microstructure were compared between groups in the present study. AFD is sensitive to both the location and the orientation of fiber bundles, which is especially relevant for regions containing multiple fiber populations such as the motor cortex (Raffelt et al., 2012), whereas FA was included to facilitate between-study comparisons. FA and DTI are well-known to be limited in crossing fiber regions. A recent systematic review and meta-analysis found evidence for a link between physical activity, cardiorespiratory fitness, exercise (PACE) and white matter microstructure indexed by FA in numerous tracts across the brain (Maleki et al., 2022). This is particularly the case for older populations, where a greater number of studies have been conducted (Domingos et al., 2021; Maleki et al., 2022). Indeed, we know of only one study where white matter microstructure was compared between groups of high- and low-fit individuals, as confirmed with VO_2 peak (Herting et al., 2014). In that study, tract based spatial statistics revealed no difference in FA between high- and low-fit adolescent males aged 15-18 in ROIs that included the CST. Tractography analysis, however, revealed significantly higher streamline counts in high-fit individuals in the CST, but no difference in FA (Herting et al., 2014). These data are in line with the present results, where no difference was observed between groups in both FA and AFD in participants aged 18-35.

A link between white matter microstructure and PA in young adults has been reported, however, in the form of correlations between indexes of PA and FA. Herting and collaborators (2014) reported a negative correlation between VO_2 peak and FA in the left CST (age:15-18) whereas Opel et al. (2019) found widespread *positive* correlations between FA and endurance in tracts that included the CST (mean age: 28.8 years). An absence of correlation between FA and activity levels measured with actigraphy has also been reported in the fornix and parahippocampal cingulum (Bracht et al., 2016; mean age: 25.5 years). Finally, no association between FA and measures of fitness was reported in a large study (1,065 participants) of young adults (22-36 years; Best et al., 2020a)

Age appears to be an important factor in mediating the relationship between CRF and WM microstructure. Hayes and collaborators (2015) found stronger correlations between FA and

VO$_2$ peak in older (55-82 years) compared to younger (18-31 years) individuals. Indeed, no significant correlation between FA and CRF was observed in the young group for any of the WM tracts (Hayes et al., 2015). Similar findings were obtained in a large study on the effects of aging, where CRF was more strongly associated with FA in older than in younger participants (Mace et al., 2021).

The present results similarly show that high levels of fitness in young adults are not associated with changes in cortical thickness in the M1 hand representation. Previous literature has reported the presence of a positive relationship between CRF and cortical volume and thickness (Erickson et al., 2010, 2011,2014; Jonasson et al., 2017; Olivo et al., 2021). In young adults, data are scarce but a recent large-scale (n= 1,195) study in young adults (age 22-36) showed that fitness was not associated with cortical thickness (Best, 2020b). Conversely, VO$_{2max}$ was reported to be negatively correlated with CT in numerous cortical areas in adolescents and young adults (age 16-26). This is contradicted by intervention studies, where 6 months of aerobic exercise in young adults (19-27 years) was found to increase CT in distributed brain areas (Bashir et al., 2021). Similar findings were reported by Zhu and collaborators (2021) in young adults (18-20 years), where 9 weeks of aerobic exercise increased CT in lateral occipital cortex and cuneus. Similarly to what has observed for white matter microstructure, age appears to be an important factor mediating the relationship between CRF and CT. Indeed, VO2 peak was found to correlate positively with CT in older adults whereas a negative correlation was reported for young adults (Williams et al., 2017).

Taken together, structural data in the present study suggest that increased CRF associated with increased PA does not induce structural alterations in the primary motor cortex of young adults. These results provide partial support for the age-dependent hypothesis of the association between CRF and neuroplasticity, whereby the effects of an active lifestyle on brain function and anatomy are present in childhood, diminish in young adulthood and become increasingly salient with advancing age (Chaddock-Heyman et al., 2014; Hötting and Röder, 2013; Hayes et al., 2015; Best et al., 2020a,b; Mace et al., 2021). A similar pattern has been observed for cognition, where a recent meta-analysis showed that aerobic interventions in children and older adults, but

not adolescents and young adults, are associated benefits in executive function (Ludyga et al., 2016).

Similarly to grey and white matter, metabolite levels were not significantly different between high- and low-fitness groups in the present study. In older adults, higher levels of fitness have been shown to be associated with lower NAA concentration in frontal cortex (Erickson et al., 2012). Similar findings were obtained in middle-aged individuals, where endurance athletes showed higher NAA levels compared to sedentary individuals in frontal (Gonzales et al., 2013). In young adults, a single bout of high-intensity exercise has been shown to increase GABA (Maddock et al., 2016; Coxon et al., 2018) and glutamate (Maddock et al., 2016). The absence of GABA and glutamate differences between highly trained athletes and sedentary individuals in the present study parallels TMS data, as SICI and ICF have been associated with inhibitory and excitatory neurotransmission (Ziemann et al., 2015).

To our knowledge, this is the first study to characterize the neurophysiological, neurometabolic and neuroanatomical correlates of intense, repeated physical activity in young adults. The present study benefited from an objective measure of CRF (VO_{2max}) that confirmed fitness levels based on participant self-report. High-fitness participants were also carefully selected to be involved in high-intensity aerobic exercise that does not require specific hand muscle activity and groups were equivalent in terms of BMI. The study also benefited from multimodal imaging that focused on a single, well-defined area and was centered on a population of young adults that has been relatively little studied. Taken together, the data suggest that brain changes associated with increased CRF are relatively limited, at least in primary motor cortex, in contrast to what has been observed in older population. This reflects a similar pattern to what has been observed in some cognitive functions (Ludyga et al., 2016). Whether a history of intense and repeated exercise in young adulthood may translate into greater benefits of PA in older age is an open and important issue that should be addressed in future studies with longitudinal designs.

The present study has limitations that should be acknowledged. First, participants in the high-fitness group were older than low-fit individuals and when age was added as a covariate to the I/O statistical analysis, the effect was no longer significant. Age has been shown to be an important factor mediating brain response to PA (Hayes et al., 2015; Williams et al., 2017) but it

is unlikely that a 4-year age difference in groups of young adults accounted for the observed effects or lack thereof. Furthermore, although aging has been shown to modify some aspects of motor cortex excitability, a recent meta-analysis found no significant difference between young and old adults on TMS measures of SICI and ICF (Bhandari et al., 2016) and analysis of a large dataset reported no link between age and CSE, SICI or ICF (Corp et al., 2021). As a result, it is unlikely that age differences accounted for the difference in CSE between groups. This is further supported by the fact that age was correlated to VO_{2max} in the present sample. In light of the very unlikely effect of age on CSE measures (Corp et al., 2021), the VO_{2max} difference between groups appears to be the more parsimonious explanation of the present findings. Second, MEP data were not normalized to M-wave values, not controlling for possible differences in the volume of muscle fibers between groups. Third, TMS measures of GABA-B receptor activity were not included in the present protocol. As a result, group differences measured with the cortical silent period and long interval intracortical excitability, may have been missed. However, although one study reported reduced LICI following acute aerobic exercise (Mooney et al., 2016), most did not (e.g. Singh et al., 2014; Stravinos et al., 2017; Andrews et al., 2020; Morris et al., 2020). Finally, imaging and TMS measures were limited to a single area to limit the number of comparisons, accounting for the small sample size, and to allow the better integration of data. As a result, the present data do not rule out the possibility that PA is associated with anatomical and functional changes outside of motor cortex in young adults.

Acknowledgements

Conflict of interest

None.

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Concluding Discussion Chapter

This study aimed to characterize physiological, anatomical, and neurochemical correlates of PA in healthy young adults and determine whether repeated physical activity is associated with long term changes in their brain similar to the effects of acute AE. TMS revealed increased CSE, reflected by a steeper I/O curve at rest in highly active athletes compared to sedentary individuals. All other variables were not significantly different between the two groups, namely intracortical excitability, WM microstructure of the CST (FA and AFD), CT, and neurometabolic (NAA, GABA, Glx) concentrations in M1.

Physiological effect of AE

As mentioned above, corticospinal excitability indexed with I/O curve amplitude and slope was the sole measure that differed between high and low fitness individuals. This finding confirms our hypothesis of greater CSE in high fitness individuals. It aligns with the previous study of Cirillo and collaborators (2009) that reported higher I/O slope in physically active participants when data acquired before and after paired associative stimulation (PAS) were combined. Moreover, individual activity level has been shown to moderate acute increases in CSE following exercise (Lulic et al., 2017) and PAS-induced plasticity (Cirillo et al., 2009), suggesting the presence of long-term changes in M1 excitability. On the other hand, there are some differential findings in the literature. In one study, I/O curve was found to be lower in high-compared to low-PA individuals (Hassanlouie et al., 2017). Subsequently, this effect was shown to be limited to the trained muscle (Rozand et al., 2019). This discrepancy may be explained by at least two factors: the muscle group from which measurements were taken and the level of activation. The present study assessed CSE in a hand muscle that is not specifically solicited by the athletes in the high-PA group (e.g., running, cycling, and swimming). This is contrary to the study of Hassanlouie and collaborators (2017) where lower limb muscles were involved in achieving high levels of PA but also have less corticospinal projections compared to upper limb muscles. The present study also obtained measures at rest rather than in a state of low activation, which increases the net excitability of the motor system.

Some studies have reported no difference in I/O curve amplitude between athletic and sedentary individuals (Lulic et al., 2017; Hand et al., 2021). Hand et al. (2021) suggested that maximum TMS intensities and participant characteristics could explained the absence of group differences. In terms of TMS intensities, differences between groups have been reported at high TMS intensities. For example, Hassanlouie and collaborators (2017) found that the largest between-groups effect occurred at 150% active motor threshold. In the present study, maximum TMS intensity was 140% rMT. The absence of differences in I/O curve amplitude aligns with the finding that there is no correlation between CRF level and CSE in young, healthy individuals (Macdonald et al., 2019). However, the I/O curve slope was found to correlate positively with the VO_{2max} scores in the present study, thereby confirming our secondary hypothesis. The difference may be due to different CSE measures, more precisely the area under the curve (Macdonald et al., 2019) versus the slope and amplitude of the I/O curve. Nonetheless, the present findings suggest that PA-induced increases in CRF may contribute to changes in CSE.

The mechanism underlying exercise-related changes in CSE remains to be determined. A possible interplay of molecular, cellular, structural, and functional components could explain the induced plasticity and its related cognitive and behavioral changes as suggested by models accounting for the effect of PA on neuroplasticity (El-Sayes et al., 2019; Nicolini et al., 2021). First, the mechanisms involved in acute versus long-term PA likely differ. While BDNF may contribute to long-term changes, modulation of neurotransmitter concentrations may underlie short-term changes in CSE (Lulic et al., 2017). It has been shown that a single session of AE increased the release of serotonin (Kitaoka et al., 2010) and norepinephrine (Meeusen et al., 1997) which have been shown to modulate the excitability of motor neurons (Heckman et al., 2009). AE also increases glutamate concentrations (Maddock et al., 2011) which is positively associated with steeper MEP recruitment curves (Stagg et al., 2011). Previous work has also shown an increase in lactate levels after intense AE which corresponds to increasing primary motor cortex excitability (Coco et al., 2010). However, fitness was not found to be correlated with neurotransmitter concentrations such as Glx and GABA (Harasym et al., 2020). As mentioned above, the brain-derived neurotrophic factor (BDNF) is believed to be implicated in long-term AE related changes in CSE. Supporting this idea, meta-analyses have confirmed the presence of increased BDNF levels after acute exercise (Dinoff et al., 2017; Fernandez-

Rodriguez et al., 2022) and related to chronic PA (Dinoff et al., 2016; Garcia-Suarez et al., 2021; Wang et al., 2022; Zhou et al., 2022). Studies examining BDNF levels in athletes specifically are scarce and results are mixed showing both lower (Babaei et al., 2014) and higher (Correira et al., 2011) levels in comparison to sedentary individuals. Additionally, BDNF is negatively correlated with long-term physical (Chan et al., 2008; Cho et al., 2012) and VO_{2max} (Currie et al., 2009; Jung et al., 2011; Cho et al., 2012; Babaei et al., 2014). Studies have also found that high frequency repetitive TMS, believed to increase CSE (Maeda et al., 2000), decreases BDNF serum levels (for meta-analysis, see Jiang and He., 2019). This effect is observed both in the primary motor cortex (Gaede et al., 2014) and dorsolateral prefrontal cortex (Schaller et al., 2014). These findings support the idea that decreased levels of BDNF may be related to the effect of PA on CSE. More precisely, lower peripheral BDNF in high fitness individuals may be due to more efficient uptake and utilization of BDNF in an athlete's central nervous system (Nofuji et al., 2012). Measuring BDNF level is beyond the objective of the present study but could be an area of interest for future studies. Another proposed long-term mechanism is the physiological changes associated with higher PA and fitness, where higher excitability may be due to the increased stroke volume (Wilmore et al., 2001), brain perfusion (Ding et al., 2006), and muscle adaption (Petriz et al., 2017).

In terms of other TMS measures, no group differences were found in the present study. Motor threshold was similar between high fitness and low fitness individuals, which replicates previous findings for physically active individuals (Hassanlouei et al., 2017; Lulic et al., 2017; Rozand et al., 2019; Hand et al., 2021) and following acute exercise (Yamazaki et al., 2019; El Sayes et al., 2020; Nicolini et al., 2020). No group differences were found for SICI, similarly to other studies that have compared SICI in active and sedentary individuals (Cirillo et al., 2009; Lulic et al., 2017; Hand et al., 2021). However, it has been shown repeatedly that SICI decreases immediately after various levels of exercise intensities (Yamaguchi et al., 2012; Singh et al., 2014; Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; Opie and Semmler, 2019; Yamazaki et al., 2019; Neva et al., 2021; for a review see Alibazi et al., 2021). Thus, SICI seems to be modulated by acute exercise, but its effect does not appear to persist. Similarly, ICF was not found to differ in the present study between the two groups. This is in line with the pattern found in other studies examining the long-term effects of PA by comparing active and

sedentary individuals (Lulic et al., 2017; Hand et al., 2021). With regards to the effects of acute exercise on ICF, results have been mixed, as studies have reported increases (Singh et al., 2014; Neva et al., 2017; Morris et al., 2020), decreases (Lulic et al., 2017; El-Sayes et al., 2019) and no change (Andrew et al., 2020; Nicolini et al., 2020, see a review by Nicolini et al., 2021).

Taken together, the present data show that regular participation in cardiovascular exercise is associated with changes in primary motor cortex excitability that differ from those associated with acute exercise (Alibazi et al., 2021; Nicolini et al., 2021). While motor threshold is not impacted by acute or regular PA, there is a differential effect for SICI and ICF. More precisely, acute PA seem to impact SICI and ICF whereas no persisting effect if observed for regular PA. Most notably is the steeper I/O curve found among high fitness participants, which suggests that repeated PA has cumulative effects on M1 neurophysiology that go beyond baseline levels. It may partly explain the modulating effect of fitness on physiological response following a single session of AE: a greater response in plasticity (Cirillo et al., 2009) and CSE (Lulic et al., 2017) found in highly active compared to sedentary individuals.

Anatomical effect of AE

Starting with WM, two measures of CST white matter microstructure (FA and AFD) were compared between groups in the present study. AFD is sensitive to both the location and the orientation of fiber bundles, which is especially relevant for regions containing multiple fiber populations such as the motor cortex (Raffelt et al., 2012), whereas FA was included to facilitate between-study comparisons. In the present study, no difference was observed between groups for both measures of WM (FA, AFD) in participants aged 18-35. Our hypothesis is therefore confirmed. It was expected that no differences would be found in a younger sample as the impact of PA on WM seem to be mediated by age. Recent systematic reviews and meta-analyses found evidence for a link between physical activity, cardiorespiratory fitness, exercise (PACE) and white matter microstructure indexed by FA in numerous tracts across the brain, especially in older adults (Domingos et al., 2021; Maleki et al., 2022).. To our knowledge, there is only one study examining young adults where white matter microstructure was compared between groups of high- and low-fit individuals, as confirmed with VO_2 peak (Herting et al., 2014). No difference in FA between high- and low-fit adolescent males aged 15-18 in ROIs that included

the CST was revealed by tract based spatial statistics. Although tractography analysis uncovered significantly higher streamline counts in high-fit individuals in the CST, no difference in FA was found. Some studies that have examined the correlation between PA and WM microstructure indexed by FA in young adults. Brancht and collaborators (2016) used actigraphy to measure activity levels in a sample of young adults (mean age: 25.5) and did not find a correlation between activity level and FA in the fornix and parahippocampal cingulum. Furthermore, a large study (n = 1065) of young adults from 22 to 36 years old reported no association between FA and physical fitness measured by grip strength and submaximal physical endurance (Best et al., 2020a). In contrast, some studies have reported significant correlations between PA and FA. For example, Herting and collaborators (2014) reported a negative correlation between VO_{2peak} and FA in the left CST (age:15-18). This is believed to reflect tissue properties like glial cell number and more crossing fibers which contribute to lower FA in high fitness individuals rather than less myelination and axonal organization (Herting et al., 2014). Widespread *positive* correlations between FA and endurance in tracts that included the CST (mean age: 28.8 years) have also been reported (Opel et al., 2019). Nevertheless, previous studies suggest that age is an important factor in mediating the relationship between CRF and WM microstructure such that its effect is only observable in older adults. For example, Hayes and collaborators (2015) found stronger correlations between FA and VO_2 peak in older (55-82 years) compared to younger (18-31 years) individuals. Indeed, no significant correlation between FA and CRF was observed in the young group for any of the WM tracts (Hayes et al., 2015). Similar findings were obtained in a large study on the effects of aging. Mace and collaborators (2021) reported that CRF was more strongly associated with FA in older than in younger participants suggesting that the neuroprotective effects of CRF on WM microstructure is age dependent.

In terms of GM indexed by cortical thickness (CT), the present results again show no group differences between high- and low-fitness groups in the M1 hand representation. Previous literature examining older adults has reported the presence of a positive relationship between CRF and cortical volume and thickness (Erickson et al., 2010, 2011,2014; Jonasson et al., 2017; Olivo et al., 2021). Tarumi et al. (2021) have examined middle-aged adults and found lower CT in the insula and right superior frontal gyrus in high- compared to the low-fitness individuals. In young adults, data are scarce but a recent large-scale (n= 1,195) study in young adults (age 22-

36) showed that fitness was not associated with CT (Best, 2020b). However, this is contradicted by intervention studies. It was found that 6 months of AE in young adults (19-27 years) can increase CT in distributed brain areas such as the left pericalcarine area, left superior parietal aera, and the right lateral occipital gyrus (Bashir et al., 2021). Similar results were reported for an even shorter AE program (9 weeks) in young adults (18-20 years) where an increase in CT was found in lateral occipital cortex and cuneus (Zhu et al., 2021). The discrepancy is likely due to brain regions investigated. Best et al. (2020b) computed an average CT score across 68 cortical parcellation whereas other studies including the present study examined specific brain regions. More research is needed to determine whether PA differentially affects specific cortical regions and whether the frequency of PA could be a moderator of changes in CT. In the present study, results suggest that CRF is unrelated to CT in the M1 hand region. However, it is not possible to generalize these findings to other cortical regions. Moreover, age appears to be an important factor mediating the relationship between CRF and CT. This is further supported by the finding that VO_2 peak correlates positively with CT in older adults and negatively in young adults (Williams et al., 2017).

In summary, structural data in the present study suggest that higher CRF associated with regular PA does not induce WM and GM structural alterations in the primary motor cortex of young adults, thereby confirming our hypothesis. This partially supports the age-dependent hypothesis of the association between CRF and neuroplasticity. In other words, the effects of an active lifestyle on brain function and anatomy may be present in childhood, diminish in young adulthood and become increasingly salient with advancing age as a potential neuroprotective mechanism (Chaddock-Heyman et al., 2014; Hötting and Röder, 2013; Hayes et al., 2015; Best et al., 2020a,b; Mace et al., 2021). A similar pattern has been observed for cognition, where a recent meta-analysis showed that aerobic interventions in children and older adults, but not adolescents and young adults, are associated with benefits in executive functions (Ludyga et al., 2016).

Metabolic effect of AE

For metabolic measures, it was expected that NAA would be higher in high fitness group, but no differences would be found for GABA and Glx between groups. In the present study, results partially align with the hypothesis with no significant differences in all three neurotransmitters between high- and low-fitness groups. In older adults, higher levels of fitness have been shown to be associated with an offset of age-related decline in NAA concentration in frontal cortex and is related with better working memory (Erickson et al., 2012). In middle-aged individuals, results show that endurance athletes have higher NAA levels compared to sedentary individuals in the frontal cortex, which may suggest greater neuronal integrity or faster metabolism (Gonzales et al., 2013). Our findings in young adults do not support previous literature investigating middle-aged and older adults. This discrepancy may be explained by age, but further studies directly comparing young and older individuals are needed.

As for GABA and Glx concentrations, the actual effect of acute and chronic PA is yet to be determined as there is only some preliminary findings. In young adults, a single bout of high-intensity exercise has been shown to increase GABA (Maddock et al., 2016; Coxon et al., 2018) and glutamate (Maddock et al., 2016). However, fitness level was not found to be correlated with glutamate and GABA concentrations in postmenopausal women (Harasym et al., 2020). This provides some evidence that acute and regular exercise do not affect GABA and Glx concentrations in a similar manner. More studies examining simultaneously fitness level and neurotransmitter concentrations pre-and post AE can provide further clarifications. In this study, the absence of GABA and glutamate differences between highly trained athletes and sedentary individuals parallels TMS data, as SICI and ICF have been associated with inhibitory and excitatory neurotransmission (Ziemann et al., 2015).

Strength and Limitations

The present study benefited from numerous strengths. First, an objective measure of CRF (VO$_{2max}$) was used that confirmed fitness levels based on participant self-report. Moreover, high-fitness participants were carefully selected to be involved in high-intensity aerobic exercise that does not require specific hand muscle activity and groups were equivalent in terms of BMI. The

study also benefited from multimodal imaging that focused on a single, well-defined area (M1) and was centered on a population of young adults that has been relatively little studied.

There are also limitations that should be acknowledged. First, participants in the high-fitness group were on average 4 years older than low-fit individuals and when age was added as a covariate to the I/O statistical analysis, the effect was no longer significant. Past studies have suggested that age is an important factor mediating brain response to PA (Hayes et al., 2015; Williams et al., 2017). However, it is unlikely that a 4-year age difference in groups of young adults accounted for the observed effects or lack thereof. Although aging has been shown to modify some aspects of motor cortex excitability, a recent meta-analysis found no significant difference between young and old adults on TMS measures of SICI and ICF (Bhandari et al., 2016). Furthermore, analysis of a large dataset reported no link between age and CSE, SICI or ICF (Corp et al., 2021). As a result, it is unlikely that age differences accounted for the difference in CSE between groups. This is further supported by the fact that age was correlated to VO_{2max} in the present sample. Indeed, the VO_{2max} difference between groups is a more parsimonious explanation of the present findings on CSE. Second, MEP data were not normalized to M-wave values. More precisely, possible differences in the volume of muscle fibers between groups was not controlled for and is a limitation of this study. Third, TMS measures of GABA-B receptor activity were not included in the present protocol. As a result, group differences measured with the cortical silent period and long interval intracortical excitability may have been missed. However, although one study reported reduced long interval intracortical excitability following acute aerobic exercise (Mooney et al., 2016), most did not (e.g. Singh et al., 2014; Stravinos et al., 2017; Andrews et al., 2020; Morris et al., 2020). Finally, imaging and TMS measures were limited to a single area limiting its generalizability to brain areas beyond the motor cortex. However, this decision is necessary to limit the number of comparisons, accounting for the small sample size, and to allow the better integration of data. As a result, it is possible that this study overlooked the anatomical and functional changes associated with PA outside of the motor cortex in young adults.

Conclusion

To our knowledge, this is the first study to characterize the neurophysiological, neurometabolic and neuroanatomical correlates of intense, repeated physical activity in young adults. Taken together, the effects of acute AE do not appear to persist at rest even with rigorous PA. Our findings suggest that brain changes associated with increased CRF are relatively limited in young adults, at least in the primary motor cortex, in contrast to what has been observed in the older population. This reflects a similar pattern to what has been observed in some cognitive functions (Ludyga et al., 2016). With the majority of healthcare professionals agreeing that discussing the subject of physical activity with their patient is part of their job, understanding the impact of physical activity on individuals in different age groups is relevant and could render PA intervention programs more flexible and personalized (Cunningham & O'Sullivan, 2021). While the current literature appears to suggest that a history of intense and repeated exercise in young adulthood may translate into greater benefits of PA in older age, evidence is needed from future studies with longitudinal designs.

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