Somatosensory brain function and gray matter regional volumes differ according to exercise history: Evidence from monozygotic twins

Pekka Hautasaari[1], Andrej M. Savić[2,3] Otto Loberg[4], Eini Niskanen[5], Jaakko Kaprio[6], Urho M. Kujala[1] and Ina M. Tarkka [1]

[1]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [2]University of Belgrade, School of Electrical Engineering, Belgrade, Serbia and [3]Tecnalia Serbia Ltd., Belgrade, Serbia, [4]Department of Psychology, University of Jyväskylä, Jyväskylä, Finland, [5]Department of Applied Physics, University of Eastern Finland, Kuopio, Finland, [6]Department of Public Health, University of Helsinki, and National Institute for Health and Welfare, and Institute for Molecular Medicine (FIMM) University of Helsinki, Helsinki, Finland

Address for correspondence:

Ina M. Tarkka, PhD
University of Jyväskylä
Rautpohjankatu 8
40014 Jyväskylä,
Finland
Tel. +358 408053596
E-mail: Ina.Tarkka@jyu.fi

ABSTRACT

Associations between long-term physical activity and cortical function and brain structure are poorly known. Our aim was to assess whether brain functional and/or structural modulation associated with long-term physical activity is detectable using a discordant monozygotic male twin pair design. Nine monozygotic male twin pairs were carefully selected for an intrapair difference in their leisure-time physical activity of at least three years duration (mean age 34±1 y). We registered somatosensory mismatch response (sMMR) in EEG to electrical stimulation of fingers and whole brain MR images. We obtained exercise history and measured physical fitness and body composition. Equivalent electrical dipole sources of sMMR as well as gray matter (GM) voxel counts in regions of interest (ROI) indicated by source analysis were evaluated. SMMR dipolar source strengths differed between active and inactive twins within twin pairs in postcentral gyrus, medial frontal gyrus and superior temporal gyrus and in anterior cingulate (AC) GM voxel counts differed similarly. Compared to active twins, their inactive twin brothers showed greater dipole strengths in short periods of the deviant-elicited sMMR and larger AC GM voxel counts. Stronger activation in early unattended cortical processing of the deviant sensory signals in inactive co-twins may imply less effective gating of somatosensory information in inactive twins compared to their active brothers. Present findings indicate that already in 30's long-term physical activity pattern is linked with specific brain indices, both in functional and structural domains.

Key words: Twin research; Brain electrophysiology; Somatosensory cortex; Mismatch negativity; Brain structure; Physical activity

1. Introduction

Physical activity is known to have many beneficial physiological effects on the human body, e.g. cardiovascular system, endocrine system and skeletal muscle function enhance because of physical activity and, in addition, physical activity has a significant role in reducing risk for several chronic diseases (Kujala, Kaprio, Sarna, & Koskenvuo, 1998; Reiner, Niermann, Jekauc, & Woll, 2013). However, less is known about the effects of physical activity on brain structure and function in healthy adults. Recently we showed that increased levels of physical activity that are associated with beneficial alterations of several known cardio-metabolic disease risk factors were associated with structural modulation cortical gray matter (GM) volumes independent of genetic background (Rottensteiner et al., 2015). Our aim in the present study is to investigate further electrophysiological functional differences in early sensory processing and their possible link to regional brain structures using a monozygotic twin pair design to adjust for known and unknown, including familial and/or genetic confounders of the association between physical activity and brain function and structure. We recruited young healthy male twins who were discordant long-term, for the past 3 years, in their physical activity habits. Our cohort was selected in order to avoid effects of chronic diseases, medications or possible prodromal phases of diseases.

Exercise has an effect on brain structure and cognitive function in humans (Hillman, Erickson, & Kramer, 2008; Ruscheweyh et al., 2011). Accumulating evidence suggests connections between better executive functioning and increased volume in prefrontal and insular cortex (Ruscheweyh et al., 2011) and between exercise and increased hippocampal (Erickson et al., 2011), prefrontal and temporal GM as well as anterior white matter (WM) volume (Hillman et al., 2008). Most of previous research has been conducted in older adults.

Much less has been done with children and especially among young adults on exercise effects on brain. In our recent study we detected larger GM volume in non-dominant striatal and prefrontal structures based on whole brain MRI analysis in active young healthy adult male twins compared to their inactive twin brothers (Rottensteiner et al., 2015).

Mismatch negativity (MMN) is a comprehensively studied component of the auditory evoked potential most often registered using EEG (for review, see (Näätänen, Paavilainen, Rinne, & Alho, 2007)). It is generated by a cortical automatic change-detection process and it is elicited by any discernible auditory change when the ongoing auditory input differs from the preceding auditory stimulus (Näätänen et al., 2007). Less frequently studied somatosensory mismatch response (sMMR) is a corresponding change detection mechanism where various stimuli can be used to elicit sMMR including electrical or vibratory stimuli (Akatsuka, Wasaka, Nakata, Kida, & Kakigi, 2007; Spackman, Boyd, & Towell, 2007). Regardless of the stimulus type, violations to previous stimulus array are necessary to elicit the mismatch response (Akatsuka et al., 2005; Kekoni et al., 1997). SMMR determinants are not yet widely studied however, we recently detected differences between young and elderly healthy adults using electrical stimuli in a location mismatch design in the hand (Strömmer, Tarkka, & Astikainen, 2014). Our previous finding suggested attenuated later phase of SMMR in the elderly compared to young adults. SMMR is, by definition, an early precognitive, sensory-driven, automatic activation of change detection system. Of high relevance is the interesting recent report by Popovich and Staines (2015). They investigated the effect of acute bout of exercise in several components of somatosensory evoked potential in attended and unattended conditions (Popovich & Staines, 2015). Their oddball design involved attention paid to the specific finger where deviant stimuli were delivered allowing afterwards analysis during attention or ignore (unattended) conditions. Their unattended

condition resulted in enhanced N140 component in the parietal area. This component may resemble an early part of sMMR of our previous work however, we never requested any voluntary response in our experiments (Strömmer et al., 2014). Popovich and Staines (2015) allocated the effect they found of acute bout of moderate intensity aerobic exercise to improvement of selective attentional processing by enhancing involuntary shifts of attention from task-irrelevant stimuli post-exercise (Popovich & Staines, 2015). That may explain the effect after one acute exercise session however, it does not answer the question regarding effects of long-term physical activity. Popovich and Staines (2015) also analyzed later component, which they call LLP component, (175-250 ms window) and show suppressed LLP after acute exercise in unattended condition. They allocated this suppression to increased sensory gating of task-irrelevant stimuli (Popovich & Staines, 2015). Their amplitude modulations (N140 and LLP) occurred within the same time window as our sMMR (Strömmer et al., 2014; Tarkka et al., 2016). Our recent data implied modulation in few electrode locations on the somatosensory cortical area, where inactive individuals showed larger components, and we allocated this difference between inactive and active ones to enhanced gating of aberrant somatosensory stimuli in active co-twin compared to inactive co-twin (Tarkka et al., 2016).

There is wide inter-individual variability in known metabolic and cardiorespiratory responses to regular physical activity, e.g. in plasma triglycerides, fasting insulin levels and cardiorespiratory fitness levels (Bouchard et al., 2012). Twin studies provide a pathway to study associations between physical activity vs. inactivity in functional and structural measures in strong study design where genetic background and mostly also childhood environment is controlled. In the present study, we analyse in detail cerebral sources of sMMR and related brain structures in MR images in a rare set of healthy twin pairs who are

long-term discordant in physical activity. We aim to recognize if possible functional differences are in any way reflected in structural brain indices.

2. Methods

2.1. Participants

Participants were a subgroup from FITFATTWIN (Rottensteiner et al. 2014) study. A total of 18 healthy men from nine monozygotic twin pairs participated such that each pair was long-term discordant in their leisure-time physical activity. The mean age of participants was about 35 years. In FITFATTWIN study we identified pairs who were long-term discordant for physical activity in order to investigate the effects of physical activity. We selected only men because before this age pregnancies have a major influence on physical activity fluctuations and irregularities related to menstrual cycle also influence many biological parameters targeted in our study. FITFATTWIN study participants were initially identified from FinnTwin16 Cohort, which is a population based, longitudinal study of Finnish twins born between October 1974 and December 1979 (Kaprio, Pulkkinen, & Rose, 2002). Selection of the twin pairs to the present study is described in detailed in Rottensteiner et al. 2015 (Rottensteiner et al., 2015). In short, the twins participated in web-based questionnaire after which there was a telephone interview and finally interview at the laboratory and medical examination. Physical activity levels and pairwise discordance was based on structured retrospective physical activity interview (Kujala et al., 1998; Leskinen et al., 2009; Waller, Kaprio, & Kujala, 2008) which we conducted and which takes into account leisure-time physical activity, including commuting activity, one-year intervals over the past six years. This information was used to define pairwise discordance. The mean leisure-time

metabolic equivalent (MET) index during the past three years (3-yr-LTMET index as MET hours/day) was calculated and used as a criterion to assess leisure-time physical activity level. Weight, height, waist circumference and maximal oxygen uptake (VO_{2max}) were measured, body mass index (BMI) was calculated, and the whole body composition was determined after an overnight fast using dual-energy X-ray absorptiometry (DXA Prodigy; GE Lunar Corp., Madison, Wisconsin) (Table 1.).

(Table 1. around here)

Study procedure and test protocols were approved by the Ethical Review Board for Human Research of the Central Finland Health Care District (9/29/2011) and the study was conducted following the tenets of the Declaration of Helsinki. All participants volunteered, received no financial benefit and provided a written informed consent prior to participation.

2.2. SMMR protocol

Somatosensory electrical stimuli were delivered (Digitimer Ltd., model DS7A, Welvyn Garden City, UK) to left index and little fingers through flexible metal ring electrodes (stimulating cathode electrode placed above the proximal phalanx and anode electrode above the distal phalanx, Technomed Europe Ltd, Maastricht, Netherlands) to elicit somatosensory mismatch response, sMMR, as an automatic location deviance detection. The somatosensory stimulation was divided into two parts: in the first part standard stimuli were applied to the index finger and deviant stimuli to the little finger and in the second part standard and deviant stimuli locations were reversed thus producing mismatch in location during the flow of stimuli independent from finger. Stimulus intensity was set twice the individual sensory threshold separately for each finger. Electrical stimulus duration was 200 μs. Total of 1000 stimuli were delivered, 10 % were randomly delivered deviants. The inter-stimulus interval was 600 ms. Both co-twins were recorded on the same day. Participants were listening to an

engaging radio play and they were asked to ignore stimuli and concentrate on the play. Participants were observed via a video camera during recording and they were asked questions of the contents of the radio play afterwards.

EEG was continuously recorded with 128-channel sensor net with Cz reference (Electrical Geodesics, Inc., Portland, Oregon) and for analysis re-referenced to average reference. The sampling rate was 500 Hz with 0.1 Hz - 200 Hz bandpass filtering at recording. For offline analysis, EEG data was bandpass filtered in a range 1Hz - 35 Hz and segmented to 450 ms epochs (100 ms baseline preceding the stimulus onset and 350 ms post stimulus onset). Epochs containing artifacts with high amplitude potential shifts and eye-blinks and/or movement artifacts were automatically rejected. Noise-free epochs were baseline corrected and averaged to form the deviant wave form event-related potential (ERP) and then same amount of standard stimuli as the individual's deviant stimuli were picked from those standards that follow deviants in order to form the standard wave form for each participant. The minimum number of accepted deviants was 66 per participant (Table 1).

2.3. ERP analysis

Grand averages were formed for deviant and standard stimulus conditions each for inactive and active co-twins. Topographic voltage maps were plotted from deviant and standard grand average wave forms. Further data processing was performed with Brain Electrical Source Analysis (BESA, Besa GmbH, Gräfelfing, Germany). Spatio-temporal multiple dipole source models were developed. In this kind of a model, each source potential described the temporal variations in each dipole moment (i.e. its strength), while the equivalent dipole source maintained a stationary location and orientation in the modeling time window (0-350 ms from the stimulus onset). The proportion of the data not explained by the model was displayed in

residual variance (RV). An ellipsoidal head model with four shells was used. First the grand average waveform with highest amplitude was chosen as a starting point for modeling because source activities are easiest to dissociate when amplitudes are high and signal-to-noise ratio is good. Thus first model was developed for the deviant wave form grand average data set of the active twins. This was a seven-dipole model, where six dipoles explained cerebral activity and one dipole accounted for residual eye movements. Dipole 1 modeled major activity between 220-300 ms peaking with 20 nAm and dipoles 2 and 3 modeled unilateral (contralateral to stimulation) activity starting already at 24 ms with 9 nAm and 11 nAm peak currents, respectively. Dipoles 4 and 5 modeled bilateral activities between 100-300 ms in deeper brain areas peaking with 9 nAm and 7 nAm currents, respectively. Finally dipole 6 modeled unilateral (ipsilateral to stimulation) activity between 74-272 ms peaking with 8 nAm. Dipoles 1, 2, 3 and 5 were completely free during fitting and dipole 4 was symmetric to dipole 5 and dipole 6 was symmetric to dipole 2, and finally dipole 7, collecting residual eye movement activity, was fixed in location with free orientation. We applied this model to the data of the deviant grand average of inactive twins, and in addition, to the standard grand average wave forms of both groups. Always when applying first model to other data sets, the equivalent electrical dipole source orientations were fitted but no source locations were allowed to change. We tested that further fitting or adding more dipoles did not result in any substantial improvement of the model. As the locations were kept similar when applying the model in other data sets, the possible individual differences were observed in modulation of dipolar source potentials and in varying RVs. The differences in dipole moments were applied in statistical models.

2.4. MRI recording and preprocessing

Brain magnetic resonance imaging (MRI) scans were acquired using a 1.5 T whole body magnetic resonance (MR) scanner (Siemens Symphony, Siemens Medical Systems, Erlangen, Germany) on the same day as other data was collected. The 3D T1-weighted MPRAGE images of whole brain were collected with the following parameters: TR = 2180 ms, TE = 3.45 ms, TI = 1100 ms, flip angle = 15˚, slice thickness = 1.0 mm, in-plane resolution 1.0 mm × 1.0 mm, and matrix size = 256 × 256. Voxel-based morphometric (VBM) analyses were performed with VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) for SPM8 (Wellcome Trust Center for Neuroimaging, UCL, UK) running under Matlab R2010a (The Mathworks Inc., Natick, MA, USA). First, the MR images were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Images were then normalized to the Montreal Neurological Institute brain template using a high-dimensional DARTEL algorithm. Nonlinearly modulated GM images were created to preserve relative differences in regional GM volume. Finally, the GM volumes were spatially smoothed with 12 mm full width at half maximum Gaussian kernel. GM, WM and CSF volumes were compared between co-twins as well as GM voxel counts of four regions of interest (ROI), suggested by the source model, from both hemispheres were compared between co-twins. The ROIs were defined using the WFUPickAtlas-tool (Wake Forest University, School of Medicine) implemented in SPM8 (Maldjian, Laurienti, Kraft, & Burdette, 2003; Maldjian, Laurienti, & Burdette, 2004). The locations of WFU atlas ROIs used here for comparison between co-twins are given in Fig. 4.

2.5.Statistical analysis

Wilcoxon Signed Rank Test was used to compare voxel counts in MRI ROIs. For dipole moment comparison statistical analysis point-to-point on source waveforms was performed in SPSS 22 with repeated measures ANOVA with 5(time) x 2(group) factorial design. Only

group effects are reported. Significance was set at $p \leq 0.05$. Source waveform results include effect sizes in η_p^2 (partial eta-squared).

3. Results

The characteristics of the 18 twins from nine twin pairs are shown in Table 1. Inactive and active co-twins differed in their fat% and VO_2max, as anticipated. The mean activity level of the active twins was 321% higher than that of their inactive brothers (3-yr-leisuretime MET), while their fitness levels were 132% higher (VO_{2max}) (Rottensteiner et al., 2015; Tarkka et al., 2016). We did not see any difference in the number of successful ERP recordings and brain segmented morphologic volumes between active and inactive co-twins. SMMR grand average waveforms of inactive and active co-twins are depicted in Fig. 1, where all 128 channels are superimposed to allow visualisation of similarities and differences between the co-twins in an illustrative window from -100 to 500 ms. In Fig.1, 0 denotes the stimulus onset and selected time points (90 ms, 150 ms, 244 ms and 280 ms) are shown in topographic maps to facilitate comparison.

(Figure 1. around here)

Equivalent electrical dipole source model developed in BESA is shown in Fig. 2, where the same model is illustrated in sagittal (A) and verticofrontal (B) planes. The model consisted of 7 source dipoles (SD), though the dipole explaining eye activity is not visible in the planes shown in Fig. 2. The 3D dipole location coordinates of the model are given in Table 2 as well as the approximate brain areas which the dipole coordinates represent. The model RV in the grand average of the deviant of active co-twins was 6.9% and the same model, when introduced in standard grand average, gave RV 25.1%. When this model was

introduced in the grand average of the deviant of inactive co-twins the RV was 5.7% and when it was introduced in standard grand average of inactive co-twins RV was 17.8%. When the model was introduced in any data sets, SD orientations were fitted but locations were not. The subsequent relatively minor orientation variations are not shown. Source wave forms of the models for deviant stimulus-elicited sMMRs were compared between inactive and active co-twins. For source SD2 we found significant difference during 280 to 290 ms post stimulus ($F(1, 16) = 5.345$, $p = 0.034$, $\eta_p^2 = 0.250$) where inactive co-twins had stronger amplitudes. In source SD3 there was significant difference between 148-158 ms after stimulus onset ($F(1, 16) = 8.200$, $p = 0.011$, $\eta_p^2 = 0.339$) where again inactive co-twins had stronger amplitudes. Source SD4 differed at two periods: first at 86 to 96 ms ($F(1, 16) = 5.780$, $p = 0.029$, $\eta_p^2 = 0.265$) where again inactive co-twins had stronger amplitudes. The later difference in SD4 was in the window from 252 to 262 ms ($F(1, 16) = 5.538$, $p = 0.032$, $\eta_p^2 = 0.257$) where active co-twins had stronger amplitudes. Source SD1 did not show differences. Also the standard stimulus equivalent dipole source waveforms were compared, and there for source SD6 we found significant difference during 252 to 262 ms ($F(1, 16) = 4.811$, $p = 0.043$, $\eta_p^2 = 0.231$) where active co-twins had stronger amplitudes. Fig. 3 details the differences in SD moments.

(Table 2 and Figures 2 and 3 around here)

Total GM, WM and CSF volumes estimated from non-normalized images did not differ between the co-twins in structural MRI analysis (see Table 1). Multiple dipole source model suggested ROIs (anterior cingulate, postcentral gyrus, frontal medial gyrus and superior temporal gyrus) where GM voxel count was performed. The exact 3D regional counts in MRI were performed using WFU Atlas, see cortical surface rendering of ROIs in Fig. 4. GM voxel count differed in one ROI, the right anterior cingulate, (inactive 544±9 vs. active 536±12, $p=0.046$) between inactive and active co-twins where inactive co-twins showed

larger voxel count (see Table 3 for all tested ROIs). Right anterior cingulate ROI is illustrated in averaged MR image in Fig. 5.

(Table 3. and Figures 4 and 5 around here)

4. Discussion

Our present results demonstrate that long-term physical activity selectively modulates specific early sensory functional brain responses and may selectively modify cortical structures. Three-dimensional source analysis indicated short time windows where specific sMMR cerebral sources were stronger, and GM voxel count in structural MR image was higher in the right anterior cingulate ROI, both distinctions in inactive co-twins compared to their active co-twins. The purpose of studying young, healthy male twins is to see whether possible dissimilarities in physical activity, at an age when chronic diseases, medications or prodromal disease processes are unlikely yet to be present, are associated with functional and/or structural modulation in the brain. The monozygotic twin design with discordant brothers provides a unique experimental opportunity allowing adjustment for known and unknown confounders of the association between physical activity and brain markers.

Previously we have shown that sMMR is reliably electrically elicited by a location difference in the hand and its modulations can be observed in ageing and in persons in different physical activity categories (Strömmer et al., 2014; Tarkka et al., 2016). The cerebral sources of auditory mismatch negativity (MMN), the apparent close relative of sMMR, have been located in bilateral temporal cortices and frontal cortex (Giard, Perrin, Pernier, & Bouchet, 1990; Naatanen & Kahkonen, 2009; Näätänen et al., 2007). In the

present study, we developed a 3D source model to approximate the cerebral sources of the electrically registered sMMR. Previously, equivalent current dipole source for the sMMR component in the window of 150-250 ms was located in the primary (SI) or secondary somatosensory cortex (SII) contralateral to stimulated hand by Akatsuka et al. (2007) in their magnetoencephalograhic study (Akatsuka et al., 2007). Kekoni et al. (1992) have also localized somewhat earlier middle-latency somatosensory magnetic fields in contralateral SI and SII (Kekoni et al., 1997). We, however, attempted to incorporate the sources of cortical activity from stimulus onset to 350 ms in order to describe the complete process of detecting sensory mismatch. Our model was developed for the deviant waveform even though mismatch negativity studies often investigate difference waveforms. In contrast to difference waveform analysis, our model approximates sources in a natural condition where most of the ongoing brain processes are taken into consideration within the modeled window.

Our source model has seven dipoles, six of which are in the brain. SD1 source located in the right ventral anterior cingulate gyrus, location associated with large variety of phenomena related to executive control with numerous projections to motor areas (Devinsky, Morrell, & Vogt, 1995). SD:s 2, 3 and 6 located in areas more specifically related to somatosensory processing as SD 2 and 6 were located in postcentral gyrus, part of the area known as primary somatosensory cortex, SI, responsible for processing sensation of touch (Noback, Strominger, Demarest, & Ruggiero, 2005). Furthermore, SD 3 located in frontal medial gyrus in the right hemisphere, area with connections to postcentral gyrus and functional links to spatial attention and top-down control of attentional focus (Fox et al., 2014). SD4 and SD5 were located in left and right superior temporal gyri (bilaterally in BA 22), in areas which are heavily implicated in auditory processing, but may also contribute to amodal, likely multisensory, and memory-related aspects of MMN response (Näätänen et al., 2007).

Those sMMR differences, that indicated larger automatic neural activation in inactive co-twins compared to their active brothers, located in contralateral SI and SII regions and in the frontal medial gyrus (Fig. 3, Source Dipole 2, Source Dipole 3). The SI and SII activity likely cover primary and secondary somatosensory processing and also some somatosensory associative function, however, difference observed in activation in frontal medial gyrus may well indicate more complex automatic sensory mismatch processing. Frontal medial gyrus is known to contribute to a number of associative and executive functions and is active also in cognitive task when subjects have to decide "where" in the body the target is (Talati & Hirsch, 2005). This region is implicated in motor planning and non-motor tasks such as decision making, discrimination and especially in convergence of sensory information for high-level processes related to coordination of motor activity (Bak, Glenthoj, Rostrup, Larsson, & Oranje, 2011; Noback et al., 2005). Thus, frontal medial gyrus may play a role in automatically alerting inactive co-twins more than the active co-twins of deviant information ascending from the body. Sensory gating using different electrical stimulation paradigm has been applicably studied in psychiatry where source modeling has implicated frontal medial gyrus as an important player in gating (Bak et al., 2011; Jensen, Oranje, Wienberg, & Glenthoj, 2008). Thus it may be that amplitude differences we have observed are explained by differences in sensory gating emerging from different levels of physical activity.

First source dipole (SD1) of the present model located close to midline and likely accounted for activity in rather large bilateral region in ventral anterior cingulate. No difference was observed in the source moment of this dipole associated with level of physical activity. This dipole mainly accounted for late activity within the model, approximately from 220 to 280 ms. As the electrical stimulus intensity in the fingers were twice sensory

threshold, the stimuli were distinctive and not pleasant. It is plausible that SD1 accounted for activity registering the unpleasantness of stimuli as ventral anterior cingulate area is known for processing painful stimuli (Apkarian, Bushnell, Treede, & Zubieta, 2005; Devinsky et al., 1995; Tarkka & Treede, 1993). Anterior cingulate is activated in various acute pain stimulus paradigms (Apkarian et al., 2005) and thus it is conceivable that co-twins responded similarly to the unpleasantness of electrical stimuli but their interpretations varied depending on their accustomed level of physical activity. Tesarz et al (2013) recently elegantly showed that pain inhibitory system may be less responsive in athletes than in non-athletes (Tesarz, Gerhardt, Schommer, Treede, & Eich, 2013). Applied to our condition, their conclusion may support our view of the present data, i.e. both twins recognized the unpleasantness similarly but active co-twins automatically assessed it less meaningful. Popovich and Staines (2015) found that only one acute bout of exercise modulated late somatosensory component (especially LLP in their work) in attended and unattended conditions, and they suggested that this modulation was associated with improvement in selective attentional processing and sensory gating of task-irrelevant stimuli (Popovich & Staines, 2015). Our findings on sMMR occurred in the same time window with corresponding results to Popovich and Staines's unattended condition and our inactive twins showed stronger amplitudes compared to their active co-twins. However, our data shows long-term exercise effect as the co-twins were discordant in their physical activity for at least three years.

As the functional modeling of sMMR revealed distinctions between co-twins, a comparison of structural brain images of co-twins was performed. It was based on the regions where active sources were identified (see Table 3). Atlas-based ROIs were used in GM voxel count comparison where a difference in the right hemisphere anterior cingulate was detected indicating higher voxel count in inactive co-twins. We were astonished that only right

anterior cingulate region showed this structural difference. Yet it should be remembered that these atlas ROIs are rather large (Fig. 4.) and inevitably these areas participate in many different functions which may or may not modulate GM morphology in young healthy men. Our data imply that anterior cingulate region is, at least to some extent, functionally involved in somatosensory deviant detection and it shows morphological difference associated with long-term exercise history. We can speculate that physical activity may have somewhat corresponding structural brain effects as is suggested by Fox et al. (2014) analyzing morphometric neuroimaging studies in meditation practitioners (Fox et al., 2014). That large meta-analysis found eight brain regions consistently altered in meditators compared to non-meditators, including anterior and mid cingulate and sensory cortices and insula. Sensation regulation is connected with anterior cingulate (Apkarian et al., 2005; Fox et al., 2014) and it is likely that the unpleasantness of electrical stimuli was automatically assessed, at least in part, in this region.

Establishing modulations in both MR revealed morphology and functional source analysis in healthy twin males who differ only in their long-term exercise history leads towards emerged point of view in brain research, namely brain plasticity in adults. Most studies assess cortical plasticity during recovery processes after brain insults, such as cerebrovascular stroke (Julkunen et al., 2016; Nudo & McNeal, 2013; Nudo, 2013; Tarkka, Könönen, Pitkänen, Sivenius, & Mervaala, 2008), however many principles found in recovery processes may also apply to any intensive long-term activity, in our case physical exercise. Number of factors influence dose-response of physical exercise in brain plasticity, ranging from molecular and cellular cascades to points of saturation of effect, most of which are poorly known. However, it seems likely that behavioral experience, in the present case it being mostly aerobic exercise, is a powerful modulator of brain plasticity.

In conclusion, we showed multiple brain areas involved in sensory discrimination and integration of sensory inputs in the early time period where conscious processing of stimuli was most unlikely. Furthermore, we demonstrated differences between monozygotic co-twins, discordant in physical activity, in the tested automatic sensory processing. Our experimental design verified that attentional or motivational factors did not contaminate our result. Though we control for familial and genetic confounders, we cannot firmly establish the direction of causation, even though we consider physical activity as the more likely driver of the neurophysiological changes than vice versa. The small number of monozygotic twin pairs discordant in long-term physical activity is clearly a limitation of the present study and thus more research is needed to confirm the present results. It is, however, very difficult to identify larger numbers of twin pairs sufficiently discordant for leisure-time physical activity and fitness who are also healthy and free of medications and other potential confounders. We essentially screened all available pairs from five birth cohorts aged in the mid-thirties in Finland. We had only structural MR images in the present study, and thus it would be interesting to relate electrically elicited sMMR and functional MR imaging, yet any brain structural differences between healthy monozygotic twins is noteworthy.

Acknowledgements

Authors wish to thank the participants of the study and laboratory personnel and students at the University of Jyväskylä who contributed to this study. Support from the Juho Vainio Foundation (Helsinki) is acknowledged (to I M Tarkka). Data collection in the twin cohort has been supported by National Institute of Alcohol Abuse and Alcoholism (grants AA-12502, AA-00145, and AA-09203 to R J Rose) and the Academy of Finland (grants 100499, 265240, 263278 and 264146 to J Kaprio).

References

Akatsuka, K., Wasaka, T., Nakata, H., Inui, K., Hoshiyama, M., & Kakigi, R. (2005). Mismatch responses related to temporal discrimination of somatosensory stimulation. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology, 116*(8), 1930-1937. doi:10.1016/j.clinph.2005.04.021

Akatsuka, K., Wasaka, T., Nakata, H., Kida, T., & Kakigi, R. (2007). The effect of stimulus probability on the somatosensory mismatch field. *Experimental Brain Research, 181*(4), 607-614. doi:10.1007/s00221-007-0958-4

Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain (London, England), 9*(4), 463-484. doi:S1090-3801(04)00148-X [pii]

Bak, N., Glenthoj, B. Y., Rostrup, E., Larsson, H. B., & Oranje, B. (2011). Source localization of sensory gating: A combined EEG and fMRI study in healthy volunteers. *NeuroImage, 54*(4), 2711-2718. doi:10.1016/j.neuroimage.2010.11.039 [doi]

Bouchard, C., Blair, S. N., Church, T. S., Earnest, C. P., Hagberg, J. M., Hakkinen, K., . . . Rankinen, T. (2012). Adverse metabolic response to regular exercise: Is it a rare or common occurrence? *PloS One, 7*(5), e37887. doi:10.1371/journal.pone.0037887 [doi]

Devinsky, O., Morrell, M. J., & Vogt, B. A. (1995). Contributions of anterior cingulate cortex to behaviour. *Brain : A Journal of Neurology, 118 (Pt 1)*(Pt 1), 279-306.

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., . . . Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory.

Proceedings of the National Academy of Sciences of the United States of America, 108(7), 3017-3022. doi:10.1073/pnas.1015950108 [doi]

Fox, K. C., Nijeboer, S., Dixon, M. L., Floman, J. L., Ellamil, M., Rumak, S. P., . . . Christoff, K. (2014). Is meditation associated with altered brain structure? A systematic review and meta-analysis of morphometric neuroimaging in meditation practitioners. *Neuroscience and Biobehavioral Reviews, 43*, 48-73. doi:10.1016/j.neubiorev.2014.03.016 [doi]

Giard, M. H., Perrin, F., Pernier, J., & Bouchet, P. (1990). Brain generators implicated in the processing of auditory stimulus deviance: A topographic event-related potential study. *Psychophysiology, 27*(6), 627-640.

Hillman, C. H., Erickson, K. I., & Kramer, A. F. (2008). Be smart, exercise your heart: Exercise effects on brain and cognition. *Nature Reviews.Neuroscience, 9*(1), 58-65. doi:10.1038/nrn2298

Jensen, K. S., Oranje, B., Wienberg, M., & Glenthoj, B. Y. (2008). The effects of increased serotonergic activity on human sensory gating and its neural generators. *Psychopharmacology, 196*(4), 631-641. doi:10.1007/s00213-007-1001-y [doi]

Julkunen, P., Kononen, M., Maatta, S., Tarkka, I. M., Hiekkala, S. H., Saisanen, L., . . . Jakala, P. (2016). Longitudinal study on modulated corticospinal excitability throughout recovery in supratentorial stroke. *Neuroscience Letters, 617*, 88-93. doi:S0304-3940(16)30082-9 [pii]

Kaprio, J., Pulkkinen, L., & Rose, R. J. (2002). Genetic and environmental factors in health-related behaviors: Studies on finnish twins and twin families. *Twin Research : The*

Official Journal of the International Society for Twin Studies, *5*(5), 366-371. doi:10.1375/136905202320906101 [doi]

Kekoni, J., Hämäläinen, H., Saarinen, M., Grohn, J., Reinikainen, K., Lehtokoski, A., & Näätänen, R. (1997). Rate effect and mismatch responses in the somatosensory system: ERP-recordings in humans. *Biological Psychology, 46*(2), 125-142. doi:S0301-0511(97)05249-6 [pii]

Kujala, U. M., Kaprio, J., Sarna, S., & Koskenvuo, M. (1998). Relationship of leisure-time physical activity and mortality: The finnish twin cohort. *Jama, 279*(6), 440-444. doi:joc71682 [pii]

Leskinen, T., Waller, K., Mutikainen, S., Aaltonen, S., Ronkainen, P. H., Alen, M., . . . Kujala, U. M. (2009). Effects of 32-year leisure time physical activity discordance in twin pairs on health (TWINACTIVE study): Aims, design and results for physical fitness. *Twin Research and Human Genetics : The Official Journal of the International Society for Twin Studies, 12*(1), 108-117. doi:10.1375/twin.12.1.108 [doi]

Maldjian, J. A., Laurienti, P. J., & Burdette, J. H. (2004). Precentral gyrus discrepancy in electronic versions of the talairach atlas. *NeuroImage, 21*(1), 450-455. doi:S1053811903005755 [pii]

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage, 19*(3), 1233-1239. doi:S1053811903001691 [pii]

Naatanen, R., & Kahkonen, S. (2009). Central auditory dysfunction in schizophrenia as revealed by the mismatch negativity (MMN) and its magnetic equivalent MMNm: A

review. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP), 12*(1), 125-135. doi:10.1017/S1461145708009322 [doi]

Näätänen, R., Paavilainen, P., Rinne, T., & Alho, K. (2007). The mismatch negativity (MMN) in basic research of central auditory processing: A review. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology, 118*(12), 2544-2590. doi:10.1016/j.clinph.2007.04.026

Noback, C., Strominger, N., Demarest, R., & Ruggiero, D. (2005). *The human nervous system, structure and function* (Sixth Edition ed.). Totowa, New Jersey, USA: Humana Press.

Nudo, R. J. (2013). Recovery after brain injury: Mechanisms and principles. *Frontiers in Human Neuroscience, 7*, 887. doi:10.3389/fnhum.2013.00887 [doi]

Nudo, R. J., & McNeal, D. (2013). Plasticity of cerebral functions. *Handbook of Clinical Neurology, 110*, 13-21. doi:10.1016/B978-0-444-52901-5.00002-2 [doi]

Popovich, C., & Staines, W. R. (2015). Acute aerobic exercise enhances attentional modulation of somatosensory event-related potentials during a tactile discrimination task. *Behavioural Brain Research, 281*, 267-275. doi:10.1016/j.bbr.2014.12.045 [doi]

Reiner, M., Niermann, C., Jekauc, D., & Woll, A. (2013). Long-term health benefits of physical activity - a systematic review of longitudinal studies. *BMC Public Health, 13*, 813-2458-13-813. doi:10.1186/1471-2458-13-813; 10.1186/1471-2458-13-813

Rottensteiner, M., Leskinen, T., Niskanen, E., Aaltonen, S., Mutikainen, S., Wikgren, J., . . . Kujala, U. M. (2015). Physical activity, fitness, glucose homeostasis, and brain

morphology in twins. *Medicine and Science in Sports and Exercise, 47*(3), 509-518. doi:10.1249/MSS.0000000000000437 [doi]

Ruscheweyh, R., Willemer, C., Kruger, K., Duning, T., Warnecke, T., Sommer, J., . . . Floel, A. (2011). Physical activity and memory functions: An interventional study. *Neurobiology of Aging, 32*(7), 1304-1319. doi:10.1016/j.neurobiolaging.2009.08.001 [doi]

Spackman, L. A., Boyd, S. G., & Towell, A. (2007). Effects of stimulus frequency and duration on somatosensory discrimination responses. *Experimental Brain Research, 177*(1), 21-30. doi:10.1007/s00221-006-0650-0

Strömmer, J. M., Tarkka, I. M., & Astikainen, P. (2014). Somatosensory mismatch response in young and elderly adults. *Frontiers in Aging Neuroscience, 6*, 293. doi:10.3389/fnagi.2014.00293 [doi]

Talati, A., & Hirsch, J. (2005). Functional specialization within the medial frontal gyrus for perceptual go/no-go decisions based on "what," "when," and "where" related information: An fMRI study. *Journal of Cognitive Neuroscience, 17*(7), 981-993. doi:10.1162/0898929054475226 [doi]

Tarkka, I. M., Könönen, M., Pitkänen, K., Sivenius, J., & Mervaala, E. (2008). Alterations in cortical excitability in chronic stroke after constraint-induced movement therapy. *Neurological Research, 30*(5), 504-510.

Tarkka, I. M., Savic, A., Pekkola, E., Rottensteiner, M., Leskinen, T., Kaprio, J., & Kujala, U. M. (2016). Long-term physical activity modulates brain processing of somatosensory

stimuli: Evidence from young male twins. *Biological Psychology*, doi:S0301-0511(16)30029-1 [pii]

Tarkka, I. M., & Treede, R. D. (1993). Equivalent electrical source analysis of pain-related somatosensory evoked potentials elicited by a CO2 laser. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society, 10*(4), 513-519.

Tesarz, J., Gerhardt, A., Schommer, K., Treede, R. D., & Eich, W. (2013). Alterations in endogenous pain modulation in endurance athletes: An experimental study using quantitative sensory testing and the cold-pressor task. *Pain, 154*(7), 1022-1029. doi:10.1016/j.pain.2013.03.014 [doi]

Waller, K., Kaprio, J., & Kujala, U. M. (2008). Associations between long-term physical activity, waist circumference and weight gain: A 30-year longitudinal twin study. *International Journal of Obesity (2005), 32*(2), 353-361. doi:0803692 [pii]

Figure Legends

Fig. 1. SMMR grand average wave forms of deviant stimuli in inactive (A) and active (B) co-twins. All 128 channels are superimposed, average reference is used and topographic voltage distribution maps are shown as 10 ms mean values at selected time points (86-96ms, 148-158ms, 252-262 ms and 280-290 ms), where later equivalent dipole source analysis indicated significant differences between co-twins. 0 is the onset of stimulation.

Fig. 2. Seven-dipole source model generated from grand average deviant waveform and presented in average MR image in sagittal (A) and verticofrontal (B) planes. Six dipoles are visible in these depicted planes, one dipole accounting for eye movement activity is not visible here. SD1=red, SD2=light purple, SD3=green, SD4=magenta, SD5=brown, SD6=blue. See Table 2 for three-dimensional source location coordinates.

Fig. 3. Source moments (not ERPs) of the developed source model explaining deviant data sets and detected significant differences between groups are shown: Source SD2 for deviant (first from left, light purple in Fig. 2), difference during 280-290 ms from stimulus onset, Source SD3 for deviant (second from left, green in Fig. 2), difference during 148-158 ms from stimulus onset, Source SD4 for deviant (third from left, magenta in Fig. 2), differences during 86-96 and 252-262 after stimulus onset. Standard stimuli data were also modeled and source SD6 (fourth from left, light blue in Fig. 2) shows standard stimulus data sets where difference during 252-262 ms after stimulus onset was found. Significant differences are indicated with gray bars and zero time-point is the stimulus onset.

Fig. 4. The WFU Atlas regions of interest (ROIs), which were initially suggested by the spatio-temporal source model, were used in analysing possible structural differences in

individual MR images between inactive and active co-twins. ROIs have been rendered on cortical surface in such a way that the stronger colours indicate more superficial locations, whereas weaker colours indicate more deeper regions.

Fig. 5. Structural MR images of co-twins differed in GM voxel count in right anterior cingulate ROI. Only the above ROI shown in green gave higher GM voxel count in inactive co-twins.

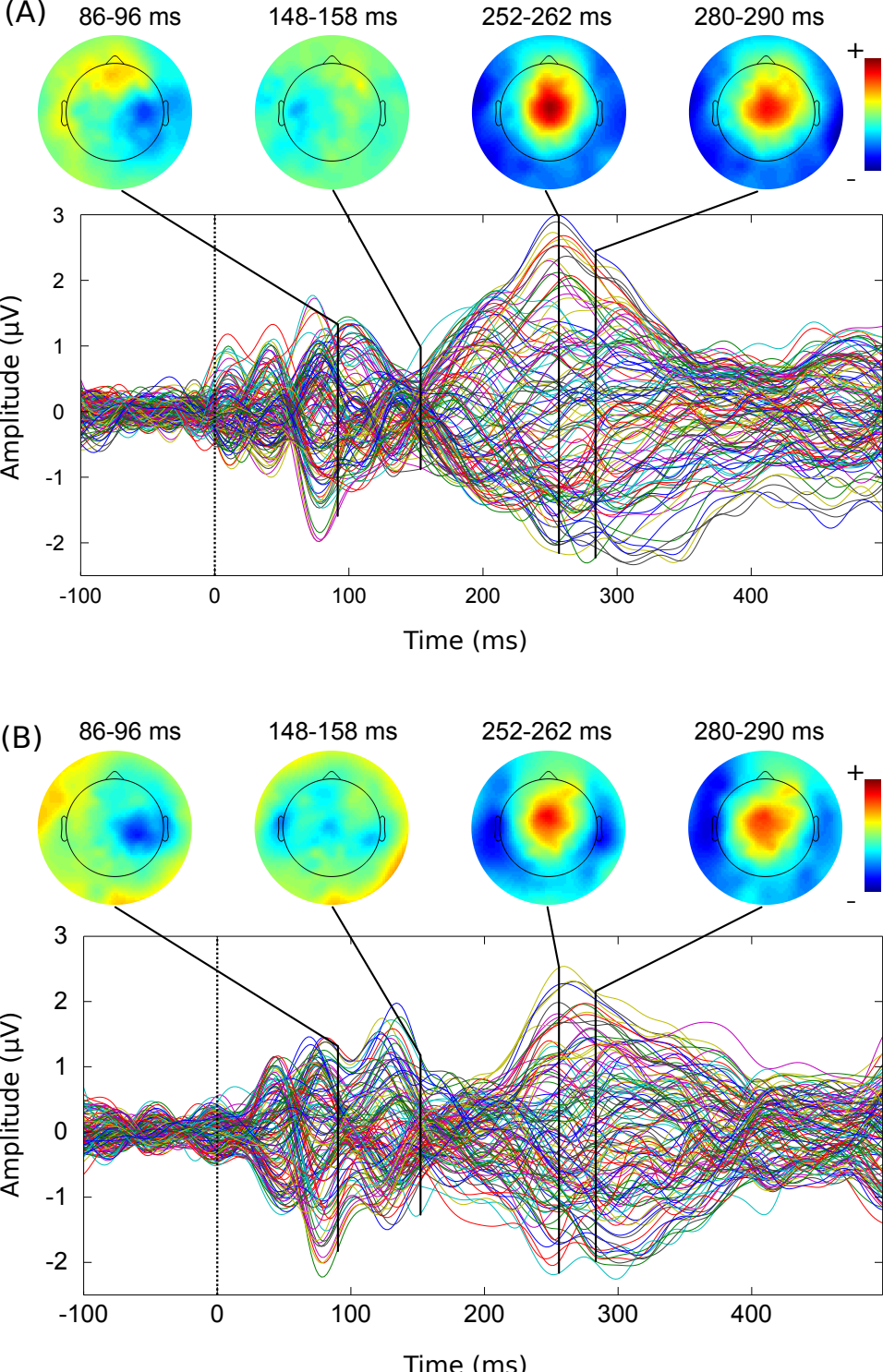
(A)
86-96 ms
148-158 ms
252-262 ms
280-290 ms
+
-
Amplitude (µV)
3
2
1
0
-1
-2
-100
0
100
200
300
400
Time (ms)
(B)
86-96 ms
148-158 ms
252-262 ms
280-290 ms
+
-
Amplitude (µV)
3
2
1
0
-1
-2
-100
0
100
200
300
400
Time (ms)

Figure 2

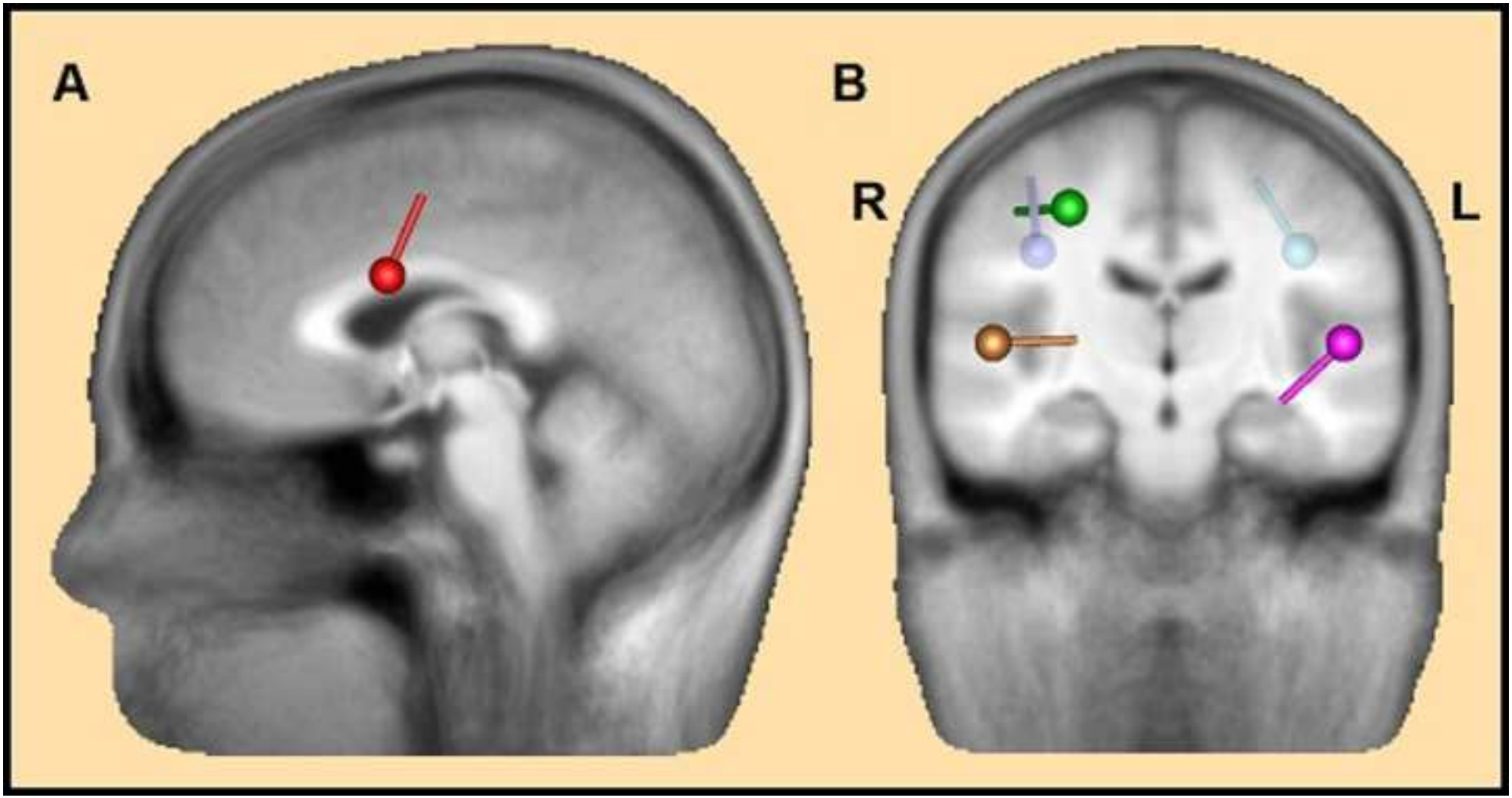

Figure 3

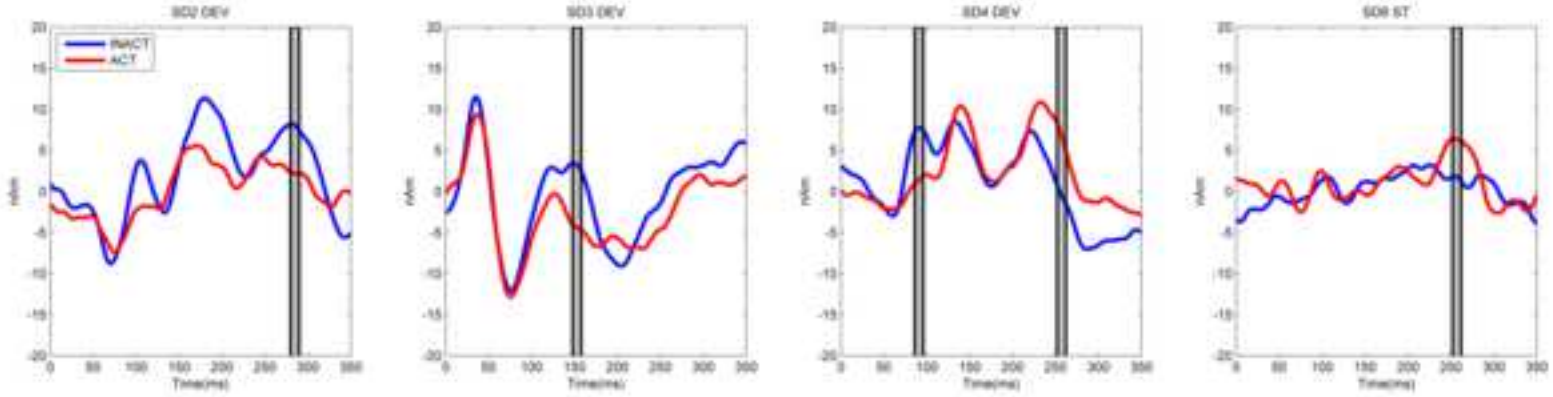

Figure 4

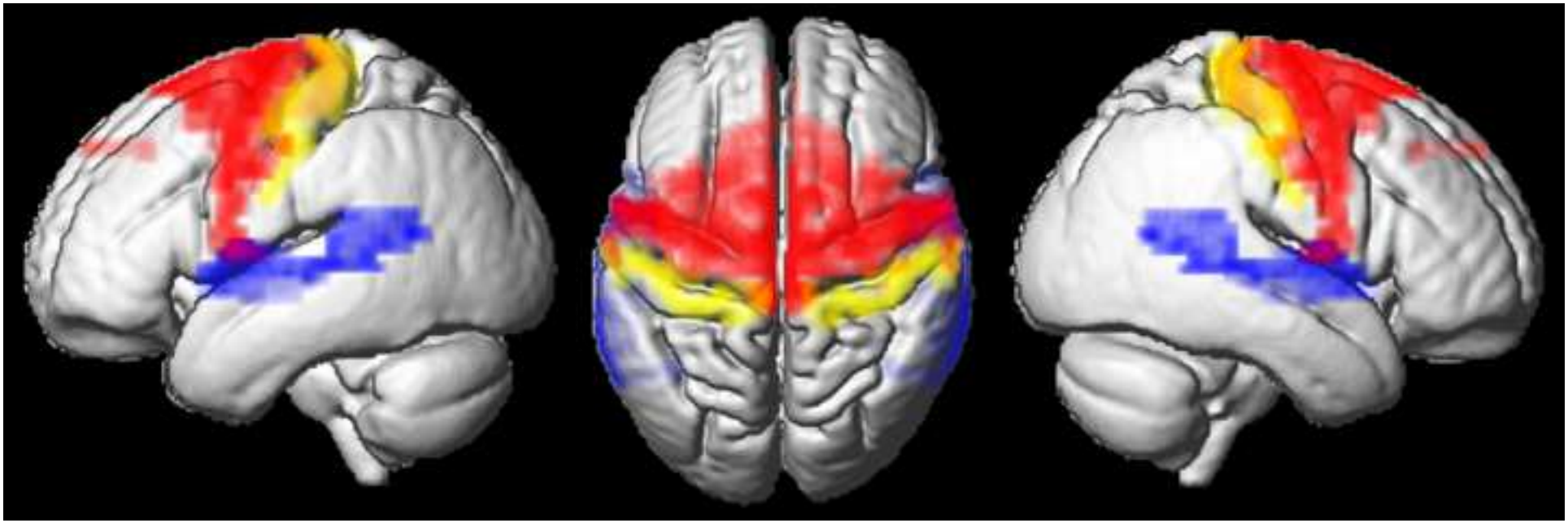

Figure 5

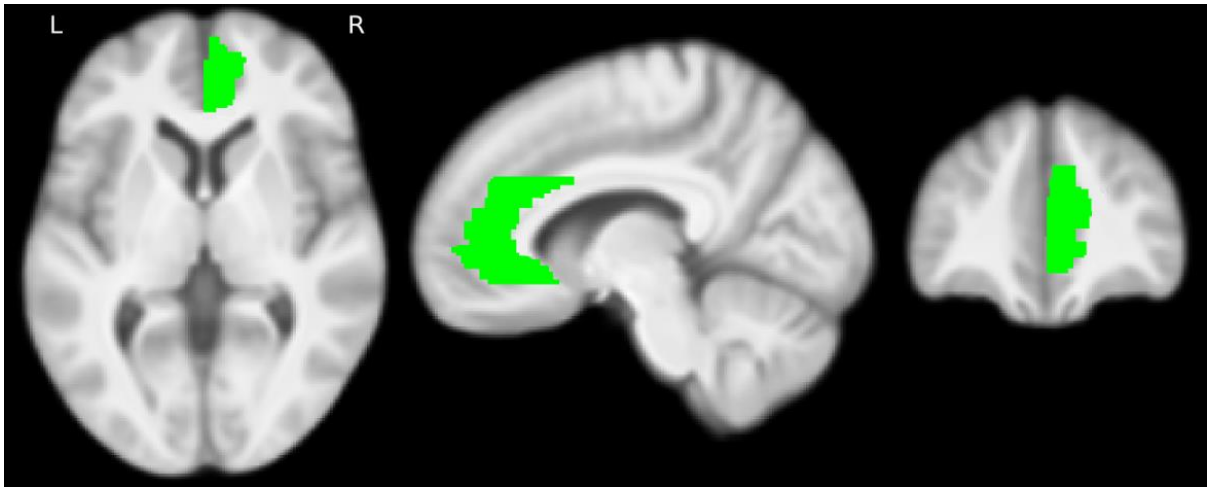

Table 1. Participant characteristics, 18 individuals (9 monozygotic male twin pairs), means and (±SD).

	Inactive co-twin	**Active co-twin**	**p-value[#]**
Age, y	34.3 (1.4)	34.1 (1.5)	0.686
Height, cm	178.5 (5.3)	179.7 (5.7)	0.012*
Weight, kg	78.0 (13)	75.9 (9)	0.424
BMI	24.3 (3)	23.4 (2)	0.269
Fat%	23.8 (5)	20.3 (4)	0.040*
Waist circ., cm	88.7 (9)	85.2 (7)	0.123
VO_2max, ml/kg/min	37.2 (3.5)	43.1 (4)	0.008**
3-yr-MET	1.4 (1.0)	4.5 (2.1)	0.003***
SMMR standards, n	92 (7)	90 (10)	
SMMR deviants, n	91 (6)	90 (8)	
GM volume, ml	668.3 (31)	675.3 (38)	0.815
WM volume, ml	685.0 (49)	696.1 (41)	0.606
CSF volume, ml	229.0 (36)	227.6 (39)	0.963
Ant. cingulate, voxel	544 (9)	536 (12)	0.046*[l]

[#] Mann-Whitney U-test. *p<.05 **p<.01 ***p<.005

[l] Wilcoxon Signed Rank -test

Table 2. Source location coordinates of the source model generated for the grand average deviant wave form of the active twins. Six equivalent electrical source dipoles (SD) localized in the brain and seventh dipole modeled the remaining eye movements (after eye movement correction). Approximate brain regions are given in Talairach labels and Brodmann areas are in parenthesis.

Fitting window Component	**Source location (x, y, z)**	**Brain region, Talairach (Brodmann Area)**
SD 1	2.9, 24.6, 54.5	Ventral anterior cingulate (R) (BA 24)
SD 2	32.7, -6.5, 65.5	Postcentral gyrus (R) (BA 3)
SD 3	24.8, 9.9, 74.6	Frontal medial gyrus (R) (BA 6)
SD 4	-43.8, 3.7, 38.6	Superior temporal gyrus (L) (BA 22)
SD 5	43.8, 3.7, 38.6	Superior temporal gyrus (R) (BA 22)
SD 6	-32.7, -6.5, 65.5	Postcentral gyrus (L) (BA 3)
SD 7	30.1, 66.5, 6.2	-

Table 3. Four regions of interest (ROI) in each hemisphere were selected and compared from whole brain structural MR images of the brains of co-twins. The gray matter voxel counts in ROIs were compared between inactive and active individuals within each twin pair using Wilcoxon Signed Rank Test. For the ROIs Brodmann areas are given in parenthesis after Talairach labels. Note, that only right anterior cingulate shows a difference.

Brain region Talairach, right	p-value	Brain region Talairach, left	p-value
Anterior cingulate (BA24)	0.046*	Anterior cingulate (BA24)	0.612
Postcentral gyrus (BA3)	0.204	Postcentral gyrus (BA3)	0.401
Frontal medial gyrus (BA6)	0.270	Frontal medial gyrus (BA6)	0.574
Superior temporal gyrus (BA22)	0.262	Superior temporal gyrus (BA22)	0.575

*$p<0.05$