Resting-state network plasticity induced by music therapy after traumatic brain injury

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Traumatic brain injury (TBI) is characterized by a complex pattern of abnormalities in resting-state functional connectivity (rsFC), and neuropathology focused on network dysfunction. Here we report a fMRI study of brain network changes induced during a randomised controlled trial of neurological music therapy in 23 moderate/severe TBI patients. Our ROI-to-ROI approach used four networks as sources: the frontoparietal (FPN), dorsal attention (DAN), default mode (DMN), and salience (SAL) networks. These networks include high-degree nodes or network hubs, and have all been associated with cognitive impairment after TBI. Furthermore, we investigated the correlation between brain network changes and executive function (EF). Lastly, we implemented a seed-to-voxel analysis to cross-link whole-brain rsFC with brain morphometry results obtained in our previous study of this data (1). The neurological music therapy increased the coupling between the FPN and DAN as well as between these networks and primary sensory networks that were engaged during musical training. By contrast, the DMN was less connected with sensory networks after the intervention. Similarly, there was a shift towards a less connected state within the FPN and SAL networks, which are typically hyperconnected following TBI. Improvements in EF were correlated with rsFC within the FPN and between the DMN and sensorimotor networks. Finally, the increase in grey matter volume in frontal regions was associated with greater rsFC in areas implicated in music processing. This study is the largest of its kind, and suggests that rsFC in response to music-based rehabilitation may provide sensitive biomarkers of cognitive recovery after TBI.

Introduction

Each year there are over 50 million diagnosed cases of traumatic brain injury (TBI), and it has been estimated that approximately half of the world’s population will sustain at least a minor TBI during their lifetime (2). The consequences of TBI can be fatal; it is the leading cause of mortality in young adults and a major cause of death and disability across all ages worldwide. Despite the wide variety of symptoms that can follow a TBI, the most prominent cognitive impairments affect the domains of attention, memory, communication, and executive functioning (EF) (2–6). In fact, deficits in EF are deemed to be the core symptoms of TBI (7, 8). Although there is no consensus on the exact definition of EF, it is thought to encompass several cognitive processes including the core of set shifting, inhibition, and updating (9). Given the heterogeneous and complex nature of TBI, there is an urgent need to develop novel and motivating rehabilitation strategies that target multiple deficits simultaneously, yet with a primary focus on EF.

It has been shown that musical training enhances EF and increases the engagement of the cognitive control network (10–19). Since brain injury patients are still able to enjoy and participate in musical activities (20), neurological music therapy can potentially contribute to restore the EF deficits observed in TBI patients. Until recently, this question had been addressed by only three studies exploring the cognitive effects of music-based interventions after TBI (21–23). Evidence from these studies indicated that music-based rehabilitation can indeed lead to cognitive recovery after brain injury, especially in the domain of mental flexibility. However, these studies presented important limitations with regard to the sample size, lack of proper randomized controlled designs, and inclusion of patients with brain injury not caused by trauma.

We have conducted the first-ever randomized controlled trial (RCT) of neurological music therapy in a sample of 40 moderate/severe TBI patients, where different domains...
of EF, attention, and memory were systematically analysed. We used a single-blind crossover design with 3 time-points (baseline/3-months/6-months) for neuropsychological assessment and s/fMRI acquisition. The neurological music therapy consisted of 20 individual therapy sessions held by a trained music therapist over a 3-month period (see Methods section for more details) and was targeted primarily to the rehabilitation of EF, attention, and working memory. In a previous publication, we reported that the music-based intervention induced a cognitive improvement in general EF performance as well as in set shifting (1).

In addition to demonstrating this improvement in neuropsychological performance, in (1) we conducted a voxel-based morphometry (VBM) analysis to investigate the volumetric changes induced by the music therapy. This analysis was motivated by previous work showing that environmental enrichment, such as that provided by musical activities, can increase cognitive reserve and promote adaptive structural neuroplasticity changes in TBI (24–26) and also in stroke patients (27). Our VBM results indicated that TBI patients showed an increase in grey matter volume (GMV) in different brain regions involved in music processing and cognitive function after the intervention. A therapy-induced increase in GMV was seen especially in the right inferior frontal gyrus (IFG) and was correlated with enhanced set shifting ability.

Resting-state functional connectivity (rsFC) is characterized by task-free spontaneous fluctuations in brain activity that occur synchronously across spatially distant brain regions in humans and non-human primates (28, 29). These fluctuations can be measured with the blood-oxygen-level-dependent response at low frequencies (usually under 0.15Hz) and are spatially organized in resting-state networks (RSNs) (30) that mirror activity evoked during cognitive tasks (31, 32). Examining rsFC after TBI is an active area of investigation motivated by the fact that diffuse axonal injury, which is a common pathology reported in all severities of TBI (33, 34), damages the structural connectivity that partly underlies functional connectivity across RSNs (4). This phenomenon makes the study of rsFC after brain injury especially relevant as the loss of integration of information in large-scale brain networks impairs performance in high-level cognitive functions (30, 35–38).

rsFC studies of TBI patients have revealed both increases and decreases in network connectivity, including the default mode (DMN) and salience (SAL) networks as well as multiple sensory and cognitive networks across the spectrum of injury severity (39–44). For example, decreased rsFC has been observed within five network pairs (DMN-basal ganglia, attention-sensorimotor, frontal-DMN, attention-frontal, and sensorimotor-sensorimotor) (43); also within the motor-striatal network, in contrast to increased connectivity within the right frontoparietal network (FPN) (42). In several cases, these abnormalities correlated with post-concussive symptoms (39, 40) and cognitive impairment (45).

Despite the complex abnormalities of interactions within and between RSNs following TBI, it is possible to identify some distinctive patterns. Within networks, reduced rsFC within the nodes of the DMN predicts attentional impairment (36). Such association could be driven in turn by damage to the cingulum bundle connecting the nodes of the DMN. The temporal coordination between networks, which is important for efficient high-level cognitive function, has also shown consistent abnormalities after TBI. According to an influential model of cognitive control (46, 47), the switching from automatic to controlled behaviour is mediated by the interaction between the SAL and DMN networks, including deactivation of the DMN to attend unexpected external events. Indeed, TBI patients exhibit a failure to appropriately deactivate the DMN, which is associated with impaired response inhibition to a stop signal (36).

The paradoxical yet well-documented finding that functional connectivity may increase secondary to TBI (40, 41, 44, 48–52) has given rise to the ‘hyperconnectivity’ hypothesis to explain the evolution of brain network reorganization after neurological disruption (53). In this context, hyperconnectivity is defined as enhanced functional connectivity in the number or strength of connections. It is thought to predominantly affect RSNs with high-degree nodes, also known as network hubs, such as the FPN, DMN and SAL networks. Although this hyperconnected state may be adaptive in the short-term in order to re-establish network communication through network hubs, it has been argued that it may have negative long-term consequences due to the chronic enhancement of brain resource utilization, and increased metabolic stress. In the case of TBI patients, this may lead to late pathological complications including Alzheimer’s disease (54, 55), where amyloid beta deposition has been linked to the neurodegeneration of posterior DMN hubs with high metabolic rate (56–58).

In the present study, we extend our previous findings by analysing rsFC from the same sample of moderate/severe TBI patients (see Methods for more details) as the music therapy RCT (1). We used a seed-to-target approach to analyse the reconfiguration of RSNs induced by the neurological music therapy, selecting seeds from the nodes of four key networks: the DMN, SAL, FPN and dorsal attention (DAN) networks.

The current work is grounded in two main hypotheses: (H1) the intervention leads to enhanced coordination activity between attention and executive function (DAN, FPN)-supporting networks, and sensory networks that are engaged during the music therapy and (H2) the intervention leads to reduced connectivity of nodes from the SAL, DMN and FPN networks. In addition, we examined the relationship between the changes in rsFC within and between networks, and the therapy-induced improvement in EF shown previously with neuropsychological testing (1). We anticipated that the FPN would be less connected in those TBI patients with better EF performance; as the hyperconnectivity hypothesis predicts. Lastly, in a similar vein to the approach adopted by Han et al. (59), we conducted an exploratory seed-to-voxel analysis to elucidate the link between whole-brain rsFC and GMV.
enhancements induced by the neurological music therapy.

Methods

We conducted a single-blinded cross-over RCT (trial number: NCT01956136) with a 6-month follow-up phase. Upon recruitment, patients were randomly assigned to one of two groups: AB and BA. During the first 3-month period, the AB group received neurological music therapy in addition to standard care, whereas the BA group received only standard care. During the second 3-month period, the BA group received the music therapy intervention and standard care and the AB group received only standard care. Baseline measurements were administered at time point 1 (TP1), and follow-up measurements were conducted at the 3-month crossover point (TP2) and at the 6-month completion point (TP3).

Subjects and study design. The subjects analysed herein are a subset of our previous study (1). In the RCT, 40 TBI patients were recruited through the Brain Injury Clinic of the Helsinki University Central Hospital (HUCH), Validia Rehabilitation Helsinki, and the Department of Neurology of Lohja Hospital during 2014–2017. The inclusion criteria were: 1) diagnosed [Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10)] TBI fulfilling the criteria of at least moderate severity [Glasgow Coma Scale (GCS) score: ≤ 12 and/or post-traumatic amnesia (PTA) ≥ 24h]; 2) time since injury ≤ 24 months at the time of recruitment; 3) cognitive symptoms caused by TBI (attention, executive function, memory); 4) no previous neurological or severe psychiatric illnesses or substance abuse; 5) age 16–60 years; 6) native Finnish speaking or bilingual with sufficient communication skills in Finnish; 7) living locally (in the Helsinki–Uusimaa region of Finland); and 8) understanding the purpose of the study and being able to give an informed consent.

Randomization of patients was performed using an online random number generator (https://www.random.org/) by a person not involved in patient recruitment or assessments. To ensure steady allocation to both groups across the trial, the randomization was done in batches of two consecutive patients. The randomization was stratified for lesion laterality (left/right/bilateral). The trial was conducted according to the Declaration of Helsinki and was consistent with good clinical practice and the applicable regulatory requirements. The trial protocol was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (reference number 338/13/03/00/2012) and all subjects signed an informed consent.

Of the 40 TBI patients enrolled to the trial, there were three drop-outs before TP2 and another three drop-outs before TP3. The dropouts were mainly due to lack of energy and motivation. Of the remaining 34 patients, one was excluded from the analyses due to intensive self-implemented piano training, which was not part of the trial protocol, and 10 were excluded from the analyses due to lack of fMRI data owing to MRI contraindications of the patients preventing scanning, technical difficulties, and geometric distortion artifacts in fMRI. This yielded a total sample of 23 patients (AB: n = 15, BA: n = 8) for the present study.

Intervention. The neurological music therapy intervention consisted of 20 individual therapy sessions (2 times/week, 60 min/session) held by a trained music therapist (authors S.L., M.H., and M.A.) at Validia Rehabilitation Helsinki. The intervention required no previous musical experience and was adaptable to different degrees of injury across TBI patients. The intervention focused on active musical production with drums and piano. Each session included three 20 min modules: (i) rhythmical training (playing sequences of musical rhythms and coordinated bimanual movements on a djembe drum and on own body), (ii) structured cognitive-motor training (playing musical exercises on a drum set with varying levels of movement elements and composition of drum pads), and (iii) assisted music playing (learning to play own favorite songs on the piano with the help of the therapist and using a special musical notation system called Figure Note). The difficulty level of the exercises was initially adjusted, and then increased in a step-wise manner within and across the sessions, to meet the skill level and progression of the patient. Musical improvisation was also included in all modules and encouraged throughout the therapy to facilitate creative expression.

Neuropsychological assessment. An extensive battery of neuropsychological tests was used to measure the potential cognitive outcomes of the music therapy intervention. The battery is fully described in our previous article (1). Briefly, it comprised standard neuropsychological tests of general EF [Frontal Assessment Battery (FAB) (60)], reasoning ability [Wechsler Adult Intelligence Scale IV (WAIS-IV) / Similarities and Block design subtests (61)], and verbal memory [Wechsler Memory Scale III (WMS-III) / Word Lists II subtests (62) and WAIS-IV / Digit Span subtest (61)] as well as computerized tests measuring different EF subcomponents, including set shifting [Number-Letter Task (NLT) (63)], working memory updating [Auditory N-back Task (64)], and inhibition [Simon Task (65)], and sustained attention [Sustained Attention to Response Task (SART)(66)]. In addition, a self-report questionnaire measuring the severity of executive deficits [Behavioural Rating Inventory of Executive Function – Adult version (BRIEF-A) (67)] was included.

In the present study, we analysed the fMRI data by correlating with the general EF (FAB total score), set shifting (NLT switching cost errors), and self-reported executive deficits (BRIEF-A / Self-Monitor and Inhibition scales), as these instruments showed a significant positive effect of the music therapy intervention in our previous publication (1) (as well as in another publication currently under preparation). The FAB (60) consists of six subtests exploring conceptualization, mental flexibility, motor programming, sensitivity to interference, inhibitory control, and environmental autonomy. The computerized tests of executive function (EF) were chosen to reflect aspects of EF (set shifting, updating, inhibi-
tion) defined by Miyake and colleagues (68), as well as sustained attention. Briefly, set shifting was measured with the Number-Letter Task (NLT) (63); updating with an Auditory N-back task (64); inhibition with the Simon Task (65); and sustained attention with the Sustained Attention to Response Task (SART) (66). In the NLT, switching costs for both reaction times and error percentages were calculated by comparing performance on switching and nonswitching trials.

**MRI Data Acquisition.** Patients underwent structural MRI (sMRI) and resting-state functional (rs-fMRI) scanning, using an 8-channel SENSE head coil, in a 3T Philips Achieva MRI scanner (Philips Medical Systems) of the HUS Helsinki Medical Imaging Center at Helsinki University Central Hospital. In sMRI, we acquired 2 T1-weighted structural images, using: (A) a magnetization-prepared rapid acquisition gradient echo (MPRAGE, hereafter referred to as T1) sequence [repetition time (TR) = 9.9 ms; echo time (TE) = 4.60 ms; flip angle (FA) = 8°; field of view (FOV) = 24.0 × 24.0 cm, matrix = 272 × 272, 187 slices, slice thickness 0.88 mm with no gap]; and (B) a fluid-attenuated inversion recovery (FLAIR) sequence [repetition time (TR) = 8 ms; echo time (TE) shortest; angle = 50°; field of view (FOV) = 9.8 × 9.7 cm, matrix = 228 × 226, 300 slices, slice thickness 0.60 mm with no gap]. In rs-fMRI, images were acquired with a T2*-weighted image sequence (TR/TE = 2000/35 ms; FA = 90°, FOV = 23.0 × 23.0 cm, matrix = 80 × 80, 31 slices, slice thickness 4 mm with 0.5 mm gap). During the rs-fMRI acquisition, the patients were instructed to remain still, eyes open and fixated to a cross.

**rs-fMRI Preprocessing.** Data were preprocessed using a standard pipeline in Statistical Parametric Mapping software (SPM12, Wellcome Department of Cognitive Neurology, University College London) running under Matlab Release 2017a (The MathWorks, Inc., Natick, MA). The preprocessing methods applied included slice timing correction, realignment, segmentation, normalization to the MNI template (final voxel size 1 mm isotropic), and smoothing with a Gaussian kernel of 8 mm full width at half maximum (FWHM). Focal brain lesions were detected in 11 patients. As the presence of lesions may influence the normalization algorithm, cost function masks (CFM) were defined for these 11 lesioned patients, to achieve optimal normalization with no postregistration lesion shrinkage or out-of-brain distortion (69). Binary masks of the lesioned areas were obtained by manually drawing, on a slice-by-slice basis, the precise boundaries of the lesion directly into the T1 image from the TP1 session with MRicron May 2, 2016 release (https://www.nitrc.org/projects/mricron). Accuracy of the CFM was validated by an expert neuroradiologist (author J.P.) who assessed multiple modalities of neuroimaging data acquired at TP1 (MPRAGE, FLAIR).

Next, within-subject T1 images from all time-points (TP1, TP2, TP3) were coregistered using the T1 images from TP1 as reference to ensure that they remained in spatial alignment with the T1 images and CFM from this acquisition. All images and CFM were oriented to the anterior commissure before this coregistration step. Unified segmentation with medium regularization was applied to the T1 images (masked with CFM for those patients with visible lesions on the T1); grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) probability maps were obtained for each individual (70). This procedure is common practice for patients with lesions (71).

After preprocessing, outlier identification was performed using the Artifact Detection Tools (ART) toolbox (https://www.nitrc.org/projects/artifacts_s_sdetect). We identified outliers as scans that exceeded 3 standard deviations from the global mean BOLD signal, or with framewise displacement >2 mm.

**rs-fMRI Denoising.** We used denoising to minimize the variability due to physiological, outlier, and residual subject-motion effects. The preprocessed output files from SPM and ART were imported into the CONN toolbox v(18.b) (https://www.nitrc.org/projects/conn), including the following subject- and session-specific files: T1 and rs-fMRI scans; segmented GM, WM, and CSF images; 6 realignment parameters after rigid body motion correction; and the regressor files containing the outliers. We used the default denoising pipeline in the CONN toolbox which comprises two steps in consecutive order: (1) linear regression of potential confounding effects in the BOLD signal, and (2) temporal band-pass filtering. Step 1 implements an anatomical component-based noise correction procedure (aCompCor), and includes: noise components from cerebral white matter and cerebrospinal areas (72); estimated subject-motion parameters and first-order derivatives (73); identified outlier scans (74); constant and first-order linear session effects (75). After evaluation of denoised outputs (residual BOLD time series), the number of noise components extracted from the white matter was increased to 10. In step 2, frequencies below 0.01Hz were filtered from the BOLD signal to reduce the slow signal drift.

**Longitudinal analysis of seed-based connectivity associated with GMV changes.** Several studies have shown that structural and brain connectivity changes may serve as biomarkers of plasticity in adults with chronic TBI (59, 76). In our previous study, we demonstrated that neurological music therapy was able to induced morphometric changes in chronic TBI patients (1). In particular, our findings indicated that GMV in the right inferior frontal gyrus (IFG), among other regions, increased significantly in AB and BA groups during the intervention versus control period as well as before and after the intervention period (1). In addition, the increase in GMV in the right IFG correlated with cognitive improvement in set shifting (1).

In the current study, we selected the statistically significant clusters in the right IFG from the intervention period analysis to assess their seed-based connectivity in resting-state, because it was the only region to significantly correlate with therapy-induced EF improvement. The seeds included two
clusters in the right IFG triangular part in standard space, that were imported into the CONN toolbox to compute seed-to-voxel functional connectivity. For more information regarding peak coordinate and size of the seeds see Table 4 in our previous study (1).

Seed-based connectivity (SBC) maps (77) between each seed and every voxel in the brain were obtained for each subject and session. SBC maps were computed as the Fisher-transformed Pearson’s bivariate correlation coefficients between a ROI BOLD timeseries and the BOLD time series of each individual voxel, using the equation:

\[
\begin{align*}
    r(x) &= \frac{\int S(x,t)R(t)dt}{\sqrt{\int R^2(t)dt x \int S^2(x,t)dt}} \\
    Z(x) &= \tanh^{-1}(r(x))
\end{align*}
\]

where \(S\) is the BOLD time series at each voxel, \(R\) is the average BOLD time series within a ROI, \(r\) is the spatial map of Pearson correlation coefficients, and \(Z\) is the SBC map of Fisher-transformed correlation coefficients for this ROI.

**Connectivity pattern analysis within and between large-scale networks.** To further understand functional connectivity (FC) changes induced by the neurological music therapy from the perspective of large-scale networks, we used the 8 resting-state networks (RSNs) comprising 32 regions of interest (ROIs) or nodes in the CONN toolbox, which are derived from an independent component analysis (ICA) of 498 subjects from the Human Connectome Project (77). The 8 RSNs included the default mode (DMN), salience (SAL), frontoparietal (FPN), dorsal attention (DAN), sensorimotor (SM), language (LAN), visual (VIS) and cerebellar (CER) networks. All of these networks have been widely reported in the resting-state literature.

We identified changes in both between- and within-network connectivity from those networks that have shown alterations after TBI (4) or are associated with EF, memory, and attention, which are the most commonly affected cognitive domains in TBI patients (3–6). In particular, we selected ROIs from the DMN, SAL, FPN, and DAN networks to examine the FC (a) within their nodes, and (b) between their nodes and the rest of the RSNs included in the CONN toolbox.

We used the same connectivity measure as in the SBC connectivity analysis described in the previous section: the Fisher-transformed bivariate correlation coefficient between a pair of ROIs time series (77). Each pair of ROIs \((i,j)\) constitutes an element in the ROI-to-ROI connectivity (RRC) matrix that can be calculated as follows:

\[
    r(i,j) = \frac{\int R_i(x,t)R_j(t)dt}{\sqrt{\int R_i^2(t)dt x \int R_j^2(x,t)dt}}
\]

\[
    Z(i,j) = \tanh^{-1}(r(i,j))
\]

where \(R\) is the BOLD time series within each ROI, \(r\) is a matrix of correlation coefficients, and \(Z\) is the RRC symmetric matrix of Fisher-transformed correlation coefficients.

**Resting-state functional connectivity and cognitive performance.** We investigated the link between rsFC and cognitive performance improvements after therapy. We performed Pearson’s bivariate correlations between, on one hand, the SBC or ROI-to-ROI (R2R) FC changes (found significant in the main analyses), and on the other hand, neuropsychological tests measuring general EF (FAB total score), set shifting (NLT switching cost errors), and the Self-Monitor and Inhibition subscales of BRIEF-A. The chosen performance measures were those which showed a statistically significant improvement after the music therapy (described in (1) and in an article in preparation).

**Quality assurance.** We visually inspected all structural and functional MRI scans to ensure that patients had no significant brain atrophy. In rsMRI preprocessing, the quality of the preprocessed data was visually inspected using the quality assurance (QA) plots available in the CONN toolbox (QA normalization, registration, segmentation, and framewise displacement). In rsMRI denoising, we inspected the distribution of connectivity values for each patient and session after denoising, and adjusted the number of noise components extracted from white matter to ensure that this distribution peaked around 0.

**Group-level analysis.** All group-level analyses were performed in the CONN toolbox using the General Linear Model framework. In the SBC analysis, to compare pre-post intervention increases in FC, we performed a paired T-test between every voxel in the brain and the clusters that showed increased GMV after the intervention. For the AB group, the beta maps from TP1 were entered as the pre-intervention condition and the beta maps from TP2 were entered as the post-intervention condition. For the BA group, the beta maps from TP2 were entered as the pre-intervention condition and the beta maps from TP3 were entered as the post-intervention condition. We focused on the pre-post intervention effect as this was the only analysis with significant brain-behaviour relationships in our previous study (1).

In the R2R approach with RSN, we performed two group-level analyses using paired T-tests (pre- and post-intervention effect for the AB and BA groups) and mixed-model ANOVA (AB>BA×TP2>TP1 interaction). For the AB group, the denoised ROI timeseries from TP1 were entered as the pre-intervention condition, and the denoised ROI time series from TP2 were entered as the post-intervention condition. For the BA group, the denoised ROI time series from TP2 were entered as the pre-intervention condition, and the denoised ROI time series from TP3 were entered as the post-intervention condition. For the within-networks analysis, we examined the R2R connectivity of the ROIs within each of the selected 4 RSNs (DMN, SAL, FPN, and DAN). For the between-network analysis, we examined the connectivity be-
between the ROIs of the each of these RSNs (DMN, SAL, FPN, DAN) with every other RSN included in the CONN toolbox.

The statistical parametric maps obtained were corrected for multiple comparisons based on Gaussian Random Field Theory (78). The maps were first thresholded using a “height” threshold of $p < 0.001$ and the resulting clusters thresholded with a cluster-level False Discovery Rate (FDR)-corrected alpha threshold of $p < 0.05$ (79).

## Results

The quality assurance procedure resulted in 145 rs-fMRI volumes from 23 participants (AB group, n=15; BA group, n=8). The scans were acquired at three time-points: TP1 (baseline), TP2 (3-months) and TP3 (6-months).

**Seed-based resting-state functional connectivity.** Previously, using voxel-based morphometry (VBM) we found that GMV in the right inferior frontal gyrus (IFG) increased significantly in both groups during the intervention period, and that this was positively correlated with enhanced set shifting ability (1). To examine the changes in functional connectivity associated with this increase in GMV, we performed seed-based connectivity (SBC) using as sources the two clusters in the right IFG that resulted from our previous voxel-based morphometry analysis (1). In the SBC analysis (results shown in Figure 1), a paired T-test of pre- versus post-intervention rsFC (pooling together AB and BA groups) revealed that the right IFG increased its connectivity with the right inferior parietal lobule (IPL; peak voxel $x = 38, y = -46, z = 48$; Figure 1A) and the left Rolandic operculum (peak voxel $x = -54, y = -2, z = 4$; Figure 1B) with $p < 0.05$ after FDR-correction at cluster level. There were no other significant clusters.

**Between- and within-network resting-state functional connectivity.** To investigate the positive effect of the neurological music therapy on the coordinated activity between large-scale resting-state networks (RSNs) that are important for high-level cognitive functions, we performed a ROI-to-ROI (R2R) analysis focusing primarily on the frontoparietal (FPN), dorsal attention (DAN), salience (SAL) and default mode (DMN) networks as source ROIs. Figure 2 shows the results of the between-network connectivity for these four RSNs, in a paired t-test of pre- versus post-intervention pooling together data from AB and BA groups (Figure 2A-C), and in the AB $>$ BA $\times$ TP2 $>$ TP1 interaction (Figure 2D) with $p < 0.05$ FDR-corrected at the source level.

In the pre- versus post-intervention comparison (Table S1), we found that the FPN increased its temporal coupling with the sensorimotor (SM) and DAN after the music-based intervention. Likewise, when the DAN was used as a source, we observed increased between-network connectivity with several nodes of the visual (VIS) network induced by the intervention. In particular, the lateral prefrontal cortex in the left hemisphere from the FPN increased its functional connectivity with the right sensorimotor lateral node from the SM network (Figure 2A) and the right intraparietal sulcus from the DAN network. In addition, the latter was also highly connected with the visual occipital and bilateral visual lateral nodes from the VIS network. By contrast, in the AB $>$ BA $\times$ TP2 $>$ TP1 interaction (Table S1), we found that the spontaneous fluctuations between the medial prefrontal cortex from the DMN network and the sensorimotor superior node from the SM network were less coordinated after the intervention.

Next, we compared the connectivity within networks (Figure 3). In this case, only the pre- versus post-intervention comparison pooling together AB and BA groups (Table S2) yielded significant results with $p < 0.05$ FDR-corrected at the source level. The FPN and SAL networks showed a reduction in the functional connectivity between several of their constituents nodes. Specifically, the lateral prefrontal cortex node in the left hemisphere exhibited less coupling with its contralateral counterpart and with the posterior parietal cortex node in the left hemisphere. Similarly, within the SAL network, the supramarginal gyrus node in the left hemisphere showed a decreased coupling with its contralateral counterpart and with the anterior insula node in the right hemisphere.

**Correlation between neuropsychological outcomes and resting-state functional connectivity.** Next, we investigated how the changes in RSNs induced by the neurological music therapy (see above) were associated with the parallel improvement in cognitive function, specifically in general EF (FAB score), set shifting (NLT switching cost errors), and self-reported executive deficits (BRIEF-A Self-Monitor and Inhibition subcales). We found statistically significant associations between the therapy-induced cognitive improvement and within-network and between-network changes in RSNs after the intervention (Figure 4). Decreased within-network FC in the left and right lateral prefrontal cortex nodes of the FPN correlated significantly with increased FAB scores ($p = 0.040, r = -0.372$, Figure 4A, left) and decreased BRIEF-A Self-Monitor scores ($p = 0.013, r = 0.485$, Figure 4A, right). Decreased between-network FC between the DMN (medial prefrontal cortex node) and the SM (superior sensorimotor cortex node) networks correlated significantly with increased FAB scores ($p = 0.003, r = -0.559$, Figure 4B, left) and marginally significantly also with decreased NLT switching cost errors ($p = 0.052, r = 0.347$, Figure 4B, right). There were no other significant correlations. Together, these results indicate that those patients who showed a larger reduction in connectivity within the FPN and between the DMN and SM networks exhibited greater improvement in general EF (higher FAB scores) and set shifting ability (less NLT errors) as well as greater reduction in executive deficits in self-monitoring (smaller BRIEF-A Self-Monitor scores). Thus, network-specific patterns of functional connectivity induced by the music-based intervention are associated with improvement in EF.
Fig. 1. Changes in seed-based connectivity induced by the neurological music therapy intervention. A. Top: R IFG mask used as seed with peak coordinate x=50, y=30, overlaid on the Montreal Neurological Institute (MNI) template. A. Bottom: Statistical map showing the p<0.05 FDR-corrected cluster in the R parietal Inf. B. Top: R IFG mask used as seed with peak coordinate x=54, y=22, overlaid on the MNI template. B. Bottom: Statistical map showing the p<0.05 FDR-corrected cluster in the L Rolandic Operculum. C. Effect size plots displaying the functional connectivity (mean beta values) of each significant cluster in the pre-intervention relative to the post-intervention period. IFG: Inferior Frontal Gyrus, Parietal Inf.: inferior parietal lobule, R: right, L: left.

Discussion

We used resting-state functional connectivity (rsFC) methods to examine brain network changes induced by neurological music therapy after moderate and severe TBI. We conducted a ROI-to-ROI analysis with nodes from four networks [default mode (DMN), salience (SAL), frontoparietal (FPN), and dorsal attention (DAN)] as seeds and all network ROIs included in the CONN toolbox as targets. This analysis showed that music therapy strengthened network connectivity between FPN and DAN, and between these networks and the sensorimotor (SM) and visual (VIS) networks, respectively. In contrast, the music therapy reduced the connectivity between the nodes of DMN and SM network. The within-network connectivity revealed specific nodes of the FPN and SAL wherein coupling decreased in the pre- versus post-intervention comparison. Importantly, the decrease in the FPN and DMN-SM connectivity was paralleled by cognitive improvement in executive function (EF). Finally, using a seed-based connectivity analysis, we demonstrated that the right inferior frontal gyrus (IFG), in which we previously observed increased grey matter volume (GMV) induced by the music therapy (1), showed high connectivity with left frontal and right parietal regions, implicated in music processing (see Sarkamo et al. (80) for a review). These results reflect substantial functional neuroplasticity changes in resting-state networks, which taken together show a shift from a hyper-connected to a more normal state of connectivity; we believe this represents a shift in executive functioning from a compensatory increase in cognitive control and attentional mechanisms to more adaptive sensory-integrative.

Changes in cross-modal integration. Our first hypothesis (H1) was that neurological music therapy enhances the rsFC between primary sensory networks and higher-order networks as a consequence of iterated interaction between the sensory-cognitive systems recruited by music production, and perception (81). This hypothesis was conceptually inspired by the ‘global workspace’ (or cross-modal integration) notion empirically supported by the convergence of unimodal sensory networks into cortical hubs that contribute to perceptual integration in the human brain (82). Our results confirmed H1 as we detected an increased coupling between the FPN and SM networks. The stepwise functional connectivity performed by Sepulcre et al. (82) highlighted the dorso-lateral prefrontal cortex, which anatomically corresponds to the lateral prefrontal cortex node in the FPN, as one of the cortical hubs reached by seeds in the primary somatosensory cortex. Similarly, the intraparietal sulcus (IPS) node in the DAN was more connected with occipital nodes in the VIS network. This is not surprising given that visual streams have previously been postulated to converge in the IPS (83). We also found an increase in rsFC between the FPN and DAN, which may be related to improved regulation of perceptual attention, as recent work using meta-analytic tools has revealed (84).

Reduced connectivity in network hubs. Our second hypothesis (H2) proposed that the DMN, SAL, and FPN connectivity would be downregulated by the neurological music therapy. Our reasoning was that the repetitive engagement of cognitive control and EF during the intervention (14, 17) would counteract the increased connectivity and cognitive load associated with TBI. Brain injury has a detrimental ef-
Fig. 2. Changes in between-network connectivity induced by the neurological music therapy intervention. Nodes are overlayed on a rendered semitransparent brain generated using CONN. Adjacency matrices display the mean post- minus pre-intervention (A–C) Fisher-transformed Z-score correlation values for each node. The bar plots (D) show the effect size of the AB > BA and TP2 > TP1 interaction represented by the Fisher-transformed Z-score correlation values for each node. FPN: frontoparietal; SM: sensorimotor; DAN: dorsal attention; VIS: visual; DMN: default mode network; FEF: frontal eye field; IPS: intraparietal sulcus; LPFC: lateral prefrontal cortex; LAT: lateral; MED: medial; MPFC: medial prefrontal cortex; OCC: occipital; PCC: posterior cingulate cortex; PPC: posterior parietal cortex; Sup: superior; R: right, L: left.

Affective on automatic processes and increases the supervisory demand for the integration of information at all levels (85, 86), leading to the symptoms of fatigue commonly reported by these patients (87). The need to compensate for the excessive cognitive demand that arises from focal lesions and diffuse axonal injury is consistent with the hyperconnectivity hypothesis (48, 53, 88).

One prominent line of evidence in favour of the latter hypothesis comes from the observation that hyperconnectivity secondary to neurological damage, including TBI (48, 51, 52, 89), is centered around nodes with a hub connectivity profile. Network hubs can be defined in terms of their structural (90–93) or functional connectivity (82, 94–96), and are characterized by a high degree of connectivity with the rest of the brain, thus making a strong contribution to the global integration of information (97). Although functional connectivity does not reflect direct anatomical connections, graph measures derived from both methodologies show strong convergence with regards to the brain regions classified as hubs. These include regions overlapping with the nodes of the DMN, SAL, and FPN, such as the superior parietal and superior frontal cortex, the anterior and posterior cingulate cortex as well as anterior portions of the anterior insula (82). According to the hyperconnectivity hypothesis, a major goal of the increase in functional connectivity following injury is to re-establish network communication through network hubs in order to maximize information transfer and minimize behavioural impairments (53).

Here, we found that the neurological music therapy decreased the functional connectivity within the FPN, involving a reduction in the bilateral communication between the lateral prefrontal cortices, and between the lateral prefrontal cortex and the posterior parietal cortex in the left hemisphere. Considering the hyperconnectivity hypothesis, this finding accords with recent evidence indicating that the bilateral prefrontal cortices are the subcomponents of the FPN with the highest average degree at 3 and 6 months after moderate/severe TBI (48). Several other studies reported a similar increase in connectivity in frontal regions and FPN, among mild TBI patients (41, 42, 51). Our results also showed that the anterior insula in the right hemisphere was less connected with the supramarginal gyrus in the left hemisphere and that interhemispheric connectivity in the latter was equally diminished after the intervention. This within-network connectiv-
Fig. 3. Changes in within-network connectivity induced by the neurological music therapy intervention. Nodes are overlaid on a rendered semitransparent brain generated using CONN. Adjacency matrices display the mean post-minus pre-intervention Fisher-transformed Z-score correlation values for each node. AINS: anterior insula; IPS: intraparietal sulcus; LAT: lateral; LPFC: lateral prefrontal cortex; MPFC: medial prefrontal cortex; OCC: occipital; PPC: posterior parietal cortex; RPFC: rostral prefrontal cortex; SMG: supramarginal gyrus; Sup: superior; R: right, L: left.

ity reduction in the SAL network, including the anterior insula, is again consistent with the hyperconnectivity reported by Hillary et al. (48). The increase in functional connectivity of the anterior insula salience network after TBI has also been supported by other longitudinal (36) and cross-sectional studies (40). Regarding the DMN, our analysis revealed a decreased connectivity between the medial prefrontal cortex and the SM network. Given the interference that the activation of the DMN may exert on attentional switching (46), it may be the case that this reduced connectivity facilitates the sensorimotor coupling with other brain regions involved in music production and perception. The negative trend with EF would lend support to this idea (though, since this correlation was only marginally significant, it should be interpreted...
with caution).

One clinically-relevant implication from these findings is that the music therapy effectively targeted network hubs from the FPN and SAL networks, and hence may have contributed to the shift from a hyperconnected to a normal state. Within the hyperconnectivity hypothesis framework, it has been argued that while this hyperconnectivity may be adaptive in the short-term, chronic hyperconnectivity may render network hubs vulnerable to late pathological complications due to the chronically-increased metabolic stress. Although the exact mechanisms and cognitive consequences of hyperconnectivity remain to be elucidated, this proposal has found some support from longitudinal studies examining the temporal evolution of TBI recovery (48, 88). For example, Roy et al. (88) performed a cost-efficiency analysis and found a peak in network strength in the frontal DMN and temporoparietal networks at 6-months postinjury, with some residual hyperconnectivity observable after one year but with diminished overall cost. In this context, the neurological music therapy could potentiate the cost-efficiency rebalancing in the TBI recovery trajectory. Though beyond the scope of this paper, this prediction could be directly tested by computing graph theory measures including cost, degree, as well as local and global efficiency.

Indirect support for this idea comes from our correlational results indicating improved performance in EF as a function of rsFC decrease within the FPN. The neurological music therapy was primarily designed to target a number of EFs (action planning and monitoring, inhibitory control, shifting), which are reflected by the three outcome measures (FAB, NLT, BRIEF-A) showing a correlation with the therapy-induced change in rsFC. In this context, it might be that the repetitive practice during the music intervention reduces the cognitive challenge and transfers to better executive functioning, thus reducing the need for a hyperconnected state to efficiently accomplish the task. This would fit well with the observation that the increased activity in the right prefrontal cortex and anterior insula cortex in a sample of TBI patients normalizes following practice of a working memory task (86).

**Relationship between brain morphometry and rsFC changes.** The simultaneous combination of both structural and rsFC measures in the context of rehabilitative strategies for TBI has only recently started to be explored (76). However, it offers an interesting possibility since both brain morphometry (98) and rsFC (99) are malleable to musical training. By cross-linking results from our previous voxel-based morphometry publication with rsFC, we were able to identify changes in GMV co-occurring with whole-brain rsFC. We focused on the right IFG, as this was the region showing a significant relationship between GMV increases and improved performance in set shifting (1). Using two right
resting-state networks after TBI. Our results lend support to the idea that the music intervention facilitated the integration of primary sensory information, by increasing the rsFC between multimodal and higher-level cognitive networks (FPN, DAN). The shift towards a less connected state within the FPN and SAL networks is also in line with the notion that chronic hyperconnectivity in network hubs after TBI can be maladaptive in the long-term. The relationship between improved performance in EF and lesser connectivity in the FPN and the DMN-SM networks, which might be linked to reduced interference, further supports this idea. Finally, the co-occurrence of changes in brain morphometry and rsFC connectivity, in a circuit supporting music processing, suggests a complex picture with interrelated plasticity across MRI modalities. Overall, our findings suggest that rsFC changes in brain networks can serve as sensitive biomarkers for the efficacy of music-based rehabilitation after TBI.

Bibliography

1. S. T. Siponkoski, N. Martinez-Molina, L. Kuusela, S. Laitinen, M. Holma, M. Ahlro, P. Jordan-Kilkki, K. Ala-Kaupalla, M. Petaks, J. Piekola, A. Rodrigues-Ferrans, M. Lane, A. Ylen, P. Rantanen, S. Koskenniemi, J. Lipsanen, and T. Sarkamo. Music therapy enhances executive functions and prefrontal structural neuroplasticity after traumatic brain injury: Evidence from a randomized controlled trial. J Neurotrauma, 37(4):818–834, 2020. ISSN 1557-9042 (Electronic) 0897-7151 (Linking). doi: 10.1089/neu.2019.641.9.
2. A. R. Maas, D. K. Menon, P. D. Adelson, N. Andelic, M. J. Bell, A. Belli, P. Bragg, A. Brazina, A. Buki, R. M. Cheon, G. Citeri, M. Coburn, D. J. Cooper, A. T. Corder, E. Czitrom, M. Czysnyka, R. Diaz-Austain, J. P. Dreier, A. C. Duhamel, A. Ercio, T. A. van Eissen, V. L. Fein, G. Gao, J. Giacino, L. E. Gonzalez-Lara, R. L. Groen, D. Gupta, J. A. Hartings, S. Hill, J. Y. Jiang, N. Katharanathan, E. J. O. Kompanje, L. Lanyon, S. Launays, F. Le, H. Leven, H. F. Lingons, M. Maleege, M. Majdan, G. Mantley, M. Masca, C. McDowen, S. Mondello, V. Newcombe, A. Palotie, D. J. Parizel, F. Peul, R. Pier, S. Polinder, L. Puybasset, T. E. Rasmussen, R. Rossant, P. Smielewski, J. Soderberg, S. J. Stanworth, M. Stein, N. von Steinbuchel, W. Stewehr, E. J. Steyerberg, N. Stocchetti, A. Symot, B. T. Tive, O. Tervuov, A. Theodum, D. Tibboel, W. Videtta, K. W. W. Wang, W. H. Williams, L. Wilson, and K. Yaffe. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. Lancet Neurol, 16(12):1087–1094, 2017. ISSN 1474-4422. doi: 10.1016/S1474-4422(17)30774-1.
3. D. T. Stuss. Traumatic brain injury: relation to executive dysfunction and the frontal lobes. Curr Opin Neurol, 24(6):584–90, 2011. ISSN 1350-7540. doi: 10.1097/WCO.0b013e328347cb69.
4. D. J. Sharp, G. Scott, and R. Leech. Network dysfunction after traumatic brain injury. Nat Rev Neurol, 10(6):156–66, 2014. ISSN 1759-4766 (Electronic) 1759-4758 (Linking). doi: 10.1038/nrneurol.2014.15.
5. T. E. Ham and D. J. Sharp. How can investigation of network function inform rehabilitation after traumatic brain injury? Curr Opin Neurol, 25(6):662–69, 2012. ISSN 1350-7540. doi: 10.1097/WCO.0b013e3283598dcf.
6. P. W. Burgess and D. T. Stuss. Fifty years of prefrontal cortex research: Impact on assessment. J Int Neuropsychol Soc, 23(9–10):755–767, 2017. ISSN 1355-6177. doi: 10.1017/jin.2017.100.9.
7. K. M. Kirpan, B. Levine, D. T. Stuss, and D. R. Dawson. Executive function and coping at one-year post traumatic brain injury. J Clin Exp Neuropsychol, 29(1):36–46, 2007. ISSN 1380-3935 (Print) 1380-3935 (Online). doi: 10.1080/13803930600437861.
8. K. Ciccone, H. Levin, J. Malat, D. Stuss, and J. Whyte. Cognitive rehabilitation interventions for executive function: moving from bench to bedside in patients with traumatic brain injury. J Cogn Neurosci, 18(7):1212–22, 2006. ISSN 0898-929X (Print) 0898-929X (Online). doi: 10.1162/jocn.2006.18.7.1212.
9. N. P. Friedman and A. Miyake. Unity and diversity of executive functions: Individual differences as a window on cognitive structure. Cortex, 86:188–204, 2017. ISSN 0010-9452. doi: 10.1016/j.cortex.2016.04.023.
10. S. M. Carpenter, S. Moreno, and A. Mcintosh. Short-term music training enhances empathy, distributed neural communication during music and linguistic tasks. J Cogn Neurosci, 28(10):1603–12, 2016. ISSN 0898-929X. doi: 10.1162/jocn_a_00989.
11. A. C. Jaschke, H. Honing, and E. J. A. Scherder. Longitudinal analysis of music education on executive functions in primary school children. Front Neurosci, 12:103, 2018. ISSN 1662-4548 (Print) 1662-453x (Online). doi: 10.3389/fnins.2018.00103.
12. V. Putkinen, M. Tervaniemi, K. Saarikivi, and M. Huhtanen. Promises of formal and informal musical activities in advancing neurocognitive development throughout childhood. Ann N Y Acad Sci, 1337:153–62, 2015. ISSN 0077-8923. doi: 10.1111/nyas.12566.
13. S. Moreno, E. Bialystok, R. Barac, E. G. Scheinberg, N. J. Copeda, and T. Chau. Short-term music training enhances verbal intelligence and executive function. Psychol Sci, 22(1):1425–33, 2011. ISSN 0956-7976. doi: 10.1177/0956797611416999.
14. M. Sachs, J. Kaplan, A. Der Sarkissian, and A. Habibi. Increased engagement of the cognitive control network associated with music training during an fMRI stroop task. PLoS One, 12(10):e0187254, 2017. ISSN 1932-6203. doi: 10.1371/journal.pone.0187254.
15. A. Habbib, A. Damasio, B. Bar, M. Elliott Sachs, and H. Damasio. Music training and child development: a review of recent findings from a longitudinal study. *Ann NY Acad Sci*, 2018. ISSN 0077-8923. doi: 10.1111/1364-0964.14306.

16. L. Moradzadeh, G. Blumenthal, and M. Wiseheart. Musical training, bilingualism, and executive function: a closer look at task switching and dual-task performance. *Cogn Sci*, 39(5):99–1020, 2015. ISSN 1551-7090 (Electronic) 0360-0799 (Print). doi: 10.1007/s10564-010-0685-4.

17. J. Zuck, C. Benjamin, A. Kenyon, and N. Gaab. Behavioral and neural correlates of execu- tional functioning in musicians and non-musicians. *PLoS One*, 9(6):e98968, 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0098968.

18. J. A. Bugos, C. T. Perrettis, S. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.

19. J. F. Strong and B. T. Mast. The cognitive functioning of older adult instrumental musicians. *Aging Ment Health*, 11(6):472–7, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108505.

20. J. A. Bugos, W. M. Perettis, C. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.

21. J. A. Bugos, W. M. Perettis, C. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.

22. J. A. Bugos, W. M. Perettis, C. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.

23. J. A. Bugos, W. M. Perettis, C. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.

24. J. A. Bugos, W. M. Perettis, C. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.

25. J. A. Bugos, W. M. Perettis, C. T. Brophy, and P. H. Bedenbaugh. Individualized video piano instruction enhances executive functioning and working memory in older adults. *Aging Ment Health*, 11(6):464–71, 2007. ISSN 1360-7458 (Print) 1360-7466 (Online). doi: 10.1080/1360786060108504.
