Brain White Matter Organisation in Adolescence is Related to Childhood Cerebral Responses to Facial Expressions and Harm Avoidance

Matilde Taddei M.Sc.[a], Marco Tettamanti Ph.D.[b], Annalisa Zanoni Ph.D.[a], Stefano Cappa M.D.[a,b,c], Marco Battaglia M.D.[a,c,d]

[a]The Academic Centre for the Study of Behavioural Plasticity, Vita-Salute San Raffaele University, 20127 Milan, Italy; [b]Department of Nuclear Medicine and Division of Neuroscience, San Raffaele Scientific Institute, 20132 Milan, Italy; [c]Department of Clinical Neurosciences, San Raffaele Hospital, 20132 Milan, Italy; [d]Laval University and Institut Universitaire en Santé Mentale de Québec, Québec, Canada. The first two authors contributed equally to this paper.

Correspondence to: Marco Battaglia, the Academic Centre for the Study of Behavioural Plasticity, Vita-Salute San Raffaele University, 20 via S. D'Ancona 20127 Milan, Italy.
Telephone +39 02 2643 5389. E-mail: marco.battaglia@unisr.it

Abstract

While white matter structural integrity is likely to influence the responses to social-emotional stimuli and emotional regulation during development, no longitudinal data are available on such relationships.

We investigated the relationships between white matter Fractional Anisotropy (FA) derived by DTI voxel-based Tract Based Spatial Statistics (TBSS) and tractography measured at age 14-15, and cerebral event-related N400 amplitudes in response to happy, neutral and angry facial expressions and Cloninger's Harm Avoidance (HA) measured at age 7-9.

Whole-skeleton TBSS analyses revealed reduced FA associated to smaller N400 amplitudes in response to anger, and to higher HA. Region-of-Interest TBSS analyses showed high correlations (ranging -0.69-0.82, $p<0.01$-0.001) between FA and N400 amplitudes across the Inferior Longitudinal, the Inferior Frontoccipital, and the left Uncinate Fasciculus, and between FA and Harm Avoidance in right Uncinate Fasciculus (-0.67, $p<0.01$). Tractography showed that these relationships were mainly present in the left Inferior Longitudinal and the right Inferior Fronto-Occipital Fasciculus for N400 amplitudes, and in the right Uncinate Fasciculus for HA.

Ventral limbic pathways' white matter organisation affects the neural responses to expressions -such as anger- that are perceptually more challenging and/or communicate social rejection, and compounds the neural pathways that predispose to avoidant behaviour and shyness with strangers.

Key words: development, event related potentials, facial expressions, fractional anisotropy, harm avoidance

1. INTRODUCTION

In humans, the ability to decode basic facial expressions of emotions (EoE) manifests early in life and is essential for optimal interpersonal communication. While the earlier studies investigated the universals of EoE categorisation (Ekman and Friesen 1971), current research is focusing on why some EoE are better discriminated than other (Battaglia et al., 2010; Chen et al., 2011), and why individuals vary in their neural and behavioural responses to EoE from childhood onwards (Adolphs 2002a; Cohen Kadosh et al., 2011; Monk 2008; Perez-Edgar et al., 2007).

At the perceptual level, EoE differ from each other for readability: normally-developing children (Battaglia et al., 2004; Battaglia et al., 2005; Herba et al., 2006), as well as adults (Chen et al., 2011; Orgeta and Phillips 2008; Shimamura et al., 2006) and neural networks (Dailey et al., 2002) process and identify the joy and surprise EoE with remarkable accuracy. On the contrary, the anger and disgust EoE are often mistaken and are the most challenging to identify (Chen et al., 2011; Dailey et al., 2002; Herba et al., 2006).Hence, a proportion of the variability within the processes of EoE decoding should be attributed to features (e.g., structural-perceptual ambiguity/similarity) that pertain primarily to the facial affects as stimuli, and are relatively independent of the viewer's attributes (Battaglia et al., 2010).

However, inasmuch as EoE belong to the wide class of emotional behaviour, a proportion of variability in the human responses to EoE (including neurofunctional activation and accuracy in discrimination) should be attributed to the viewers' personal features and experiences. In adherence with these expectations, children's and adolescents' decoding abilities and neural responses to EoE seem to be influenced by disparate elements, such as macro-social factors (Elfenbein and Ambady 2002), early adversities (Fries and Pollak 2004), an individual's cognitive-emotional profile and proneness to social anxiety (Battaglia et al., 2004; Horley et al., 2003; Stirling et al., 2006; Ball et al., 2011) and some polymorphic genes (Battaglia et al., 2005; Battaglia et al., 2012; Perez-Edgar et al., 2010).

By contrasting specific EoE with psychobiological readouts and behavioural indicators of individual differences, one can study relatively specific developmental pathways of adaptation/maladaptation in interpersonal contexts. Brain event-related potentials (ERP) are often favoured to assess children's neural responses to EoE because they are non-invasive, inexpensive, and yield well-replicated findings. The ERP responses to EoE are well developed by the age of 8 years (Batty and Taylor 2006), with the later (300-400 msec after stimulus presentation) complexes attaining stability from middle childhood onwards (Batty and Taylor 2002; Coch et al., 2002; Pollak et al., 2001). These 'endogenous' waveforms are thought to represent manifestations of human facial information processing (Posamentier and Abdi 2003; Schweinberger and Burton 2003) in the absence of verbal-semantic information (Olivares et al., 2003). Consistent with these premises, we found that the N400 ERP amplitudes acquired at age 8-9 in response to EoE that communicate social rejection (i.e., angry EoE) or ambiguity (i.e., neutral EoE) predict a higher degree of social shyness and behavioural inhibition cross-sectionally (Battaglia et al., 2005), while longitudinally the ERP N400 waveform evoked by the angry expressions at age 8-9 predicted social anxiety disorder at age 14-15 (Battaglia et al., 2012).

By adopting measures of structural integrity of neural interconnections, one can now investigate more precisely the relationships that tie the presentation of social-emotional stimuli (including EoE) to individual differences in behaviour and in neurofunctional responses. This approach can add substantial information to the field, given that the processes that underlie EoE decoding, face recognition, and social communication are mediated by neural systems distributed across multiple brain regions (Haxby et al., 2002; Jehna et al., 2011) via the involvement of different white matter (WM) tracts (Gschwind et al., 2011). Contemporary magnetic resonance techniques include diffusion tensor imaging (DTI), and DTI-based tractography, which allow for tracing the WM tracts' virtual trajectories (Basser et al., 2000). Specifically, DTI-derived Fractional Anisotropy -FA- represents a measure of the directionality and coherence of water diffusion in WM tissues and

constitutes an index of WM micro-structural properties' organisation (Mori and Zhang 2006; Phan et al., 2009; Pierpaoli and Basser, 1996).

By focusing on some WM tracts selected *a priori* for their involvement in social/emotional stimuli processing, some cross-sectional DTI studies found reduced WM organisation in adults with heightened trait anxiety (Kim and Whalen 2009), social phobia (Baur et al., 2011a; Baur et al., 2011b; Phan et al., 2009), and temperamental disposition to anticipation of harm (Westlye et al., 2011). Specifically, reduced WM organisation was reported in cortico-limbic WM microstructures (Kim and Whalen 2009; Phan et al., 2009; Westlye et al., 2011)-including the uncinate fasciculus (Baur et al., 2011a; Baur et al., 2011b; Phan et al., 2009)- that overlap at least partially with the circuitries at the basis of anxiety (Kim et al., 2011; Martin et al., 2009; Monk 2008).

No study, however, has directly addressed the relationship between measures of brain responses to social/emotional stimuli and WM organisation. In the only study that neared such design, Kim & Whalen (Kim and Whalen 2009) regressed the amygdalar reactivity to EoE on FA measures in order to anatomically isolate an amygdala-prefrontal cortex WM pathway associated with EoE decoding. Moreover, there are no published developmental studies in this field, and no study addressed the longitudinal relationship between temperamental dispositions toward internalizing conditions and indices of WM microstructural organisation.

We based our longitudinal design on the speculation that the ERP neurofunctional responses to EoE depend upon the efficiency of WM tracts that connect the brain areas implicated in the processing of social-emotional stimuli (Adolphs 2002b). In order to address this hypothesis, we investigated the association between the ERP waveforms evoked by EoEs presentation at age 8-9, and the degree of WM structural organisation –as indexed by FA- at age 14-15, when myelination has reached a considerable degree of development and stability (Casey et al., 2005). Since Harm Avoidance –a temperamental disposition towards cautiousness and shyness with strangers (Cloninger 1987)- has been found associated with reduced WM organisation in adult subjects (Westlye et al., 2011), we

also investigated whether the same relationship could be substantiated earlier in life, in a sample of general population adolescents.

2. MATERIALS AND METHODS

2.1 Subjects

The sampling method is described in details elsewhere (Battaglia et al., 2005; Battaglia et al., 2011). Briefly, subjects belonged to a sample followed up longitudinally across three different time points, as shown in Table 1. At time 1, 149 general population Caucasian children (mean age: 7.5 ± 0,5) were characterized by their ability to discriminate facial EoE (Battaglia et al., 2004) and various psychometric indexes of shyness/inhibition were collected, including Harm Avoidance. One year after (Time 2), 49 children (mean age: 8.8 ± 0.7) of the original 149 seen at Time 1, participated to an ERP study of responses to EoE (Battaglia et al., 2005). Six years later (Time 3), all these 49 children were invited to the third phase of the study, which encompassed fMRI and DTI measurements: 7 were unavailable due to relocation, 38 accepted, and 4 refused. Amongst the 38 acceptant subjects, 11 had to be excluded for the presence of orthodontic apparels, 4 withdrew for intervening health/family problems, 2 eventually dropped off for sickness/unexpected constraints on the experiment day, and 1 child did not undergo the DTI session because of tiredness at the end of the fMRI session. This left 20 participants (mean age 15 ± 0,7) for the present study. There were no significant differences between the 20 participants and the 29 non-participants for several key demographic and psychometric variables in the study. Moreover, there were no significant differences in N400 pattern comparing participants vs. non-participants to the present study (see *Table S1*).

Our study was approved by the local ethical committee of the Vita-Salute San Raffaele University of Milan, Italy. After a detailed description of the study, written informed consent was obtained from all participants.

2.2 Electro-Physiological and Behavioural Measures

2.2.1 ERP N400 amplitude

A full description of how we acquired the ERP waveforms in responses to EoE and of the general results is available elsewhere (Battaglia et al., 2005). Briefly, when participants to the present study were 8-9 years-old, we acquired visual ERPs in a task of implicit processing of standardized faces of other children of similar age (models aged 8-9 years). Participants to the original ERP study were 49 children (53% boys, 91% right-handed): according to the Italian version of the Schedule for Affective Disorders and Schizophrenia for School-age Children (K-SADS) interview, 8% had Social Phobia, 6% had Specific Phobia, 4% had Separation Anxiety Disorders and 2% had Generalized Anxiety Disorder. No children met the criteria for a diagnosis of Depression, Panic Disorder, Attention Deficit/Hyperactivity Disorder, Obsessive-Compulsive Disorder, Conduct Disorder, Oppositional Disorder or Tic Disorder. No one was taking medications.

Stimuli consisted of 6 black-and-white pictures of a boy and a girl, standardized for size, contrast, and luminosity, displaying 3 emotions: joy, anger, and a neutral expression. Each experimental session consisted of 2 trials, during which each stimulus was presented 20 times (total: 120 stimuli in a complete session), to ensure sufficient ERP acquisition. Participants were instructed to watch each stimulus carefully (total time on screen, 1300 milliseconds), and to click a mouse as soon as a blue circle appeared superimposed around the centre of the picture (700 milliseconds after the appearance of the stimulus).

Electroencephalographic activity was recorded at Fz, C3, Cz, C4, and Pz electrodes, referred to linked mastoids. The ERP averages were constructed from artifact-free epochs for each trigger code and for each electrode. Amplitudes were measured according to the distance between peaks and troughs for each identified waveform.

Analyses of the waveforms generated by the EoEs showed an enhanced late negativity occurring after stimulus presentation at a mean of 394 milliseconds for the Pz electrode, which we identified as N400 (Battaglia et al., 2005). Such centroparietal maximum amplitudes for a face-evoked ERP

N400 wave can be found in other studies following similar methods (e.g., Eimer 2000; Bentin and Deouell, 2000). Preliminary analyses showed no evidence for violation of sphericity assumptions: a check for the homogeneity of covariance matrices yielded non-significant Bartlett $\chi 2$ on all dependent variables and the covariate (boxM=19.98,$\chi 220$=16.15, p=0.70, Battaglia et al., 2005). The N400 waveform –together with other late ERP components- has been long studied as a possible marker of semantic-linguistic processing (Kutas Hillard 1980, Kutas and Federmeier 2011). Several studies found an ERP N400 component in response to other types of stimuli (Barett and Rugg 1990), including –but not limited to- human faces (Olivares 2003; Eimer 2000), and facial expressions (Balconi and Pozzoli 2005; Battaglia 2005). These latter studies made a case in favour of the hypothesis that the N400 maps the cerebral networks underlying face emotion processing, and some authors consider the N400 as a temporal correlate of a cortico-amygdala pathway of emotional processing (Williams et al., 2004). Even though the N400 is not exclusive to faces and its neural sources include a widely distributed set of brain regions (Kutas and Federmeier 2011), the centroparietal regions involved in N400 production are interconnected with emotion processing networks including the amygdala and the prefrontal cortex (Adolphs 2002b; Lang et al., 1998; Sprengelmeyer et al., 1998).

2.2.2 Harm Avoidance Scale

A modified 12 item version of the Harm Avoidance (HA) scale of Cloninger's Junior Temperament and Character Inventory, Parent version (Cloninger et al., 1994), was filled by the participants' teachers when children were attending the 2nd /3rd grades (Battaglia et al., 2004). The HA scale measures temperamental disposition toward cautiousness in the face of uncertainty and anticipation of harm; it is originally organised into four subscales: Fear of Uncertainty, Worry and Pessimism, Shyness With Strangers, and Fatigability. Individuals who score high in HA are described as cautious, tense, fearful, worried, shy, and easily fatigable (Cloninger 1987). HA is moderately-to-highly heritable (Stallings et al., 1996) and is relatively stable from childhood into

adult life (Caspi et al., 2003). High HA scores are predictive of heightened risk for internalizing disorders, such as anxiety disorders (Battaglia et al., 1996; Farmer et al., 2003).

2.3 Diffusion Weighted Data Acquisitions

Magnetic resonance DTI volumetric scans were acquired on a 3T Achieva Philips body scanner (Philips Medical Systems, Best, NL) using an 8 channels-sense head coil (sense reduction factor=2). For each subject, we acquired a DTI scan, comprising 50 contiguous axial slices, and with the following parameters: TR: 13021 msec, TE: 50 msec, voxel size: $1.8 \times 1.8 \times 2.3$ mm^3, diffusion gradients along 35 non-collinear directions, 2 b-values (0–1000 s/mm^2).

2.4 DTI Data Analyses

We have used two different procedures to investigate the association between FA measures in adolescence and 1) ERP N400 amplitudes in response to facial EoE, 2) HA scores. First, we performed tract-based spatial statistics (TBSS (Smith et al., 2006)) voxelwise analysis. Second, to better characterise the effects played by specific WM tracts, we performed tractography analysis (Basser et al., 2000) of those tracts in which the TBSS analysis revealed a significant association.

2.4.1 DTI Pre-processing

Diffusion-weighted images were processed by the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL; version 4.1, Oxford University; for an overview of the program see: Woolrich (Woolrich et al., 2009) and Smith (Smith et al., 2004)). Images were first inspected for motion artifacts: 2 subjects had to be excluded, because of excessive movements (see legend to *Table S1*); in 3 other subjects, we removed a maximum of 4 gradient orientation images containing movement artifacts. Using the Eddy Current Correction tool in the FSL Diffuson Toolbox (FDT), images were affine registered to the b_0 image as a reference. This was made to minimize distortions such as stretch and shears, and to decrease simple head motions (Horsfield,

1999). Images were brain extracted (non-brain tissues were deleted from the whole head images) by the Brain Extraction Tool (Smith, 2002).

Diffusion Tensors were calculated at each voxel with DTIFIT in FDT, by fitting the tensor model to the diffusion data via a simple least squares procedure. By this procedure we derived the tensor eigenvalues that describe the diffusion coefficients in the primary, secondary and tertiary diffusion directions, leading to the estimation of FA (Pierpaoli and Basser, 1996). We focused on FA, a highly sensitive measure of the directionality of water diffusion (Alexander et al., 2007; Basser and Pierpaoli, 1996), thought to represent a better proxy of WM directional coherence and organisation compared to other DTI indices, such as Mean Diffusivity or Radial Diffusivity (Mori and Zhang 2006; Pierpaoli and Basser 1996).

2.4.2 TBSS image Pre-processing

To align FA images, we employed TBSS (version 1.2) in FSL (Smith et al., 2006). By voxelwise non-linear registration, every FA image is aligned to all other images in a one-by-one order, so that the "most representative" image (i.e. the one which minimizes the amount of warping required for alignment) can be identified and employed as the target image. Then, the target image was affine-aligned to the MNI152 standard space (McConnell Brain Imaging Centre, Montreal Neurological Institute), and every image was transformed into a $1x1x1mm^3$ MNI152 space. By these procedures, a standard-space version was obtained for each subject's FA image. Next, a mean of the standard-space version FA images of all subjects was created and entered into the skeletonisation procedure, to generate the group mean FA skeleton (henceforth “mean FA skeleton”). As such, the mean FA skeleton embodies the centres of all the tracts common to all the subjects in the study group. For the creation of the mean FA skeleton an FA value minimum threshold of 0.25 was chosen, to ensure maximal inter-subjects' tract correspondence and to exclude voxels that were likely to primarily map grey matter or cerebro-spinal fluid. The standard-space version of each subject's FA image was then projected onto the mean FA skeleton. The

subject-specific skeleton FA images obtained by this procedure were entered into the subsequent voxelwise General Linear Model (GLM) analyses (see sections 2.4.3 and 2.4.4).

2.4.3 TBSS Whole Skeleton FA Analysis

To examine the association of N400 amplitudes in response to each of the 3 EoE (joy, neutrality and anger) and HA with the FA values at each voxel of the whole skeleton images, we set 4 General Linear Model analyses by the FEAT toolbox in FSL, using the 'Single-Group Average with Additional Covariate' option. Each General Linear Model included the HA score or the N400 amplitudes in response to 1 of the 3 EoE as the explanatory covariate, while age- and sex were added as confounding covariates, as appropriate for developmental studies of WM (Bava et al., 2011; Westlye et al., 2010). All explanatory covariates had been normalized prior to GLM analyses. We applied the "*randomise*" permutation-based nonparametric inference routine (version 2.1), a permutation program which enables modelling and inferential testing based on a standard General Linear Model, with the number of permutations set at 500. To control for multiple testing, we used the TFCE (Threshold-Free Cluster Enhancement) option included in the randomise routine. This method is thought to be generally robust (Smith and Nichols 2009) and avoids the need for an arbitrary initial cluster-forming threshold. The significance threshold was set at $p = 0.05$.

2.4.4 TBSS ROI Analysis

We used the John Hopkins University White Matter Tractography Atlas (Mori et al., 2005) in FSLView to define ROI masks for each of the following WM ventral associative tracts in the two hemispheres: the right and left Inferior Longitudinal Fasciculus (ILF); the right and left Inferior Fronto-Occipital Fasciculus (IFOF), the right and left Uncinate Fasciculus (UF). All these tracts were selected because of their probable involvement in social-emotional stimuli processing and face recognition (Catani and Thiebaut de Schotten 2008; Philippi et al., 2009).

With the *fslmaths* command in FSL, we then calculated the intersection between each ROI mask and the mean FA skeleton image, yielding one skeletonised ROI image for each WM tract.

For each skeletonised ROI, we defined two General Linear Model analyses (Single-Group Average with Additional Covariate option) in exactly the same manner as for the whole skeleton FA images (see section 2.4.3): one including the HA scores and another including N400 amplitudes in response to anger. Happy and Neutral EoE were excluded from this and from subsequent analyses due to non-significant results in the whole skeleton FA analyses (vide infra). The significance threshold was set at p =0.05 (TFCE corrected).

2.4.5 Correlation coefficients and linear regression plots

In order to obtain the correlation coefficients and linear regression plots, which are not available in the TBSS output, for the whole skeleton and ROI-based skeleton effects (see Results, sections 3.1 and 3.2), we used the R software (R 2.11.1, R Foundation for Statistical Computing, 2010). First, we extracted with the *fslmeants* and *fslmaths* commands in FSL one single FA value for each subject, consisting in the average FA within all the voxels that were significant in each GLM group analysis. Second, the extracted sets of subject-specific FA values were tested for non-normality distribution with the Shapiro-Wilk test (Shapiro and Wilk 1965). Finally, we computed in R 2.11.1 the correlation and linear regression plots between each set of subject-specific FA values and, respectively, N400 or HA (see Figure 1 and Table 2). In case of non-normal distributions, correlation and linear regression analyses were made after rank transformation of the data.

2.4.6 Tractography Analysis

To further analyze and better characterize the effects played by specific WM tracts, a tractography analysis was performed bilaterally for the 3 associative tracts (UF, ILF, IFOF) included in the TBSS analyses, thus yielding 6 tract FA values for each subject. Eddy current-corrected and brain-extracted brain volumes were processed with the Diffusion Toolkit program (DTK (Wang et al., 2007)) to perform data reconstruction and fiber tracking. The Interpolate Streamline tractography algorithm (FA threshold = 0.15, angle threshold = 45°) was used to estimate the propagation of streamlines from “seed” ROIs, manually-defined in Trackvis (Wang et al., 2007).

For each of the 6 tracts, we used a two ROIs approach method, as described by Catani and Thiebaut de Schotten (2008), which yields high inter-experimenter reliability.

Temporal, occipital and frontal ROIs were manually drawn onto each subject's native FA map. Depending on the obligatory passages of tracts, we set the following combinations of two inclusive ROIs: temporal and occipital ROIs for ILF; occipital and frontal ROIs for IFOF; frontal and temporal ROIs for UF. When necessary, exclusive ROIs were drawn to remove artifacts: this was applied to those streamlines which did not belong to the tract of interest, as expectable from anatomy. To optimize ROIs drawing consistency, for each subject we drew each ROI 3 times, yielding 3 different virtual reconstructions of each tract. For each tract, we thus obtained 3 mean FA values. The average of these 3 mean FA values was used for linear regression analysis. Before statistical processing, all distributions were preliminary tested for non-normality distribution with the Shapiro-Wilk test (Shapiro and Wilk 1965).

The relationship between the extracted FA values, and HA scores or N400 'anger' amplitudes, was tested by applying a Linear Regression to each tract separately in R 2.11.1 . In case of non-normal distributions, we employed correlation and regression analyses after a rank transformation of the data.

3. RESULTS

3.1 TBSS Whole Skeleton Results

The whole skeleton analyses yielded significant and negative correlations between FA and the N400 amplitudes in response to angry expressions, and between FA and HA. No significant correlations were found for the N400 amplitudes in response to happy and neutral faces.

These results indicate that lower FA is associated with reduced ERP N400 amplitudes in response to angry faces (Figure 1 :A), and with higher scores at the temperamental scale of Harm Avoidance (Figure 1 :B). The results were confirmed by the analyses of extracted FA indices (see legend to Figure 1 for the correlation scores).

3.2 TBSS ROI-based Skeleton Results

TBSS ROI-based skeleton analyses yielded a significant negative correlation between N400 amplitudes in response to angry expressions and FA values in the left UF, in the left and right ILF, and in the left and right IFOF (Figure 2 :A-E). Moreover, we found a significant negative correlation between HA and FA values in the right UF (Figure 2 :F). The results were confirmed by the analyses of FA indices extracted from significant voxels in each ROI (Table 2).

3.3 Tractography Results

The regression analyses in which the N400 amplitude in response to angry expressions was the independent variable, and the 6 FA values obtained by the application of tractography were the dependent variables, showed a significant effect for the left ILF ($p=0.019$, $\beta=-2.655$, adjusted $R^2=0.287$) and the right IFOF ($p=0.007$, $\beta=-3.13$, adjusted $R^2=0.37$).

Analogous regression analyses with the HA score as the independent variable showed a significant effect for the right UF ($p=0.02$, $\beta=-2.53$, adjusted $R^2=0.24$).

4. DISCUSSION

Our data show a longitudinal relationship that links white matter fibres' microstructural organisation in adolescence to cerebral responses to hostile facial expressions and temperamental disposition to cautiousness/shyness with strangers in childhood. Specifically, reduced fractional anisotropy at age 14-15 correlated with smaller ERP N400 amplitudes acquired at age 8-9 in response to angry faces, and higher Harm Avoidance measured at age 7-8.

Significant and substantial-to-high correlations between FA and the ERP N400 amplitudes emerged from the estimations of TBSS whole skeleton WM structural organisation, and from the TBSS ROI analyses of tracts that subserve the processing of visual and emotional stimuli and face encoding (Catani and Thiebaut de Schotten 2008; Fox et al., 2008; Philippi et al., 2009). Tractography

revealed a clearer definition of the relationship between ERP responses to angry expressions and FA, by showing specific effects at the levels of the left ILF and the right IFOF.

The fact that the angry expression was the only among 3 different EoE categories to sustain the correlations between ERP responses and FA indexes may not be surprising, since anger is less accessible to decoding than most other EoE (Battaglia et al., 2010; Chen et al., 2011), and its processing –even when implicit- can challenge one's neural resources. The ILF and IFOF connect the occipital areas to the temporal lobe (Catani and Mesulam 2008) and to the frontal cortex (Catani 2007) respectively, both tracts carrying visual information. The ILF is likely to be involved in face recognition (Catani et al., 2003; Catani and Thiebaut de Schotten 2008; Fox et al., 2008), visual perception (ffytche and Catani 2005; ffytche 2008) and visual memory (Ross 2008); the IFOF is likely to play an important role in visual processing (Fox et al., 2008), attention (Doricchi et al., 2008), and facial emotions recognition (Philippi et al., 2009).

At the electrophysiological level, EoE processing is associated with a time-sensitive series of activations, whereby the later, more complex phases of elaboration subserve the conceptual knowledge of the specific emotion being depicted by an EoE (Adolphs 2002b). The ERP waveforms 300-400 msec after stimulus onset - including the ERP N400- are thus thought to map these later phases of EoE processing (Adolphs 2002b). Diminished ERP amplitudes could indicate diminished engagement reflective of increased facility of processing (Kutas and Federmeier 2009). This occurs when a stimulus is primed, or within a supportive context. If this were the case, reduced N400 amplitudes in response to anger could indicate a priming effect, selective for this stimulus, among shy/socially anxious subjects. Alternatively, reduced N400 amplitude could reflect smaller postsynaptic potentials, and/or activation of fewer neurons within a given brain area (Kutas and Federmeier 2011) in response to anger. Thus, diminished cortical engagement could map less than optimal stimulus processing (Eimer 2000; Felmingham et al., 2003) and a relative impairment of EoE reading. This latter interpretation is in much better harmony with empirical data showing an association between social anxiety and worse abilities to process and identify angry EoE in both

adult (Horley et al., 2003) and children (Battaglia et al., 2004; 2010) populations. In this light, the negative correlation between ERP N400 amplitudes and FA indicates that reduced WM microstructural organisation can underlie a reduced cortical activation during EoE decoding.

We also found that higher Harm Avoidance during childhood correlates with decreased FA in adolescence, providing developmental support to the findings of a recent large cohort study of adults (Westlye et al., 2011). In relation to HA, we found reduced FA in the TBSS whole skeleton WM, while both the TBSS ROI analyses and tractography showed specific effects in the right UF. The UF is a prominent tract that connects the lateral amygdala to the inferofrontal and anterotemporal cortices (Phan et al., 2009); it is thought to functionally belong to the limbic system and to participate in emotional processing (Catani and Mesulam 2008; Catani and Thiebaut de Schotten 2008; Gaffan and Wilson 2008). The negative correlation between FA in the right UF and Harm Avoidance among adolescents may thus indicate that part of the disposition towards cautiousness and inaccurate evaluation of danger (Cloninger 1987) that are proper of this temperamental trait can be explained by reduced WM structural organisation. It has been speculated that a consequence of reduced WM organisation may include a relative functional imbalance between the emotional appraisal role played by the amygdala, and the integrative and regulatory functions played by the frontal cortices, which mediate threat perception/evaluation (Phan et al., 2009; Westlye et al., 2011). As expectable with a temperamental trait, our data show that these relationships can be traced already in adolescence, while the fact that our FA data have been collected at age 14-15, when the myelination process has reached considerable stability, yields a degree of firmness to our results.

In sum, these results show that individual differences for a) cortical activation to EoE, and b) temperamental disposition towards avoidant behaviour in the face of novelty, are amenable to differences in FA measured in relatively well-separated WM pathways. While individual variability in HA correlates with FA in the right UF -a tract involved in emotional regulation- the N400

amplitude in response to anger correlates mainly with FA of tracts that deal with the early encoding of the visual-perceptual characteristics of the stimulus, namely the ILF and IFOF.

At least three potential limitations apply. First, as it is often the case with developmental longitudinal studies that employ complex technologies, the sample size is relatively small, implicating reduced power and the need for replication in larger longitudinal samples. However, after accurate control, we found no clear indications of participation bias in this sample (*Table S1*). Second, this study is correlational in nature. We suggest a direction of causality that goes from reduced FA to decreased cortical (ERP) engagement. While an opposite direction of causality appears implausible, our reasoning remains inferential. Third, we hypothesized that reduced WM microstructural organisation leads to imbalanced cortico-amygdalar cross-talk as a possible pathway to developing social anxiety. Further evidence from additional functional connectivity paradigms in this sample would have been useful to support this view. However, our fMRI findings with this same study group (Battaglia et al., 2012) showed amygdalar hyperactivity in response to angry EoE among the more socially anxious participants. Moreover, studies of adults with social phobia during simulated social interaction (Reiman 1997; Tillfors et al., 2001; Van Ameringen et al., 2000) found patterns of decreased cortical activation plus exaggerated amygdalar activation, a datum consistent with this view. Functional connectivity case-control studies also provided results compatible with the hypothesis of an imbalanced cortico-amygdalar cross-talk in adults with social phobia, both in response to facial EoE (Danti et al., 2010) and at rest (Liao et al., 2010; Hahn et al., 2011).

5. CONCLUSIONS

Overall, our data indicate that reduced WM organisation in ventral limbic tracts may compound the etiopathogenetic pathways that –by affecting the neural responses to socio-emotional stimuli and the regulation of emotions- alter the risk for internalising conditions such as social anxiety (Battaglia et al., 2005; Battaglia et al., 2012; Phan et al., 2009)- and generalised anxiety (Westlye et al., 2011) disorder.

Disclosure Statement: All Authors declare no actual or potential conflict of interest within three (3) years of beginning the work submitted that could inappropriately influence their work.

Role of the funding source: This study was supported by a National Alliance for Research in Schizophrenia and Depression 2006 Independent Investigator Award (Dr Battaglia) by an Italian Ministry Of Health Strategic Research 2008-2010 Grant (To M.B.) and by an Italian Ministry of University and Research, Rome (Co-Fin grant 11/2001-113555_004 to M.B.); MTa, is in the San Raffaele University International Ph D Program in Developmental Psychopathology, supported in part by the CARIPLO Foundation 'Human Talents' Grant for Academic Centres of Excellence in Post-Graduate Teaching (to M.B.).

Acknowledgments: We are grateful to Flavio Dell' Acqua Ph.D. for precious help with the tractography analysis, Eleonora Bertoletti M.Sc., Valentina Lampis Ph.D. and Simona Scaini M.Sc. for help in subjects' recruitment, and children and their families for participation to the study.

References

Adolphs R., 2002a. Recognizing emotion from facial expressions: Psychological and neurological mechanisms. Behav.Cogn.Neurosci.Rev. 1(1):21-62.

Adolphs R., 2002b. Neural systems for recognizing emotion. Curr.Opin.Neurobiol. 12(2):169-177.

Alexander A.L., Lee J.E., Lazar M., Field A.S., 2007. Diffusion tensor imaging of the brain. Neurotherapeutics 4(3):316-329.

Ball T.M., Sullivan S., Flagan T., Hitchcock C.A., Simmons A., Paulus M.P., Stein M.B., 2011. Selective effects of social anxiety, anxiety sensitivity, and negative affectivity on the neural bases of emotional face processing. Neuroimage .

Basser P.J. and Pierpaoli C., 1996. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J.Magn.Reson.B 111(3):209-219.

Basser P.J., Pajevic S., Pierpaoli C., Duda J., Aldroubi A., 2000. In vivo fiber tractography using DT-MRI data. Magn.Reson.Med. 44(4):625-632.

Battaglia M., Przybeck T.R., Bellodi L., Cloninger C.R., 1996. Temperament dimensions explain the comorbidity of psychiatric disorders. Compr.Psychiatry 37(4):292-298.

Battaglia M., Zanoni A., Ogliari A., Crevani F., Falzone L., Bertoletti E., Di Serio C., 2010. Identification of gradually changing emotional expressions in schoolchildren: The influence of the type of stimuli and of specific symptoms of anxiety. Cogn Emot 24:1070-1079.

Battaglia M., Ogliari A., Zanoni A., Citterio A., Pozzoli U., Giorda R., Maffei C., Marino C., 2005. Influence of the serotonin transporter promoter gene and shyness on children's cerebral responses to facial expressions. Arch.Gen.Psychiatry 62(1):85-94.

Battaglia M., Ogliari A., Zanoni A., Villa F., Citterio A., Binaghi F., Fossati A., Maffei C., 2004. Children's discrimination of expressions of emotions: Relationship with indices of social anxiety and shyness. J.Am.Acad.Child Adolesc.Psychiatry 43(3):358-365.

Battaglia M., Zanoni A., Taddei M., Giorda R., Bertoletti E., Lampis V., Scaini S., Cappa S., Tettamanti M., 2012. Cerebral responses to emotional expressions and the development of social anxiety disorder: A preliminary longitudinal study. Depress.Anxiety 29(1):54–61.

Batty M. and Taylor M.J., 2006. The development of emotional face processing during childhood. Dev.Sci. 9(2):207-220.

Batty M. and Taylor M.J., 2002. Visual categorization during childhood: An ERP study. Psychophysiology 39(4):482-490.

Baur V., Hanggi J., Rufer M., Delsignore A., Jancke L., Herwig U., Beatrix Bruhl A., 2011a. White matter alterations in social anxiety disorder. J.Psychiatr.Res. 45(10):1366-1372.

Baur V., Bruhl A.B., Herwig U., Eberle T., Rufer M., Delsignore A., Jancke L., Hanggi J., 2011b. Evidence of frontotemporal structural hypoconnectivity in social anxiety disorder: A quantitative fiber tractography study. Hum.Brain Mapp. in press.

Bava S., Boucquey V., Goldenberg D., Thayer R.E., Ward M., Jacobus J., Tapert S.F., 2011. Sex differences in adolescent white matter architecture. Brain Res. 1375:41-48.

Casey B.J., Tottenham N., Liston C., Durston S., 2005. Imaging the developing brain: What have we learned about cognitive development? Trends Cogn.Sci. 9(3):104-110.

Caspi A., Harrington H., Milne B., Amell J.W., Theodore R.F., Moffitt T.E., 2003. Children's behavioral styles at age 3 are linked to their adult personality traits at age 26. J.Pers. 71(4):495-513.

Catani M., 2007. From hodology to function. Brain 130(Pt 3):602-605.

Catani M. and Mesulam M., 2008. The arcuate fasciculus and the disconnection theme in language and aphasia: History and current state. Cortex 44(8):953-961.

Catani M. and Thiebaut de Schotten M., 2008. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. Cortex 44(8):1105-1132.

Catani M., Jones D.K., Donato R., Ffytche D.H., 2003. Occipito-temporal connections in the human brain. Brain 126(Pt 9):2093-2107.

Chen W., Lander K., Liu C.H., 2011. Matching faces with emotional expressions. Front.Psychol. 2:206.

Cloninger C.R., 1987. A systematic method for clinical description and classification of personality variants. A proposal. Arch.Gen.Psychiatry 44(6):573-588.

Cloninger, C.R., Przybeck, T.R., Svrakic, D., Wetzel, R.D. (Eds.), 1994. The temperament and character inventory, St Louis: Washington University.

Coch D., Maron L., Wolf M., Holcomb P.J., 2002. Word and picture processing in children: An event-related potential study. Dev.Neuropsychol. 22(1):373-406.

Cohen Kadosh K., Cohen Kadosh R., Dick F., Johnson M.H., 2011. Developmental changes in effective connectivity in the emerging core face network. Cereb.Cortex 21(6):1389-1394.

Dailey M.N., Cottrell G.W., Padgett C., Adolphs R., 2002. EMPATH: A neural network that categorizes facial expressions. J.Cogn.Neurosci. 14(8):1158-1173.

Danti S., Ricciardi E., Gentili C., Gobbini M.I., Pietrini P., Guazzelli M., 2010. Is social phobia a "mis-communication" disorder? brain functional connectivity during face perception differs between patients with social phobia and healthy control subjects. Front.Syst.Neurosci. 4:152.

Doricchi F., Thiebaut de Schotten M., Tomaiuolo F., Bartolomeo P., 2008. White matter (dis)connections and gray matter (dys)functions in visual neglect: Gaining insights into the brain networks of spatial awareness. Cortex 44(8):983-995.

Eimer M., 2000. Effects of face inversion on the structural encoding and recognition of faces. evidence from event-related brain potentials. Brain Res.Cogn.Brain Res. 10(1-2):145-158.

Ekman P. and Friesen W.V., 1971. Constants across cultures in the face and emotion. J.Pers.Soc.Psychol. 17(2):124-129.

Elfenbein H.A. and Ambady N., 2002. Is there an in-group advantage in emotion recognition? Psychol.Bull. 128(2):243-249.

Farmer A., Mahmood A., Redman K., Harris T., Sadler S., McGuffin P., 2003. A sib-pair study of the temperament and character inventory scales in major depression. Arch.Gen.Psychiatry 60(5):490-496.

Felmingham K.L., Bryant R.A., Gordon E., 2003. Processing angry and neutral faces in post-traumatic stress disorder: An event-related potentials study. Neuroreport 14(5):777-780.

ffytche D.H., 2008. The hodology of hallucinations. Cortex 44(8):1067-1083.

ffytche D.H. and Catani M., 2005. Beyond localization: From hodology to function. Philos.Trans.R.Soc.Lond.B.Biol.Sci. 360(1456):767-779.

Fox C.J., Iaria G., Barton J.J., 2008. Disconnection in prosopagnosia and face processing. Cortex 44(8):996-1009.

Fries A.B. and Pollak S.D., 2004. Emotion understanding in postinstitutionalized eastern european children. Dev.Psychopathol. 16(2):355-369.

Gaffan D. and Wilson C.R., 2008. Medial temporal and prefrontal function: Recent behavioural disconnection studies in the macaque monkey. Cortex 44(8):928-935.

Gschwind M., Pourtois G., Schwartz S., Van De Ville D., Vuilleumier P., 2011. White-matter connectivity between face-responsive regions in the human brain. Cereb.Cortex in press.

Hahn A., Stein P., Windischberger C., Weissenbacher A., Spindelegger C., Moser E., Kasper S., Lanzenberger R., 2011. Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. Neuroimage 56(3):881-889.

Haxby J.V., Hoffman E.A., Gobbini M.I., 2002. Human neural systems for face recognition and social communication. Biol.Psychiatry 51(1):59-67.

Herba C.M., Landau S., Russell T., Ecker C., Phillips M.L., 2006. The development of emotion-processing in children: Effects of age, emotion, and intensity. J.Child Psychol.Psychiatry 47(11):1098-1106.

Horley K., Williams L.M., Gonsalvez C., Gordon E., 2003. Social phobics do not see eye to eye: A visual scanpath study of emotional expression processing. J.Anxiety Disord. 17(1):33-44.

Horsfield M.A., 1999. Mapping eddy current induced fields for the correction of diffusion-weighted echo planar images. Magn.Reson.Imaging 17(9):1335-1345.

Jehna M., Neuper C., Ischebeck A., Loitfelder M., Ropele S., Langkammer C., Ebner F., Fuchs S., Schmidt R., Fazekas F., and others., 2011. The functional correlates of face perception and recognition of emotional facial expressions as evidenced by fMRI. Brain Res. 1393:73-83.

Kim M.J. and Whalen P.J., 2009. The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. J.Neurosci. 29(37):11614-11618.

Kim M.J., Gee D.G., Loucks R.A., Davis F.C., Whalen P.J., 2011. Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. Cereb.Cortex 21(7):1667-1673.

Lang P.J., Bradley M.M., Cuthbert B.N., 1998. Emotion, motivation, and anxiety: Brain mechanisms and psychophysiology. Biol.Psychiatry 44(12):1248-1263.

Liao W., Qiu C., Gentili C., Walter M., Pan Z., Ding J., Zhang W., Gong Q., Chen H., 2010. Altered effective connectivity network of the amygdala in social anxiety disorder: A resting-state FMRI study. PLoS One 5(12):e15238.

Martin E.I., Ressler K.J., Binder E., Nemeroff C.B., 2009. The neurobiology of anxiety disorders: Brain imaging, genetics, and psychoneuroendocrinology. Psychiatr.Clin.North Am. 32(3):549-575.

Monk C.S., 2008. The development of emotion-related neural circuitry in health and psychopathology. Dev.Psychopathol. 20(4):1231-1250.

Mori S. and Zhang J., 2006. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51(5):527-539.

Mori, S., Wakana, S., Nagae-Poetscher, L.M., van Zijl, P.C. 2005. MRI atlas of human white matter. Elsevier, Amsterdam.

Olivares E.I., Iglesias J., Rodriguez-Holguin S., 2003. Long-latency ERPs and recognition of facial identity. J.Cogn.Neurosci. 15(1):136-151.

Orgeta V. and Phillips L.H., 2008. Effects of age and emotional intensity on the recognition of facial emotion. Exp.Aging Res. 34(1):63-79.

Perez-Edgar K., Bar-Haim Y., McDermott J.M., Gorodetsky E., Hodgkinson C.A., Goldman D., Ernst M., Pine D.S., Fox N.A., 2010. Variations in the serotonin-transporter gene are associated with attention bias patterns to positive and negative emotion faces. Biol.Psychol. 83(3):269-271.

Perez-Edgar K., Roberson-Nay R., Hardin M.G., Poeth K., Guyer A.E., Nelson E.E., McClure E.B., Henderson H.A., Fox N.A., Pine D.S., and others., 2007. Attention alters neural responses to evocative faces in behaviorally inhibited adolescents. Neuroimage 35(4):1538-1546.

Phan K.L., Orlichenko A., Boyd E., Angstadt M., Coccaro E.F., Liberzon I., Arfanakis K., 2009. Preliminary evidence of white matter abnormality in the uncinate fasciculus in generalized social anxiety disorder. Biol.Psychiatry 66(7):691-694.

Philippi C.L., Mehta S., Grabowski T., Adolphs R., Rudrauf D., 2009. Damage to association fiber tracts impairs recognition of the facial expression of emotion. J.Neurosci. 29(48):15089-15099.

Pierpaoli C. and Basser P.J., 1996. Toward a quantitative assessment of diffusion anisotropy. Magn.Reson.Med. 36(6):893-906.

Pollak S.D., Klorman R., Thatcher J.E., Cicchetti D., 2001. P3b reflects maltreated children's reactions to facial displays of emotion. Psychophysiology 38(2):267-274.

Posamentier M.T. and Abdi H., 2003. Processing faces and facial expressions. Neuropsychol.Rev. 13(3):113-143.

Reiman E.M., 1997. The application of positron emission tomography to the study of normal and pathologic emotions. J.Clin.Psychiatry 58 Suppl 16:4-12.

Ross E.D., 2008. Sensory-specific amnesia and hypoemotionality in humans and monkeys: Gateway for developing a hodology of memory. Cortex 44(8):1010-1022.

Schweinberger S.R. and Burton A.M., 2003. Covert recognition and the neural system for face processing. Cortex 39(1):9-30.

Shapiro S. and Wilk M., 1965. An analysis of variance test for normality (complete samples). Biometrika 52(3-4):591-611.

Shimamura A.P., Ross J.G., Bennett H.D., 2006. Memory for facial expressions: The power of a smile. Psychon.Bull.Rev. 13(2):217-222.

Smith S.M., 2002. Fast robust automated brain extraction. Hum.Brain Mapp. 17(3):143-155.

Smith S.M. and Nichols T.E., 2009. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 44(1):83-98.

Smith S.M., Jenkinson M., Johansen-Berg H., Rueckert D., Nichols T.E., Mackay C.E., Watkins K.E., Ciccarelli O., Cader M.Z., Matthews P.M., and others., 2006. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. Neuroimage 31(4):1487-1505.

Smith S.M., Jenkinson M., Woolrich M.W., Beckmann C.F., Behrens T.E., Johansen-Berg H., Bannister P.R., De Luca M., Drobnjak I., Flitney D.E., and others., 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23 Suppl 1:S208-19.

Sprengelmeyer R., Rausch M., Eysel U.T., Przuntek H., 1998. Neural structures associated with recognition of facial expressions of basic emotions. Proc.Biol.Sci. 265(1409):1927-1931.

Stallings M.C., Hewitt J.K., Cloninger C.R., Heath A.C., Eaves L.J., 1996. Genetic and environmental structure of the tridimensional personality questionnaire: Three or four temperament dimensions? J.Pers.Soc.Psychol. 70(1):127-140.

Stirling L.J., Eley T.C., Clark D.M., 2006. Preliminary evidence for an association between social anxiety symptoms and avoidance of negative faces in school-age children. J.Clin.Child.Adolesc.Psychol. 35(3):431-439.

Tillfors M., Furmark T., Marteinsdottir I., Fischer H., Pissiota A., Langstrom B., Fredrikson M., 2001. Cerebral blood flow in subjects with social phobia during stressful speaking tasks: A PET study. Am.J.Psychiatry 158(8):1220-1226.

Van Ameringen M., Mancini C., Farvolden P., Oakman J., 2000. The neurobiology of social phobia: From pharmacotherapy to brain imaging. Curr.Psychiatry Rep. 2(4):358-366.

Wang, R., Benner, T., Sorensen, A.G., Wedeen, V.J., 2007. Diffusion toolkit: A software package for diffusion imaging data processing and tractography. Proc. Intl. Soc. Mag. Reson. Med. 15, 3720.

Westlye L.T., Bjornebekk A., Grydeland H., Fjell A.M., Walhovd K.B., 2011. Linking an anxiety-related personality trait to brain white matter microstructure: Diffusion tensor imaging and harm avoidance. Arch.Gen.Psychiatry 68(4):369-377.

Westlye L.T., Walhovd K.B., Dale A.M., Bjornerud A., Due-Tonnessen P., Engvig A., Grydeland H., Tamnes C.K., Ostby Y., Fjell A.M., 2010. Life-span changes of the human brain white matter: Diffusion tensor imaging (DTI) and volumetry. Cereb.Cortex 20(9):2055-2068.

Williams L.M., Liddell B.J., Rathjen J., Brown K.J., Gray J., Phillips M., Young A., Gordon E., 2004. Mapping the time course of nonconscious and conscious perception of fear: An integration of central and peripheral measures. Hum.Brain Mapp. 21(2):64-74.

Woolrich M.W., Jbabdi S., Patenaude B., Chappell M., Makni S., Behrens T., Beckmann C., Jenkinson M., Smith S.M., 2009. Bayesian analysis of neuroimaging data in FSL. Neuroimage 45(1 Suppl):S173-86.

Table 1

PHASES OF THE LONGITUDINAL STUDY

	Time 1 (Battaglia et al. 2004)	**Time 2** (Battaglia et al., 2005)	**Present (Time 3)**
Sample	N=149	N=49	N=20
Mean age ± SD	7,5 ± 0,5	8.8 ± 0.7	15 ± 0,7
Main measurements	Behavioural; Assessment of Harm Avoidance	ERP	DTI

Phases of the Longitudinal Study. For each phase, the outcome measures that are relevant to the present study are presented, together with sample sizes and mean age of the study participants.

Table 2

TBSS ROI-BASED SKELETON RESULTS

A)	**Pearson r**	**Adjusted R^2**
Inferior Longitudinal Fasciculus R	-0.69*	0.43
Inferior Longitudinal Fasciculus L	-0.82**	0.65
Inferior Frontoccipial Fasciculus R	-0.80**	0.62
Inferior Frontoccipital Fasciculus L	-0.79**	0.59
Uncinate Fasciculus L	-0.70*	0.46
B)		
Uncinate Fasciculus R	-0.71*	0.47

Correlations between extracted white matter FA indices and: A) N400 amplitudes in Response to Angry Expressions; B) HA scores.

*p <0.01 **p <0.001

TBSS, Tract Based Spatial Statistics; **FA**, Fractional Anisotropy; **HA**, Harm Avoidance; **R**, right; **L**, left.

FIGURE LEGENDS

Figure 1

TBSS Whole Skeleton Results. Spatial distribution and scatter plots of significant correlations between white matter FA in the whole TBSS skeleton and: A) Normalized N400 amplitude in response to angry faces (n=16; Pearson r =-0.79; P<0,001; adjusted R^2 =0.61). (The N400 amplitudes in response to facial expressions acquired at age 8-9 at the Pz electrode are shown superimposed to the scatterplot of the correlations between FA and N400); B) Normalized Harm Avoidance score (n=18; Pearson r =-0.87; P<0,001; adjusted R^2 =0.73). The correlation between N400 amplitude and HA was =0.21, P=NS.

The significant voxels (p<0.05, TFCE-corrected) are superimposed (in a dark-blue/light-blue colourmap for the N400 response and in a red/orange colourmap for HA) on the whole TBSS skeleton (in green colour) and on the MNI152 template image (in a grey colourmap), both as 3D representations and as axial sections. Coordinates along the z-axis for axial slices are given in mm.

Figure 2

TBSS ROI-based Skeleton Results. Significant correlations between white matter FA and normalized N400 amplitude in response to angry faces in A) the left inferior fronto-occipital fasciculus ROI; B) the right inferior fronto-occipital fasciculus ROI; C) the left inferior longitudinal fasciculus ROI; D) the right inferior longitudinal fasciculus ROI; E) the left uncinate fasciculus ROI. F) Significant correlations between white matter FA and normalized Harm Avoidance score in the right uncinate fasciculus ROI.

The significant voxels (p<0.05, TFCE-corrected; see Table 2 for significance scores) are superimposed (in a dark-blue/light-blue colourmap for the N400 response and in a red/orange colourmap for HA) on the TBSS skeletonised ROIs (in green colour) and on the MNI152 template image (in a grey colourmap) as 3D representations.

Figure 1

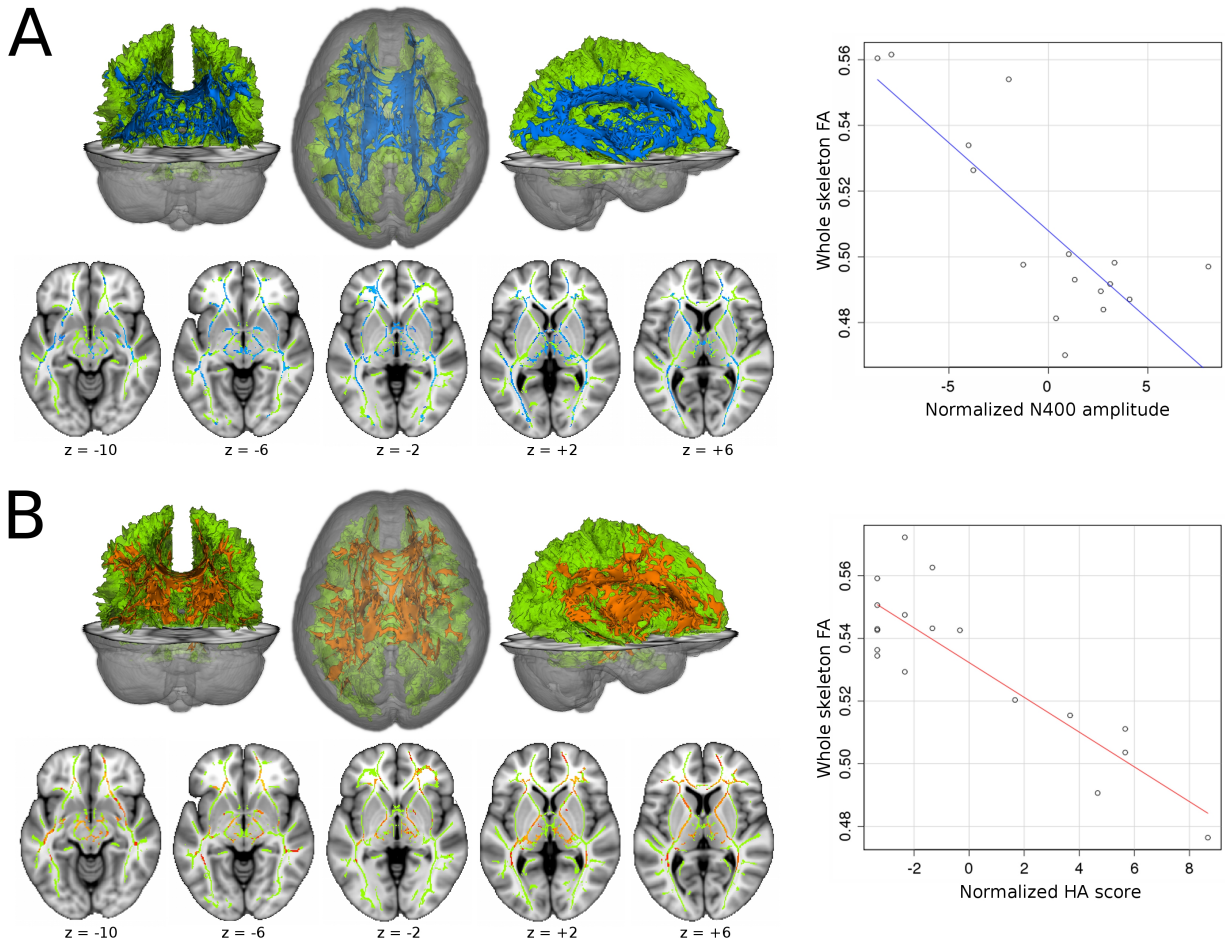
A
z = -10
z = -6
z = -2
z = +2
z = +6
Whole skeleton FA
0.48
0.50
0.52
0.54
0.56
-5
0
5
Normalized N400 amplitude
B
z = -10
z = -6
z = -2
z = +2
z = +6
Whole skeleton FA
0.48
0.50
0.52
0.54
0.56
-2
0
2
4
6
8
Normalized HA score

Figure 2