The role of the insula in the generation of motor tics and the experience of the premonitory urge-to-tic in Tourette syndrome

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Abstract

Tourette syndrome (TS) is a neurological disorder of childhood onset that is characterised by the occurrence of motor and vocal tics. TS is associated with cortical-striatal-thalamic-cortical circuit [CSTC] dysfunction and hyper-excitability of cortical limbic and motor regions that are thought to lead to the occurrence of tics. Importantly, individuals with TS often report that their tics are preceded by 'premonitory sensory/urge phenomena' (PU) that are described as uncomfortable bodily sensations that precede the execution of a tic and are experienced as a strong urge for motor discharge. While the precise role played by PU in the occurrence of tics is largely unknown, they are nonetheless of considerable theoretical and clinical importance, not least because they form the core component in many behavioural therapies used in the treatment of tic disorders. Several lines of evidence indicate that the insular cortex may play a particularly important role in the generation of PU in TS and 'urges-for-action' more generally. In the current study we utilised voxel-based morphometry techniques together with 'seed-to-voxel' structural covariance network (SCN) mapping to investigate the putative role played by the right insular cortex in the generation of motor tics and the experience of PU in a relatively large group of young people TS. We demonstrate that clinical measures of motor tic severity and PU are uncorrelated with one another, that motor tic severity and PU scores are associated with separate regions of the insular cortex, and that the insula is associated with different structural covariance networks in individuals with TS compared to a matched group of typically developing individuals.

Introduction

Tourette syndrome (TS) is a neurological disorder of childhood onset that is characterised by the presence of chronic vocal and motor tics (Cohen, Leckman, & Bloch, 2013). Tics are involuntary, repetitive, stereotyped behaviours that occur with a limited duration (Cohen et al., 2013). Motor tics can be simple or complex in appearance, ranging from repetitive movements to coordinated action sequences. Verbal tics can consist of repetitive sounds, words or utterances, the production of inappropriate or obscene utterances, or the repetition of another's words. Tics occur in bouts, typically many times in a single day, and are the most common form of movement disorder in children. TS is estimated to affect approximately 1% of individuals aged 5-18 years (Cohen et al., 2013).

Individuals with TS perceive a relatively constant demand to suppress their tics, particularly in social situations, and while the voluntary suppression of tics is possible in many cases, TS patients typically report that it can be uncomfortable and stressful to suppress tics, and that the urge to tic becomes uncontrollable after a period of suppression. Importantly, in the context of the current study, the majority (~90%) of individuals with TS report that their tics are often preceded by 'premonitory sensory/urge phenomena' (PU) that are described as uncomfortable cognitive or bodily sensations that occur prior to the execution of a tic and are experienced as a strong urge for motor discharge. Individuals who experience PU often report that: these experiences are more bothersome than their tics; that expressing their tics give them relief from, and temporarily *abolishes*, their PU; and that they would not exhibit tics if they did not experience PU. For this reason, it has been proposed that PU should be considered as the driving force behind the occurrence of tics, and that tics are a learnt response to the experience of PU (Cavanna, Black, Hallett, Voon, 2017). Furthermore, PU are of particular clinical importance because they form the core component of behavioural therapies that are currently used in the treatment of tic disorders (Cohen et al., 2013).

Surprisingly, our understanding of PU and their relationship to tics is currently limited, and there are grounds for thinking that the occurrence of tics and the occurrence of PU are independent processes or only loosely associated. First, not all individuals with TS report experiencing PU. In particular, children under 10 years of age, who present with simple tics, do not typically report being aware of PU (Cohen et al., 2013). Second, tics have been observed during sleep, including slow-wave sleep, indicating

that at least some tics are involuntary (Cohrs et al., 2001). Third, the occurrence of tics - and an individual's ability to suppress them - may occur independently of the awareness of PU (Ganos et al., 2012). Finally, the generation of tics and the genesis of PU in TS have been linked to different brain networks (Bronfeld, Israelashvili, & Bar-Gad, 2013; Conceicao, Dias, Farinha, & Maia, 2017; Jackson, Parkinson, Kim, Schuermann, & Eickhoff, 2011; McCairn, Iriki, & Isoda, 2013) . Previous studies have indicated that the urge-for-action more generally may activate a common set of brain areas across a wide range of behavioural domains (e.g., the urge to blink, the urge to yawn, the urge to micturate, the urge to scratch an itch, etc.), that includes the urge to tic in Tourette syndrome (TS) (Jackson, Parkinson, Kim, et al., 2011). Jackson and colleagues conducted a quantitative meta-analysis of functional brain imaging studies that investigated the 'urge-for-action' associated with everyday behaviours such as yawning, swallowing, and micturition, and demonstrated that the right anterior insula and the mid-cingulate cortex were the only regions consistently activated across brain imaging studies associated with the perception of the urge for action in different behavioural domains (Jackson, Parkinson, Kim, et al., 2011). Importantly, these authors proposed that the right insula plays a central role in a neural circuit that represents bodily sensations, generates urges for action, selects an action based upon an estimation of the likely outcomes associated with that action, and determines whether the conditions giving rise to the urge-for-action have been resolved once an action has been initiated.

Consistent with this proposal, functional brain imaging studies indicate that PU may be particularly associated with brain activity within the insular cortex (Bohlhalter et al., 2006; Neuner et al., 2014) which has been linked to interoceptive awareness (Craig, 2009). Consistent with this view, Ganos and colleagues (2015) reported that interoceptive awareness was strongly associated with PU scores in TS, and that increased tic severity scores were associated with increases in PU (Ganos et al., 2015). Brain imaging studies have also demonstrated that functional connectivity of the anterior insular cortex of the right hemisphere is associated with both the urge to tic and tic severity in TS (Tinaz, Malone, Hallett, & Horovitz, 2015) and that cortical thickness within the insular cortex is reduced relative to a matched group of typically developing controls, and that cortical thickness within the insula is inversely associated with the strength of PU in young adults with TS. In the current study we focus on the relationship between the insular cortex, measured using structural magnetic resonance imaging

together with the analysis of structural covariance networks, and clinical measures of tic severity and PU.

Human brain imaging studies have identified a number of functional brain networks, often referred to as ICNs (intrinsic cortical networks) that reflect correlated brain activity across anatomically separate brain areas. Recent evidence indicates that these networks are dominated by common organisational principles and stable features, and may largely reflect enduring individual characteristics, including the consequence of brain health conditions (Gratton et al., 2018). Similarly, neuroimaging studies have repeatedly demonstrated covariance of cortical thickness or grey matter (GM) volume over widespread, distributed, brain regions, and these structural covariance networks (SCNs) have also been shown to be highly heritable and to reflect differences in age and disease status (Alexander-Bloch, Giedd, & Bullmore, 2013).

It has been proposed that structural covariance between brain regions may reflect brain areas that are functionally co-active; reflect common patterns of maturational change - including shared long-term trophic influences; share patterns of gene co-expression (Romero-Garcia et al., 2018; Zielinski, Gennatas, Zhou, & Seeley, 2010) and are selectively vulnerable to specific brain health conditions (Seeley, Crawford, Zhou, Miller, & Greicius, 2009). Importantly, recent studies have demonstrated that SCNs closely mirror the functional ICNs revealed using resting-state functional magnetic resonance imaging [fMRI] (Kelly et al., 2012; Seeley et al., 2009) and co-degenerate in distinct human neurodegenerative conditions (Cauda et al., 2018; Seeley et al., 2009). This suggests that analysis of SCNs, while currently under-utilised to study brain networks in neurodevelopmental conditions, may be a particularly useful method for investigating alterations in brain network development in children and adolescents for whom the use of conventional fMRI approaches is especially challenging. In this study we chose to investigate specifically how SCNs associated with the insular cortex may be altered in children and adolescents with Tourette syndrome (TS) relative to a group of age- and gender-matched typically developing individuals.

Materials and Methods

This study was approved by an appropriate local ethical review committee. Written informed consent was acquired from all participants and where appropriate from their parents/caregivers. No part of the study procedures were pre-registered prior to the

research being conducted and none of the study analyses were pre-registered prior to the research being conducted. We report how we determined our sample size, all data exclusions (if any), all inclusion/exclusion criteria, whether inclusion/exclusion criteria were established prior to data analysis, all manipulations, and all measures in the study. Finally, The conditions of our ethics approval do not permit public archiving of individual MRI data or clinical biographical data.

Participants

In total 76 volunteers took part in this study. 39 had a confirmed diagnosis of TS (TS group) and 37 formed our control group (CS group) of age- and sex-matched, typically developing, individuals with no history of neurological disorders. The TS group were recruited either from the Child and Adolescent Psychiatry Clinic at the Queens Medical Centre in Nottingham or by advertising through the Tourettes Action charity or regional TS support groups. The CS group were recruited from local schools, by local advertising, and recruitment at science fairs. All volunteers were provided with a small inconvenience allowance for their participation.

After magnetic resonance imaging (MRI) the scans from twelve participants were found to be un-usable and data from these individuals were excluded from further analyses. The participants who remained included 28 individuals in the TS group (3 females; mean age 14.62 ± 3.4 years) and 36 controls (3 females; mean age 14.38 ± 3.2 years). 10 individuals with TS had a confirmed or suspected clinical diagnosis of a co-occurring neuropsychiatric condition in addition to their TS (attention deficit/hyperactivity disorder [ADHD] = 2; obsessive-compulsive disorder [OCD] =2; and autism spectrum disorder [ASD] = 6). 10 patients were medicated at the time of scanning. Details of the TS group are reported in Table 1.

Table 1 about here

Diagnosis, symptom severity and screening

Diagnosis of TS was confirmed by an experienced clinician. In addition, all participants underwent comprehensive screening for current symptoms of TS by a highly experienced and trained research nurse. Measures of the current severity of tics were obtained using the Yale Global Tic Severity Scale (YGTSS) (Leckman et al., 1989). The YGTSS is a semi-structured clinician-rated measure assessing the nature of motor and vocal tics present over the past week. The YGTSS is a commonly used

clinical assessment scale and has been found to have good psychometric properties (Leckman et al., 1989; Storch et al., 2005). It consists of three subscales: impairment rating, motor tic rating and vocal tic rating. Motor and vocal tic ratings are made up of the composite answers from questions relating to number, frequency, intensity, complexity and interference of tics reported in the previous week and observed during the interview. The current frequency and severity of premonitory sensory/urge phenomena [PU] was measured using the Premonitory Urge for Tics Scale (PUTS) (Woods, Piacentini, Himle, & Chang, 2005). The PUTS is a self-report measurement where items assess the intensity and frequency of PSP (on a scale of 1 – 4). 9 of the 10 items on the PUTS scaled were scored based on recommendation, and thus scores could range from 9 to 36 (Woods et al., 2005). Participants were screened for any indication of symptoms of ADHD, OCD and Autism using the Connors-3 Parent Report (Conners, 2008), Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) (Scahill et al., 1997) and Social Communication Questionnaire (SCQ) (Berument, Rutter, Lord, Pickles, & Bailey, 1999), respectively. Based on these measures, a further eight patients were categorised as being at high risk of having OCD and/or ADHD. All participants also completed the Wechsler's Abbreviated Scale of Intelligence (WASI-II) (Wechsler, 1999) used to assess intellectual ability. Two subtests were used (the verbal and performance subtests). Participants would be excluded from the study if their WASI score was < 70 (none were).

Image acquisition

Whole-brain, high-resolution, T1-weighted structural MRI brain images were acquired for each participant. Scanning was conducted at the Sir Peter Mansfield Imaging Centre (SPMIC), Nottingham, UK using a 3T Philips Achieva MRI scanner with a 32-channel SENSE head-coil and running a MPRAGE sequence (180 contiguous axial slices, 8.6 ms repetition time [TR], 4.0 ms echo time [TE], 256 x 224 x 180 matrix size, 1x1x1 mm raw voxel size, and a scan duration of 225 seconds). Prior to acquisition, participants were asked to lie as still as possible with their eyes open. Foam padding was added for extra stability and to reduce head movements. All participants wore also noise-cancelling headphones.

MR data pre-processing

Pre-processing of all MRI images was accomplished using SPM12 and the Computational Anatomy Toolbox (CAT12; http://www.neuro.uni-jena.de/cat/). First, raw structural T1-w MRI scans were oriented to have the origin lying on the AC-PC line using automated registration. Intensity normalisation, bias and noise-correction was conducted using the Spatially Adaptive Non-Local Means (SANLM) tool in CAT12 and the images were spatially normalised using DARTEL (affine and non-linear registration, [Ashburner, 2007]) to standard space and segmented into different tissue classes (grey matter, white matter and CSF). The images are then modulated - which involves scaling by the amount of contraction done during the normalisation step – to ensure that GM in the normalised images remains the same as in the raw images. Finally, all de-noised, normalised, segmented and modulated GM maps were smoothed using an 8-mm full-width at half maximum (FWHM) Gaussian kernel. Prior to pre-processing, all scans were visually inspected and any images with any visible artifact were excluded, in addition to using CAT12's retrospective QA framework. Total intracranial volume (TIV) was estimated from all subjects and subsequently used as a covariate in the final statistical model to control for any individual differences in brain volume. The GM maps were co-registered to the AAL2 atlas to enable an anatomically defined region-of-interest (ROI) for the right insular cortex to be generated.

Structural covariance

For the structural covariance analysis all high-quality re-oriented images were entered into the Computational Anatomy Toolbox (CAT12; http://www.neuro.uni-jena.de/cat/) using the 'Segment data' module and the default settings. The images were segmented into tissue types (GM, WM and CSF), spatially normalised to an MPRAGE images template in MNI space using DARTEL and affine registration (Ashburner, 2007), modulated, smoothed using an 8 mm FWHM Gaussian kernel, and corrected for covariates of no interest (age, IQ and TIV) as outlined above.

The anatomically-defined (AAL2 atlas) right insular cortex was initially isolated and three empirically defined regions-of-interest (ROI) were then defined based upon a K-means clustering analysis (Kelly et al., 2012) of the Z-transformed cross-subject Pearson correlation between individual voxels within the right insular region and motor tic and PUTS scores for the TS group. The K-means clustering analyses examined between 1-9 clusters and silhouette values were calculated and then used to examine

the separation distance between the resulting clusters and identify the best solution. Specifically, the silhouette value for each point is a measure of how similar that point is to points in its own cluster, compared to points in other clusters. High silhouette values indicate that a given point is a good match to its own cluster and a poor match to other clusters. Based upon the average silhouette values for the different solutions, a 3-cluster solution was selected as most appropriate identifying 3 separate regions of the right insula (See Figure 2a below): an anterior-dorsal region that was positively associated with PUTS scores; a posterior region that was negatively associated with motor tic severity scores; and an anterior-ventral that was uncorrelated with either measure. Based upon the results of this K-means analysis we selected the anterior-dorsal and posterior insula regions as ROI for the structural covariance network analyses.

Using the segmented whole-brain GM maps and a 'seed-to-voxel' approach, we computed the structural covariance between the mean GM values for voxels within our empirically-defined insular cortex (seed) ROIs and the GM values obtained for all voxels in the GM maps. This analysis yielded a covariance map for each group in which the value at each voxel reflected the cross-subject Pearson correlation coefficient between the mean GM value for the seed region (the respective insular cortex ROI) and the GM value at that particular voxel. The correlation coefficients were converted to Z scores using Fisher's r-to-Z transformation and the whole-brain Z(r) maps for each group were then statistically compared at group-level using the following equation

(1) Observed Z = (Z1 – Z2) / [1/(N1 - 3) + 1/(N2 - 3)]^0.5

where, for each voxel: Z1 is the Z(r) value for that voxel for the CS group; Z2 is the Z(r) value for that voxel for the TS group; N1 the number of participants in the CS group; and, N2 the number of participants in the TS group. The computed Z-maps were corrected for multiple comparisons using FDR [p-FDR < 0.05] (Benjamini & Hochberg, 1995) and a cluster threshold of $K_E \geq 100$ voxels was applied. Labelling of statistically significant clusters was accomplished using the Brain Anatomy Toolbox (Eickhoff et al., 2005). By definition, two regions 'covary' positively when increased GM values in one region is associated with increased GM values in another region. We defined *negative* covariance as an increase in GM values in one region that is associated with a reduction in GM values in a separate region. It is important to note that between

group differences in 'negative covariance' must be interpreted with caution. The analysis of MRI data in this study was conducted using the CAT12 (computational anatomy toolbox) running in SPM12. Some additional analyses were conducted using in-house Matlab code which cannot be placed in the public domain as they contain biographical and clinical data to which access is limited to the immediate research team by our ethical approval.

Results:

Preliminary analyses: group differences

An independent samples t-test confirmed that there was no significant difference in age between the TS (mean =14.62 ± 3.43) and CS (mean = 14.38 ± 3.23) groups ($t(62) = -0.28$, $p= 0.78$). However, further independent samples t-tests revealed that there was a significant between-group difference in total intracranial volume [TIV] (TS mean = 1544.97 ± 97.15; CS mean =1640.88 ± 158.71; $t(62)=2.81$, $p=0.007$), with controls having a higher TIV than individuals with TS, and a significant between-group difference in IQ (TS mean = 111.36 ± 13.93; CS mean = 118.58 ± 12.28; $t(62)=2.20$, $p=0.03$) with controls exhibiting a slightly higher average IQ. However, it should be noted that both groups exhibited above-average IQ scores. For the analysis the adjusted grey matter volumes were used after co-varying out age, IQ and TIV.

Relationship between clinical measures of tic severity and premonitory urge scores

To examine the association between tic severity (measured using the subscales of the YGTSS) and premonitory urges (measured using the PUTS), we conducted four separate Pearson correlation analyses. Scatterplots illustrating the results of these analyses are presented in Figure 1. The analyses revealed that premonitory urge (PUTS) scores were uncorrelated with: YGTSS impairment score ($p = 0.13$); YGTSS severity (i.e., motor + phonic tics) score ($p = 0.34$); YGTSS motor tic severity score ($p = 0.99$); YGTSS phonic tic severity score ($p = 0.58$).

Insert Figure 2 about here

Relationship between right insula grey matter volume and clinical measures

To examine the association between the right insular GM values and clinical measures of tic severity (measured using the YGTSS) and premonitory urges (measured using the PUTS), we conducted two separate converging analyses.

First, for each voxel in the right hemisphere insular cortex ROI (defined using the AAL2 atlas), we computed separate Pearson correlation coefficients for the grey matter volume (GMV) values across the TS group for that voxel and motor tic severity scores and PUTS scores. These coefficient values were then Z-transformed for further analyses using a K-means clustering approach as outlined above (see Methods section). This analysis yielded that a 3-cluster solution that separated right insula voxels into a posterior cluster negatively associated with motor tic severity scores, an anterior-dorsal cluster positively associated with PUTS scores, and an anterior-ventral cluster that was uncorrelated with either measure. The spatial distribution of these three clusters is illustrated in Figure 2a and a scatterplot illustrating the association of voxels within each cluster with the two clinical scales is presented in Figure 2b.

Second, to obtain converging evidence we identified the spatial location of voxels within the right insular cortex that were significantly correlated with YGTSS motor tic scores and PUTS scores. In each case the initial correlation threshold was set at $r = 0.3$ and the statistical threshold was then corrected for multiple comparisons using the false-discovery rate [FDR] with alpha set at $p < 0.05$ (Benjamini & Hochberg, 1995). This analysis revealed two clusters of voxels that were significantly associated with one or other of the clinical measures. Specifically, a posterior insula (Area IG 2) cluster was identified that was negatively associated with YGTSS motor tic severity scores (cluster size = 33 voxels, MNI coordinates: X=43, Y=-15, Z=11; $r = -0.49$, $p < 0.05$ FDR-corrected), and an anterior-dorsal (Area IG 2) cluster was identified that was positively associated with PUTS scores (cluster size = 44 voxels, MNI coordinates: X=43, Y=13, Z=1; $r = 0.43$, $p < 0.05$ FDR-corrected). The spatial distribution of these two clusters is illustrated in Figure 2c.

Structural covariance network analyses

We investigated whether there were statistically significant between-group differences in the structural covariance networks (SCNs) linked to the seed regions of the insula that were identified above using K-means clustering. As noted above (Methods), we did this by comparing the covariance maps for each group (in which the value at each voxel reflected the cross-subject Pearson correlation coefficient between the mean GM value for the seed region and the GM value at that particular voxel) and computing a Z statistic at each voxel (corrected for multiple comparisons using FDR

(Benjamini & Hochberg, 1995) and subject to a cluster threshold of $K_E \geq 100$ voxels) which represented the difference between the correlation coefficients .

Insert Table 2 about here

Anterior-dorsal insula

Analysis of the SCNs associated with the anterior-dorsal region of the right insular cortex that was identified by way of K-means clustering as being positively associated with premonitory urge (PUTS) scores revealed five clusters of 100+ voxels where structural covariance with the anterior-insular seed region was significantly increased in the TS group relative to the group of matched controls. Note that in this case, these clusters exhibited significantly larger correlations with the seed region than was the case for the typically developing controls (i.e., Z difference scores). These clusters included: the cerebellum; the periaqueductal grey region of the brain stem; and the occipital cortex. Further details are presented in Table 2a.

The analysis also revealed four clusters of 100+ voxels in which the structural covariance difference (Z) values were significantly negative in the TS group relative to the group of matched controls. Note that in this case, the cluster regions were either strongly negatively correlated with the seed region in the TS group and then positively correlated or uncorrelated with seed region in the control group; or else strongly positively correlated with the seed region in the control group and then negatively correlated or uncorrelated with seed region in the TS group. These clusters were located within the temporal lobe bilaterally, and in the right somatosensory cortex. Further details are presented in Table 2b.

Insert Table 3 about here

Posterior insula

Analysis of the SCNs associated with the posterior region of the right insular cortex that was identified by way of K-means clustering as being negatively associated with YGTSS motor tic severity scores revealed one cluster of 100+ voxels in which structural covariance with the posterior insula seed region was significantly increased in the TS group relative to the group of matched controls. This cluster was located in the left occipital cortex. Further details are presented in Table 3a.

The analysis also revealed 11 clusters of 100+ voxels in which the structural covariance difference (Z) values were significantly more negative in the TS group relative to the group of matched controls. Again, this is due to cluster regions being strongly positively correlated with the seed region in the control group and then negatively correlated or uncorrelated with seed region in the TS group, or strongly negatively correlated with the seed region in the TS group and positively correlated or uncorrelated with seed region in the control group. These clusters included: the left fusiform gyrus; bilateral temporal lobe (area TE); bilateral inferior parietal lobe (areas PF and PFm); right inferior frontal gyrus; right hippocampus; bilateral thalamus; and right cerebellum. Further details are presented in Table 3b.

Discussion

In this study we utilised voxel-based morphometry techniques together with 'seed-to-voxel' structural covariance network (SCN) mapping to investigate the putative role played by the right insular cortex in the generation of motor tics and the experience of PU in a relatively large group of young people TS. Our findings demonstrate the following. First, that clinical measures of tic severity and premonitory urge (PU) are uncorrelated with one another. Second, that motor tic severity and PU scores are associated with anatomically separate regions of the right insular cortex. Specifically, whereas motor tic severity scores are negatively associated with the GM volume values in a posterior region of the right insular cortex, premonitory urge (PUTS) scores are positively associated with GM volume values in a more anterior-dorsal region of the right insula. Third, when we examined the structural covariance networks (SCNs) associated with these two regions of the right insula cortex, we found that these networks differed in individuals with TS compared to a matched group of typically developing individuals. These results are discussed below.

Relationship between clinical measures of tic severity and PU

In contrast to recent studies, we found no statistically significant correlation between tic severity, as measured by the YGTSS, and estimates of premonitory urges measured using the PUTS. For example, in two recent papers by (Draper, Jackson, Morgan, & Jackson, 2016; Ganos et al., 2015) it was reported that tic severity scores and PUTS scores were positively associated with one another. However, it should also be noted

that other studies have previously reported that these measures were uncorrelated (e.g. (Ganos et al., 2012; Müller-Vahl, Riemann, & Bokemeyer, 2014; Steinberg et al., 2010).For this reason, it seems appropriate to conclude that tic severity and premonitory urges are not always strongly associated with one another and may reflect largely independent phenomena. This would be consistent with reports that: not all individuals with TS report experiencing PU (Cohen et al., 2013); tics can be observed during sleep; and the occurrence of tics - and an individual's ability to suppress them - may occur independently of the awareness of PU (Ganos et al., 2012). It is also consistent with the suggestion that the generation of tics and the genesis of PU in TS may be associated with different brain networks (Bronfeld et al., 2013; Conceicao et al., 2017; Jackson, Parkinson, Kim, et al., 2011; McCairn et al., 2013).

Anatomical separation of clinical measures of tic severity and PU within the right insular cortex

Anatomical and functional imaging studies in both humans and non-human primates have investigated the functional differentiation of the insular cortex. For example, Kurth and colleagues conducted a meta-analytic study of 1700+ functional brain imaging experiments, and identified four functionally distinct regions of the human insula: a posterior region associated with sensorimotor function; a ventral mid-insula region associated with olfacto-gustatory function; an anterior-ventral region associated with social-emotional function; and, an anterior-dorsal region associated with cognition (Kurth, Zilles, Fox, Laird, & Eickhoff, 2010). These authors also noted that the majority of functions tested overlapped within the anterior-dorsal insula and they proposed that this region might operate to allow the functional integration of brain networks linked to different functional systems, so as to construct a coherent experience of the world (Kurth et al., 2010). This proposal is consistent with the viewpoint expressed by Craig (2009) that the anterior insula may constitute the final stage of a hierarchical processing chain within the insular cortex (particularly the right insula) that is associated with the awareness of bodily states (particularly the right insular cortex) and the maintenance of homeostasis (Craig, 2009).

Kelly and colleagues carried out a parcellation of the right and left insular cortices based upon three distinct neuroimaging modalities: tasked-based meta-analytic functional connectivity analyses; resting-state (i.e., task-independent) functional connectivity; and, structural covariance analyses (Kelly et al., 2012). They

demonstrated that: each neuroimaging modality yielded a highly similar convergent parcellation of the insular cortex across multiple spatial scales; the parcellations were similar for left and right hemispheres; and, were consistent with previous parcellation schemes (Deen, Pitskel, & Pelphrey, 2011; Kurth et al., 2010).

The above functional parcellation of the human insula is also consistent with previous parcellation of the posterior, anterior-basal and anterior-dorsal insula in non-human primates based upon both cytoarchitectonic differences (Augustine, 1996; Mesulam & Mufson, 1982) and differing patterns of connectivity (Mesulam & Mufson, 1985). Specifically, whereas the anterior insular cortex is densely connected with limbic, temporal, and frontal cortical areas, the posterior insula is by contrast densely connected with primary and secondary sensorimotor cortical areas (Mesulam & Mufson, 1985).

In the current study we used K-means clustering to separate the right insular cortex into three regions based upon the relationship between the GM volume values of the voxels in these regions and clinical scores measuring motor tic severity (YGTSS) and premonitory urges (PUTS). We reported that a posterior region of the right insula was negatively associated with motor tic severity scores and uncorrelated with premonitory urge score. This finding is consistent with the insula parcellation studies outlined above that have previously reported that the posterior region of the insula is associated with sensorimotor function (Augustine, 1996; Kelly et al., 2012; Kurth et al., 2010; Mesulam & Mufson, 1982; Mesulam & Mufson, 1985). By contrast, we reported that GM volume values within the anterior-dorsal region of the right insula was positively associated with premonitory urge scores and uncorrelated with motor tic severity. This finding is consistent with previous parcellation studies indicating that the anterior-dorsal region of the insula may be associated with cognitive function (Kelly et al., 2012; Kurth et al., 2010) and particularly with the proposal that this region may play a role integrating different functional systems to produce a coherent experience of the world (Kurth et al., 2010) and with awareness of bodily sensations (Craig, 2009). Our finding is also entirely consistent with our previous demonstration that the right anterior-dorsal insula is associated with awareness of the urge-for-action across a number of behavioural domains (e.g., yawning, swallowing, micturition) including the urge-to-tic in Tourette syndrome (Jackson, Parkinson, Kim, et al., 2011), and with our proposal that the anterior-dorsal insula participates in a neural circuit that: represents body sensations; generates urges-for-action; selects an appropriate action based upon a computation of

the likely outcomes of that action; and determines whether the circumstances giving rise to the urge-for-action have been satisfactorily resolved (Jackson, Parkinson, Kim, et al., 2011) [for a related proposal see Lerner et al. (2009)].

Between-group differences in structural covariance networks (SCNs) associated with the right insula

As noted above, previous studies have reported that structural covariance networks (SCNs) closely mirror the intrinsic functional connectivity networks identified using resting-state fMRI (e.g., (Kelly et al., 2012; Seeley et al., 2009), and it has been proposed that SCNs likely reflect brain areas that are functionally co-active and subject to common patterns of maturational change and gene co-expression (Romero-Garcia et al., 2018; Zielinski et al., 2010). Such SCNs are selectively vulnerable to specific brain health conditions (Seeley et al., 2009) and co-degenerate in specific neurodegenerative conditions (Cauda et al., 2018; Seeley et al., 2009).

Craig (2003, 2009) has proposed that sensory information contributing to a representation of the current physiological state of our body is processed in the posterior insula, and then passed to the anterior insula where it may become accessible to conscious awareness, leading to subjective experience of bodily states. Importantly, (Craig, 2009) has argued for a functional asymmetry between the right and left anterior insula, with the right anterior insula playing a more prominent role in arousal, negative effect, and awareness of uncomfortable bodily sensations. Consistent with this proposal, we and others have confirmed that the right anterior insula is particularly associated with urges-for-action (Berman, Horovitz, Morel, & Hallett, 2012; Jackson, Parkinson, Kim, et al., 2011; Lerner et al., 2009), and specifically with the urge-to-tic in Tourette syndrome (Jackson, Parkinson, Kim, et al., 2011). For this reason, in the current study we chose to investigate specifically whether the SCNs associated with the right anterior-dorsal and right posterior insular regions would differ between a group of individuals with Tourette syndrome with a history of motor tics and premonitory urges and a matched group of typically developing individuals who present without either tics or premonitory urges.

<u>Right anterior-dorsal insula seed</u>

Areas of increased structural covariance in TS

Our analyses of the SCNs associated with the right anterior-dorsal 'seed' region revealed that three areas exhibited significantly increased structural covariance with the right anterior-dorsal seed in the TS group relative to a group of matched typically developing individuals. These were the occipital cortex bilaterally, the left and right cerebellum (lobules VIIb, VIIIa, IX), and the brainstem (periaqueductal grey).

Occipital cortex The finding of increased structural covariance of the right insula cortex with the occipital cortex bilaterally is consistent with previous findings that the occipital cortex is activated immediately prior to and during tic execution (Neuner et al., 2014) and its activity is strongly associated with suppression of the urge-to-blink (Berman et al., 2012; Lerner et al., 2009). In particular it has been reported previously that during blink suppression the occipital cortex is de-activated relative to periods of spontaneous blinking although the functional significance of this finding requires clarification (Lerner et al., 2009). By contrast, there is accumulating evidence to implicate both the cerebellum and the periaqueductal grey (PAG) in Tourette syndrome and the pathophysiology of tics.

Cerebellum Previous structural MRI (VBM) studies have reported alterations in cerebellar morphology in individuals with TS, involving crus I and lobules VI, VIIB and VIIIA (Tobe et al., 2010). Furthermore, that study demonstrated that reduced GM volumes were associated with increased tic severity, thereby implicating the cerebellum in the generation of tics in TS. This suggestion is also supported by functional MRI studies of brain activity immediately prior to and during tic generation, that have repeatedly demonstrated that the cerebellum is activated bilaterally immediately before and during tic execution (Bohlhalter et al., 2006; Neuner et al., 2014). Further compelling evidence for cerebellar involvement in the generation of tics comes from a recent study using an animal model of tic generation based upon striatal disinhibition (McCairn et al., 2013). In this model, tic-like movements are produced following localised injections of a GABA-antagonist into the striatum of the animal. McCairn and colleagues conducted multisite, multielectrode electrophysiological recordings of single-unit activity and local field potentials (LFPs) from a number of brain areas including: the cerebellum; basal ganglia; and primary motor cortex, while the animal exhibited tic-like movements. A key finding from their study was that, while changes in LFPs preceded the occurrence of tic-like movements in many of the brain areas recorded from with different latencies, the occurrence of tic-like movements was most closely associated with LFPs in the primary motor cortex and cerebellum (McCairn et

al., 2013). The authors propose that the primary motor cortex and in particular the cerebellum may act as a gate to release overt tic-like movements. In a study investigating GABA-A receptor binding in individuals with TS it was shown that GABA-A receptor binding was significantly increased bilaterally in the cerebellum (and, incidentally, within the PAG) in individuals with TS. As Purkinje cells are the sole output of the cerebellar cortex and function to inhibit their cortical targets, it is likely that any alteration in the number or the efficacy of Purkinje cells might lead to changes in the inhibitory output of the cerebellum in TS and thereby contribute to the occurrence of tics.

Periaqueductal grey (PAG) The mid-brain PAG nuclei are thought to participate in a range of neurobiological functions that include modulation of pain and anxiety responses and the initiation and control of stereotyped movements. In particular, the PAG is thought to play a key role within Newman's (1999) social behaviour network (SBN) which consists of an interconnected set of subcortical nuclei that regulate a range of male and female behaviours that include mating behaviour, aggressive behaviours, and parenting. Within the SBN, the PAG is thought to function to initiate stereotyped actions through the activation of downstream brainstem and spinal circuits (Newman, 1999). This may include the activation of brainstem circuits used to initiate contextually appropriate social signals through stereotyped facial movements and vocalisations (Albin, 2018).

The PAG has been consistently implicated in the generation of motor and vocal tics in TS (Albin, 2018; Devinsky, 1983) and neuroimaging studies have confirmed structural and functional alterations of the PAG in TS. Garraux and colleagues conducted a voxel-based morphology study of individuals with TS compared to a group of typically developing controls. They reported that GM volume within a mid-brain region that the authors identified as the left PAG was significantly increased in the TS group relative to controls (Garraux et al., 2006). In addition, a positron emission tomography study that examined GABA-A binding abnormalities in TS demonstrated that there was increased GABA-A binding within the left PAG in individuals with TS (Lerner et al., 2012). Once again it is likely that alteration in the number or the efficacy of inhibitory GABA-ergic cells within the PAG in TS may contribute to the occurrence of tics. Particularly as this structure has been linked to the modulation and initiation of stereotyped facial movements and vocalisations.

Areas of reduced structural covariance in TS

Our analyses of the SCNs associated with the right anterior-dorsal insula 'seed' region also revealed two cortical regions with significantly *decreased* structural covariance values relative to matched controls. These areas included the temporal lobe bilaterally and the right somatosensory cortex. For the reasons outlined above, decreases in structural covariance values must be interpreted with caution. In this case the observed differences resulted from the fact that while the GM values for the temporal lobe and somatosensory cortical areas were strongly positively associated with those of the anterior-dorsal insula seed area for the typically developing control group, these same areas were either uncorrelated or negatively correlated with the insular seed region in the TS group.

Temporal lobe Anatomical and functional studies have previously demonstrated that the anterior insula is densely connected to temporal lobe regions (e.g., Mesulam and Mufson, (1985)). Given that SCNs closely mirror the functional intrinsic connectivity networks revealed using resting-state fMRI (Kelly et al., 2012), and the proposal that structural covariance between brain regions reflects brain areas that are functionally co-active and share common patterns of maturational change (Romero-Garcia et al., 2018), we might speculate that our finding of reduced structural covariance between the anterior insular and temporal lobe might reflect decreased functional connectivity between these regions in TS. The temporal lobes have been reported in previous structural brain imaging studies of TS to be an area of significant GM thinning (Sowell et al., 2008), and GM structure in this area was found to be correlated with tic severity (Worbe et al., 2010), and in functional brain imaging studies it was reported that the temporal lobes were abnormally connected with the basal ganglia (Worbe et al., 2012).

Sensorimotor cortex By contrast, structural and functional alterations of sensorimotor cortex in TS, including patterns of connectivity to this region, are a very prominent feature of TS (e.g., (Bohlhalter et al., 2006; Draganski et al., 2010; Draper et al., 2016; Neuner et al., 2014; Sowell et al., 2008; Thomalla et al., 2009; Tinaz et al., 2014; Worbe et al., 2010). Key findings include that the sensorimotor cortex is most often associated with: reductions in GM volume and/or cortical thinning in TS (Draper et al., 2016; Sowell et al., 2008; Worbe et al., 2010); increased structural connectivity in adults with TS (Thomalla et al., 2009; Worbe et al., 2015); and increased functional connectivity in adults with TS (Tinaz et al., 2014). Furthermore, in many cases these

alterations in sensorimotor morphology (e.g. Sowell et al. (2008), Draper et al. (2016)), structural connectivity (e.g., Worbe et al. (2015)), and functional connectivity (Worbe et al., 2012) were associated with increased tic severity.

Right posterior insula seed

Our analyses of the SCNs associated with the right posterior insula 'seed' region revealed that only one area, the occipital cortex, with significantly increased structural covariance in the TS group relative to a group of matched typically developing individuals. By contrast, our analysis revealed a number of areas with significantly *decreased* structural covariance values relative to matched controls. These areas included: bilateral temporal (area TE) and parietal (areas PF and PFm) lobes; bilateral thalamus; left occipital cortex; right inferior frontal cortex; and right hippocampus.

Areas of increased structural covariance in TS

The finding of increased structural covariance of the right posterior insula cortex with the occipital cortex bilaterally is consistent with previous findings that the occipital cortex is activated immediately prior to and during tic execution (Neuner et al., 2014), and is associated with suppression of the urge-to-blink (Berman et al., 2012; Lerner et al., 2009).

Areas of reduced structural covariance in TS

Temporal lobes As noted above, anatomical and functional studies have demonstrated that the insula is connected to temporal lobe regions (e.g., Mesulam and Mufson (1985)) and reduced structural covariance between the posterior insular and temporal lobe may reflect decreased functional connectivity between these regions in TS. However, as was noted above, temporal lobe regions do not feature prominently in neuroimaging investigations of structural abnormalities in TS (e.g. Worbe et al. (2010), Draganski et al. (2010); Greene et al. (2017)) or in studies that have investigated the functional antecedents of tics in TS (e.g., Bohlhalter et al. (2006); Neuner et al. (2014)), so the functional significance of this finding remains to be clarified.

Supramarginal gyri The supramarginal gyrus region of the inferior parietal cortex is part of the somatosensory association cortex and has long been associated with representation of bodily space (Critchley, 1953), and in particular with maintaining and updating a current representation of the body schema (Parkinson, Condon, & Jackson,

2010; Parkinson et al., 2011; Pellijeff, Bonilha, Morgan, McKenzie, & Jackson, 2006). Previous neuroimaging studies of cortical morphology in TS have reported reduced cortical thickness within the supramarginal gyrus region of the parietal lobe and that cortical thickness in this area is associated with tic severity (Sowell et al., 2008; Worbe et al., 2010) particularly for complex tics (Worbe et al., 2010). In addition, functional brain imaging studies that have investigated the neural antecedents of tics in TS have demonstrated that the supramarginal gyrus is activated immediately before and during the generation of tics in TS (Bohlhalter et al., 2006; Neuner et al., 2014). In the current study we observe that while GM volume in the supramarginal gyrus is strongly positively correlated with GM volume in the posterior insula seed region for the matched control group, it is non-significantly negatively correlated (-0.29) in the TS group.

Right inferior frontal lobe In the current study we demonstrated that structural covariance between the right inferior frontal cortex and the right posterior insula is significantly reduced in the TS group relative to matched controls. The inferior frontal cortex, particularly within the right hemisphere, is generally thought to play major role in the inhibitory control of other brain areas, including cognitive control over motor outputs (for reviews see Aron (2007), Munakata et al. (2011)). Importantly, in the context of TS, it has been proposed that structural and functional alterations in this region may be responsible for the attenuation or remission of tic symptoms that are often observed during adolescence (Plessen, Bansal, & Peterson, 2009). Consistent with this proposal, Jackson and colleagues demonstrated in a multi-modal brain imaging study of TS, that the microstructure of the white-matter (WM) tracts leading to inferior frontal cortex was altered in adolescents with TS relative to matched controls, and that performance on an inhibitory control task was strongly predicted by the WM microstructure of these same tracts (Jackson, Parkinson, Jung, et al., 2011).

Bilateral thalamus We demonstrated that structural covariance between the right posterior insula and the thalamus is reduced in the TS group relative to matched controls. Structural and functional alterations of thalamic nuclei in TS, including changed patterns of functional connectivity (Worbe et al., 2012), are a prominent feature of TS (Bohlhalter et al., 2006; Makki, Behen, Bhatt, Wilson, & Chugani, 2008; Miller et al., 2010; Neuner et al., 2014). Key findings include: that the thalamus is activated immediately before and during the generation of tics in TS (Bohlhalter et al., 2006; Neuner et al., 2014); that the GM volume of the thalamus is significantly

increased in TS (Greene et al., 2017; Miller et al., 2010); that GABA-A binding is decreased bilaterally within the thalamus in individuals with TS relative to matched controls (Lerner et al., 2012); that structural connectivity of the thalamus is altered in children with TS (Makki et al., 2008; Sigurdsson, Pépés, Jackson, Draper, Morgan, Jackson, 2018); and, that functional connectivity to the thalamus is increased in adults with TS and is negatively correlated with severity (Worbe et al., 2012).

Right hippocampus In the current study we showed that structural covariance between the right posterior insula and the right hippocampus was reduced in the TS group relative to matched controls. More specifically, whereas the GM values in the seed region were significantly negatively correlated with the right hippocampal GM values in the TS group, they were non-significantly positively correlated for the control group. As is the case for several of the areas identified above, structural and functional alterations to the hippocampus have previously been reported in TS. Specifically, increases in hippocampal and associated frontal cortex GM volume have been demonstrated in children with TS and linked to increased cognitive control over tic expression (Peterson et al., 2007). Similarly, hippocampal GM volume has been shown to be associated with tic severity (Ludolph et al., 2006) and obsessive-compulsive symptomology in TS (Peterson et al., 2007; Worbe et al., 2010). However, the direction of morphological alterations to hippocampal volume have not always been consistent. Thus, Worbe and colleagues reported a large study of adults with TS in which right hippocampal GM volumes were reduced relative to matched controls, particularly for those presenting with co-occurring OCD, and it was suggested that alterations in hippocampal volume may contribute to impairment in the regulation of aversive emotional states such as fear and anxiety Worbe et al. (2010).

In addition to morphological changes involving the hippocampus, previous studies have demonstrated decreased GABA-A binding in the right hippocampus in TS (Lerner et al., 2012) and also altered functional connectivity involving the hippocampus (Worbe et al., 2012). Specifically, whereas for typically developing individuals the hippocampus is identified a key hub within the limbic cortico-striatal-thalamic cortical network, no hubs were identified for the group of individuals with TS. Note that within a network, hubs are nodes that facilitate the efficient integration of information throughout separated parts of the network.

Limitations of this study

Throughout this paper we have assumed that structural covariance networks (SCNs) closely mirror the intrinsic connectivity networks (ICNs) measured and robustly demonstrated using resting-state fMRI. This assumption is based upon a number of studies, referred to above, that have directly compared seed-based SCNs and ICNs and have demonstrated their very close alignment, particularly with reference to the connectivity of the insular cortex (e.g., Kurth et al., 2010; Kelly et al., 2012). However, given that our study investigates SCNs in adolescents and young adults with TS – a period during which the brain is known to undergo considerable maturation – it is possible that during this period there could be differences in the maturation structural and functional brain networks and that these may be exacerbated by the presence of TS. For this reason, as we not that as we have not directly measured functional brain connectivity in this study, we advocate caution in drawing strong conclusions based upon our findings on functional brain connectivity differences in TS.

Conclusion

In summary, in the current study we used voxel-based morphometry together with 'seed-to-voxel' structural covariance mapping to investigate the role played by the right insular cortex in the generation of motor tics and the experience of PU in a group of young people TS. We demonstrated that in contrast to previous reports, clinical measures of tic severity and premonitory urge (PU) were uncorrelated, indicating that tic severity and premonitory urges are not always strongly associated with one another and may reflect largely independent phenomena. Consistent with this view we also showed that motor tic severity and PU scores were associated with anatomically separate regions of the right insular cortex. Specifically, motor tic severity scores were associated with the GM volume values in a posterior region of the right insular while premonitory urge (PUTS) scores were associated with GM volume values in a more anterior-dorsal region of the right insular cortex. Finally, we examined the structural covariance networks associated with each of these two regions of the right insula and demonstrated that these networks differed in individuals with TS compared to a matched group of typically developing individuals. This suggests that analysis of SCNs, while currently under-utilised in the study brain networks in neurodevelopmental conditions, we suggest that analysis of structural covariance networks may be a particularly useful method for investigating alterations in brain network development

in children and adolescents, in particular for those for whom the use of conventional fMRI approaches is especially challenging.

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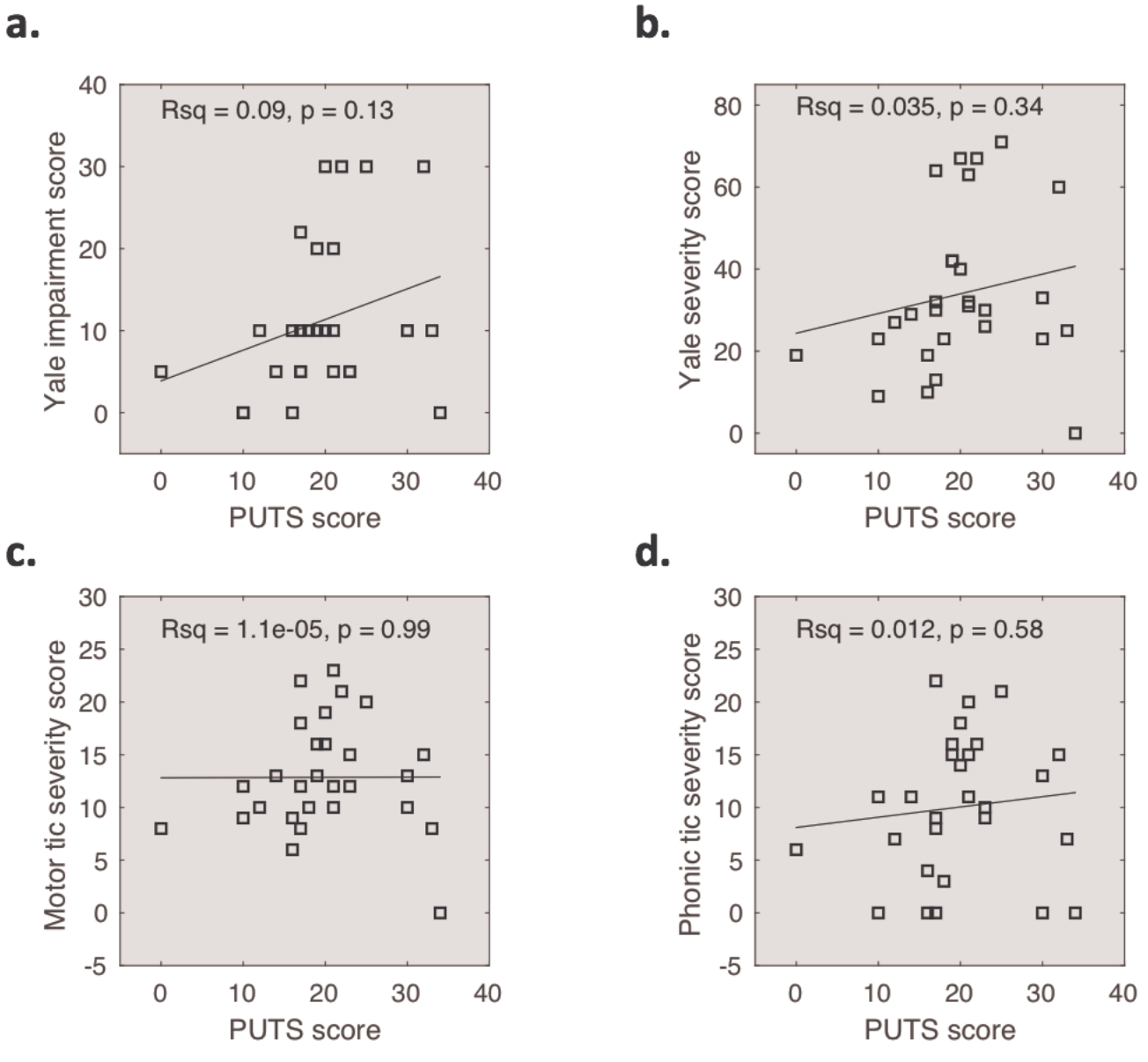
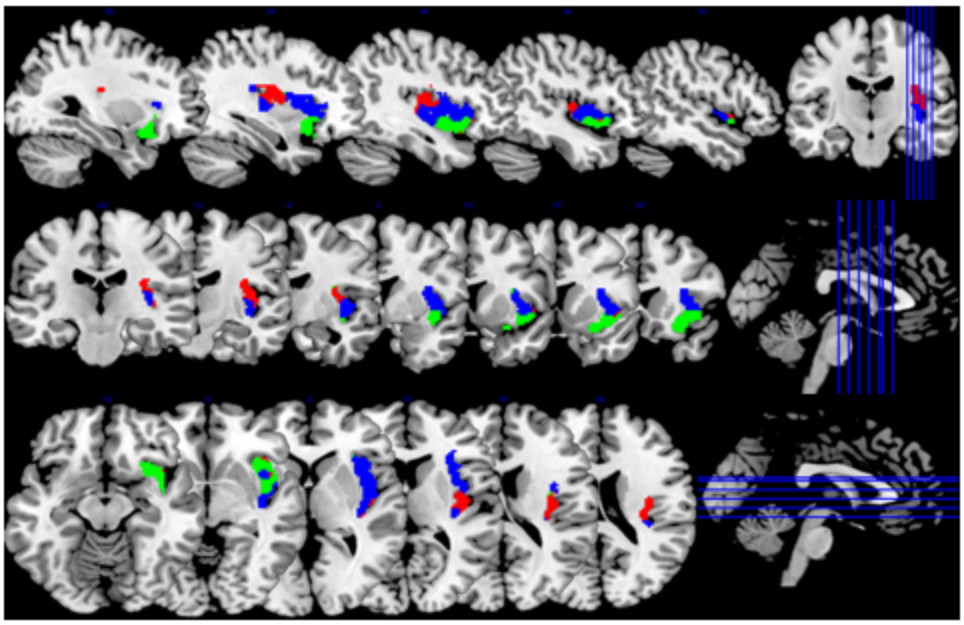
Figure captions

Figure 1: Illustrates associations between clinical measures. **a.** Scatterplot showing the association between tic severity, measured using the YGTSS impairment score, and premonitory urges, measured using the PUTS. **b.** Scatterplot showing the association between tic severity, measured using the YGTSS severity score, and premonitory urges, measured using the PUTS. **c.** Scatterplot showing the association between tic severity, measured using the YGTSS motor tic score, and premonitory urges, measured using the PUTS. **d.** Scatterplot showing the association between tic severity, measured using the YGTSS phonic tic severity score, and premonitory urges, measured using the PUTS.

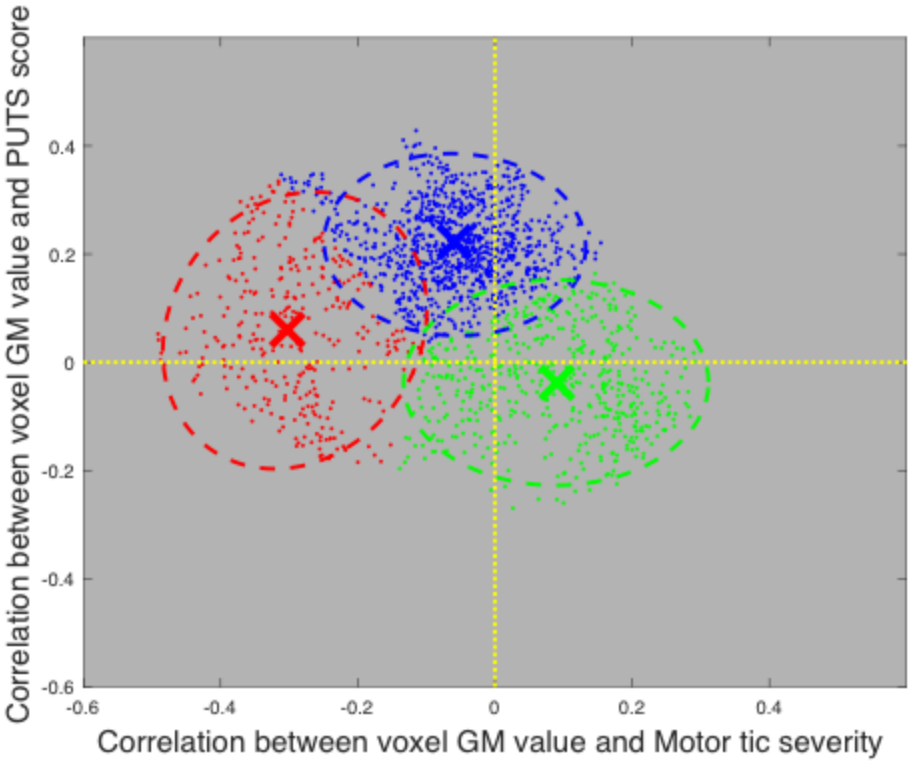
Figure 2: **a.** Illustrates the results of the K-means clustering analysis which resulting in the partitioning of the right insular cortex into three separate regions based upon the association between the GM value of each voxel with motor tic severity and PUTS score. These regions consist of a posterior region (red) negatively correlated with motor tic severity, an anterior-dorsal region (blue) positively correlated with premonitory urges, and an anterior-ventral region (green) that was uncorrelated with either. **b.** Scatterplot showing the association for each individual voxel between that voxel's GM value and motor tic severity and premonitory urge score and the results of the K-means analysis into the three clusters outlined above. The 'X' symbol reflects the centre-of-mass and the broken circle represents the 95% confidence interval ellipse for each cluster. c. Illustrates right insula voxels that are significantly negatively correlated with motor tic severity score (red) and significantly positively correlated with premonitory urge (blue) score.


a.
Rsq = 0.09, p = 0.13
Yale impairment score
PUTS score
b.
Rsq = 0.035, p = 0.34
Yale severity score
PUTS score
c.
Rsq = 1.1e-05, p = 0.99
Motor tic severity score
PUTS score
d.
Rsq = 0.012, p = 0.58
Phonic tic severity score
PUTS score

a.



b.


Correlation between voxel GM value and PUTS score
0.4
0.2
0
-0.2
-0.4
-0.6
-0.6
-0.4
-0.2
0
0.2
0.4
Correlation between voxel GM value and Motor tic severity

c.

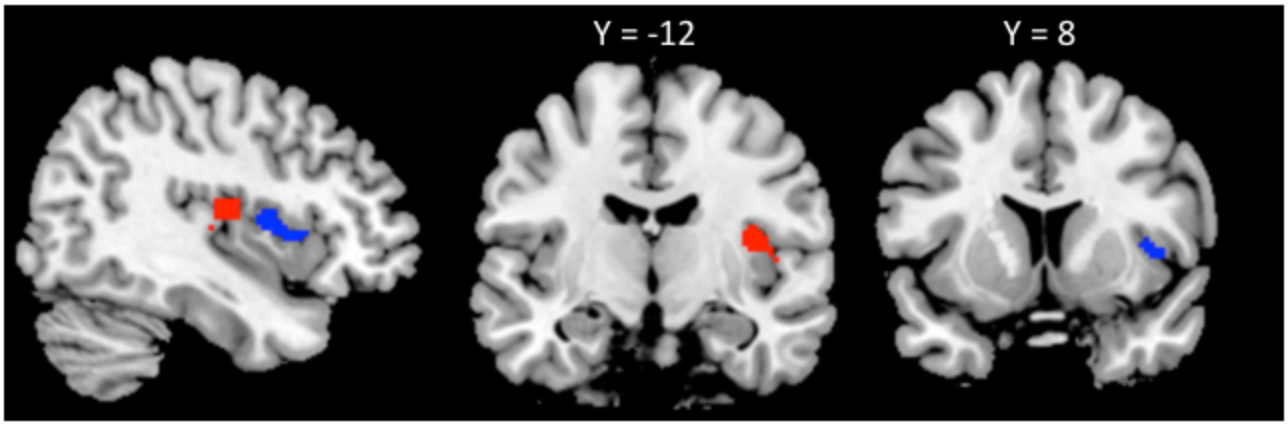

Y = -12
Y = 8

Table 1. Details of TS participants

ID	Gender	Age	IQ	TIV	YGSS	Motor	Phonic	Impairment	PUTS	Comorbidity
TS013	M	19.29	135	1672.99	32/100	18/25	9/25	5/50	17/36	-
TS030	M	17.03	103	1534.19	23/100	12/25	11/25	0/50	10/36	-
TS031	M	20.12	111	1393.68	67/100	21/25	16/25	30/50	22/36	-
TS034	M	16.56	118	1572.93	0/100	0/25	0/25	0/50	34/36	-
TS043	M	14.09	123	1521.32	63/100	23/25	20/25	20/50	21/36	-
TS048	M	16.82	118	1560.25	19/100	9/25	0/25	10/50	16/36	-
TS058	M	10.04	96	1488.58	10/100	6/25	4/25	0/50	16/36	ADHD
TS061	M	11	99	1604.86	26/100	12/25	9/25	5/50	23/36	-
TS066	M	16.16	102	1579.58	42/100	16/25	16/25	10/50	19/36	OCD
TS069	M	13.47	133	1678.11	19/100	8/25	6/25	5/50	9/36	-
TS071	M	15.61	118	1545.65	31/100	10/25	11/25	10/50	21/36	-
TS075	M	16.25	112	1602.50	30/100	12/25	8/25	10/50	17/36	-
TS084	F	22.78	126	1495.58	25/100	8/25	7/25	10/50	33/36	ASD, ANXIETY, ASPERGERS, DEPRESSION
TS087	M	12.79	119	1509.92	30/100	15/25	10/25	5/50	23/36	-
TS088	M	13	109	1527.10	71/100	20/25	21/25	30/50	25/36	-
TS094	F	18.75	116	1292.99	32/100	12/25	15/25	5/50	21/36	ASD
TS095	M	14.49	115	1452.84	23/100	13/25	0/25	10/50	30/36	-
TS096	M	8.61	126	1544.50	23/100	10/25	3/25	10/50	18/36	ADHD
TS097	M	15.28	112	1494.73	33/100	10/25	13/25	10/50	30/36	OCD
TS101	M	10.48	129	1699.19	40/100	16/25	14/25	10/50	20/36	ASD
TS102	M	15	111	1552.28	13/100	8/25	0/25	5/50	17/36	-
TS103	M	18.96	113	1510.52	64/100	22/25	22/25	22/50	17/36	-
TS105	M	11.35	115	1596.10	42/100	13/25	15/25	20/50	19/36	-
TS110	M	13.22	85	1631.39	9/100	9/25	0/25	0/50	10/36	ASD
TS113	M	13.1	92	1580.11	67/100	19/25	18/25	30/50	20/36	ASD
TS119	M	9.67	107	1470.05	29/100	13/25	11/25	5/50	14/36	-
TS127	F	13.01	75	1355.28	27/100	10/25	7/25	10/50	12/36	ASD
TS139	M	12.36	100	1710.96	60/100	15/25	15/25	30/50	32/36	-

Abbreviations. Motor, phonic and impairment scores were measured using the YGTSS (Yale Global Tic Severity Scale); PUTS (Premonitory Urge for Tics Scale). TIV (Total intercranial volume). ADHD (attention deficit/hyperactivity disorder); ODC (obsessive compulsive disorder); ASD (autism spectrum disorder).YGTSS scores were measured on the day of testing.

Table 2a: *Regions in which the structural covariance values for the anterior-dorsal Insula cluster were significantly larger in individuals with TS relative to those for matched controls (Z >= 2.3, p < 0.05 FDR-corrected, K (cluster-size >= 100).*

							MNI coordinates		
Cluster	Size	z-value	CS r(Z)	TS r(Z)	Region	Hemisphere	X	Y	Z
1	1722	3.65	-0.45	0.5	Cerebellum (Lob IX)	L/R	2	-52	-47
2	156	3.36	0.11	0.55	Occipital cortex (V3)	L/R	-7	-94	35
3	147	2.6	0.07	0.51	Cerebellum (VIIIa, VIIb)	L	-40	-41	-45
4	137	2.78	-0.17	0.48	Occipital cortex (V4)	R	39	-83	-1
5	106	3.2	-0.39	0.41	Brainstem (PAG)	L/R	-3	-25	-17

Table 2b: *Regions in which the structural covariance values for the anterior-dorsal Insula cluster were significantly lower in individuals with TS relative to those for matched controls (Z >= 2.3, p < 0.05 FDR-corrected, K (cluster-size >= 100).*

							MNI coordinates		
Cluster	Size	z-value	CS r(Z)	TS r(Z)	Region	Hemisphere	X	Y	Z
1	2476	4.76	0.52	-0.6	Temporal lobe	L	-65	-27	-23
2	1426	3.88	0.47	-0.55	Temporal lobe	R	71	-7	-17
3	637	3.68	0.64	-0.22	Somatosensory cortex (3b,1)	R	57	-19	39
4	392	3.33	0.53	-0.3	Temporal pole	R	47	11	-21

Table 3a: *Regions in which the structural covariance values for the posterior Insula cluster were significantly larger in individuals with TS relative to those for matched controls (Z >= 2.3, p < 0.05 FDR-corrected, K (cluster-size >= 100).*

							MNI coordinates		
Cluster	Size	z-value	CS r(Z)	TS r(Z)	Region	Hemisphere	X	Y	Z
1	101	3.76	0.52	-0.6	Calcarine gyrus	L	16	-90	17

Table 3b: *Regions in which the structural covariance values for the posterior Insula cluster were significantly lower in individuals with TS relative to those for matched controls (Z >= 2.3, p < 0.05 FDR-corrected, K (cluster-size >= 100).*

							MNI coordinates		
Cluster	Size	z-value	CS r(Z)	TS r(Z)	Region	Hemisphere	X	Y	Z
1	804	3.53	0.53	-0.25	Fusiform gyrus	L	-35	-67	-11
2	641	3.25	0.56	-0.23	Temporal lobe (TE)	L	-69	-23	-7
3	495	3.6	0.57	-0.29	Inf. parietal lobe (PF)	R	51	-33	39
4	347	3.33	0.33	-0.45	Cerebellum (VIIa, Crus1)	R	-15	-93	-17
5	204	3.32	0.36	-0.43	Inf. temporal gyrus	R	63	-13	-5
6	187	3.08	0.73	0.1	Inf. frontal gyrus	R	33	25	-15
7	168	2.83	0.33	-0.39	Inf. parietal lobe (PFm)	L	-47	-57	45
8	156	3.6	0.51	-0.3	Thalamus	L/R	-1	-23	21
9	153	2.72	0.16	-0.51	Hippocampus	R	35	-37	3
10	130	2.8	0.56	-0.05	Insular cortex	R	43	5	-3
11	122	3.11	0.31	-0.41	Fusiform gyrus	L	-34	-25	-27